PETER J. MORRIS
STUART J. KNECHTLE

KIDNEY TRANSPLANTATION

Principles and Practice

SIXTH EDITION

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Preface to the First Edition

Renal transplantation is now an accepted treatment of patients in end-stage renal failure. A successful transplant restores not merely life but an acceptable quality of life to such patients. The number of patients in end-stage renal failure in the Western World who might be treated by hemodialysis and transplantation is considerable and comprises some 30-50 new patients/million of population. Unfortunately in most, if not all, countries the supply of kidneys for transplantation is insufficient to meet the demand. Furthermore, hemodialysis facilities are usually inadequate to make up this deficit so that many patients are still dying of renal disease who could be restored to a useful and productive life. Nevertheless, few of us would have imagined even 10 years ago that transplantation of the kidney would have become such a relatively common procedure as is the case today, and indeed well over 30,000 kidney transplantations have been performed throughout the world.

Transplantation of the kidney for the treatment of renal failure has been an attractive concept for many years. As long ago as 1945, three young surgeons at the Peter Bent Brigham Hospital in Boston, Charles Hufnagel, Ernest Landsteiner and David Hume, joined the vessels of a cadaver kidney to the brachial vessels of a young woman who was comatose from acute renal failure due to septicemia. The kidney functioned for several days before it was removed, and the woman regained consciousness. Shortly afterwards, the woman's own kidneys began to function and she made a full recovery. The advent of the artificial kidney at that time meant that this approach to the treatment of acute renal failure was no longer necessary, but attention was soon given to the possibility of transplanting kidneys to patients with end-stage renal failure who were requiring dialysis on the newly developed artificial kidney to stay alive.

Although the first experimental kidney transplants in animals were reported first in Vienna by Dr. Emerich Ulmann in 1902 and then in 1905 by Dr. Alexis Carrel in the United States, the problem of rejection was not mentioned by either author. Later in 1910, Carrel did discuss the possible differences between an autograft and a homograft. The vascular techniques developed by Carrel for the anastomosis of the renal vessels to the recipient vessels are still used today. But in 1923, Dr. Carl Williamson of the Mayo Clinic clearly defined the difference between an autografted and homografted kidney and even published histological pictures of a rejecting kidney. Furthermore, he predicted the future use of tissue matching in renal transplantation.

> It is unfortunate that the lower animals, such as the dog, do not possess a blood grouping like that of man. In the future it may be possible to work out a satisfactory way of determining the reaction of the recipient's blood serum or tissues to those of the donor and the reverse; perhaps in this way we can obtain more light on this as yet relatively dark side of biology.

The recognition that allogeneic tissues would be rejected was further established in later years by Drs. Gibson and Medawar, who treated burn patients with homografts in Glasgow during the Second World War. Indeed, it was the crash of a bomber behind the Medawars' house in Oxford during the early years of the war that first stimulated his interest in transplantation, especially of skin.

In his address at the opening of the new Oxford Transplant Unit in 1977, Sir Peter Medawar recounted this event.

> Early in the war, an R.A.F. Whitley bomber crashed into a house in North Oxford with much serious injury and loss of life. Among the injured was a young man with a third degree burn extending over about 60% of his body. People burned as severely as this never raised a medical problem before: they always died; but the blood transfusion services and the control of infection made possible by the topical use of sulphonamide drugs now made it possible for them to stay alive. Dr. John F. Barnes, a colleague of mine in Professor H. W. Florey's School of Pathology, asked me to see this patient in the hope that being an experimental biologist I might have some ideas for treatment. With more than half his body surface quite raw, this poor young man was a deeply shocking sight; I thought of and tried out a number of ingenious methods, none of which worked, for ekeing out his own skin for grafting, trying to make one piece of skin do the work of ten or more. The obvious solution was to use skin grafts from a relative or voluntary donor, but this was not possible then and it is not possible now.
>
> I believe I saw it as my metier to find out why it was not possible to graft skin from one human being to another, and what could be done about it. I accordingly began research on the subject with the Burns Unit of the Glasgow Royal Infirmary, and subsequently in the Zoology Department in Oxford. If anybody had then told me that one day, in Oxford, kidneys would be transplanted from one human being to another, not as a perilous surgical venture, but as something more in the common run of things, I should have dismissed it as science fiction; yet it is just this that has come about, thanks to the enterprise of Professor Morris and his colleagues.

Nevertheless in 1951, David Hume in Boston embarked on a series of cadaver kidney transplants in which the kidney was placed in the thigh of the recipient. All but one of these kidneys were rejected within a matter of days or weeks, the one exception being a patient in whom the kidney functioned for nearly 6 months and enabled the patient to leave the hospital! This event provided hope for the future as no immunosuppressive therapy had been used in this patient. At this time, the problems of rejection of kidney allografts in the dog were being clearly defined by Dr. Morton Simonsen in Copenhagen and Dr. William Dempster in London, but in 1953, a major boost to transplantation research was provided by the demonstration, by Drs. Rupert Billingham, Lesley Brent and Peter Medawar, that tolerance to an allogeneic skin graft in an adult animal could be produced by injecting the fetus with donor strain tissue, thus confirming experimentally the clonal selection hypothesis of Burnet and Fenner in the recognition of self and non-self. The induction of specific unresponsiveness of a host to a tissue allograft has remained the ultimate goal of transplant immunologists ever since.

Then in 1954, the first kidney transplant between identical twins was carried out successfully at the Peter Bent Brigham

Hospital which led to a number of further successful identical twin transplants in Boston and elsewhere in the world over the next few years.

There still remained the apparently almost insoluble problem of rejection of any kidney other than an identical-twin kidney. The first attempts to suppress the immune response to a kidney allograft employed total body irradiation of the recipient and were carried out by Dr. Merril's group in Boston, two groups in Paris under the direction of Drs. Kuss and Hamburger, respectively, and by Professor Shackman's group in London. Rejection of a graft could be suppressed by irradiation, but the complications of the irradiation were such that this was really an unacceptable approach, although an occasional relatively long-term acceptance of a graft provided encouragement for the future.

Then came the discovery by Drs. Schwartz and Dameshek in 1959 that 6-mercaptopurine could suppress the immune response of rabbits to human serum albumin. Shortly afterwards, they showed that the survival of skin allografts in rabbits was significantly prolonged by the same drug. This event ushered in the present era of renal transplantation, for very quickly Roy Calne in London and Charles Zukoski working with David Hume in Virginia showed that this same drug markedly prolonged the survival of kidney allografts in dogs. And indeed, 6-mercaptopurine was first used in a patient in Boston in 1960. Elion and Hitchings of the Burroughs Wellcome Research Laboratories in New York State then developed azathioprine, which quickly replaced 6-mercaptopurine in clinical practice as it was less toxic. With the addition of steroids, the standard immunosuppressive therapy of today was introduced to the practice of renal transplantation in the early sixties.

Not that this meant the solution of the problems of renal transplantation for this combination of drugs was dangerous and mortality was high in those early years. But there was a significant number of long-term successful transplants, and as experience grew, the results of renal transplantation improved. Another major area of endeavor in renal transplantation at that time was directed at the study of methods of matching donor and recipient for histocompatibility antigens with the aim of lessening the immune response to the graft and so perhaps allowing a decrease in the immunosuppressive drug therapy. Although this aim has only been achieved to any great extent in siblings who are HLA identical, tissue typing has made a significant contribution to renal transplantation, perhaps best illustrated by the recognition in the late sixties that the performance of a transplant in the presence of donor-specific presensitization in the recipient leads to hyperacute or accelerated rejection of the graft in most instances. Nevertheless, the more recent description of the Ia-like system in man (HLA-DR) may have an important impact on tissue typing in renal transplantation. The present decade also has seen an enormous effort directed at immunological monitoring in renal transplantation and at attempts to induce experimental specific immunosuppression. We have solved most of the technical problems of renal transplantation; we have been left with the problem of rejection and the complications arising from the drug therapy given to prevent rejection.

Although the contributions in this book cover all aspects of renal transplantation, certain subjects, as for example immunological monitoring before transplantation, transplantation in children and cancer after renal transplantation, have received considerable emphasis as they do represent developing areas of great interest, and I must take responsibility for this emphasis. For in the seventies we have seen many of the principles and practice of renal transplantation become established and the areas of future investigation become more clearly defined. With an ever-increasing demand for renal transplantation, more and more people in many different disciplines, doctors (surgeons, physicians, pathologists, virologists, immunologists), nurses, scientists and ancillary staff are becoming involved in renal transplantation either in the clinic or in the laboratory. It is to these people I hope this book will be of value.

Oxford, November 1978 **PETER J. MORRIS**

Preface to the Sixth Edition

It is now 7 years since the fifth edition of this book was published, and it is fair to say that since the first edition, published in 1979, each edition has reflected the rapid and continuing advances in renal transplantation. All chapters have been rewritten and updated, many by new contributors. As always, subjects of relevance come and go. In this edition, a separate chapter on transplantation in the highly sensitized recipient and across the ABO blood barrier appears, whereas a chapter on fine needle aspiration cytology of the transplanted kidney as well as a chapter on renal xenotransplantation, which appeared for the first time in the fifth edition, have been discarded. Xenotransplantation remains a major area of research endeavour; however, there is no clinical application in sight at this time, which perhaps seemed more likely at the time of the fifth edition. Overall, the format is much the same as before, with many new contributors and also, above all, a new editor—Stuart Knechtle has joined Peter Morris for the first time in the production of this edition.

We continue to see evidence of the advances in immunosuppression, but there is also a recognition of the increasing morbidity associated with using immunosuppression long term. Furthermore, the long-term results of renal transplantation are not as good as the short- and medium-term results might have led one to anticipate. Thus, there is now a major emphasis on chronic allograft nephropathy, which may be due to a host of injurious events, and the possibility of its prevention or treatment. Considerable attention has been given to calcineurin-sparing and steroid-sparing protocols using the more powerful new immunosuppressive agents in an attempt to achieve this goal, and several approaches are contained within the various chapters on immunosuppressive agents.

This edition, like the fifth edition, illustrates the continuing progress in all aspects of renal transplantation, but disappointingly there is little to describe in the way of induction of tolerance in clinical practice, which is to some extent due to the lack of appropriate biomarkers of tolerance or immunosuppression. We suspect that the next edition will have a whole chapter on biomarkers of immunosuppression, but at the moment their role is more speculative than actual. Patient and graft survival figures continue to improve in the short and medium term, graft survival now being around 90% or even better at 1 year. This is quite remarkable in view of the increasing number of high-risk patients undergoing transplantation, as well as the greater use of marginal donors. Without doubt, this is a tribute not only to the work of the scientists and clinicians who have made this possible but also to the thousands of patients who have participated in this evolution of what has been described as one of the medical miracles of the 20th century.

Sir Peter J. Morris
2008

Stuart J. Knechtle
2008

Chapter 1

Kidney Transplantation: A History

David Hamilton

The modern period of transplantation began in the late 1950s, but two earlier periods of interest in clinical and experimental transplantation were the early 1950s and the first 2 decades of the 20th century. Hamilton[21] provides a bibliography of the history of organ transplantation. Table 1-1 summarizes landmarks in kidney transplantation.

EARLY EXPERIMENTS

Interest in transplantation developed in the early part of the 20th century because experimental and clinical surgical skills were rapidly advancing, and many of the pioneering surgeons took an interest in vascular surgical techniques as part of their broad familiarity with the advance of all aspects of surgery. Payr's demonstration of the first workable, although cumbersome, methods of vascular suturing led to widespread interest in organ transplantation in Europe. Many centers were involved, notably Vienna, Bucharest, and Lyon. The first successful experimental organ transplant was reported by Ullmann in 1902. Emerich Ullmann (1861-1937) (Fig. 1-1) had studied under Edward Albert before obtaining a position at the Vienna Medical School, which was then at its height. Ullmann's article shows that he managed to autotransplant a dog kidney from its normal position to the vessels of the neck, which resulted in some urine flow. The animal was presented to a Vienna medical society on March 1, 1902, and caused considerable comment.[53] At this time, Ullmann was Chief Surgeon to the Spital der Baumhertigen Schwestern, and his experimental work was done in the Vienna Physiology Institute under Hofrath Exner. Exner's son Alfred had already tried such a transplant without success. In the same year, another Vienna physician, Alfred von Decastello, physician assistant at the 2nd Medical Clinic, carried out dog-to-dog kidney transplants at the Institute of Experimental Pathology.[14]

Ullmann and von Decastello had used Payr's method, and later in 1902 Ullmann demonstrated a dog-to-goat kidney transplant that, to his surprise, passed a little urine for a while. Neither Ullmann nor von Decastello continued with this work, although von Decastello was noted for his work on blood groups, and Ullmann published extensively on bowel and biliary surgery.

In Lyon, the department headed by Mathieu Jaboulay (1860-1913) had a major influence (Fig. 1-2). In his research laboratories, his assistants Carrel, Briau, and Villard worked on improved methods of vascular suturing, leading to Carrel's famous article credited with establishing the modern method of suturing.[9] Carrel left to work in the United States, and in the next 10 years he published extensively on organ grafting, successfully carrying out autografts of kidneys in cats and dogs and showing that allografts eventually failed after functioning briefly. He was awarded a Nobel Prize for this work in 1912.

HUMAN KIDNEY TRANSPLANTS

Jaboulay, Carrel's teacher, had carried out the first recorded human kidney transplant in 1906,[27] although Ullmann later claimed an earlier attempt in 1902.[54] Jaboulay was later to be better known for his work on thyroid and urological surgery, but, doubtless encouraged by the success of Carrel and others in his laboratory, he carried out two xenograft kidney transplants using a pig and goat as donors, transplanting the organ to the arm or thigh of patients with chronic renal failure. Each kidney worked for only 1 hour. This choice of an animal donor was acceptable at that time in view of the many claims in the surgical literature for success with xenograft skin, cornea, or bone.

More is known of the second and third attempts at human kidney transplantation. Ernst Unger (1875-1938) (Fig. 1-3) had a thorough training in experimental work and set up his own clinic in 1905 in Berlin, being joined there by distinguished colleagues. He continued with experimental work and by 1909 reported successful transplantation of the kidneys en masse from a fox terrier to a boxer dog. The urine output continued for 14 days, and the animal was presented to two medical societies. By 1910, Unger had performed more than 100 experimental kidney transplants. On December 10, 1909, Unger attempted a transplant using a stillborn child's kidney grafted to a baboon. No urine was produced. The animal died shortly after the operation, but postmortem examination showed that the vascular anastomosis had been successful. This success and the new

Table 1–1	**Landmarks in Kidney Transplantation**
1902	First successful experimental kidney transplant[53]
1906	First human kidney transplant—xenograft[27]
1933	First human kidney transplant—allograft[56]
1950	Revival of experimental kidney transplantation[15,47]
1950-1953	Human kidney allografts without immunosuppression, in Paris[16,31,46] and Boston[26]
1953	First use of live related donor, Paris[32]
1954	First transplant between identical twins, Boston[38]
1958	First description of leukocyte antigen Mac[12]
1959-1962	Radiation used for immunosuppression, in Boston[37] and Paris[19,29]
1960	Effectiveness of 6-MP in dog kidney transplants[5,59]
1960	Prolonged graft survival in patient given 6-MP after irradiation[30]
1962	First use of tissue matching to select a donor and recipient[13,30,51]
1966	Recognition that positive crossmatching leads to hyperacute rejection[28,51]
1967	Creation of Eurotransplant[43]
1967	Development of kidney preservation
1973	Description of the transfusion effect[4]
1978	First clinical use of cyclosporine
1978	Application of matching for HLA-DR in renal transplantation[5]
1987	First of new wave of immunosuppressive agents appears (tacrolimus)
1997	Transgenic pigs produced

6-MP, 6-mercaptopurine.

knowledge that monkeys and humans were serologically similar led Unger to attempt, later in the same month, a monkey-to-human transplant.[55] The patient was a young girl dying of renal failure, and the kidney from an ape was sutured to the thigh vessels. No urine was produced. Unger's report concluded that there was a biochemical barrier to transplantation, a view mistakenly advocated by the basic science of the day; his main contributions thereafter were in esophageal surgery. (For a biography of Unger, see Winkler.[58])

These early experiments established that kidney transplants were technically possible. Methods of study of renal function were primitive then; without routine measurement of blood urea and without any radiological methods, subtle studies of transplant function were impossible. This impossibility plus the uncertainty of the mechanism of allograft rejection led to a diminished interest in organ transplantation after about 10 years of activity. By the start of World War I, interest in organ transplantation had almost ceased and was not resumed in the European departments of surgery after the war. Carrel had switched his attention to studies of tissue culture. Interest elsewhere also was low; in Britain and the United States, scarce research funds were being applied to fundamental biochemistry and physiology, rather than applied projects of clinical relevance. Transplantation immunology faded away after the bright start in the capable surgical hands of Carrel, Murphy's sound grasp of immunosuppression, and Landsteiner's awareness of the serological detection of human antigens. Carrel, Murphy, and Landsteiner all worked at the Rockefeller Institute in New York.

In 1914 in a remarkable lecture to the International Surgical Society, Carrel did anticipate the future development of transplantation. His colleague, J. B. Murphy, at the Rockefeller Institute, had found that radiation or benzol

Figure 1–1 Emerich Ullmann (1861-1937) in 1902 carried out the first experimental kidney transplants in dogs. (Courtesy of The Vienna University, Institute for the History of Medicine.)

treatment would increase the "take" of tumor grafts in rats, and Carrel realized the potential of these findings:

It is too soon to draw any definite conclusions from these experiments. Nevertheless it is certain that a very important point has been acquired with Dr. Murphy's discovery that the power of the organism to eliminate foreign tissue was due to organs such as the spleen or bone marrow, and that when the action of these organs is less active a foreign tissue can develop rapidly after it has been grafted.

It is not possible to foresee whether or not the present experiments of Dr. Murphy will lead directly to the practical solution of the problem in which we are interested.

The surgical side of the transplantation of organs is now completed, as we are now able to perform transplantations of organs with perfect ease and with excellent results from an anatomical standpoint. But as yet the methods cannot be applied to human surgery, for the reason that homoplastic transplantations are almost always unsuccessful from the standpoint of the functioning of the organs. All our efforts must now be directed toward the biological methods which will prevent the reaction of the organism against foreign tissue and allow the adapting of homoplastic grafts to their hosts.[10]

THE MIDDLE YEARS

Until the revival of interest in transplantation in the 1950s, the 1930s and 1940s were a stagnant period in clinical science. The great European surgical centers had declined; in North America, only at the Mayo Clinic was there a cautious program of experimental transplantation without building on Carrel's work, notably failing to make attempts at

Figure 1–2 Mathieu Jaboulay (1860-1913) and his surgical team at Lyon in 1903. Until his death in a rail accident, Jaboulay made numerous surgical contributions and encouraged Alexis Carrel's work on vascular anastomosis. In 1906, Jaboulay reported the first attempt at human kidney transplantation

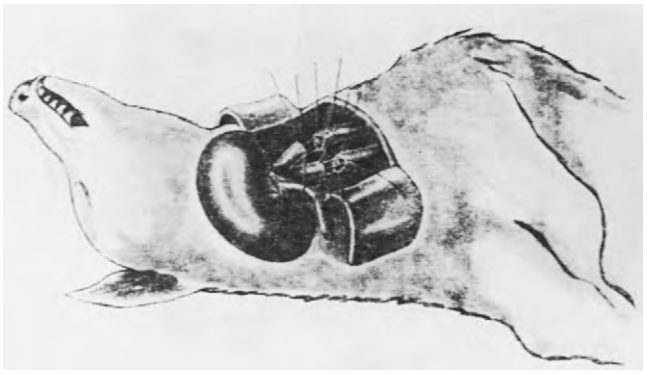

Figure 1–4 Yu Yu Voronoy (1895-1961) had experience with dog allografts before carrying out the first human kidney allograft in 1933 at Kherson in the Ukraine. His experimental animal model is shown here.

immunosuppression. In transplantation circles, such as they were, there was not even the confidence to counter the vivid claims of Voronoff to rejuvenate human patients via monkey gland grafts, and the endless reports of successful human skin grafts were not examined critically.

The main event of this period was an isolated and little known event—the first human kidney allograft. It was performed in the Ukraine by the Soviet surgeon Yu Yu Voronoy.[56] Voronoy was an experienced investigator, and he eventually performed six such transplants up to 1949. Voronoy (1895-1961) trained in surgery at Kiev under Professor V. N. Shamov and obtained experience there with serological methods of blood transfusion, then in their developmental stage. He used these methods to detect complement-fixing antibodies after testis slice transplants, and later he had some success with the same methods applied to kidney grafts (Fig. 1-4). In 1933, Voronoy transplanted a human kidney of blood group B to a patient of blood group O with acute renal failure as a result of mercuric chloride poisoning. The donor kidney was obtained from a patient dying as a result of a head injury and was transplanted to the thigh vessels under local anesthetic; the warm time for the kidney was about 6 hours. There was a major mismatch for blood groups, and despite a modest exchange transfusion, the kidney never worked. The patient died 2 days later; at postmortem, the donor vessels were patent. By 1949, Voronoy reported six such transplants, although no substantial function had occurred in any. (For a biography of Voronoy, see Hamilton and Reid.[22])

Figure 1–3 A contemporary cartoon of Ernst Unger (1875-1938) at work at the Rudolf Virchow Hospital, Berlin. (Courtesy of the Rudolf Virchow Hospital.)

POST–WORLD WAR II

The sounder basis of transplantation immunology, which followed Medawar's pioneer studies during World War II, led to a new interest in human transplantation. In 1946, a human allograft kidney transplant to arm vessels under local anesthetic was attempted by Hufnagel, Hume, and Landsteiner at the Peter Bent Brigham Hospital in Boston. The brief period of function of the kidney may have helped the patient's recovery from acute renal failure; it marked the beginning of that hospital's major interest in transplantation and dialysis.[33]

In the early 1950s, the interest in experimental and clinical kidney transplantation increased. With a growing certainty that immunological mechanisms were involved, the destruction of kidney allografts could be reinvestigated. Simonsen, then an intern in Ålborg in Denmark, persuaded his surgical seniors to teach him some vascular surgery; using dog kidney transplants, he reported on the mechanism of kidney rejection.[47] Dempster in London also re-examined this question.[15] Both workers found that the pelvic position of the kidney was preferable to a superficial site, and both concluded that an immunological mechanism was responsible for failure. Dempster found that radiation, but not cortisone, delayed rejection. Both workers considered that a humoral mechanism of rejection was likely.

In the early 1950s, two groups simultaneously started human kidney transplantation. In Paris, with encouragement from the nephrologist Jean Hamburger, the surgeons Küss (five cases),[31] Servelle (one case),[46] and Dubost (one case)[16] reported on kidney allografts without immunosuppression in human patients, placing the graft in the now-familiar pelvic position. The Paris series included a case reported by Hamburger of the first live related kidney transplant, the donor being the mother of a boy whose solitary kidney had been damaged in a fall from a height. The kidney functioned immediately, but was rejected abruptly on the 22nd day.[32] In the United States, the Chicago surgeon Lawler had been the first to attempt such an intra-abdominal kidney allograft in 1950; it was met with the intense public interest and professional skepticism that were to characterize innovative transplantation thereafter.

A series of nine cases, closely studied, was recorded from Boston, using the thigh position of the graft, and for the first time hemodialysis had been used in preparing the patients, employing Merrill's skill with the early Kolff/Brigham machine. David Hume (Fig. 1-5) reported on this Boston experience in 1953. Modest unexpected survival of the kidney was obtained in some of these cases and served to encourage future careful empirical surgical adventures, despite advice from scientists to wait for elegant solutions. Although small doses of adrenocorticotropic hormone or cortisone were used, it was thought that the endogenous immunosuppression of uremia was responsible for these results, rather than the drug regimen. Many of Hume's tentative conclusions from this short series were confirmed later, notably that prior blood transfusion might be beneficial, that blood group matching of graft and donor might be necessary, and that host bilateral nephrectomy was necessary for control of post-transplant blood pressure.[26] The first observation of recurrent disease in a graft was made, and accelerated arteriosclerosis in the graft vessels was noted at postmortem. Other cases were reported from Chicago, Toronto, and Cleveland in the early 1950s, but because no sustained function was achieved, interest in clinical and experimental renal allograft transplantation waned, despite increasing knowledge of basic immunological mechanisms in the laboratory.

Figure 1–5 David M. Hume (1917-1973) pioneered human kidney transplantation at the Peter Bent Brigham Hospital, Boston, and the Medical College of Virginia. He died in an air crash at the age of 55.

The technical lessons learned from the human allograft attempts of the early 1950s allowed confidence in the surgical methods, and in Boston, on December 23, 1954, the first transplant of a kidney from one twin to another with renal failure was performed. From then on, many such transplantations were performed successfully in Boston.[38] Although sometimes seen now merely as a technical triumph, valuable new findings emerged from this series. Some workers had predicted that in the short-term, the activity of the inactive bladder could not be restored, and that in the long-term human kidney grafts would decline in vitality as a result of denervation or ureteric reflux. Other workers were convinced that a single kidney graft could not restore biochemical normality to an adult, and that in any case the existing changes caused by chronic renal failure were irreversible. All of these gloomy predictions were neutralized by the success of the twin kidney transplants, and the greatest triumph came when one such recipient became pregnant and had a normal infant, delivered cautiously by cesarean section, with the anxious transplanters in attendance. Many of the twin recipients are still alive today, although the good results were tempered by failures caused by the prompt return of glomerulonephritis in some transplanted kidneys. This complication was later much reduced by immunosuppression. Other lessons learned were that the hazard of multiple donor renal arteries provided a need for pretransplant angiography of the kidneys in living donors, although it still was not thought necessary to perfuse or cool the donor organ. Lastly, there was the first airing of the legal aspects of organ donation, particularly the problem of consent in young, highly motivated related donors. (For an account of this period, see Murray and colleagues.[40])

IMMUNOSUPPRESSION AND THE MODERN ERA

In 1948, the first patients crippled with rheumatoid arthritis were given the Merck Company's Cortone (cortisone) at the Mayo Clinic, and intense worldwide interest in the pharmacological actions of adrenal cortical hormones followed. Careful studies by Medawar's group in the early 1950s suggested a modest immunosuppressive effect of cortisone, but when Medawar shortly afterward showed profound, specific, and long-lasting graft acceptance via the induction of tolerance, the weak steroid effect was understandably sidelined and thought to be of no clinical interest. Induction of tolerance in adult animals (rather than newborns) was accomplished by lethal irradiation and bone marrow infusion, and with this strong lead from the laboratory, it was natural that the first attempts at human immunosuppression for organ transplants were with preliminary total-body irradiation and allograft bone marrow rescue. These procedures were carried out in Paris, Boston, and elsewhere in the late 1950s.

This regimen was too difficult to control, and graft-versus-host disease was inevitable. It was found unexpectedly that sublethal irradiation alone in human patients was quite

immunosuppressive, however, and this approach was used until 1962, the year of the first general availability of azathioprine (Imuran). In Boston, 12 patients were treated in this way, but with only one long-term survival in a man receiving his transplant from his nonidentical twin.[37] In Paris, similar success was obtained with sibling grafts.[19,29] These isolated kidney survivals after a single dose of radiation gave further hope and showed again that the immunology of humans, dogs, and mice is different. These cases also showed that if a human organ could survive the initial crucial rejection period, it could be protected or adapted to the host in some way, possibly shielded by new endothelium, by enhancement, or, as suggested later, by microchimeric tolerance induced by mobile cells in the graft.

CHEMICAL IMMUNOSUPPRESSION

In 1958, at the New England Medical Center, attempts were made at human bone marrow transplantation for aplastic anemia and leukemia. To enable the marrow grafts to succeed, irradiation of the recipient was used. Results were poor, and mortality was high. Dameshek and Schwartz looked for alternatives to irradiation and reasoned that an anticancer drug, such as 6-mercaptopurine (6-MP) or methotrexate, might be of use for immunosuppression in their patients. (For an account of this period, see Schwartz.[44]) Their important paper in 1959, showing a poor immune response to foreign protein in rabbits treated with 6-MP,[45] was noticed by Roy Calne, then a surgeon in training at the Royal Free Hospital, London, and David Hume, new Chairman of Surgery at the Medical College of Virginia. Calne had been disappointed at the failure of irradiation to prolong kidney allograft survival in dogs and, similar to others looking for an alternative, he found that 6-MP was successful.[5] Zukoski and colleagues[59] in Richmond found the same effect.

In 1960, Calne visited Boston for a period of research with Murray, and Hitchings and Elion of Burroughs Wellcome, then at Tuckahoe, provided him with new derivatives of 6-MP. Of these, BW57-322 (later known as azathioprine [Imuran]) proved to be more successful in dog kidney transplants and less toxic than 6-MP.[7]

In 1960 to 1961, 6-MP was used in many human kidney transplants. In London at the Royal Free Hospital, three cases were managed in this way, but without success, although one patient receiving a live related transplant died of tuberculosis rather than rejection.[23] In Boston, no lasting human kidney function was obtained, but in Paris, Küss and associates[30] reported one prolonged survival of a kidney from a nonrelated donor when 6-MP was used with intermittent prednisone in a recipient who also had received irradiation as the main immunosuppressive agent (Fig. 1-6). This case was the first success for chemical immunosuppression.

This change in approach, giving lifelong, risky medication with toxic drugs, although an obvious development in retrospect, was accepted with reluctance because it meant leaving aside, at least in the short-term, the hopes from the work of the transplantation immunologists for the elegant, specific, one-shot, nontoxic tolerance regimen. Many workers thought that entry into this new paradigm was only a temporary diversion.

In 1961, azathioprine became available for human use; the dosage was difficult to judge at first. The first two Boston cases using the drug did not show prolonged survival of the grafts, but in April 1962 the first extended successes with human kidney allografts were obtained.[39] Shortly afterward, at the bedside rather than in the laboratory, it was discovered that steroids, notably prednisolone, when given with azathioprine had a powerful synergistic effect. The regular use of both together became a standard regimen after reports by Starzl and colleagues[49] and Goodwin and coworkers,[18] and this combined therapy continued to be the routine immunosuppressive method despite many other suggested alternatives, until azathioprine was displaced by cyclosporine much later. Use of the combined immunosuppression and the increasing use of live related donors (rather than occasional twin or free or cadaver kidneys), along with the remarkably good results reported in 1963 from Denver[49] and Richmond,[25] greatly encouraged the practice of transplantation. (For an account of this period, see Starzl.[48])

Figure 1–6 R. Küss (*right*) and M. Legrain (*center*) in 1960 with their first long-term kidney transplant survivor. The patient and her brother-in-law donor (*center right*) are shown with the staff of the unit at the Hôpital Foch. Immunosuppression with irradiation and mercaptopurine was used. (Courtesy of Prof. M. Legrain.)

A TIME OF OPTIMISM

The mid-1960s was a period of great optimism. The rapid improvement in results seemed to indicate that routine success was at hand. Looking to the future, calculations were made that suggested that enough donor organs would be available in the future if all large hospitals cooperated, and such donations did start to come from outside the transplantation pioneer hospitals. Transplantation societies were set up, and specialist journals were started. The improvements in regular dialysis treatment meant an increasing pool of patients in good health suitable for transplantation, and this allowed for better and planned preparation for transplantation. With a return to dialysis being possible, heroic efforts to save a rejected kidney were no longer necessary. Management of patients improved in many aspects, and the expected steroid long-term effects were met and managed (primarily by the demonstration that low-dose steroids were as effective as high-dose steroids). The need for cooling of donor organs was belatedly recognized, many tests of viability were announced, and transport of organs between centers began. Bone disease and exotic infections were encountered and treated, but the kidney units were affected by a hepatitis B epidemic in the

mid-1960s, which affected morale and status. The narrow age limit for transplantation was widened, and in Richmond the first experience with kidney grafts in children was obtained.

Recipients of kidney transplants re-entered the normal business of life and became politicians, professors, pilots, and fathers and mothers of normal children. Other good news in the United States came when the federal government accepted the costs of regular dialysis and transplantation in 1968. There were always unexpected findings, usually reported from the pioneer units with the longest survivors. Cautiously, second kidney transplants were performed at Richmond when a first had failed; these did well, and the matter became routine. Chronic rejection and malignancy first were reported in kidney transplant recipients from Denver. As a result of the optimism, experimental heart transplantation started, the first human livers were grafted, and there was a revival of interest in xenotransplantation. Although the attempts of Reemtsma and coworkers,[42] Hume,[24] and Starzl[48] at transplantation with chimpanzee or baboon kidneys ultimately failed, rejection did not occur immediately, and the cases were studied closely and described.

In the search for better immunosuppression, there was great excitement when laboratory studies by Woodruff and Medawar produced a powerful immunosuppressive antilymphocyte serum, and production of a version suitable for human use started. Initial results were favorable, but the antilymphocyte serum had an unspectacular role thereafter, supplanted from 1975 onward to a large extent by the production of monoclonal antibodies. Hopes for another biological solution to transplantation were raised in 1969 when French and Batchelor[17] found an enhancing serum effect in the new experimental model of rat kidney transplantation made possible by the development of microsurgical methods, but it proved impossible to mimic the effect in humans.

TISSUE TYPING

The greatest hopes resided in the evolution of tissue typing methods, which entered routine use in 1962 (Fig. 1-7).[13,20] The increasing identification of the antigens of the HLA system seemed to promise excellent clinical results in the future from close matching made possible when choosing from a large pool of patients. Sharing of kidneys in Europe started in 1967 at van Rood's suggestion,[43] and in North America, Amos and Terasaki set up similar sharing schemes on both coasts of the United States. Others followed throughout the world, and these organizations not only improved the service but also soon gathered excellent data on kidney transplant survival. The need to transport kidneys within these schemes encouraged construction of perfusion pumps designed to increase the survival of organs and the distance they could be transported.[1] Much work on perfusion fluids was done until the final intracellular type of fluid devised by Collins in 1969 allowed a simple flush and chill to suffice for prolonged storage.[11] Although the hopes for typing were not fully realized, such schemes had other benefits in obtaining kidneys when urgently required for patients with rarer blood groups, for children, or for highly sensitized patients. Such patients had been recognized by the new lymphocytotoxicity testing using a crossmatch between donor cells and recipient serum. First noted by Terasaki and associates[51] and described in more detail by Kissmeyer-Nielsen and colleagues[28] in 1966, such pretransplant testing explained cases of sudden failure and led to a marked diminution in hyperacute rejection.

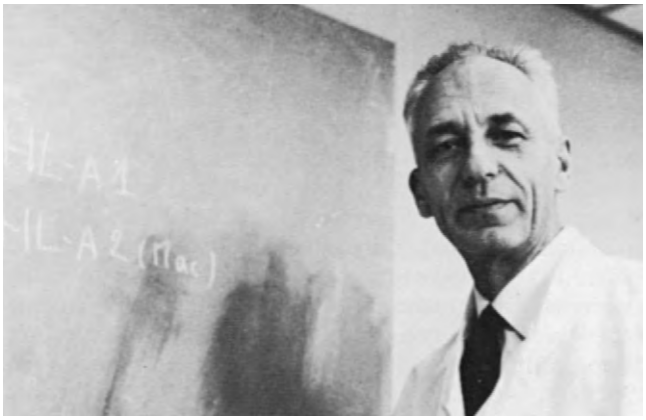

Figure 1–7 Jean Dausset first described an antigen MAC, later known as HL-A2, defined by numerous antisera from multitransfused patients, and which later was shown to be part of the major histocompatibility complex in humans (HLA).

THE 1970s PLATEAU

The 1970s was a period of consolidation, of improvements in data collection such as the valuable European Dialysis and Transplant Association surveys, and increased sophistication in HLA typing methods and organ-sharing schemes. Cadaver organ procurement generally increased as a result of wider involvement of the public and medical profession, although the number of patients waiting for transplantation persistently exceeded the organs available, and donation declined transiently during times of public concern over transplantation issues. Governments took initiatives to increase donations; in Britain, the Kidney Donor Card was introduced in 1971, becoming a multidonor card 10 years later. In hospital practice, methods of resuscitation and intensive care improved, and the concept of brain death was established to prevent prolonged, pointless ventilation, although its immediate application to transplantation provoked controversy. Despite many new claims for successful methods of immunosuppression, such as trials of splenectomy, thymectomy, and thoracic duct drainage, as well as a new look at cyclophosphamide, no agent except antithymocyte globulin became established in routine use.

Although patient survival after kidney transplantation continued to increase, the 1970s did not show the expected increase in cadaver graft survival. Some groups reported decreased survival figures; this paradox was solved partly by the demonstration that blood transfusion during regular dialysis, which had been discouraged because of the risk of sensitization, was beneficial to the outcome of kidney transplantation,[41] an observation made some years earlier by Morris and coworkers.[35]

The 1970s ended with two innovations that revived hopes of reaching the goal of routine, safe, and successful kidney transplantation. Ting and Morris[52] reported the successful clinical application of HLA-DR matching, and Calne and associates[8] revived memories of the excitement of the early days of the use of azathioprine by introducing into clinical

practice the first serious rival to it in 20 years, cyclosporine, which had been discovered to be a powerful immunosuppressive agent by Borel.[3] Cyclosporine replaced the earlier drug regimens and was the dominant agent in use until the 1990s. Transplantation had grown to a sufficiently large clinical service that it was worth the attention of the pharmaceutical companies, and in the 1990s steady production of new agents occurred—tacrolimus, mycophenolate mofetil, rapamycin, FTY720, brequinar, and others. Any drug with promise was marketed aggressively, and sponsored trials became a routine part of clinical life.

The improved results of transplantation meant that the procurement of organs became a more dominant issue. Comparisons of transplantation practice throughout the world showed remarkable differences in attitudes to use of live related donors and cadaver organs, depending on religion and cultural traditions. Kidney transplantation had started as a difficult surgical and scientific challenge confined to a few academic centers in the developed world, but its success had led to the technique becoming a routine service in all parts of the world.[4] In some nations not sharing Western attitudes, the donor shortage meant the appearance of undesirable commercial developments in renal transplantation, such as the purchase of kidneys from living unrelated donors (discussed in more detail in Chapter 39).[34]

WAITING FOR XENOGRAFTS

As the demand for kidney transplants continued to exceed supply, other initiatives appeared and included study of nations and areas with high donation rates (e.g., Spain), the regulated use of properly motivated unrelated individuals, and a return to use of marginal cadaver kidneys, notably from non–heart beating donors. As all attempts to increase donor supply fell short of the ever-rising target, the radical alternative of the use of animal organs was examined afresh. Profound immunosuppression alone was ineffective and, at first, methods of removing natural antibody from recipient plasma were tried to deal with the hyperacute phase of xenograft organ rejection. Although the traditional hopes for xenografting of human patients had assumed that concordant species such as the monkey would be used, a new strategy using genetic engineering methods first used a line of transgenic pigs, a distant species discordant with humans, with a modified endothelium that reduced the complement-mediated immediate reaction.[2]

These new hopes for xenografts raised old fears among the public and legislators, notably regarding disease transmission. Although this had been a familiar problem in human-to-human transplantation and had been met regularly and dealt with, governments required reassurances about xenotransplantation with the added threat of retrovirus transmission. Hopes faded that these early developments would evolve into a sophisticated routine. Instead, the kidney transplanters could only watch, with detached interest, the emergence of stem cell use in cellular transplantation.

CONCLUSION

Kidney transplantation was the first of the organ transplant procedures to develop because of availability of live donors and the crucial backup of dialysis. When radical new ideas are to be tested, pioneers still turn to kidney transplantation. Kidney transplantation is where it all started, with good reason, and it will always be a test bed for major innovation.

In the early 1990s, Murray[36] was awarded the Nobel Prize in Medicine for his pioneer work in renal transplantation and in the development of many new immunosuppressive agents, including drugs and monoclonal antibodies. The future promises to be exciting. Nowhere is the excitement of the past reflected better than in the recollections of 35 of the pioneers of transplantation gathered together by Terasaki.[50]

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Chapter 2

Immunology of Graft Rejection

Margaret J. Dallman

Transplantation remains the treatment of choice for patients with renal failure. In most cases, this procedure entails the use of an organ from a genetically disparate individual and inevitably results in a response in the host and in the graft. Some responses occur as a result of the trauma associated with organ harvest, perfusion, and surgery, whereas others involve specific recognition by the immune system of antigenic differences between donor and recipient. The cumulative effect of these events is a destructive response that, if uncontrolled, leads to loss of the transplant, as originally highlighted by workers such as Little and Tyzer.[201] The immunological nature of tissue rejection as originally suggested by Gorer[105] was firmly established more than 50 years ago by Medawar[99,232,233] after the demonstration that the rejection process in humans and rodents displays marked specificity and memory for donor tissue and is accompanied by infiltration with leukocytes.

Our understanding of the immune system has evolved considerably, and we now are able to describe more fully the molecular and cellular events that result in graft rejection. With such knowledge has come an impressive range of new, primarily biological agents, including antibodies and fusion proteins that are targeted to specific aspects of the immune response in an attempt to deliver better and more selective immunosuppression. Many of these agents are currently in clinical use or trial (see Chapters 20 and 21), complementing established agents such as cyclosporine, tacrolimus, and steroids. Despite the wealth of immunosuppressive agents, clinically detectable acute rejection is common, and although this in itself may not result in graft loss, it undoubtedly contributes to slowly deteriorating renal dysfunction, which is accompanied by the histological changes of chronic allograft nephropathy (see Chapters 24 and 25). Current use of immunosuppressive agents, although becoming more sophisticated, remains heavy-handed because suitable predictive criteria and assays that can dictate an individualization of therapy in different patients are still lacking. An ability to tailor therapy remains a major challenge in transplantation and is likely to be achieved only when we can develop a full profile of the evolution of the immune response after transplantation and understand the parameters that control immunity. Developing accurate, early, noninvasive, and predictive biomarkers to trace the emerging immunity and to indicate a response to therapeutic intervention remains a crucial but elusive goal.

This chapter describes the molecular and cellular events of the immune response that are understood. It assumes a basic level of knowledge of the cells and molecules involved in immune responses. The reader is referred to other books for general descriptions of the immune system.[147,160] See Table 2-1 for terminology.

TRAUMA OF TRANSPLANTATION

The response to a transplant occurs in a series of relatively well-defined stages (Fig. 2-1), the first of which involves the severe physical assault that the graft undergoes during harvest from the donor and transplantation into the recipient and includes the hemodynamic and neuroendocrine responses to brainstem death in cadaver donors. Harvesting and preservation involve cooling the kidney to reduce its metabolic rate; perfusion with preservation solution, which is designed to reduce cold-induced cell swelling and prevent loss of potassium from the cell; storage for sometimes long periods, which results in pH changes and the accumulation of toxic products; and the surgical procedures required for transplantation to the recipient. All of these events sensitize the organ to reperfusion injury when the organ is warmed rapidly on revascularization in the recipient. Preservation solutions, alongside approaches that upregulate or provoke overexpression of heme oxygenase 1, aim to reduce these effects on the kidney (see Chapter 9); nevertheless during and shortly after the ischemic and reperfusion periods, a variety of genes become activated, and inflammatory cells begin to infiltrate the graft.

Table 2–1 **Transplant Terminology**	
Autograft (autologous transplant)	Transplantation of an individual's own tissue to another site (e.g., the use of a patient's own skin to cover third-degree burns or a saphenous vein femoropopliteal graft)
Isograft (syngeneic or isogeneic transplant)	Transplantation of tissue between genetically identical members of the same species (e.g., kidney transplant between identical twins or grafts between mice of the same inbred strain)
Allograft (allogeneic transplant)	Transplantation of tissue between genetically nonidentical members of the same species (e.g., cadaver renal transplant or graft between mice of different inbred strains)
Xenograft (xenogeneic transplant)	Transplantation of tissue between members of different species (e.g., baboon kidney into a human)

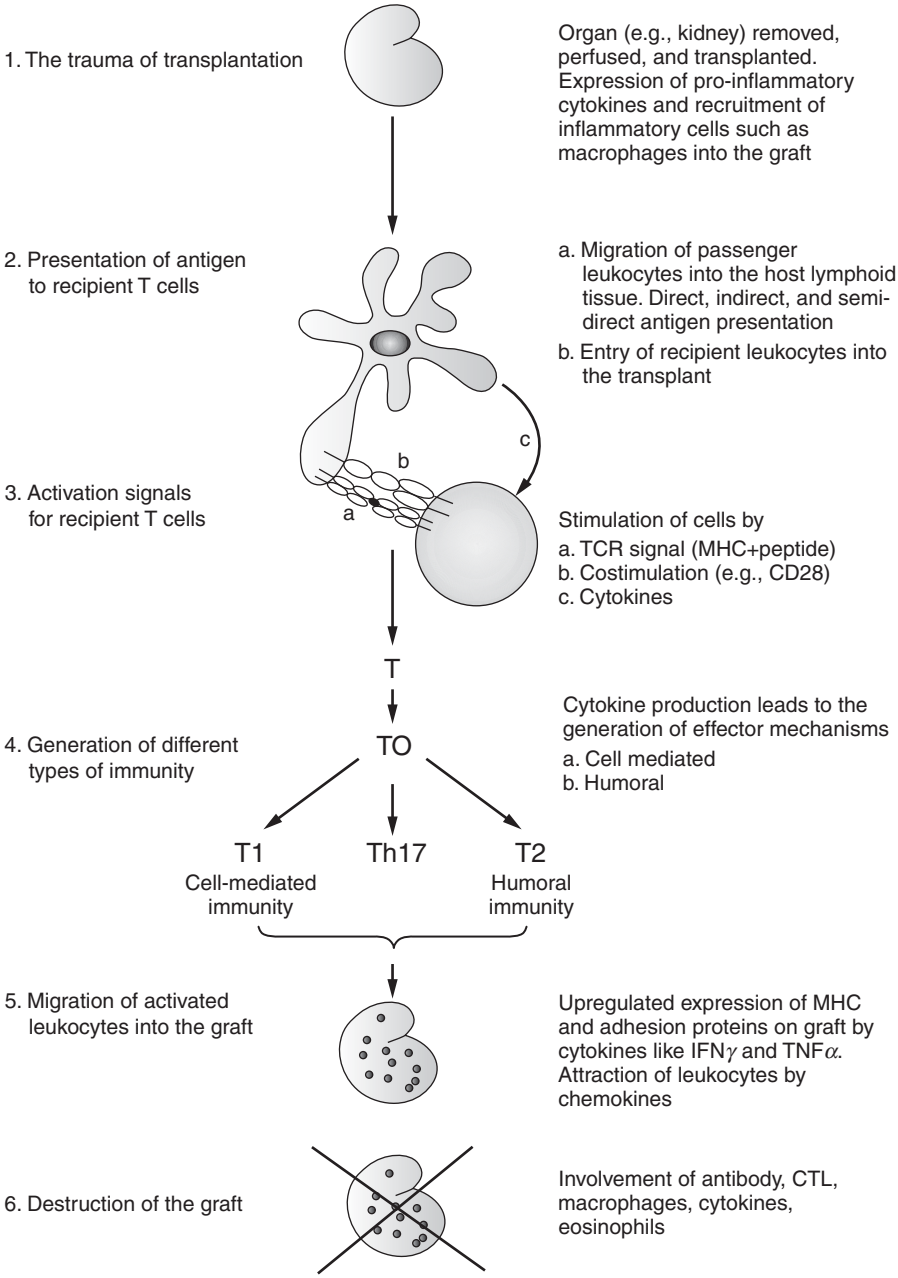


Figure 2–1 The evolution of the immune response after kidney transplantation. CTL, cytotoxic T cell; IFN, interferon; MHC, major histocompatibility complex; TCR, T cell receptor; TNF, tumor necrosis factor.

The importance of these aspects of transplantation is shown by the superior outcome of live donor transplants even in the face of significant major histocompatibility complex (MHC) mismatch,[217] the importance of cold ischemia time in graft outcome,[315] and reportedly higher rates of rejection observed in individuals with delayed graft function.[287] In experimental transplantation between identical individuals, graft histology similar to that seen in chronic allograft nephropathy may be observed after prolonged ischemia.[363]

INNATE IMMUNE RESPONSE

Cells and mediators involved in the early nonadaptive, non–antigen-specific response are components of the innate immune system that provides the body with a first-line defense against damage and invading pathogens. Activation of the endothelium together with the induction of several soluble proteins or cytokines (or transcripts of cytokines), such as interleukin (IL)-6 and IL-1, can be shown at early time points after transplantation, even of syngeneic grafts, in which there is no antigenic difference between donor and recipient and in which an antigen-specific immune response is not generated.[58,358] Probably as a result of this induction, combined with an upregulated expression of adhesion proteins on the vascular endothelium and other cells of the graft,[172] an early infiltrate of inflammatory cells, including macrophages, develops.[231] This early inflammatory response also triggers the migration out of the graft of tissue-resident, bone marrow–derived dendritic cells (DCs).[184,185] These early events in themselves do not result in graft rejection and, as noted, are observed in syngeneic grafts. The severity of the initial injury and the nature of the subsequent inflammatory infiltrate are central, however, in the stimulation of antigen-specific immunity: A maximally damaged organ generates a maximal "danger signal,"[227] which can initiate adaptive or antigen-specific immunity manifested as rejection when antigenic differences between donor and recipient exist.

Receptors of the Innate Immune System

Cells of the innate immune system bear receptors (pattern recognition receptors) that recognize and respond to molecules expressed by pathogens (pathogen-associated molecular patterns), sensing danger. Innate immune cells, such as macrophages and DCs, are activated via their cell surface and internal pattern recognition receptors to a heightened cytocidal state and antigen-presenting capacity. One group of pattern recognition receptors, the Toll-like receptors,[234] have received much attention in recent years, and there is evidence that signaling via Toll-like receptors can be important, at least in experimental transplantation.[103]

Why should such activation via pattern recognition receptors occur after apparently sterile procedures such as transplantation? Although still controversial, it seems that endogenous ligands released after tissue damage also can bind certain pattern recognition receptors, activating cells of the innate immune system.[264] The antigenic differences between donor and recipient can then be efficiently presented to cells of the adaptive immune system, and antigen-specific immunity ensues.

Other Aspects of Innate Immunity

Other cells of the innate immune system contribute not only at the early stages after transplantation (e.g., interferon [IFN]-γ produced by natural killer [NK] cells[241] is a factor in the activation of DCs) but also at the later phases of rejection (e.g., eosinophils can be involved in tissue destruction). We return to this subject later in this chapter for a fuller description of NK cells and eosinophils and their role in the effector phase of rejection.

Complement

The complement system is a humoral component of innate immunity, composed of a well-defined group of soluble proteins, enzymes, and receptors that act in a cascade fashion to mediate their effector functions. Although normally activated in the presence of infections, complement also can be activated by a variety of endogenous signals, including hypoxia and stress.[376,377] Complement activation generates numerous products that are important not only for host defense but also for regulation of inflammatory processes and adaptive immunity.[35] Conventionally thought to be a product of the liver, it is now clear that there are many extrahepatic sites of complement synthesis and that myeloid cell–derived complement component C3 is important in the generation of adaptive immunity.[374,375] In the context of transplantation, production of C3 by the donor organ in experimental and clinical studies is an important factor in the generation of alloimmunity.[23,286] It has been found that DCs themselves are able to produce C3 and that this can regulate the maturation of DCs and their ability to activate T cells. DCs from C3-deficient ($C3^{-/-}$) mice stimulated diminished T cell responses and notably seemed to be particularly good at inducing T cells with a regulatory phenotype that could be responsible for dampening immune responses.[277,391] How exactly C3 production by DCs affects T cell responses is the focus of current interest in this area.[392]

ADAPTIVE IMMUNITY—THE AFFERENT ARM

The antigen-specific or adaptive immune response to a graft occurs in two main stages. In the first, the afferent arm, donor antigens are presented to recipient T lymphocytes, which become activated, proliferate, and differentiate further while sending signals for growth and differentiation to a variety of other cells. In the second stage, or efferent arm, effector leukocytes are recruited into the organ, where they can wreak the havoc that results in tissue destruction.

Antigens That Stimulate Graft Rejection

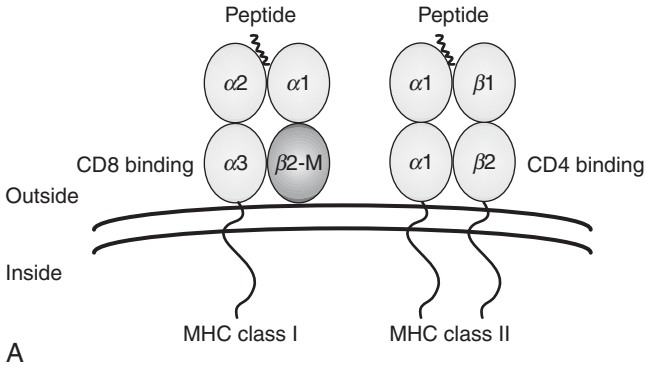
Histocompatibility antigens determine the outcome of tissue allografts between different members of the same species. In all vertebrate species, histocompatibility antigens can be divided into a single MHC and numerous minor histocompatibility (miH) systems. Incompatibility for either MHC or miH antigens between donor and recipient leads to an immune response against the graft, but more vigorous rejection occurs in the face of MHC differences. In a nonsensitized recipient, rejection of MHC-compatible organ grafts may not occur or may be delayed, although there is evidence

that multiple miH differences alone can result in cardiac allograft rejection in mice as rapidly as that seen with transplantation across a full MHC barrier.[280] It is a different matter with bone marrow, however, in which transplants between HLA-identical siblings may be rejected or cause graft-versus-host disease because of a disparity between host and donor in only one or a few minor antigens.[106,107]

Major Histocompatibility Antigens

There is substantial similarity between the MHC in different species with respect to immunogenetics and protein structure. The genes within the MHC are divided into class I, class II, and class III types[31,169]; the human MHC (HLA) is described more fully in Chapter 10. MHC class I proteins (Fig. 2-2) are cell surface glycoproteins composed of two chains—the heavy chain (molecular weight approximately 45 kD), which is highly polymorphic and encoded within the MHC by a class I gene, and a nonvariable light chain, β_2-microglobulin (molecular weight approximately 12 kD), which is encoded at another chromosomal location. In contrast to the heavy chain, β_2-microglobulin is not anchored in the membrane (Fig. 2-2A) so that it may be exchanged for, or stabilized by, β_2-microglobulin from the surrounding fluid. MHC class I proteins are expressed on most nucleated cells, albeit at variable levels, and are generally responsible for activating T cells bearing the CD8 surface protein ($CD8^+$ cells) (see later). MHC class II proteins are encoded entirely within the MHC and are composed of two membrane-anchored glycoproteins (see Fig. 2-2A) of similar molecular weight (alpha chain, molecular weight approximately 35 kD; beta chain, molecular weight approximately 28 kD). These chains primarily stimulate T cells bearing the CD4 surface protein ($CD4^+$ cells). The tissue distribution of MHC class II proteins is far more restricted than that of class I, being expressed constitutively only by B lymphocytes, DCs, and some endothelial cells, the last being particularly the case in humans. During an immune or inflammatory response, however, many other cell types, with a few exceptions, may be induced to express MHC class II proteins.[54,68,86,91,182,225,383]

MHC class I and class II proteins form a similar three-dimensional structure at the cell surface (see Fig. 2-2B and C



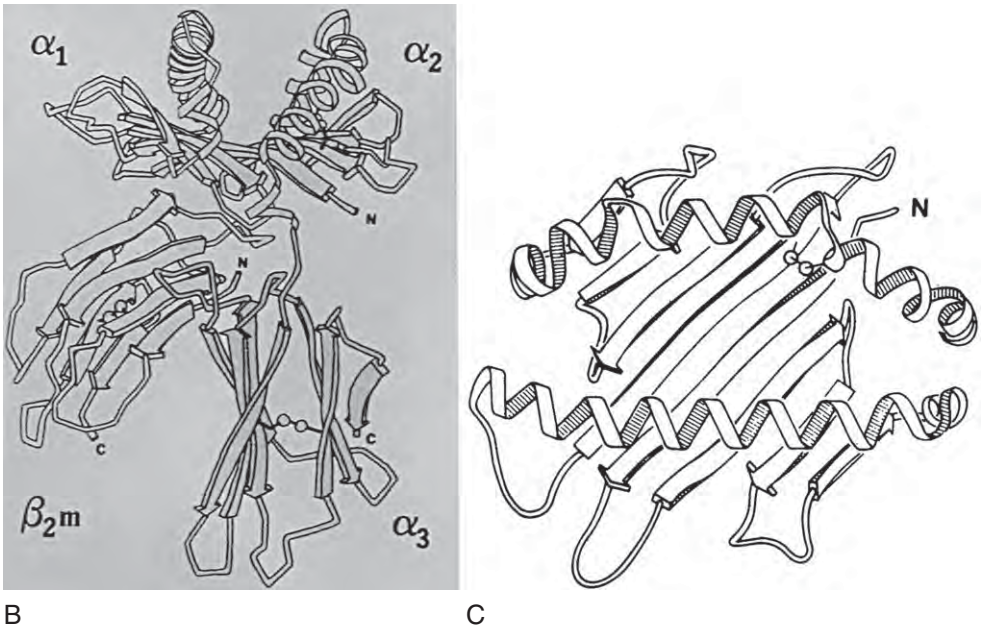


Figure 2–2 Stick diagrams of MHC class I and II and ribbon diagrams of HLA-A2. **A**, Stick diagram showing MHC class I and II as associated with the cell membrane. The MHC class I–associated molecule, β_2-microglobulin (β2-M), is not membrane-inserted. **B** and **C**, The structure of the human MHC complex class I antigen HLA-A2. The peptide groove is clearly visible lying between the two alpha helices. **B**, Side view. **C**, Bird's eye, or the T cell's, view. MHC class II proteins have a similar structure, although the ends of the groove are less closely associated allowing the peptide to extend beyond the constraints of the groove.

for ribbon diagrams of HLA-A2). Within this structure is a groove flanked by two alpha helices, and the amino acids in this groove show the highest degree of polymorphism within a species. During the synthesis and transport of MHC class I and class II proteins to the cell surface, they become associated with small peptides that fit into the groove. The groove of MHC class I has a closed structure, allowing peptides no longer than about 8 to 10 amino acids in size to be accommodated, whereas that of MHC class II has a more open structure permitting the ends of the peptides to flop out of the groove, allowing it to accommodate peptides of at least 13 and often many more amino acids in length.

A major difference between proteins of the two MHC classes is in the origin of these peptides, which are acquired primarily (although not exclusively) from the intracellular environment in the case of class I and extracellular environment in the case of class II (Fig. 2-3). The combination of MHC and peptide is recognized by the antigen receptor (T cell receptor [TCR]) on the T cell. In a pathogen-free immune system, the peptides contained within the MHC proteins originate largely from self-proteins, and many may be derived from the MHC proteins themselves. It is only when a foreign pathogen invades or a graft is in place that the MHC proteins become loaded with foreign peptides. The ability to extract peptides from within MHC proteins[289,308] has shown what types of peptides reside within the MHC groove.[330,349] It is possible to predict from the protein sequence of an antigen which peptides could be recognized in the context of different MHC antigens,[24] and how post-translational modifications of the peptides can affect binding.[79,349] It is possible, with a knowledge of the MHC and peptide sequences, to predict which amino acids in the peptide will be associated with the floor and sides of the groove, and which will be in contact with the TCR.

Several other proteins encoded within the MHC aid the assembly and loading of class I and class II proteins with their peptides (see Fig. 2-3). One type of class II protein, HLA-DM, does not appear on the cell surface, but plays a role in exchanging the class II–associated invariant chain peptide for the antigenic peptide in class II proteins before they emigrate to the cell surface.[297] The LMP (proteosome components) and TAP (transporters associated with antigen processing) genes also lie within the class II region of the MHC and are involved in processing and loading of peptides for MHC class I presentation. Understanding of such antigen processing and presentation pathways has increased[11,51,98,113,196,243-245,296]; this understanding and the structural resolution of MHC (see Fig. 2-2) and TCR proteins[17,18,97] represent some of the most important advances in immunology in the 1990s.[310]

In addition to its value regarding our knowledge of how the immune system works, our insight into the process of antigen processing and presentation has practical value as we begin to explore how peptides may be used in vaccination and tolerance strategies. Although, as mentioned, exogenously derived peptides are generally found presented by MHC class II and endogenously derived peptides by MHC class I proteins, the reverse also can be true owing to a process termed cross-presentation. Originally described for exogenously derived peptides entering the MHC class I pathway,[13] it has been shown that endogenously derived peptides can enter the MHC class II pathway.[355] This increases the diversity and origin of peptides available not only for presentation but also as potential therapeutic candidates.

Data from experiments performed between congenic strains of animals in which only MHC class I or class II antigens differ in donor and recipient show that both are important in graft rejection, although frequently grafts with only MHC class I disparities reject more slowly than grafts with class II only or class I and class II differences.[168,303] Mice with disrupted expression of either β_2-microglobulin (in which surface expression of the whole class I protein is largely prevented, class $I^{-/-}$ mice) or class II genes (class $II^{-/-}$ mice) have been generated and used as recipient (see later) or donor in transplantation experiments. The literature regarding this work is complex. In many studies, a lack of class I or class II antigens alone on donor tissue has little effect on graft survival.[6,74,135,200,221] In other experiments, graft survival may be prolonged or permanent when donor tissue lacks either class I[135,224] or class II only[32,135] or both class I and class II antigens.[32,221,268] It is clear from all of this work that results vary when different types of grafts are used, probably reflecting a greater or lesser involvement of the different T cell subsets ($CD4^+$ and $CD8^+$; see later).[32,135] The interpretation of some of these apparently straightforward experiments is complicated, however, by the suggestion that grafts from class $I^{-/-}$ mice may be reconstituted in their expression of class I by serum β_2-microglobulin in the recipient or may express residual cell surface class I protein in the absence of β_2-microglobulin.[195,200]

One notable feature of MHC protein that makes it different from any other region of the chromosome and the feature that creates serious problems for the transplant clinician is the high degree of variation or polymorphism in the class I and class II cell surface proteins that it encodes within a species. It is likely that this extensive polymorphism has evolved as a product of immune defense mechanisms against infection[169] because of the crucial role of MHC proteins in presentation of pathogen-derived peptides to the immune system, and the fact that, as described earlier, MHC proteins exhibit selectivity in the peptides that they can present. Certain species that have limited polymorphism at class I or class II loci can be devastated by infections that in closely related species with a polymorphic MHC are cleared without difficulty.[263] With two alleles at each MHC locus, most individuals can express six different MHC class I proteins and eight different MHC class II proteins. Combined with the polymorphism at this locus, this means that for transplantation between unrelated individuals, MHC-identical donors and recipients are rare, and even when they are found, miH antigens are almost undoubtedly different. It is only realistically possible clinically to graft tissue that is MHC and miH antigen identical between monozygotic twins; this is why immunosuppression is needed routinely in clinical transplantation.

As described earlier, several genes within the class II and class I regions do not encode classic MHC proteins. In addition to the genes mentioned previously, some of these encode nonclassic MHC proteins that are similar in structure to classic MHC proteins, but that are nonpolymorphic. These may have antigen-presenting capacity for specialized antigens, such as lipids (e.g., mycolic acid and lipoarabinomannan from *Mycobacterium*) or peptides of different sequence but with common characteristics (e.g., with *N*-formylated amino termini).

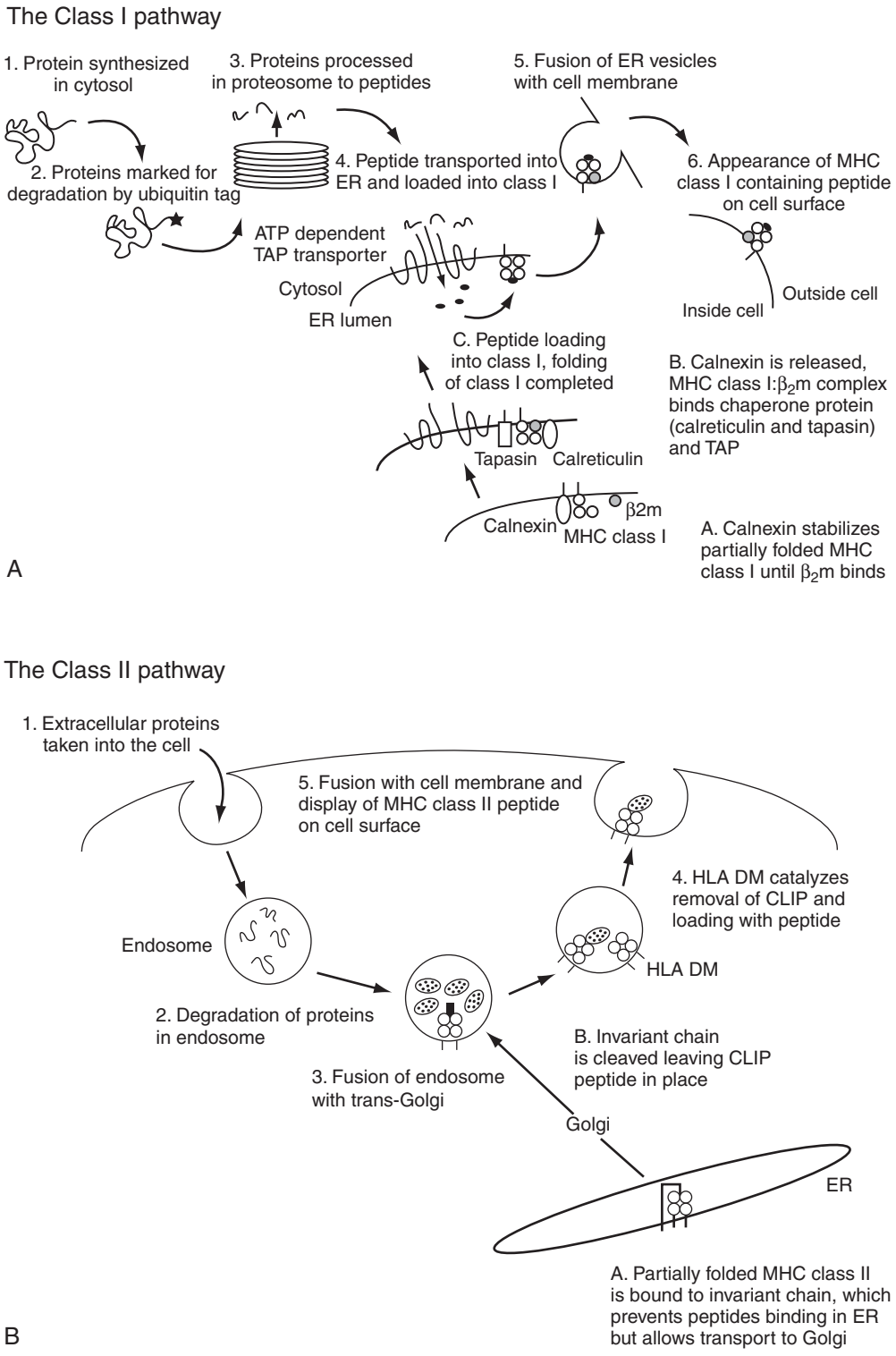


Figure 2–3 Antigen processing and presentation in the MHC class I and class II pathways. **A**, Processing of endogenous antigens occurs primarily by way of the class I pathway. Peptides are produced and loaded into MHC class I proteins as shown in steps 1 through 4. During the synthesis of MHC class I proteins (steps A through C), the alpha chain is stabilized by calnexin before β_2-microglobulin (β_2m) binds. Folding of the MHC class I/β_2-microglobulin remains incomplete, but the complex is released by calnexin to bind with the chaperone proteins, tapasin and calreticulin. Only when the TAP transporter delivers peptide to the MHC class I/β_2-microglobulin can folding of this complex be completed and transport to the cell membrane occur (steps 5 and 6). **B**, Processing of exogenous antigens occurs primarily by way of the class II pathway. Antigens are taken up into intracellular vesicles where acidification aids their degradation into peptide fragments (steps 1 and 2). Vesicles containing peptides fuse with trans-Golgi containing CLIP-MHC class II complexes (step 3). DM aids removal of CLIP and loading of peptide before the class II peptide complex is displayed on the cell surface (steps 4 and 5). MHC class II proteins are synthesized in the endoplasmic reticulum where peptide binding is prevented by invariant chain. Invariant chain is cleaved leaving the CLIP peptide still in place (steps A and B) before fusing with acidified vesicles containing peptide. In B lymphocytes and epithelial cells of the thymus, an atypical class II protein, HLA-DO, is expressed that is a dimer of HLA-DOα and HLA-DOβ. Similar to HLA-DM, it is not expressed at the cell surface and inhibits the action of HLA-DM. Its precise role is unknown. ATP, adenosine triphosphate; CLIP, class II–associated invariant chain peptide; ER, endoplasmic reticulum; MHC, major histocompatibility complex; TAP, transporters associated with antigen processing.

The class III region of the MHC is large and is increasingly well characterized.[31,130,238] Genes in this region encode proteins with a wide diversity of different functions, and although they themselves do not stimulate T cells in the same way as class I and class II proteins, many have important activities in generating and influencing immunity. Tumor necrosis factor (TNF)-α and TNF-β are encoded in the class III region, and a marker of TNF-α polymorphism associated with high TNF production has been found in heart transplant patients.[366]

Minor Histocompatibility Antigens

Although the highest degree of genetic polymorphism within a species lies within the MHC, many other loci encode proteins with a lower degree of variability, and from genetic studies it is clear that such proteins can act as transplantation antigens. They have been termed miH antigens, although their structure and distribution for many years were elusive. Although T cells could recognize and respond to cells from MHC-identical individuals, it was almost impossible to raise antibodies against the antigens involved, making biochemical characterization difficult. The knowledge that T cells recognize small peptides from antigens, together with the resourceful application of molecular techniques, allowed the characterization of the prototypic miH antigen, the male antigen or H-Y.[326,379] From such work, it is clear not only that miH antigens are a composite of peptides from low-polymorphic or nonpolymorphic proteins, presented in the MHC groove, but also that the so-called H-Y antigen is derived from a group of proteins encoded on the Y chromosome.[111,326,327,379] The former finding explains why it has been difficult to raise antibodies to miH antigens because antibodies frequently recognize conformational determinants on proteins, and peptides bound within the MHC groove may not be accessible for recognition by the antibody-producing B lymphocyte.

miH antigens may play a prominent role in graft rejection in a recipient who is given an MHC-compatible graft but in whom preexisting sensitization to miH antigens exists. This situation can be shown in the rat and mouse[81,280] and probably explains the occurrence of rejection episodes (which rarely result in graft loss) in renal transplants performed between HLA-identical siblings. Multiple miH differences have been shown to represent an immunogenic stimulus equivalent to that of the MHC in a nonsensitized recipient of a cardiac allograft in the mouse,[280] but it is difficult to gather similar data in clinical transplantation. Polymorphic tissue-specific antigens also may be common, and such systems have been shown for mouse skin[344] and rat kidney.[127] In the rat, incompatibility for kidney antigens alone is incapable of causing rejection of a renal allograft, even when the recipient has been presensitized. An endothelial-monocyte antigenic system has been shown in humans, and it has been suggested that cells sensitized to these antigens can cause graft damage. More miH antigens are being characterized, and this whole area has been reviewed extensively by other authors.[107,298,334]

Antigen Presentation

Donor Dendritic Cells and Direct Antigen Presentation

Immunization with MHC antigen in the form of a soluble membrane extract or in liposomes may not produce an immune response, whereas integrated cell surface MHC proteins may be highly immunogenic. Presentation of MHC class I antigen on cells that do not express class II antigens (e.g., red blood cells in rodents or platelets) does not produce a good primary immune response, suggesting that MHC class II antigens must be present on the immunizing cells for an optimal immune response to be generated. In some cases, presentation of incompatible class I antigens in the absence of class II antigen not only may fail to evoke a primary immune response but also may initiate a state of active suppression or tolerance (see Chapter 23).

The level of immunogenicity of MHC proteins varies considerably with the cell type on which they are found. Cells with the characteristics of bone marrow–derived leukocytes are found throughout the body in nonlymphoid and in lymphoid tissues.[52,128] As previously alluded to, these cells migrate rapidly out of a tissue after transplantation to the recipient lymphoid organs, where they are able to interact with and stimulate the host immune response.[184,185] Such tissue-resident leukocytes have the characteristics of immature DCs,[292] which on migration mature rapidly into antigen-presenting cells that are particularly potent in their ability to stimulate T lymphocytes.[340-342] Mature DCs express a high level of MHC class I and class II antigens together with a range of costimulatory proteins and cytokines (see later) and as such are able to stimulate $CD4^+$ and $CD8^+$ T lymphocytes. They are uniquely powerful in stimulating naive (previously unactivated) T cells, earning them the title of professional antigen-presenting cells, and it is generally accepted that such cells, derived from the transplant itself, can stimulate strongly adaptive immunity in the recipient (Fig. 2-4A).

This suggestion is perhaps counterintuitive when one considers that the T cell restriction is skewed (by positive selection of T cells in the thymus) toward recognition of peptide in the context of self-MHC proteins. It is clear experimentally, however, that allogeneic MHC/peptide complexes provide a uniquely strong stimulus to the immune system, and a high frequency—1% to 10%—of all T cells respond to allogeneic MHC. The allogeneic MHC contains peptides derived from the donor tissue originating mainly from normal nonpolymorphic proteins[308] or the MHC itself.[101,269] In the context of self-MHC, the former type of peptide would not normally induce an immune response because the body would be tolerant of them. When the MHC is allogeneic (i.e., when a graft is placed into an MHC-disparate recipient), the sum of the MHC plus nonpolymorphic peptide may now be recognized as nonself and stimulate a T cell. The real job of such T cells is not to respond to alloantigen, but to eliminate invading organisms. Their ability to respond to alloantigen is due to an inconvenient cross-reactivity of their receptor for self-MHC plus foreign peptide with allogeneic MHC plus self-peptide. For many T cells that have reactivity with a foreign peptide plus self-MHC, it is possible to show cross-reactivity on one or more alloantigens. Also, different peptides from the same proteins may be displayed by the foreign MHCs and self-MHCs because of the different peptide-binding capacities of each MHC groove. Peptides normally not displayed in self-MHC do not have an opportunity to induce tolerance in the recipient and may induce an immune response when presented on allogeneic MHC proteins. Alloreactive cytotoxic T lymphocytes (CTLs) induced by direct antigen presentation are able

to recognize a wide spectrum of different peptide-MHC aggregates and empty MHC molecules, as elegantly shown by Rotzschke and colleagues.[308] The unusually high number of T cells that react to any given allogeneic MHC exist because many different self-peptides are derived from the graft, and the combination of these with the allogeneic MHC stimulates many different T cell clones in the recipient.[75]

Indirect Antigen Presentation

Elimination from the graft of passenger leukocytes does not abrogate rejection completely, suggesting that there is a second route to sensitization of the recipient that requires antigen presentation by an MHC class II–expressing cell. In humans, the endothelium bears MHC class II antigens constitutively and may provide such a route, but it has also become apparent that foreign, graft-derived antigens (of MHC and non-MHC origin) can be presented to the recipient immune system by its own DCs in the process termed indirect antigen presentation (see Fig. 2-4B). This is the process by which normal antigens are displayed to the host on an antigen-presenting cell. From what we understand about antigen processing and presentation (see Fig. 2-3), it seems likely that most allogeneic MHC peptides are presented in the context of class II MHC antigens because it is this pathway that deals with proteins exogenous to the cell. Cross-presentation (see earlier) does allow for presentation of cytosolic proteins in MHC class II, however. Fangmann and coworkers[83] showed that indirect presentation may have a practical significance in transplantation responses. They showed that peptides derived from rat class I antigens were able to immunize animals via the indirect pathway for accelerated rejection of a subsequent skin graft carrying the class I antigens from which the peptides were derived.

Further information on this issue comes from experiments in which skin grafts from class $II^{-/-}$ mice are transplanted onto normal mice. Antigen-presenting cells from these grafts do not directly stimulate $CD4^+$ cells because of the absence of class II antigen, but graft rejection still occurs and is $CD4^+$ cell dependent. In this case, the $CD4^+$ cells are presumed to have been stimulated by indirect presentation of donor alloantigens on self-MHC.[6,194,195] Further experiments addressing this issue involve antigen-presenting cells from the recipient or host that are disabled by genetic manipulation such that they no longer express costimulatory molecules, which are one of the hallmarks of professional antigen-presenting cells ($B7^{-/-}$ mice)[218] (see also later in this chapter). These workers observed that the absence of B7 on donor cells had no effect on the kinetics of vascularized heart allograft rejection. Absence of such proteins on the cells of the recipient had a dramatic effect, however, and allowed long-term survival of normal, B7-expressing hearts, data that were taken by the authors to suggest that in this mouse model, costimulation provided by recipient antigen-presenting cells is much more important in the initiation of graft rejection than is the costimulation provided by donor antigen-presenting cells. The simplest interpretation is that indirect presentation is playing a more important role than direct presentation in this model, although the possibility that costimulation provided by recipient antigen-presenting cells is important, rather than antigen presentation and costimulation, cannot be discounted completely.

Some workers have long believed that indirect antigen presentation plays the dominant role in acute graft rejection,[7] and there is plentiful evidence of its importance. Indirect antigen presentation also can provide the continuing antigenic stimulus required for chronic graft rejection,[49,331,387] at a time when, because donor DCs are lost quickly from the graft, direct antigen presentation assumes a lesser or absent role.

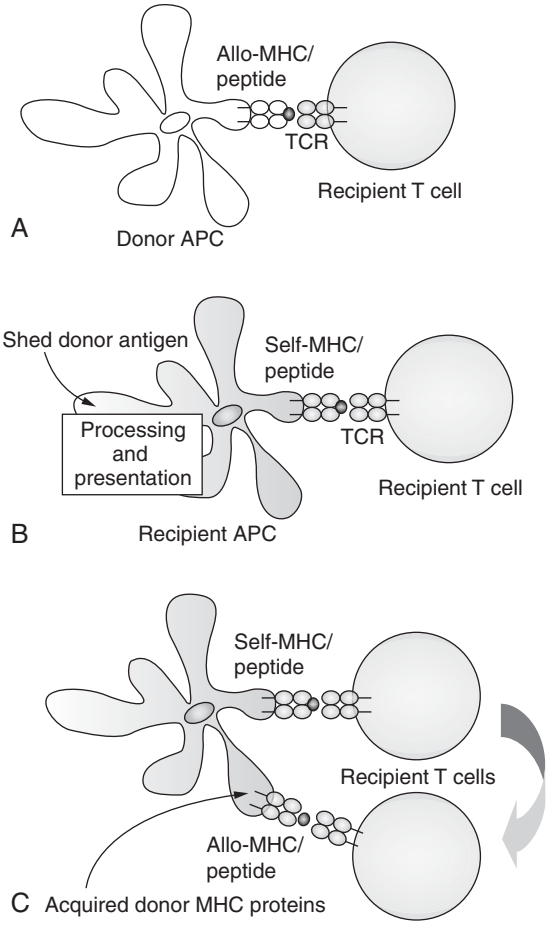


Figure 2–4 **A-C**, Direct, indirect, and semidirect pathways of antigen presentation. Sensitization of the recipient can occur by antigen presentation delivered through passenger leukocytes or dendritic cells of donor origin (direct antigen presentation) (**A**) or recipient origin (indirect or semidirect antigen presentation) (**B** and **C**). APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T cell receptor.

Semidirect Antigen Presentation

If host T cells are stimulated by recipient-derived DCs via indirect antigen presentation, the MHC restriction of the effector cell population is to host rather than donor. A problem could arise if a cytotoxic T cell, previously stimulated with self-MHC and allogeneic peptide, comes to lyse its target cell—in the case of graft rejection, the foreign transplanted tissue, which does not express self-MHC molecules. This problem is overcome if the foreign MHC on the target cell appears to be identical to the degraded foreign MHC in association with self insofar as the T cell is concerned, if the effector arm of the immune response does not require MHC restriction (e.g., macrophages, delayed-type hypersensitivity), or if the effector population is primed by the donor-derived MHC. How could the last situation arise if the T cells are primed by recipient-derived DCs? Intact proteins can be

exchanged between cells in cell culture systems, and MHC proteins transferred in this fashion can stimulate alloreactive responses.[10,126,312] Donor MHC acquired by a recipient's DCs could stimulate the T cells such that they could react with the graft itself. The importance of this reaction in stimulation of alloreactive responses in the whole animal has been highlighted in recent work,[137] although its importance in inducing graft rejection has yet to be established.

Activation and Types of Dendritic Cell

So far this chapter has concentrated on the role that DCs (or other antigen-presenting cells) play in activating T lymphocytes. T lymphocyte–DC interactions are reciprocal, however, and it is becoming increasingly clear that T cells control the maturation[333] and functional phenotype[294] of DCs. Ligation of CD40 on DCs by CD154 (CD40 ligand) on the T cell results in the upregulation of the B7 proteins, which may affect the T cell further.

Several types of DC have been described, including bloodborne conventional DCs that are delivered to lymphoid organs (and which have several different cell surface phenotypes), tissue-resident DCs such as Langerhans cells (the skin-resident passenger leukocyte), and plasmacytoid DCs. These DCs are characterized not only by the expression of different cell surface proteins but also by different functional phenotypes.[332,343] Although this chapter has concentrated on the role of DCs in activating the immune response, it is becoming increasingly apparent that some of these DC subsets may be crucial in the induction and maintenance of tolerance.[216] This view is becoming widely held, and the consequences of this for the regulation of transplantation responses is increasingly a focus of attention.[228]

Activation of Recipient T Cells

Location of T Cell Activation

After small bowel transplantation, recipient-derived leukocytes, including T lymphocytes, migrate in large numbers into the mesenteric lymph nodes and Peyer's patches of the graft, generating a marked cytokine response within 24 hours of grafting.[145,159,359,360] This situation may represent normal homing of such cells because the small bowel is so rich in lymphoid tissue. It is likely that these T cells, if not already activated, may become so within the transplant, which is rich in mature DCs.

Naive lymphocytes are thought normally to recirculate from blood into lymphoid tissues without entering peripheral tissues and as such would be unlikely to become activated in a graft. The extent to which naive T cells enter transplants other than small bowel and become activated in situ is therefore less clear—the cells neither express the adhesion proteins and chemokine receptors normally associated with homing to peripheral tissues, nor are the DCs within the graft mature. More recent experiments have reinvigorated the idea, however, that naive cells can recirculate in small numbers through peripheral tissues,[46,47] although the extent to which they can become activated within such peripheral sites is unclear. Indeed, in mice lacking secondary lymphoid tissue, graft rejection can be abrogated or seriously attenuated.[179,180] In certain chronic inflammatory situations, lymphoid neogenesis or ectopic accumulations of lymphoid cells develop within peripheral tissues, however, and can provide an environment in which naive cells can become activated. The possibility that lymphoid neogenesis also occurs and is important in the context of transplantation has been suggested by studies in a mouse cardiac transplant model in which the presence of such accumulations occurred in a high proportion of grafts undergoing chronic rejection.[8]

During acute graft rejection of organs other than small bowel, it would seem that T cells are most likely to become activated in draining or local lymphoid tissue where they can interact in an optimal fashion with donor or host-derived DCs. The contribution of naive T cell recirculation to acute graft rejection is probably minor, although its role in the longer term may become more important. The possibility that naive cells recirculate through peripheral tissues for the purposes of tolerance induction rather than activation[47] is interesting and should be considered in the context of longer term graft function and survival.

Immune Synapse

T lymphocyte activation, central to the immune response to a transplant, is a complex process. Much information has been accumulated in this area, and although the antigen signal delivered to the T cell through the TCR/CD3 complex (Fig. 2-5) is absolutely required for activation, T cells also receive many other signals via cell surface receptors without which they do not become fully able to initiate a productive immune response. It is becoming increasingly clear that the contact between antigen-presenting cells and T lymphocytes (and other cells of the immune system) involves supramolecular organization of receptors and ligands into microdomains, or immune synapses, which exhibit reproducible patterns of the receptor-ligand pairs. For instance, it has been shown that adhesion molecules cluster with TCRs on the lymphocyte.[246] In the T cell–antigen-presenting cell synapse, MHC protein initially accumulates in a ring around adhesion proteins, but on interaction with the TCR moves to a central patch[109]; this clustering of proteins involved in T cell activation seems to be crucial for consolidation or

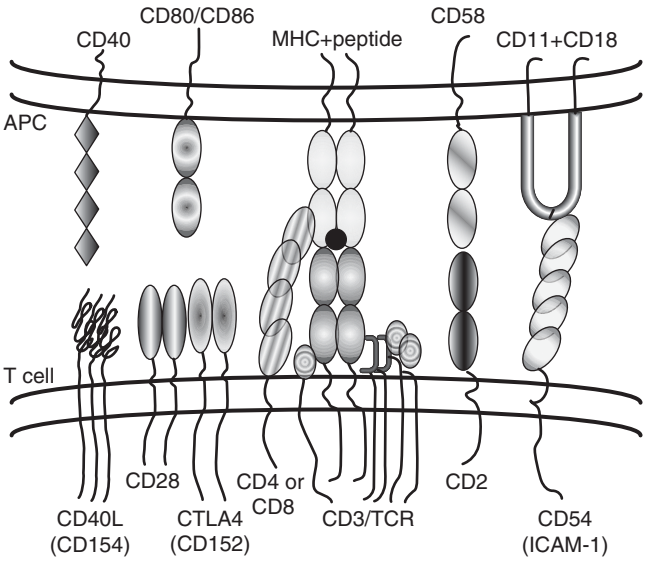


Figure 2–5 Antigen-presenting cell (APC)–T cell protein interactions that are required for T lymphocyte activation. Many cell surface proteins are involved in the interactions of T cells with their APCs. The interactions often may be bidirectional and affect APC and T cell.

maintenance of signaling or both. This is a newly emerging area of immunology, and increased knowledge in this area is likely to help in predicting more accurately the outcome of intercellular interactions.[36]

T Cell Receptor Signals

Without an interaction of the TCR with its cognate antigen, T cells remain in a quiescent or resting state and can recirculate through the lymphoid tissues for many years.[29,108] Most T cells bear a TCR composed of two similar chains, the alpha and beta chains, which are complexed with several more proteins, the gamma, delta, epsilon, and sigma chains of the CD3 complex. The TCR confers specificity of antigen/MHC binding (see Fig. 2-5), whereas the sigma chains of the CD3 complex transduce signals of activation to the T cell. Many intracellular signaling pathways are activated, resulting in de novo expression of a range of genes, including genes encoding cytokines and new cell surface proteins. The signaling pathways are increasingly well characterized and have been described fully elsewhere.[33,257,367,370] They form the target of many immunosuppressive drugs.[22,115,118,249,250]

Second or Costimulatory Signals

The fate of a $CD4^+$ T cell when in receipt of a TCR signal depends critically on whether or not it secures other so-called costimulatory or second signals. Without these second signals, a T cell may become anergic or unresponsive,[148,149,324,325] a state that also may result in an ability to prevent the activation of its neighboring T cells.[34,89,203] The fact that deprivation of second signals can result in an unresponsive or regulatory fate for T cells has attracted enormous interest because it has implications in preventing graft rejection. There are many cell surface proteins on a T cell that potentially contribute to its activation (see Fig. 2-5). CD4 and CD8 proteins act by binding to class II and class I on the antigen-presenting cell. CD4 and CD8 are linked to intracellular proteins, which are involved in transducing further signals to the T cell. A series of additional proteins on the T cell surface, such as CD54, CD2, CD11a/CD18, and CD5, act largely to increase the affinity of interaction between the T cell and its antigen-presenting cell, although they also may transduce further signals to the T cell.

The cell surface protein CD28 was the first to be described as a costimulatory protein. Now known to be a member of a family of similar proteins,[39,112,293] CD28 still attracts attention as a potential target for the regulation of transplantation responses.[188,335] Activation of the downstream signaling via CD28 results from ligation with one of the B7 family of proteins, CD80 or CD86. These proteins are expressed by antigen presenting cells such as DCs and are readily able to engage CD28 during antigen presentation. Signaling through CD28 in the context of TCR ligation results in an increase in glucose metabolism, high levels of cytokine and chemokine expression including the production of very high levels of IL-2, resistance to apoptosis, and long-term expansion of T cells. This powerful driver to activation and proliferation is counterbalanced by the presence on activated T cells of CTLA-4. Similar in structure to CD28, CTLA-4 inhibits the earliest events in T cell activation. CTLA-4 has a higher affinity for CD80 and CD86 than does CD28,[42,323] and its engagement with CD80 induces a lattice structure at the cell surface consisting of alternating CTLA-4 and CD80 homodimers.[42,323] These properties of CTLA-4 may limit the ability of CD80 to interact with and cluster CD28 at the immune synapse, potentially explaining the finding that low levels of CTLA-4 can be effective at inhibiting immune responses.

The requirement of CD28 signals for $CD4^+$ T cells in secondary immune responses or for $CD8^+$ T cells is less clear. The prevailing view for $CD4^+$ cells is that if they have not been stimulated very recently by antigen (e.g., they have developed into memory cells), they will require costimulation for reactivation, but recently activated cells also have been shown to be costimulation dependent.[119,222] Experimentally, it can be shown that in certain situations virus-reactive $CD8^+$ cells require neither costimulation through CD28 nor CD28-dependent help.[176,393] To achieve this, however, they may require prolonged TCR stimulation (e.g., provided by a replicating virus), a situation that infrequently may occur during other immune responses. Even for $CD4^+$ cells, overwhelming stimulation through the TCR may obviate the requirement for CD28-mediated costimulation. This is important in the context of clinical transplantation, where a large proportion of the alloreactive pool has previously been antigen activated as a result of its cross-reactivity with pathogen-derived peptides (see earlier) providing one possible explanation for the finding that targeting this costimulatory pathway is less effective in attenuating transplantation responses in humans than would have been predicted from rodent studies.

Mice with a disrupted *cd28* gene have impaired immune responses, but can reject skin grafts, albeit in a delayed fashion[158]; this is likely due to the plethora of other costimulatory proteins that can substitute the action of CD28.[39,293,329] The severe phenotype of CTLA-$4^{-/-}$ mice, in which animals die from lymphoproliferative disorder within a few weeks of birth, illustrates the crucial role of CTLA-4 in counterbalancing the effects of CD28. Blocking the CD28 pathway in normal animals may have effects on the generation of immune responses and may result in prolonged graft survival or tolerance of grafts.[198,276,365] The most widely used reagent for this purpose has been CTLA-4-Ig, which potentially blocks all CD28 and CTLA-4-B7 interactions. The fact that CTLA-4 seems to provide an essential signal in the resolution of immune responses indicates this is unlikely to be the optimal strategy, however, and reagents that effectively block only CD28-B7 interactions (and perhaps interactions between the other costimulatory receptor ligand pairs) should be sought.

As mentioned earlier, there are several other members of the CD28 family of costimulatory proteins, including ICOS, PD-1, and BTLA,[112] and another T cell costimulatory family of proteins, the TNF/TNFR family, which includes CD27, CD134 (OX40), and CD137 (4-1BB).[380] The ligands of these proteins are more broadly distributed, including on cells not thought to have a role in activating naive T cells. Such receptor-ligand pairs may be important in maintaining immune responses and act to sustain detrimental immunity in the setting of chronic graft rejection. Indeed, blocking ICOS has been found to reduce the pathophysiology of experimental chronic allograft rejection. All of these costimulatory proteins seem to have counterbalancing proteins analogous to CTLA-4, showing the critical nature of delivering controlled immunity.

On activation, T cells express another cell surface protein, CD154 (CD40 ligand, gp39). Interaction of this protein with its counterreceptor, CD40, is crucial for the activation of

B cells, DCs, and monocytes. Larsen and coworkers[186] showed that blocking this interaction could prolong graft survival in a mouse cardiac transplant model. Even more impressive, however, are more data from this group showing that combined blocking of CD28 and CD40 interactions can induce permanent survival of allogeneic skin grafts in mice with no long-term deterioration of graft integrity.[187] Tolerance to the graft antigens could not be shown in these mice despite the excellent survival of the transplant itself. Other groups have now taken up this approach, always with dramatic effects on the regulation of immunity. Kidney graft rejection in monkeys can be prevented completely with antibodies to CD154 CD40 ligand.[163] Similar antibodies have been developed for use in the clinical setting and are in trials. The first antibody of this type to have been used clinically has now been withdrawn, however, because of its side effects.

Initiation of the Immune Response—CD4$^+$ and CD8$^+$ Cells

As described in previous sections, the interaction of the T cell with antigen-presenting cells plays a fundamental role in initiation of the immune response. The consequences of this interaction include proliferation and differentiation of the so-called helper T cells with the concomitant production of growth and differentiation factors (or cytokines) that are required by other cells so that a potent effector response can be mounted. In many cases, these helper T cells bear the CD4 surface protein, but in certain situations CD8$^+$ cells are able to respond in the absence of CD4$^+$ cells and themselves meet all of the requirements of a helper T cell.[209,304,338] That CD4$^+$ cells frequently are required to initiate graft rejection has been shown by many workers in a variety of experimental systems,[55,116,117,205,207] although depending on the mismatch between donor and recipient, CD8$^+$ cells may be additionally required or may act independently of CD4$^+$ cells.[300,301,303,338,356]

Investigation of the effects of CD4$^+$ and CD8$^+$ cells in transplantation responses has included the use of knockout mice that are deprived of these populations through genetic manipulation. Mice lacking class I or class II antigens are severely depleted of CD8$^+$ or CD4$^+$ cells, as are mice in which either the *cd4* or the *cd8* gene has been disrupted.[4,64,65,74,174,181,221,321] The effects on graft rejection of a lack of CD4$^+$ or CD8$^+$ cells produced in this manner have not been predictable and often depend on the nature of the mismatch between donor and recipient. Further, despite an apparent depletion of CD8$^+$ cells in class I$^{-/-}$ mice, CD8$^+$ CTLs can be generated in large numbers after transplantation and may be involved in the rejection process.[4,194,321] The results of such experiments are essentially consistent with previous ideas in this area—CD4$^+$ cells seem normally to initiate graft rejection, but there are experimental models (usually when there is a dominant or sole MHC class I mismatch) in which CD8$^+$ cells also are required for rejection to proceed with normal kinetics or may act independently of the CD4$^+$ cell.

GENERATION OF EFFECTOR IMMUNITY—THE EFFERENT ARM

T1-, T2-, and Th17-Driven Immunity

After stimulation of the immune system, a response develops in which either humoral or cell-mediated immunity may be seen to dominate,[156,271] and it has become clear that cytokines play a determining role in this process (Fig. 2-6).[69,230,251-253] The cells (and the cytokines they produce) that drive a cell-mediated response have been called Th1, and the cells driving humoral immunity have been called Th2. It has been shown that both helper and cytotoxic T cell populations diverge in their cytokine production, and so here we refer here to T1 and T2 populations. More

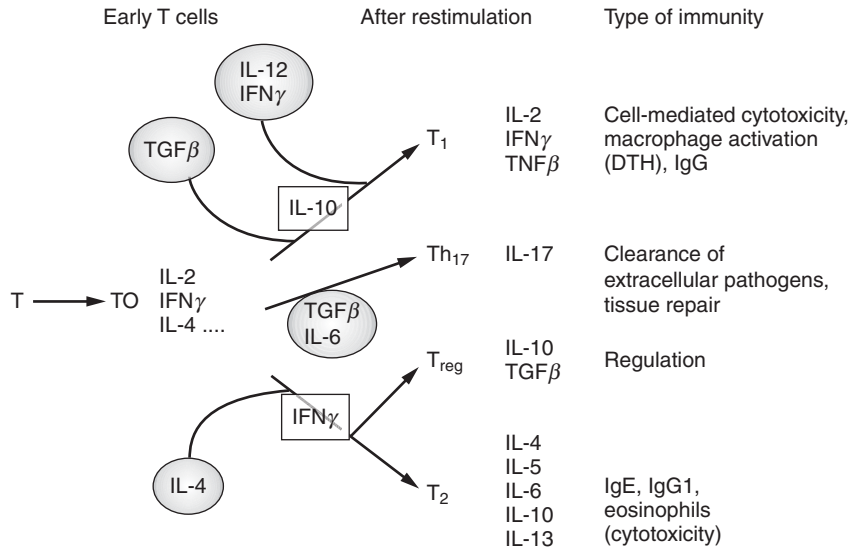


Figure 2–6 T1/T2/Th17 cell differentiation and immunity. Cytokines produced by T cells and that influence their divergence to T1, T2, and Th17 subsets are shown, defining the effector immunity generated. Cytokines that may positively (*in circles*) or negatively (*in squares*) regulate divergence of the T1, T2, and Th17 cells are shown. Cells with a regulatory or suppressive function (Treg) also may be generated de novo during an immune response, likely diverging from the T2 pathway. Such cells differ from naturally occurring Tregs, which have a CD25$^+$ cell surface phenotype, but nevertheless function in a similar fashion to control immunity. DTH, delayed-type hypersensitivity; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor.

recently, a discrete population of T cells has been described, characterized by production of IL-17 in the relative absence of IFN-γ, which also can contribute to the development of cell-mediated immunity and which may be responsible for driving the pathology in autoimmune conditions previously ascribed to an inappropriate Th1 response.[124,125,183,372,373] To date, most work on such cells has focused on the $CD4^+$ population, and whether or not $CD8^+$ cells also make a major contribution to IL-17 production remains to be determined.

Early on during an immune response, T cells seem to make a wide range of cytokines, a clear divergence of cytokine production at the population level and individual cell level being observed only after continued antigenic stimulation. IFN-γ is the prototypic cytokine of T1 cells, and a predominance of this population results in the appearance of cell-mediated immunity involving the generation of specific CTLs and activated macrophages. T2 cells make cytokines such as IL-4, IL-5, and IL-6, which are crucial for the induction of humoral immunity, for class switching to certain immunoglobulin isotypes, and eosinophilia. Th17 cells are characterized by the production of IL-17 and their involvement in a variety of experimental pathologies.[125]

Much is now understood about how such responses are determined[291] in terms of the molecular and the cellular interactions involved. The local cytokine milieu,[92,94,95,140,141,351,352] involvement of antigen-presenting cells other than DCs,[210,211] and the type of CD28 signal delivered (i.e., through either CD80 or CD86)[90,175,198,290] all have been suggested as important factors. Most recently, it has been suggested that signals delivered through the evolutionary conserved cell surface protein, Notch, can determine the ability of T cells to become T1 or T2 producers; ligation by the delta-like family of ligands, inducing IFN-γ–producing cells; and the jagged ligands inducing IL-4 producers.[2] Other data suggest, however, that ligation by delta induces IL-4–producing cells, inhibiting Th1 cell differentiation.[362,384] Although this area remains confused, it is clear that the Notch signaling pathway, first described for its role in cell fate determination during development across the species, can strongly influence differentiation of mature T cells and other cells of the peripheral immune system.[63]

Interest of the transplantation community has focused on the possibility that although a T1-driven response may inevitably be damaging and result in graft rejection, a T2-driven response may not have this effect and may be associated with the induction of tolerance to a graft.[61,62,259,282,347] Many groups have found that tolerance or reduced donor-directed reactivity is associated with a decrease in the prolonged expression of the T1-associated cytokines IL-2 and IFN-γ.[1,25,26,59,242,353] It has been tempting to speculate that this decrease is accompanied by or even due to the expansion of regulatory T2 cells.[353] There is some evidence that the expression of cytokines such as IL-4, IL-5, and IL-10 is preserved during the development of tolerance.[60,104,320,353] Cells other than T2 lymphocytes can produce such cytokines, however, meaning that their detection does not infer the presence or action of the T2 population. As described in the following sections, an immune response to a transplant is complex; humoral mechanisms and a variety of cellular mechanisms can effect graft destruction, and it is likely that any type of immunity, T1, T2, or Th17 driven, would result in graft rejection. Clones of T lymphocyte that have T2-like properties are as capable of initiating graft rejection as are clones of T1 cells,[371,390] and it has been suggested that T2 cells drive chronic graft rejection.[331] In models of true tolerance, rather than prolonged graft survival, a rapid shutdown of cytokine, rather than preferential T2 cytokine production, may be observed.[150,276]

Several groups have tried to assess the role of key cytokines by performing experiments in which their overexpression or absence is tested. Two groups have shown that tolerance can be induced using reagents that block CD28 signaling in $IL\text{-}4^{-/-}$ mice.[178,260] An important additional finding from these studies was that tolerance was induced more easily in homozygous $IL\text{-}4^{-/-}$ mice than in heterozygous $IL\text{-}4^{+/-}$ mice,[260] the implication being that the presence of IL-4 itself can be damaging to the graft. In other experiments, it was shown, again using knockout mice, that neither IL-2 nor IFN-γ is required for rejection,[171,318,339,347] but that both are required for tolerance induction.[53] Interpretation of these experiments can be complicated by the fact that cytokines often can substitute each others' function, and it is unclear whether or not the phenotype of knockout mice reflects accurately the importance of these cytokines in normal mice. IL-15 can substitute many of the actions of IL-2 and IL-13 for IL-4. In the experiments described earlier, IL-15 transcripts were found in grafts put into the $IL\text{-}2^{-/-}$ mice, and IL-13 transcripts were found in grafts transplanted into IL-4 knockout mice. The fact that $IL\text{-}2^{-/-}$ or $IFN\text{-}\gamma^{-/-}$ mice were unable to become tolerant suggests a nonredundant role for these cytokines in the induction of tolerance, a finding that a few years ago not many people would have predicted. The recent findings that, first, regulatory cells elaborate IFN-γ[386] and, second, a major population of regulatory cells expresses the IL-2 receptor constitutively[313,314] provide a reasonable explanation.

An alternative method of investigation has been used by several groups in experiments in which cytokines have been injected or overexpressed in animal transplant models in an attempt to deviate the immune system toward a T1 or T2 response. Paradoxically, given the aforementioned data, injection of IL-2 or IFN-γ can prevent the induction of tolerance.[25,59] Injection or overexpression of IL-4 cannot induce tolerance,[254] however, and although this treatment may prolong graft survival marginally, it may inhibit tolerance induction.[260]

The conclusion from all of these studies is that an effector immune response driven by either T1 or T2 cells is damaging, although in some cases the response driven by T2 cells may be less detrimental acutely than that driven by T1 cells. T2 cells may be the primary drivers of chronic rejection. The individual actions of certain cytokines still are not fully understood, but it would seem, given the data on IL-2 and IFN-γ, that such cytokines may assume different functions depending on the timing or perhaps location and origin of their production. The involvement of Th17 cells in protective immunity and immune-mediated pathology is an area of intense current investigation. The role of such cells in transplantation merits investigation, particularly because an early study indicated participation of IL-17 in rejection.[3]

Migration of Activated Cells into the Graft

To enter a site of inflammation or immune response, leukocytes must migrate across the vascular endothelium. This migration process is controlled by the elaboration of cell attractants or chemokines and by cell-cell interactions

between the leukocyte and the endothelium.[346,389] Activated and memory cells bear adhesion proteins, chemokine receptors, and addressins, which allow homing to and migration into peripheral tissues.[212,213]

Cell-Cell Interactions

The adhesion of leukocytes to the endothelium is a complex multistep process that involves a series of interactions between the surface of the leukocyte and the endothelial cell or its extracellular matrix.[29,162,346] The proteins involved fall into three groups—the selectins and members of the integrin and immunoglobulin superfamilies. Initial interaction and rolling of leukocytes along the endothelium allows the leukocyte to sample the endothelial environment, while maintaining its ability to detach and travel somewhere else. This step is largely controlled by the selectins, although, for example, α_4 integrins also may play a role at this stage. At this time, the endothelial cells begin to express IL-8 and platelet-activating factor, which induces strong leukocyte adhesion. Under the correct conditions, this interaction leads to signaling to the leukocyte, slowing down and arresting the rolling process. Shedding of L-selectin by leukocytes allows their detachment and extravasation.[229] These latter stages are regulated mainly by the β_2 integrins and adhesion proteins of the immunoglobulin superfamily.

The expression of many adhesion proteins involved in these interactions is upregulated by proinflammatory cytokines. Ischemic damage alone results in increased expression of several cytokines, and of these, IL-1 upregulates the expression of members of the selectin family.[43,283] Other adhesion proteins, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 of the immunoglobulin superfamily and E-selectin (endothelial-specific selectin), are known to be upregulated by the type of cytokines also induced by donor brain death[189] and after the trauma of transplantation. Before an immune response has been generated, the graft becomes attractive to circulating leukocytes, although, as previously mentioned, naive lymphocytes tend not to home into nonlymphoid sites. Antigen-activated lymphocytes have an altered recirculation pattern, however, and migrate into extralymphoid sites.[28,212,281] They may show tissue-selective homing and show preference for sites in which they are most likely to re-encounter their specific antigen.[319] The process seems to be facilitated further by recognition by the T cell of MHC class II/peptide complexes on the vascular endothelium.[223] This process is likely to result in the accumulation of antigen-specific lymphocytes within the site of inflammation, in this case the graft.

One practical aspect with respect to transplantation is that it may be possible to hide or block the expression of the proteins involved in leukocyte extravasation, slowing or preventing the rejection process. Blocking the adhesion proteins by using antibodies or by inhibiting their expression has been attempted in experimental and clinical transplantation settings.[48,57,129,133,139] In general, cocktails of antibodies are more potent than single antibodies,[146,388] although the results vary, and in one case a combination of antibodies to ICAM-1 and lymphocyte function-associated antigen (LFA)-1 was shown to result in accelerated rejection of rat cardiac allografts.[247] Antisense oligonucleotides have been used in an attempt to prevent the expression of ICAM-1 and have been effective in prolonging graft survival in experimental models.[345] Small molecule inhibitors also may effectively interrupt the interactions required for leukocyte adhesion and extravasation.[258,348] The possibility that these types of reagent may simultaneously be effective in blocking ischemia-reperfusion injury and in controlling the rejection process[258,265,348] is an attractive one, which merits further study.

Chemokines

Several chemokines—small soluble proteins, similar to cytokines—have been identified and form two major groups based on their structure: the CXC or alpha chemokines, which primarily attract neutrophils and T cells, and the CC or beta chemokines, which attract T cells, monocytes/macrophages, DCs, NK cells, and some polymorphs.[122,123,237,266,307] The CXC chemokines include IL-8 and IFN-γ–inducible protein, and the CC chemokines include macrophage inflammatory protein (MIP)-1α/β, RANTES, and macrophage chemoattractant protein-1 (MCP-1).

Transplantation studies have suggested that chemokines are important not only in the development of graft infiltrates[73,82,110,122,123] but also in reperfusion injury.[122,199] The indications are that they act not only as attractants for various leukocyte populations but also by augmenting the effector functions of leukocytes within the graft.[170] CCR1$^{-/-}$ mice accept MHC class II mismatched grafts without immunosuppression and MHC class I and class II mismatched grafts with low-dose immunosuppression.[96] Long-term surviving grafts do not appear to show signs of chronic dysfunction. Although CXCL10$^{-/-}$ recipients show normal rejection kinetics of a CXCL10$^{+/+}$ graft, CXCL10$^{-/-}$ grafts placed in normal recipients show prolonged survival.[121] All of these data indicate that blocking chemokine-chemokine receptor interactions could be a useful adjunct to immunosuppressive regimens. One of the most potent agents that interferes with chemokine function, FTY720, acts by sequestration of lymphocytes in the secondary lymphoid tissue,[134] rather than by directly influencing migration of cells into the graft.

DESTRUCTION OF THE GRAFT

The immune system generates many different effector mechanisms depending on the challenge it meets. In certain infections, a single mechanism seems to be essential for the clearance of the organism, and the absence of that mechanism renders the host susceptible to disease. In the clearance of lymphocytic choriomeningitis virus infections in mice, cytotoxic cells are absolutely required, and disabling this arm of immunity by disrupting the perforin gene leads to death of infected animals.[151] As seen in detail subsequently, most of the known effector mechanisms of the immune system are capable of damaging a graft such that the obliteration of any single effector mechanism has little beneficial effect on graft survival. This is most likely the reason that it is so difficult to prevent graft rejection without disabling the central components of the immune system.

Specificity of Rejection

The nature of tissue destruction during rejection reveals a lot about the processes involved; that is, graft destruction can show fine specificity for cells carrying donor alloantigens. The elegant studies of Mintz and Silvers[239,240] showed an

exquisite specificity of donor cell lysis in experiments using allophenic mice as tissue donors. Such allophenic, or tetraparental, mice are bred by fusing the embryos from mice of two different genetic origins. The tissues of the resulting mosaic offspring are composed of patches of cells from each parental type. Mintz and Silvers performed experiments using mice with different coat colors, and when skin from an allophenic donor was grafted to mice of either parental origin, only the cells of nonidentical type were rejected, leaving cells of recipient type intact and capable of hair growth. These studies have been repeated and extended in experiments performed by Rosenberg and Singer[302]; in this work, an initial large inflammatory/immune response was observed, but this resolved, remarkably leaving cells only of the recipient genotype in place. In a different type of experiment, Sutton and colleagues[350] showed that transplantation of an intimate mixture of allogeneic and syngeneic pancreatic islets resulted in destruction only of the allogeneic cells, with no evidence of bystander damage to the syngeneic islets. It is difficult to imagine how an essentially nonspecific effector mechanism, such as that involved in delayed-type hypersensitivity lesions, could mediate graft rejection in the exquisitely specific manner observed in these experiments.

Bystander destruction of tissue may be observed, however, after the activation of specific immune responses to foreign antigens.[309] Snider and Steinmuller[336] have shown that destruction of bystander tissue may occur in the immune response to miH antigens. In their experiments, cytotoxic T cell clones reactive with a variety of minor antigens (e.g., H-Y and Epa-1 antigens) were injected intradermally together with their specific antigen into a syngeneic animal, which did not express that antigen. As a result, ulcerating skin lesions developed that were radiosensitive, suggesting the involvement of a nonspecific, host-derived effector mechanism in the tissue destruction. In the experiments described earlier using donor material from tetraparental animals, if most cells in the graft were allogeneic to the recipient, the overwhelming inflammatory response could lead to destruction of the entire tissue.

From these experiments, we can conclude that antigen-specific and antigen-nonspecific effector mechanisms may be involved in graft destruction. In both types of experiment, the initial damage was mediated in a specific fashion—it was only when this initiated a massive inflammatory response that the nonspecific elements resulted in tissue destruction. The various effector systems that can damage tissue are described subsequently, and their roles in hyperacute, acute, and chronic rejection are discussed.

Antibody

The target antigens for damaging antibodies are the MHC class I and class II molecules, the ABO blood group antigens, other minor alloantigens that may be expressed selectively by the endothelium, and autoantigens including the angiotensin II type I receptor (expressed by vascular smooth muscle cells) and vimentin.[45] Antibody may cause tissue damage through antibody-dependent cellular cytotoxicity, where the antibody acts as a bridge between the target tissue and the effector cell, activating the lytic machinery cell and resulting in tissue damage.[278,279] Alternatively, antibody is able to fix complement, and complement component 4d (C4d), produced during complement activation, is detectable in tissues that are undergoing antibody-mediated rejection, even in the apparent absence of immunoglobulin. Complement fixation also results in the recruitment of macrophages and neutrophils with consequent injury to the endothelium. In addition, antibody binding activates endothelial cells, which can result in remodeling of the arteries and basement membranes. The latter damage is associated with irreversible damage and chronic graft dysfunction.[45] Activation of the endothelium results in an upregulated expression of adhesion molecules, cytokines, and chemokines and proliferation and synthesis of tissue factor (part of the extrinsic clotting system). Complement-independent activation also can occur that results in activation of the innate NFκB pathway and expression of proinflammatory cytokines.

Antibodies to ABO blood group antigens are preformed or natural antibodies. The presence of ABO blood group incompatibility between donor and recipient is generally considered a contraindication to transplantation because it induces hyperacute graft rejection, where the organ fails minutes after revascularization. After the removal of these antibodies, however, it is possible to undertake successful transplantation,[161] and with protocols for reduced intervention in the recipient,[337] this is becoming an increasingly attractive proposition (see also Chapter 22). The subsequent return of antibodies generally is not associated with antibody-mediated rejection, and this resistance of the organ to their action is termed accommodation. Accommodation is a complex process and not fully understood,[70,71,161,316] but it is thought to involve downregulation of antigen density and the development of resistance in endothelial cells to antibody-mediated injury that may involve changes in coagulation and in the expression of antiapoptotic proteins.

Patients who have been exposed to MHC antigens through transplant, blood transfusions, or pregnancy often develop antibodies reactive with those MHC antigens, which also can cause hyperacute rejection.[166,248,272,381] Hyperacute rejection of this nature is now largely a thing of the past since the introduction of pretransplant screening by the crossmatch test for antibodies directed toward donor antigens (see also Chapter 10). The conventional crossmatch test detects not only harmful MHC-directed cytotoxic antibodies but also harmless autoantibodies.[357] In most cases, it is now possible to distinguish autoreactive from alloreactive antibodies, and it has become possible to transplant an increasing number of patients across an apparent positive crossmatch, but in whom the reactivity is due to autoantibodies.[191]

All of this information suggests that we should focus attention on inhibiting the B cell response after transplantation, and newer reagents confirm that this is a valuable approach. Rituximab has been a useful addition to the immunosuppressive arsenal and is thought to act in part by depleting B cells.[317]

Cellular Mechanisms

The involvement of cell-mediated mechanisms usually is invoked in acute or chronic graft rejection, but although hyperacute rejection almost always has been attributed to antibody, in certain situations a rapid rejection may occur when the role of antibody has been excluded. In these situations, a cellular mechanism of rejection has been implicated.[164]

Natural Killer Cells

NK cells do not need prior exposure to antigen to become lytic to target cells (although their activity can be increased by certain cytokines) and as such provide a component of the primary defense mechanism of innate immunity. NK cells may be recovered from the blood or spleen and are able to lyse NK-sensitive targets, which tend to be of tumor origin.[136] Until more recently, the NK cell was not thought to play a central role in solid organ graft rejection, although its importance in bone marrow transplantation was not disputed.[255,256] Several laboratories using different experimental models have found that grafts survive indefinitely in the presence of demonstrable NK effector activity.[5,21,226] The role of NK cells in activation of the afferent arm of immunity via their interaction with DCs and their production of high levels of IFN-γ has prompted a re-evaluation of their role. Compelling data now exist to suggest that, although insufficient to cause graft rejection, NK cells can contribute to the process. In costimulation-deficient ($CD28^{-/-}$) mice, NK cell depletion prolongs allograft survival significantly.[167]

The method of target cell recognition employed by the NK cell is increasingly understood,[20,44,197,204,270,361] and the nature of the immune synapse between effector and target cell is the subject of considerable interest.[66,67] In contrast to T cells, the interaction with MHC on a target cell can result in the delivery of a negative signal to the NK cell through the so-called missing-self hypothesis,[155,202] preventing the activation of its lytic machinery. The absence of self-class I MHC antigens triggers the NK cell to attack its target, a finding that is consistent with the observation that NK cells are important in the rejection of bone marrow cells that express little or no class I antigen.[16] This fact is important to remember in any approach that considers the removal or blocking of donor MHC antigen as a strategy to overcome rejection of allogeneic or xenogeneic graft rejection. More recently, is has been shown that NK cells also can be triggered into cytotoxicity by recognition of selected antigens—the balance between signals of inhibition and activation determining whether NK cells kill or not.[165]

Specific Cytotoxic T Cell

In cell culture systems, MHC-mismatched lymphocytes proliferate and produce cytokines in response to one another in the mixed lymphocyte reaction. The resulting cytokine production allows the differentiation of precursor CTLs into effector cells that lyse target cells bearing the mismatched MHC antigens.[131,138] The fact that a powerful yet antigen-specific response is generated rapidly in mixed lymphocyte reaction has made the CTL a prime suspect as the central effector mechanism of acute graft rejection.

Considerable evidence suggests that CTLs may be involved in graft rejection. First, CTLs may be recovered from allografts that are undergoing rejection, but they are present only at low levels in grafts of animals that have been treated with cyclosporine to prevent rejection.[21,226] Second, cloned populations of CTLs are capable of causing the type of tissue damage associated with rejection.[80,368] Third, most MHC class I antigen-directed CTLs express the CD8 protein, and graft rejection often may be delayed after the depletion of $CD8^+$ cells.[40,207,214,215,356]

Conversely, graft destruction may occur in the absence of demonstrable CTL activity, and the presence of such cells within a graft may not always lead to graft destruction.[5,56] Rats given a donor-specific preoperative blood transfusion may retain a subsequent renal allograft indefinitely, but cells extracted from such grafts show high and persistent donor-specific CTL activity. The simple conclusion from these studies is that CTLs cannot always reject grafts, although the possibility that the action of these CTLs may be blocked in the graft itself, or that the activity of CTLs in cell culture does not accurately reflect their potential in the animal must be considered. These results remain intriguing, however, and provide direct evidence of the presence of cytotoxic effector cells within an organ graft that is not ultimately rejected.

CTLs are able to kill their targets through the elaboration of perforins, granzymes, and granulysin through activation of the Fas death pathway, or through secretion of the cytokine TNF-α. Their involvement in graft rejection has been questioned further by the finding that mice deficient in perforin (perforin knockouts) are able to reject tumor,[378] skin,[328] and organ[322] grafts, even when the grafts are resistant to Fas-mediated and TNF-α–mediated killing.[378] In the experiments of Schulz and colleagues,[322] grafts mismatched only at the MHC class I are rejected more slowly in perforin knockout mice, however, indicating that, in this situation at least, cytotoxic cells are important in rejection.

As alluded to earlier, even if CTLs themselves do not mediate the tissue damage that ultimately results in graft loss, they still may be important in generating the destructive response to the graft. Through the elaboration of high levels of IFN-γ and other cytokines or chemokines, they are able to recruit and activate cells involved in delayed-type hypersensitivity lesions, initiating acute or chronic rejection.

Macrophages and Delayed-Type Hypersensitivity Reactions

T cells initiate a delayed-type hypersensitivity reaction,[206] which involves an essentially nonspecific effector phase (as described by Koch in 1891 in the tuberculin skin reaction[87]), characterized by an infiltrate of lymphocytes and cells of the monocyte/macrophage lineage. Damage occurs in a tissue during a delayed-type hypersensitivity reaction through the elaboration of various noxious substances, including reactive nitrogen and oxygen intermediates and TNF-α. Support for this idea comes from situations in which CTL responses are not detectable (e.g., in irradiated rats reconstituted with $CD4^+$ cells).[208]

The high level of inflammatory mediators and the type of changes within grafts undergoing chronic rejection suggest a role for activated macrophages in this process.[38,132,273,275] Cytokines such as IL-1, TNF-α, transforming growth factor-β, and platelet-derived growth factor lead to smooth muscle proliferation; transforming growth factor-β and platelet-derived growth factor result in an increased synthesis of extracellular matrix proteins. These cytokines are products of activated macrophages and may result in the atherosclerotic and fibrotic changes associated with chronic graft failure.

Cytokines

The primary role of cytokines in an immune response to a graft is to initiate proliferation, differentiation, and homing of leukocytes in the generation of immunity. However, Certain cytokines also may directly damage tissue acutely or chronically. As described earlier, TNF-α, produced by CTLs and macrophages, may damage a graft, and blocking the

effects of TNF with neutralizing antibodies can prolong organ graft survival.[19,142-144] The minimal effects of these antibodies suggest, however, that the TNFs may not contribute centrally to graft rejection, or that when neutralized other effector mechanisms take over. Islets seem to be particularly susceptible to damage mediated by proinflammatory cytokines, such that these may be a more important component in the rejection of islet transplants.[219,220,288,382]

Eosinophils

It has been recognized for years that episodes of acute and chronic kidney allograft rejection are associated with various levels of eosinophilia,[88,173,262] but the significance of this association in terms of its contribution to rejection has not been acknowledged widely. In an experimental model of acute mouse cardiac allograft rejection in which the depletion of $CD8^+$ T lymphocytes results in a dominant T2 response, rejection seems to be mediated by eosinophils.[37] In another model, in which acute rejection of MHC class II disparate mouse skin grafts was studied, IL-5–dependent infiltration with eosinophils was observed. In this model, when Fas/FasL interactions were absent, neutralizing antibodies to IL-5 blocked eosinophilia and rejection, implicating the eosinophil as an effector cell in this system.[193] In another experimental model of skin allograft rejection, the same group showed a role for IL-5 and eosinophilia in chronic rejection, but in this system, not all of the pathology could be attributed to eosinophils.[192] In situations in which classic pathways of graft rejection are absent or are dominated by a T2-type response, the eosinophil seems to be crucial in graft destruction.[102]

Target Cells of Destructive Immunity

Damage to the vascular endothelium, which may express MHC class I and class II and autoantigens, some of which may be specific to the vasculature, is likely to result in rapid cell necrosis and graft loss.[100,157] The predominantly vascular changes that occur during rejection of an organ graft[78,177,284] suggest that this is the case. The development of antibodies reactive with donor endothelium is strongly correlated with early severe rejection.[100] It is likely that parenchymal cells also may be targets for tissue destruction, and in the kidney tubular cells elaborate cytokines and chemokines that attract and activate T cells,[295,385] but damage to the parenchyma is likely to be secondary to the initial attack on endothelium. The increase in expression of MHC class I and class II antigens together with increased adhesion molecule expression after transplantation is likely to increase susceptibility of endothelium and parenchymal cells to destruction. The marked arterial changes seen as a manifestation of acute and chronic rejection also suggest the importance of the endothelium as the main target of the response, and in the case of chronic rejection, the fibrotic changes seen histologically could be due in large part to ischemia resulting from gradual vascular obliteration.

PRIVILEGED SITES

Tissue allografts placed in certain sites may evoke a weak immune response, and the grafts may survive for prolonged periods.[14] The anterior chamber of the eye, the cornea, the brain, and the testis all show immune privilege either in that transplantation of tissue into these sites evokes a reduced immune response, or in that they themselves seem to have low immunogenicity. The classic privileged site experimentally is the cheek pouch of the Syrian hamster, in which a skin allograft survives indefinitely, provided that the host has not been specifically sensitized against donor histocompatibility antigens.[15] The historical view has been that physical and physiological barriers were critically involved in delivering immune privilege. The aforementioned sites have in common to a greater or lesser extent a lack of or abnormal lymphatic drainage, which seems to play such an important role in sensitization of the host against a free graft such as skin. More recently, it has been suggested that a much broader spectrum of sites, including the liver, the mucosal surfaces of the gastrointestinal tract, and the developing fetus, show many of the features of immune privilege.[235] The developing fetus, although antigenically different from the mother, is not usually rejected, commensal bacteria survive within the gut, and transplantation responses to the liver are diminished.

Calne and colleagues[30] first showed that outbred pigs often failed to reject orthotopic liver allografts; kidney allografts transplanted at the same time and that normally are rejected also show prolonged survival. In certain strain combinations in the rat in which an orthotopic liver allograft is not rejected, the liver allograft has been shown to abrogate an existing state of sensitization of the host against donor histocompatibility antigen.[152] Although HLA matching and crossmatching have been shown to be beneficial in liver transplantation,[76,285] usually the urgency with which the graft is required precludes the use of matching, yet these grafts survive well. The reasons for the refractoriness to immune rejection displayed by liver grafts are not fully understood and may be due partly to the size and enormous capacity for regeneration displayed by the liver. The immune response to liver transplants also differs from that to other grafts, however, and spontaneous tolerance can develop in several rat and all mouse strain combinations. An understanding of this phenomenon may help workers design new strategies of tolerance induction.[84,85,153,154]

What the liver and other sites of apparent immune privilege have in common are mechanisms to regulate or suppress immune responses negatively—it is suggested that immune privilege is a very active process involving mechanisms ranging from cytotoxicity directed at immune effector mechanisms delivered by Fas-FasL interactions[12,93,114,190,200,261] to those delivered by regulatory or suppressor cells.[41] The reader who wishes to delve deeper into the area of immune privilege is referred to an entire review volume covering this area.[235a]

CHRONIC ALLOGRAFT NEPHROPATHY

Although chronic rejection has been mentioned at various points in this chapter, most of what has been said refers to the acute processes that occur rapidly after transplantation. (See Chapter 25 for a more complete discussion of chronic allograft nephropathy.) That these may influence the likelihood of more chronic changes seems reasonable, although accumulating evidence in favor of this suggestion has not been easy.[9,354,364] As better immunosuppression reduces the loss of organ allograft to acute rejection, chronic rejection becomes more evident; currently, the greatest loss of kidney grafts is to chronic rather than acute rejection.

Multiple effector mechanisms are thought to contribute to the immunological aspects of chronic graft rejection, but it has become apparent that other factors are involved in

this process, which have nothing to do with the immune response. The development of experimental models of chronic allograft rejection has increased knowledge of the possible causative mechanisms and pointed to therapies that might prevent the development of the obliterative arterial changes of chronic rejection in the future.[132, 274] An inflammatory cellular infiltrate is always seen, comprising macrophages, eosinophils,[192] NK cells,[167,369] and T cells. The T cells comprise $CD4^+$ and $CD8^+$ cells, with usually a predominance of the former.[50,72,77,120]

Because of the predominant vascular nature of chronic rejection, alloantibody has been long thought to play a role in the development of this process, and the demonstration of donor-specific alloantibodies in patients with chronic rejection of cardiac allografts[299] and the deposition of immunoglobulin in graft vessel walls of chronically rejected organs[27,306] would be compatible with that concept. In experimental systems, B cell–deficient mice do not develop arterial lesions.[311] It also is possible, however, to show immunoglobulin and complement deposition in organs that show no evidence of rejection so that the role of antibody remains uncertain.

The graft arteriosclerosis seen in chronic rejection is concentric and affects all graft arteries, and this forms the basis of a working hypothesis for the development of chronic rejection proposed by Hayry and associates.[132] It is suggested that low-grade damage to the graft endothelium, with possible loss of endothelium, allows platelet deposition on the arterial wall, and the production of a variety of growth factors, which cause proliferation of smooth muscle cells in the media of the arterial wall and their subsequent invasion of the intima. This response-to-injury hypothesis first proposed for atherosclerosis[305] has been tested in an experimental model in the rat using an aortic allograft.[236] These grafts undergo an initial acute inflammatory reaction in the adventitia, which subsides and is followed by gradual migration of proliferating muscle cells from the vascular media to the intima and the appearance of intimal fibrosis. When induced, this allograft arteriosclerosis is not reversible by transplanting the aortic allograft into a syngeneic recipient. The development of the chronic arterial lesion is associated with cytokines (IL-1, IL-6, TNF, IFN-γ), growth factors (platelet-derived growth factor, transforming growth factor-β), and lipid mediators of inflammation (eicosanoids and platelet activation factor). The demonstration that a particular somatostatin analogue, lanreotide, which downregulates the production of several growth factors, prevents smooth muscle proliferation and the development of arteriosclerosis in the aortic allograft model suggests that these growth factors may be important effector molecules in the development of the chronic lesion.[132]

The causes of chronic rejection are immunological and nonimmunological,[267] with the immunological causes being important, and the primary target of the immunological response being the endothelium. Nonimmunological causes are attracting increasing attention, but a full discussion of these is outside the scope of this chapter.

CONCLUSION

The immune response to a tissue allograft is complex, not only in the manner by which allogeneic histocompatibility antigen is recognized but also in the response to this recognition, which generally results in graft damage. In the recognition of antigen, the DC, be it of donor or recipient origin, plays a central role, whereas the effector arm is mediated by cells and by antibody. The hierarchy of importance of all the effector mechanisms described is affected by the type and nature of the graft, the incompatibility between donor and recipient, and the type of immunosuppression used. Because all potential effector mechanisms can cause graft damage, adequate immunosuppression usually seems to require disabling the immune system at a central point. The consequence of this requirement is that patients become susceptible to infection, are at increased risk of cancer, and experience the other side effects of long-term immunosuppression. For continued success with organ transplantation, strategies that strive to reduce and tailor immunosuppression are paramount, as are strategies that aim to achieve immunological tolerance.

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Chapter 3

Nontransplant Modalities of Kidney Replacement Therapy

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Dialysis is the most well-established mode of mechanical organ replacement in use today. Dialysis attempts to replace a complex and vital organ that regulates electrolyte and fluid status and endocrine and metabolic function. It is readily acknowledged that dialysis—whether peritoneal dialysis (PD) or hemodialysis (HD)—remains a nonphysiological replacement for normal healthy kidney function. Dialysis may become necessary with acute deterioration of kidney function or in the context of a progressive decline of kidney function. Although clinical and laboratory measurements need to be considered for an appropriate assessment of renal replacement therapy need, the decision to dialyze a patient remains predominantly a clinical judgment in nearly all instances.

There are five absolute indications to begin dialysis (Table 3-1): (1) pulmonary edema resistant to diuretics; (2) hyperkalemia unable to be managed medically; (3) severe uremic symptoms, such as intractable nausea and vomiting, and mental status changes with no other obvious cause; (4) metabolic acidosis not responsive to medical management; and (5) a pericardial effusion in the presence of an elevated blood urea nitrogen (BUN) level. Only one of these five indications needs to be present for initiation of kidney replacement therapy.

Definitive recommendations regarding the optimal timing of initiation of kidney replacement therapy in patients with acute kidney injury (either with baseline normal renal function or baseline chronic kidney disease [CKD]) are unavailable and remain subject to debate and investigation. Historical data that are primarily retrospective strongly supported the prophylactic initiation of dialysis before the onset of advanced uremia. A five-center collaborative effort studying acute kidney injury in 2006 showed lower crude survival rates for patients initiating HD with BUN levels greater than 76 mg/dL.[29] Although these studies support the initiation of treatment by the time the BUN level is 80 mg/dL or greater, data addressing the question of earlier initiation of therapy are limited.

When it has been established that a patient requires dialysis therapy, the next step is to select what form of therapy is appropriate for that clinical situation. The form varies depending on the acute or chronic nature of the kidney dysfunction. Selection also is based on the patient's hemodynamic status. There are two major forms of kidney replacement therapy: HD, using a machine and artificial kidney membrane for diffusion and ultrafiltration, and PD, using the peritoneal membrane for diffusion and ultrafiltration. There are several variations of HD, including hemodiafiltration, continuous venovenous hemofiltration (CVVH), and continuous venovenous hemodialysis (CVVHD). In PD, the two major forms are continuous ambulatory peritoneal dialysis (CAPD) and continuous cyclic peritoneal dialysis (CCPD).

Patients with CKD are now staged based on level of estimated glomerular filtration rate (GFR) (Table 3-2). Kidney replacement therapy should be discussed with patients when they are in stage 4 CKD and should be offered to patients when they have reached stage 5 CKD. The modalities used for kidney replacement therapy for such patients include HD and PD. The goal for access in patients with CKD is for the patients to be educated at stage 4 of CKD in the different forms of renal replacement therapy and to have obtained the appropriate access before initiation of dialysis. In acute kidney injury, it is impossible to plan access and prepare for initiation of dialysis.

GFR is used more in the chronic setting to gauge disease progression by monitoring the trend, whereas in the acute setting, the daily increase in serum creatinine or other markers of kidney dysfunction (e.g., cystatin C) are laboratory signs of kidney failure, regardless of the calculated GFR. Electrolyte measurements and the patient's overall clinical status are more important in the decision to initiate dialysis.

Table 3–1 **Absolute Indications for Dialysis**
Hyperkalemia—unable to be controlled medically
Acidosis—unable to be corrected medically
Pulmonary edema/volume overload—unresponsive to diuretics
Pericardial effusion
Severe uremia—includes intractable nausea, emesis, mental status changes

In acute kidney injury, access and dialysis itself are initially considered for short-term use, and this influences the type of access and form of dialysis. Although more common in pediatric patients, in whom vascular access can be problematic, PD is still used in cases of acute renal failure in adults. This practice has declined with the increasing use of slow, continuous HD. HD remains the predominant form of renal replacement therapy in acute and chronic kidney failure.

HEMODIALYSIS

Process

HD is an extracorporeal therapy. HD uses the mechanism of diffusion of the patient's blood against dialysate through a membrane contained in an artificial kidney. The movement of solutes by diffusion is the result of random molecular motion that can be manipulated by the concentration gradient of the dialysate compared with the patient's plasma, and by the size of the pores of the semipermeable membrane of the dialyzer compared with the molecular weight of the solutes in the plasma.

Approximately 250 to 500 mL of blood is removed from the patient's body via a form of vascular access into tubing that attaches to a dialysis machine. This blood circulates into a dialysis membrane containing artificial semipermeable fibers and then back into tubing connected to the dialyzer through an outflow track. This filtered blood is returned to the patient through the vascular access. The dialysis machine features a pump that delivers the patient's blood to the dialyzer at a constant rate (200 to 500 mL/min). Dialysate is circulated in a single-pass fashion, countercurrent to the blood flow. This allows for solute removal by diffusion, based on concentration gradients of solutes between the blood and dialysate across the semipermeable membrane.

Dialysis membranes are classified according to their composition, biocompatibility, and pore size. The two major types of material used to form dialysis membranes are cellulose and synthetic polymers. The type of material influences membrane biocompatibility and function. Biocompatibility refers to the reactions that occur as a result of blood-membrane interactions. These include activation of complement and coagulation cascades and cell activation, in particular, peripheral blood leukocytes and platelets. Reactions can manifest as thrombosis in the dialyzer and, rarely, as acute anaphylactoid reactions.

Function refers to the ability of the dialyzer to clear the blood of particular proteins or molecules. A typical modern hemodialyzer is composed of several thousand parallel hollow fibers. The walls of these fibers are semipermeable, separating the blood in the fiber lumen from the dialysate outside. The total internal surface area of all the fibers is usually 0.5 to 1.2 m^2, although some dialyzers are even larger, providing greater solute transport. High efficiency in HD refers to a high rate of removal by diffusion of small-sized solutes. The high-efficiency dialyzer contains membranes with larger surface area (1.5 to 2.1 m^2) and achieves a higher rate of removal of solutes with greater blood flows. High flux connotes a high rate of removal by diffusion of "middle molecules" larger than urea; this is achieved with membranes containing larger pore sizes (60 Å compared with low-flux dialyzer pore sizes of 25 Å).

Hemofiltration membranes are always high flux and are usually made of synthetic materials (polysulfone, polyamide, cellulose acetate, polyacrylonitrile). Synthetic membranes are generally more biocompatible.

Dialysate is composed of water containing sodium, potassium, calcium, magnesium, chloride, acetate, dextrose, and bicarbonate. Optimal dialysate flow rates during HD are 800 mL/min, and the average time on HD is approximately 3 to 4 hours. Patients are exposed to 120 L or more of water during each dialysis treatment. All small-molecular-weight substances present in the water have direct access to a patient's circulation as if they had been administered by intravenous injection. For this reason, it is important that the purity of the water used for HD be known and controlled. Significant contaminants in dialysate water with their associated complications include aluminum, associated with bone complications, neurological disease, and anemia; copper, associated with hemolytic anemia; chloramine, associated with hemolytic anemia; and fluoride, associated with cardiovascular, gastrointestinal, and neuromuscular derangements (with intoxication can prove fatal).

Purifying water for HD is a stepwise process, usually conducted in a dedicated central system within a freestanding HD unit. Purification begins with softening to remove most of the calcium and magnesium. Water is then passed through a series of carbon filters to remove organic and inorganic impurities, such as chloramine and chlorine. The water is passed through a semipermeable membrane with pores that prevent passage of small-molecular-weight solutes, such as chloride, sodium, and urea. Reverse osmosis removes more than 90% of the impurities. Deionizers that exchange charged solutes for hydrogen and hydroxyl ions, removing charged solutes from water, can be used as an alternative to reverse osmosis or to refine water already treated with a reverse osmosis system. The bacterial counts should be less than 100 colonies/mL in the water and less than 500 colonies/mL in the final dialysis solution. Despite the efficiency of the dialyzer membrane as an effective barrier to bacteria and endotoxins in dialysate, maintaining the aforementioned colony counts significantly limits any

Table 3–2 **Stages of Chronic Kidney Disease**	
Stage	**Glomerular Filtration Rate (mL/min)**
1	>90
2	60-90
3	30-59
4	15-29
5	<15

potential for transmission of endotoxin or bacterial products across the dialyzer, enhancing patient safety.

Access (see Chapter 5)

Vascular access that allows for a high-flow state is necessary for adequate HD. This access can be achieved through an arteriovenous (AV) fistula, an AV graft (polytetrafluoroethylene or bovine endovascular material), or a venous catheter. Each of these forms of access has risks and benefits, but it is widely accepted that the AV fistula is the best form of vascular access for HD.

The National Kidney Foundation–Kidney Disease Outcomes and Quality Initiatives (NKF K/DOQI) guidelines recommend access placement at stage 4 CKD (estimated GFR 15 to 29 mL/min); this allows adequate time for maturation of a vascular access without need for emergent catheter placement. AV fistulas and grafts have better survival if used at the time of initiation of HD compared with their use in patients initiated on HD with a catheter. Data from the United States Renal Data System (USRDS) indicate that now approximately 40% of prevalent HD patients use AV fistulas.[4] This percentage is significantly greater in many other countries around the world.

After the creation of an AV fistula, a certain period is necessary for maturation of the fistula to occur for the fistula to be functional as a vascular access for HD. In European centers, greater than 80% of medical directors recommend using an AV fistula within 2 months of fistula creation, whereas in Canada and the United States, more than 75% of medical directors wait longer than 2 months.[35] Cannulation of the AV fistula within 14 days of creation is associated with reduced long-term fistula survival.[42]

Synthetic AV grafts and central venous catheters have more problems with flow, morbidity, and increased cost compared with AV fistulas, but there are circumstances where a synthetic graft is required for long-term HD, such as suboptimal arterial or venous anatomy for AV fistula creation. Compared with fistulas, grafts have a reduced primary failure rate, have a shorter time to use and successful cannulation, and potentially require fewer salvage procedures for the primary access. A central venous catheter may be required when immediate access to the circulation is required, or when there is insufficient time for an AV fistula or graft to mature. Many patients with catheters become difficult or impossible to convince to proceed with an AV fistula, as they have become used to a needle-free and painless initiation of HD when using a catheter. The duration of catheter dependence is inversely correlated with the likelihood of proceeding with the creation of an AV fistula or graft.

Vascular access, important for being the patient's lifeline, all too often is the cause of the HD patient's death. Infection is the second leading cause of death in dialysis patients. Death rates from septicemia have been estimated to be 100-fold to 300-fold higher than in the general population. The risk for infection-related death is greater in catheter-dependent patients. In an analysis of data from the HEMO trial, the frequency of hospitalization as a result of access-related infection was greatest among HD patients with catheters.[2,3] Several studies show a gradient of patient mortality risk by access type, with the highest risk observed with central venous catheters, and the lowest risk with AV fistulas. Prophylactic measures, such as use of antimicrobial lock solutions or exit site antibiotic ointments, may reduce the frequency of catheter-related bacteremia.

Maintenance of vascular access is a major challenge for long-term HD. A loss of flow in the fistula or graft can be devastating, with subsequent loss of the access if not addressed in a timely manner. Vascular access complications are one of the main causes associated with an increase in morbidity and mortality in end-stage renal disease (ESRD). For AV fistulas, it is important to assess potential salvage procedures early (within 4 to 6 weeks) after fistula creation. Changes in blood flow or arterial or venous pressures during HD can raise concerns for stenosis or collateral blood flow or both. Stenosis is the major cause of dysfunction in an AV fistula. Ultrasonic investigation of the fistula can determine flow states within the fistula and indicate possible stenotic areas that may benefit from intervention with fistulogram plus angioplasty and possible stenting.[7] Collateral vessels may require surgical revision. Clinically, loss of blood flow within the fistula or graft is associated with a decrease in intensity of an audible bruit along the access. This is likely due to thrombosis and can result from hypotension, a hypercoagulable state, or constriction of the graft. Swift thrombolytic intervention can save the access.

Steal syndrome is an uncommon but serious condition of arterial insufficiency distal to a fistula. The diagnosis is largely based on clinical features of numbness, pain, or a coolness of the extremity distal to the access site. In some cases, angiography may be necessary to ascertain the lesion leading to steal syndrome.[20] The cause is usually high fistula flow, but other causes, such as inflow or anastomotic stenoses, or a combination of these causes, have to be considered. The main treatment options are flow-reducing procedures or distal revascularization with selective ligation. In some cases, fistula ligation is the method of choice.

Fluid Status

Compartments

Approximately 60% of the body is composed of water with two thirds of total body water being intracellular and the rest extracellular. Extracellular fluid (ECF) can be divided further into the plasma, interstitial, and transcellular compartments. Approximately one fifth of ECF is intravascular within plasma (Fig. 3-1). When monitoring patients on HD, the focus is on the ECF, particularly the plasma compartment. In a person with normal kidney function, if the ECF compartment is volume expanded, the kidney excretes the excess sodium and water in the urine, maintaining a normal plasma volume. In ESRD when excretory capacity is diminished, sodium and water retention persists despite expansion of the ECF volume, creating total body sodium and water excess. The dysregulation of fluid volume can lead to pulmonary and peripheral edema. Elevated intravascular volume increases the intravascular pressure, leading to hypertension and cardiac hypertrophy. Regulation of total body fluid status is no small task because the kidney filters 180 L of plasma daily. Inability to regulate total body fluid status is an indication to begin renal replacement therapy.

Ultrafiltration

Ultrafiltration during HD removes water accumulated either by ingestion of fluid or by metabolism of food during the

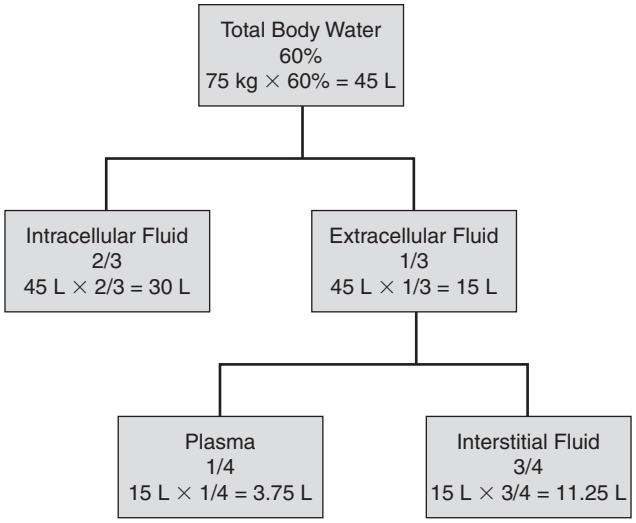

Figure 3–1 Fluid compartments in a 75-kg patient.

interdialytic period. Solutes are removed via diffusion during an HD treatment, and free water can be removed via convective forces during a treatment. Water driven by either a hydrostatic or an osmotic force is pushed through the membrane of the dialyzer. Typically, a patient dialyzed three times a week gains 1 to 4 kg of weight between treatments, most of which is water. This water needs to be removed during a 3- to 4-hour dialysis session. Normally, ultrafiltration is performed at the same rate throughout the dialysis session.

Problems can arise with excessive ultrafiltration on HD. These occur either in the amount of volume removed or with the rapidity of rate of removal; either can result in hypotension, muscle cramping, and mental status changes. Patients may develop nausea and emesis that may be erroneously attributed to uremia.

The amount of fluid that should be removed as ultrafiltrate during HD is clinically determined by assigning a dry weight to each patient. Dry weight is defined as the postdialysis weight at which the blood pressure is lowered into the presumed normal range without the development of intradialytic hypotension, and without clinical signs of pulmonary congestion or peripheral edema. The discontinuous and brief nature of routine dialysis therapy often requires high ultrafiltration rates to reach a patient's "dry weight."

Fluid Assessment

Clinical signs are the primary tool used to assess volume overload in patients. There are other methods to ascertain volume status, however. Biochemical markers of dry weight include plasma levels of atrial natriuretic peptide and cyclic guanosine monophosphate. Plasma levels of atrial natriuretic peptide are elevated in HD patients because of their inability to remove excess intravascular volume. Ultrafiltration of excess fluid during dialysis can reduce plasma atrial natriuretic peptide levels. Fluid overload also has been associated with elevated serum levels of cyclic guanosine monophosphate.

Anatomical measures of dry weight include the diameter of the inferior vena cava. Central venous and right atrial pressures reflect right ventricular function. Right ventricular function is an indicator of volume status. Central venous pressure as measured by the diameter of the inferior vena cava is an indirect measurement of total body fluid. During fluid removal with ultrafiltration during HD, intercompartmental fluid shifts occur. The diameter of the vena cava at the end of HD reflects blood volume, not total body water volume. The bedside correlate to this is ultrasonographic visualization of the internal jugular vein. Notably, a distended internal jugular vein on ultrasound potentially can indicate increased right atrial pressures, but stenosis of the distal vasculature also can imitate this and must remain in the differential diagnosis. The clinical examination is a less expensive and useful tool for estimation of dry weight because all current modalities are not exact indicators of dry weight.

The assessment of dry weight and volume status in patients undergoing HD is extremely important because of the detrimental effects of chronic fluid overload on the heart. HD patients have hypertension, subsequent left ventricular hypertrophy, and cardiomyopathy, all in part resulting from persistent hypervolemia. Sodium and fluid restriction can be used in addition to ultrafiltration to maintain a stable weight and volume status, especially during the intradialytic period (Table 3-3). Aggressive fluid restriction stimulates thirst, and patients with ESRD already have a plasma osmolality set higher than normal, further stimulating thirst. This can complicate patient adherence to dietary advice.

Electrolytes

Sodium

Sodium chloride is the most abundant molecule in the ECF. At steady state, urinary sodium excretion essentially is identical to the dietary intake of sodium. ECF volume increases linearly as the dietary intake of sodium increases.

The volume of the ECF is directly proportional to the content of sodium in the body. An increase in ECF volume increases the plasma volume. Sodium is the ion that allows us to use osmosis for dialysis. Osmosis is the movement of water across a membrane from an area of lower solute concentration to an area of higher concentration of solutes until both solutions on either side of the membrane reach equal concentrations. The osmotic pressure of a solution depends on the number of particles dissolved in a unit volume of solvent. These particles are referred to as osmoles. Osmolality refers to the number of particles (osmoles) in 1 kg of water. Tonicity refers to the solutes that remain in the ECF compartment causing water movement. Solutes such as sodium and glucose increase tonicity because they do not pass freely through cell membranes, causing

Table 3–3 **Nutritional Recommendations for Dialysis Patients**

	Hemodialysis	Peritoneal Dialysis
Sodium	<90 mEq daily	≤150 mEq daily
Potassium	<60 mEq daily	≤90 mEq daily
Phosphorus	800-1000 mg daily	800-1000 mg daily
Calcium	<2 g daily	<2 g daily
Fluid	1-1.5 L daily	≤2 L daily

water movement. The osmolality of plasma is largely a function of sodium concentration. Patients with ESRD have excess nitrogenous waste contributing to an elevated plasma osmolality. Yet, urea, an example of a nitrogenous waste product, is readily diffusible across cell membranes and does not contribute to the tonicity of plasma. This leaves sodium as the major contributor to body tonicity. The kidney is no longer able to excrete a sodium load in ESRD, resulting in sodium retention, ECF hypertonicity, and hypertension.

COMPLICATIONS OF SODIUM BALANCE

Disorders of sodium balance are disorders of ECF volume. Patients with ESRD have expanded ECF volume despite normal sodium intake. Dialysis uses dialysate, a synthetic plasma water component, to remove soluble wastes from the blood by diffusion. The average dialysate sodium concentration is 135 to 145 mmol, close to normal physiological serum levels. Sodium crosses dialysis membranes by diffusion and convection. Sodium removal can be increased by applying higher ultrafiltration volumes and by lowering dialysate sodium concentration.

Plasma volume depletion and hemodynamic instability during HD are a function of the dialysate-to-plasma tonicity gradient because it is the tonicity that determines water movement across cell membranes to influence plasma refilling and intradialysis discomfort. Supraphysiologic dialysate sodium concentrations have been used to reduce volume shifts and to maintain hemodynamic stability. These elevated dialysate sodium concentrations have caused an increase in hypertension, increase in thirst, and increase in intradialytic weight gain.

Sodium intake is the most important determinant of intradialytic weight gain in nondiabetic patients. The biggest difficulty with a sodium-restricted diet is patient adherence. The most recent dietary recommendations are a sodium restriction of 2 to 3 g a day (Table 3-4). In HD patients requiring parenteral nutrition, it may not be necessary to add sodium to the formulation, unless the patient is having significant sodium loss from gastrointestinal fluids.

Potassium

Two percent of a patient's total body potassium content is located in the extracellular compartment. This uneven distribution reflects the large potassium concentration gradient between the intracellular fluid and ECF compartments that determines cell resting membrane potential. A disruption of this ratio can have detrimental consequences on the function of excitable tissues, especially muscle (myocardium in particular) and nerve. The most prominent adverse effects of hyperkalemia include potentially lethal arrhythmias, respiratory depression, and enhanced weakness and fatigue.

In the normal state, 90% to 95% of daily potassium intake is excreted by the kidneys. Although HD is the primary method of potassium removal for ESRD patients, they too rely on gastrointestinal excretion and cellular uptake for potassium homeostasis. ESRD patients eliminate 25% of their daily potassium load via increased colonic secretion.

Persistent hyperkalemia in dialysis patients is due to excessive potassium intake, inadequate potassium elimination, or a combination of the two. Excessive potassium intake is most commonly due to dietary noncompliance. Dietary restriction in HD patients should be less than 60 mEq of potassium daily. Patients requiring parenteral nutrition may require only 10 to 40 mEq/day of potassium in their formulation.

As previously mentioned, ESRD patients depend heavily on gut elimination of potassium. The amount of potassium excreted through the gastrointestinal tract is roughly proportionate to the stool volume. Constipation has been

Table 3–4 **Management of Electrolyte Abnormalities in Dialysis Patients**

Electrolyte Abnormalities in HD and PD Patients	Complications if Untreated	Management
Hypernatremia	Hypertension	HD—decrease ultrafiltration PD—decrease dextrose concentration in dwells Medications—evaluate for sodium-containing medications (i.e., antibiotics, sodium bicarbonate) Nutrition—restrict sodium to <2 g daily
Hyperkalemia	Cardiac arrhythmias; cardiac arrest	HD—decrease K^+ bath PD—patients usually hypokalemic and may need K^+ supplementation Medications—calcium gluconate, insulin (followed by 50% dextrose), bicarbonate, albuterol, Kayexalate Nutrition—restrict K^+ <60 mEq daily; ensure patient *not* consuming K^+-containing salt substitutes
Hypercalcemia	Secondary hyperparathyroidism; calciphylaxis	HD—lower calcium bath PD—lower dialysate calcium Medications—change phosphate binder if calcium containing to non–calcium containing (i.e., sevelamer, lanthanum carbonate); begin calcimimetics (check parathyroid hormone level); stop supplemental vitamin D Nutrition—<2 g daily of calcium (including calcium-containing medications)
Hyperphosphatemia	Pruritus; calciphylaxis	HD—increase HD run time PD—increase dwell time or volume of dwell Medications—phosphate binders Nutrition—decrease dairy intake; ensure patient taking binders *with* meals; restrict to 800-1000 mg daily

HD, hemodialysis; PD, peritoneal dialysis.

reported to occur in 40% of HD patients and can predispose ESRD patients to hyperkalemia.[37] Inadequate dialysis is another common factor leading to hyperkalemia.

The indication for dialysis related to hyperkalemia is different when comparing acute kidney injury versus CKD. Patients with CKD have a diminished ability to excrete a potassium load acutely, creating more severe and prolonged hyperkalemia when challenged. Recognition that mild-to-moderate hyperkalemia is an adaptive response in CKD should lead to tolerance of a steady-state serum potassium concentration of 5 to 5.5 mmol/L in patients with CKD.[16] Many signs of hyperkalemia are more difficult to identify in this patient group. Patients who present with severe hyperkalemia may have a normal electrocardiogram or have changes that are so subtle that physicians have difficulty attributing these changes to increased serum potassium levels.[39] Individuals also may have less overt weakness. Clinical diligence is necessary, however, to monitor these patients and avoid any additional complications. Dialysis is the definitive treatment for significant hyperkalemia (see Table 3-4).

Ingestion of high-potassium foods and medications that potentially can cause hyperkalemia (Table 3-5) needs to be changed with education and alternative prescriptions. By controlling the concentration of potassium in the dialysate, it is possible to decrease elevated serum potassium levels.

Phosphorus

Phosphorus constitutes approximately 1% of an individual's total body weight. Phosphate is unevenly distributed in different compartments of the body. Only a very small amount of phosphate (approximately 1%) is present in the accessible plasma compartment of the ECF. The kidney, gastrointestinal tract, and bone are the major organs involved in phosphorus homeostasis.

Phosphate balance is disturbed in most ESRD patients because absorption from the diet exceeds the elimination through HD treatment. The positive phosphate balance of HD patients leads to a chronic phosphate load. Elevated serum phosphorus levels are associated with an increased mortality rate in patients with CKD. This increased mortality is most likely due to the development and progression of vascular calcification owing to higher serum phosphorus levels in a population already at increased risk of cardiovascular disease.

Secondary hyperparathyroidism also occurs in patients with ESRD as a result of dysregulation of stimuli affecting parathyroid hormone (PTH) (Table 3-6). ESRD with loss of renal mass impairs phosphate excretion and the synthesis of 1,25-dihydroxyvitamin D_3. Hypocalcemia and hyperphosphatemia stimulate PTH release and its synthesis and decrease the intracellular degradation of PTH. Electrolyte imbalances between calcium and phosphorus ensue, and if left untreated result in debilitating bone resorption.

Table 3–5 Hyperkalemia-Potentiating Medications*

Angiotensin-converting enzyme inhibitors
Angiotensin receptor blockers
Penicillin G
Trimethoprim
Spironolactone
Succinylcholine
Heparin

*List is incomplete.

Table 3–6 Appropriate Parathyroid Hormone Levels

Stage of Chronic Kidney Disease	Parathyroid Hormone Levels (pg/mL)
3	35-70
4	70-110
5	150-300

PHOSPHORUS MAINTENANCE

Despite adequate dialysis three times weekly, patients with ample diets remain in a positive phosphate balance. The next steps in treatment of hyperphosphatemia include phosphate binders and more efficient dialysis.

Phosphate-binding medications bind to phosphate in the gastrointestinal tract and prevent its intestinal absorption (see Table 3-4). The most widely used phosphate binders are calcium based. Calcium carbonate and calcium acetate are well-established effective phosphate binders, with calcium acetate having a smaller calcium load per equivalent phosphate-binding dose. Sevelamer hydrochloride (Renagel), lanthanum carbonate (Fosrenol), and magnesium-containing compounds are the only nonaluminum, noncalcium binders currently available. Sevelamer hydrochloride is an effective binder with a favorable side-effect profile. Lanthanum carbonate has a side-effect profile similar to that of calcium carbonate, currently showing no deleterious effects on bone (4-year follow-up) when compared with aluminum-containing binders used in the past. Magnesium binders are limited by the development of overt hypermagnesemia, gastrointestinal side effects, and the need for individualization of dialysate magnesium concentrations. Calcimimetics (cinacalcet) bind to the calcium-sensing, G protein–coupled receptor in the parathyroid gland and allosterically alter sensitivity of the calcium-sensing receptor to calcium in the gland.

Phosphate removal in HD is limited after the initial hour of dialysis clearance because of the rate-limiting step of transfer of phosphate from the intracellular to extracellular space. Only a small percentage of phosphate is distributed in the extracellular space, with most of the total body phosphate concentration located intracellularly. Increased dialysis time or increased frequency of dialysis treatments possibly can remove more phosphate as it is transferred from the intracellular space to the dialyzable, extracellular space. As a result of the kinetics of phosphate metabolism, increasing the frequency of dialysis sessions more effectively removes phosphate than increased time at individual dialysis sessions.

Calcium

Calcium is the most abundant divalent ion in the body. Of total body calcium, 99% is located in the bone, with the remaining 1% found in teeth, soft tissues, plasma, and cells. Approximately 1.2 to 1.3 kg of calcium is present in a 70-kg individual. Calcium homeostasis is maintained by

reabsorption and formation interplay between the intestine, bone, and kidney.

Approximately 1000 mg of calcium is ingested daily. Of that 1000 mg, about 400 mg is absorbed along the intestine, primarily in the duodenum and jejunum, and the remaining 600 mg is excreted in the feces. Intestinal absorption of calcium is accomplished through passive and active mechanisms, the active mechanism via vitamin D_3. Net calcium reabsorption and formation in bone is important in maintenance of plasma calcium concentration. Daily turnover occurs via activity of PTH, active vitamin D_3, and calcitonin. PTH regulates plasma calcium concentration by (1) stimulating bone resorption by activating osteoclasts, which demineralize bone; (2) increasing the synthesis of active vitamin D_3; and (3) increasing calcium reabsorption in the distal tubule of the kidney. Active vitamin D_3 promotes calcium uptake in the intestine. Calcitonin is released in response to elevated calcium concentrations and acts by directly inhibiting the activity of osteoclasts, decreasing serum calcium levels.

Patients with ESRD initiating HD are usually hypocalcemic. This hypocalcemia is due to the inability to reabsorb filtered calcium along with decreased production of 1,25-dihydroxyvitamin D_3 in the nonfunctioning kidney. Dialysate usually contains 1.75 mmol/L of calcium. This degree of calcium in the dialysate stabilizes myocardial function during dialysis and reduces the risk of hypotension in HD patients during treatment.

Treatment for hypocalcemia is with calcium and vitamin D supplementation. Patients with ESRD may already be on calcium-containing phosphate binders. Active vitamin D_3 promotes not only active absorption of calcium in the intestine, but also absorption of phosphorus. This absorption can worsen hyperphosphatemia and cause an increased calcium-phosphorus product.

The calcium-phosphorus product is the number obtained by multiplying the serum calcium by the serum phosphorus value. Current guidelines state that a calcium-phosphorus product greater than 55 mg/dL can increase the risk of soft tissue calcification or calciphylaxis. This can lead to tissue necrosis, periarticular calcification, and vascular calcification including the coronary vasculature along with an increased mortality risk.

Anemia

Normal red blood cell production is primarily regulated by circulating erythropoietin. The kidney produces 90% of circulating erythropoietin, accounting for its pivotal role in erythropoiesis. When erythropoietin binds to its receptors on bone marrow erythroid progenitor cells, their proliferation, differentiation, and development into mature erythrocytes is increased. Nonkidney erythropoietin is produced by the liver by centrilobar hepatocytes. Nonkidney erythropoietin production is rarely able to provide for significant erythropoiesis in an anephric state, however.

The pathogenesis of anemia in kidney failure is multifactorial. Erythropoietin deficiency, shortened erythrocyte survival, the presence of uremic inhibitors of erythropoiesis, hemolysis, bleeding, and iron deficiency all are contributors. Erythrocyte survival is 60 to 90 days in uremic patients versus 120 days in normal individuals and generally does not improve with dialysis therapy. NKF K/DOQI guidelines recommend a hemoglobin level of 11 mg/dL for premenopausal women and a level of 12 mg/dL for men and postmenopausal women. These target levels were selected for the reduced need for blood transfusions and improved quality of life. Anemia may predispose patients to left ventricular dilation and hypertrophy that can predispose to heart failure and mortality.[15] A normal hemoglobin target in ESRD patients may not be optimal, however, because such individuals seem to risk an increased rate of ischemic cardiac events and access complications and cerebrovascular events compared with individuals with slightly lower hemoglobin values. Therapeutic modalities for treatment of anemia in ESRD include recombinant erythropoietin, darbepoetin alfa, iron supplementation when indicated, and packed red blood cell transfusions.

Adequacy

Numerous outcome studies have shown a correlation between the delivered HD dose and patient morbidity and mortality.[18] Clinical signs and symptoms alone are unreliable indicators of HD adequacy. To ensure that ESRD patients treated with long-term HD receive a sufficient amount of dialysis, the delivered dose should be measured and monitored routinely. Adequacy is a method to quantify the optimal amount of HD that should be delivered to a patient in a dialysis session that has been widely based on the removal of urea. Urea is a small, readily dialyzed solute that is the bulk catabolite of dietary protein. It constitutes 90% of waste nitrogen accumulated in body water between HD treatments, it is easily measured in blood, and the fractional clearance of urea in body water correlates with morbidity and mortality.[27]

A dose of dialysis is best described as the fractional clearance of urea as a function of its distribution of volume. Kt/V is the formula used in the urea kinetic model that helps guide the nephrologist for proper dialysis dosing. K is the hemodialyzer clearance (in L/min), t is the duration of the dialysis session (in minutes), and V is the volume of distribution of urea in the body (in liters). Maximum solute clearance has been shown to occur in the first hour of HD, and increasing dialysis time does not equate to improved adequacy.[27] Daily dialysis has shown improved clearance as opposed to increasing time of thrice-weekly dialysis sessions. Current NKF K/DOQI recommendations are for a weekly Kt/V of 1.2 for thrice-weekly dialysis, and a Kt/V of 0.57 for short daily and nocturnal HD.

Cardiovascular Disease

ESRD patients manifest extraordinary risk for cardiovascular disease, including myocardial infarction, atherosclerotic heart disease, and congestive heart failure. In addition, patients with ESRD have a unique excess of sudden death from cardiac arrest[49]; this may be due partly to the tremendous prevalence of left ventricular hypertrophy in this group. Eighty percent of all individuals who reach stage 5 CKD (<15 mL/min) have left ventricular hypertrophy.[14]

Traditional and nontraditional risk factors are present in ESRD patients, and dialysis seems to exacerbate them. ESRD patients are elderly and have a high prevalence of diabetes. Yet, even markers of inflammation are elevated eightfold to tenfold in long-term dialysis patients compared with healthy controls.

Other cardiovascular complications common to dialysis include atrial fibrillation. Significantly, there is a twofold increased risk of death and 50% increased rate of stroke associated with atrial fibrillation in dialysis patients. Increased aortic stiffness also has been shown in ESRD patients.[31] The decreased distensibility of the large elastic arteries is likely due partly to increased collagen deposition, shown in animal models and human studies. Aortic stiffening is characterized by decreased buffering capacity of the ascending aorta to reduce the pulsatile impact of ejected blood from the heart during systole. Increased pulse wave velocity and early wave reflections back to the heart result, increasing systolic load and decreasing diastolic blood pressure and coronary perfusion, ultimately leading to myocardial hypertrophy. Aberrant aortic stiffness has been noted in patients with ESRD independently of age and blood pressure.[43]

Complications

The procedure of HD itself is not without complications. Problems can arise at any part of the HD run. Common complications include hypotension, cramping, febrile episodes, arrhythmias, nausea, and vomiting.

Hypotension during HD is a common complication that occurs in 40% of all dialysis treatments. Hemodynamic instability contributes to the morbidity associated with dialysis.[13] Maintenance of intravascular volume during HD depends on the rapid refilling of the intravascular compartment from surrounding tissue spaces. The process of HD itself, with the removal of 500 mL of blood extracorporeally to the dialysis machine and removal of fluid from ultrafiltration, creates a decrease in the intravascular volume. This decrease results in decreased cardiac filling, which leads to reduced cardiac output and ultimately hypotension. Other factors contributing to hypotension during HD include splanchnic vasodilation, commonly as a result of food ingestion, and overly warm dialysate, which also can lead to vasodilation. Patients unable to vasoconstrict adequately, owing to autonomic dysfunction such as with long-standing diabetes, have an increased risk of developing hypotension during HD. Cardiac patients with poor myocardial contractility and diastolic dysfunction also are at increased risk of developing hypotension with HD. Less common causes include infection, pericardial tamponade, myocardial infarction, cardiac arrhythmias, and air emboli. Each patient's medical history, medications, and current clinical situation should be considered before the HD prescription to prevent hypotension during HD.

In the case of intravascular volume depletion as the source of hypotension, ideally, the rate of fluid removal should be constant throughout the dialysis session. It is important to educate the patient on the importance of fluid restriction to prevent intradialytic weight gain. A goal of less than 1 kg/day prevents excessive ultrafiltration during HD. Shorter treatments with higher rates of ultrafiltration increase the risk of hypotension. If a patient requires more than 3 to 4 L of fluid removal, the dialysis time should be increased to allow for continued controlled fluid removal. Sodium modeling is a process in which the sodium content of the dialysate is higher than the patient's serum sodium (i.e., 152 mEq/L) with controlled decrements in the sodium level to approximate 140 mEq/L to maintain a stable plasma osmolality within the patient during the course of ultrafiltration on dialysis. Studies have shown that blood pressure is well supported during sodium modeling protocols, but excessive thirst is reported in the intradialytic period.[11]

Dialysis solutions are normally kept at 37°C, but temperatures can be decreased to 34°C. These lower dialysate temperatures cause vasoconstriction and have been shown to maintain blood pressures during HD. The shivering and cramping that some patients experience with this method often limit its use.

Blood volume monitors are either an optical or an ultrasonic sensor located on the inflow blood line within the HD circuit. This sensor detects changes in hematocrit during dialysis. The blood volume monitor can indirectly monitor the effects of ultrafiltration during HD on intravascular volume by associating an increase in hematocrit with a reduction in plasma volume. Continuous monitoring of blood volume can be used to predict symptoms resulting from intradialytic hypovolemia.

Splanchnic vasodilation can be prevented by prohibiting eating during dialysis and holding meals until after HD is complete. In patients with refractory cases of hypotension, such as patients with autonomic neuropathy, α-adrenergic agonists such as midodrine should be considered, which can be given 30 minutes before scheduled dialysis time. Patients on antihypertensive medications should have their blood pressure medications held on the day of their dialysis, especially if they frequently develop hypotension with HD. A higher calcium dialysate concentration should be considered in cardiac patients to help with overall myocardial contractility.[48] When an acute hypotensive episode occurs during HD, ultrafiltration should be turned off, the patient should be placed in Trendelenburg position, nasal cannula oxygen should be given, and fluid in the form of 0.9% normal saline should be administered rapidly through the venous HD line.

The exact cause of cramping during HD is unknown but is commonly attributed to aggressive ultrafiltration and taking a patient below his or her "dry weight." During HD, treatment for cramping includes stopping ultrafiltration and, in instances when cramping does not resolve, administering 250- to 500-mL normal saline boluses to restore intravascular volume. Preventive measures, especially in patients with a history of cramping during HD, include prescribing quinine sulfate at least 2 hours before the next dialysis run.

Cardiac arrhythmias during HD are related to changes in blood volume affecting coronary artery perfusion and electrolyte abnormalities. Arrhythmias related to electrolyte abnormalities are due not only to a high serum level of potassium but also to the extreme changes of potassium during the HD run. Slower declines in potassium levels during HD have shown decreased incidences of supraventricular and ventricular arrhythmias. The incidence of cardiac arrhythmias during HD is estimated to be 50%. The most common sustained cardiac arrhythmia during HD is atrial fibrillation. Atrial fibrillation can occur in 20% of HD treatments and is more common in patients with left ventricular diastolic dysfunction, particularly patients with reduced intravascular volume and patients with ongoing sepsis.[5] Multiform ventricular ectopic arrhythmias are usually nonsustained and asymptomatic, resolving with completion of HD. Atrial fibrillation usually corrects spontaneously within a few hours of HD, but requires rate control with amiodarone or other agents in symptomatic patients. Impaired cardiac function, underlying autonomic neuropathy, an elevated calcium-phosphorus product,

and elevated PTH levels all have been shown to increase the incidence of cardiac arrhythmias during HD.

PERITONEAL DIALYSIS

In PD, the patient's own peritoneal membrane is the dialysis membrane. The patient is able to transport solutes and water from blood in the peritoneal capillaries to a dialysis solution in the peritoneal cavity via the peritoneal membrane. Peritoneal membrane transport consists of three simultaneous processes: (1) diffusion down a concentration gradient between the patient's blood and peritoneal dialysate, (2) ultrafiltration resulting from the osmotic gradient between these two fluid compartments, and (3) fluid absorption via lymphatics at a relatively constant rate. Although in ESRD a patient's blood has elevated concentrations of urea, the blood remains hypotonic to peritoneal dialysate. The peritoneal dialysate consists of sodium, chloride, water, and elevated concentrations of glucose to maintain a hypertonic solution relative to the patient's blood.

Process

Although there is an intermittent regimen of PD in which the dialysis is performed periodically or several times a week, the common practice of PD is a continuous regimen in which there is a constant presence of peritoneal dialysate in the peritoneal cavity 24 hours a day, 7 days a week. There are two techniques for a continuous regimen of PD: (1) a continuous flow technique using either two catheters or a double-lumen catheter, which allows for simultaneous and continuous inflow and outflow of dialysate, and (2) an intermittent flow technique in which a single catheter provides discrete inflow, dwell, and outflow phases of dialysate, with the flow of dialysate being completely interrupted during the inflow, dwell, and outflow portion of PD. The two major forms of PD, CAPD and CCPD, combine a continuous regimen with an intermittent technique.

A standard CAPD regimen consists of four 2-L dialysate exchanges daily. This regimen involves an infusion of the dialysate into the peritoneal cavity through the abdominal catheter for approximately 10 to 20 minutes; a dwell period in which the dialysate remains within the peritoneal cavity 3 to 8 hours; and the drainage of dialysate out of the peritoneal cavity through the same catheter, which takes an estimated 10 to 20 minutes. The infusion time and dwell time are dictated by the flow through the catheter along with the patient's anatomy. The overall dwell time is defined by the transport characteristics of the peritoneal membrane. As the name states, the patient is actively dialyzing with a "dwell" in the peritoneum while ambulatory, carrying out daily activities.

CCPD is an automated form of PD that is performed while the patient sleeps. All connections and preparation of equipment take place at bedtime. The dialysate bags are placed on a "cycler," a machine that has programmed infusion, dwell, and drain time. The patient is supine for CCPD, which allows for better surface area interface between the peritoneum and the dialysate. A supine position also allows dwell amounts larger than 2 L because there is less intra-abdominal pressure with the dialysate compared with a person standing upright. Because an entire nighttime of cycling may not be enough for adequate dialysis, an additional daytime dwell of dialysate may be necessary to improve clearance and ultrafiltration. This use of CCPD with an additional exchange during the day is referred to as PD Plus. The last automated exchange is provided by the cycler, with the second exchange during the day usually provided by manual CAPD, commonly called a "pause."

Access (see Chapter 5)

PD catheters are constructed from silicone rubber or polyurethane and have one or two Dacron cuffs. The silicone rubber or polyurethane surface promotes development of squamous epithelium in the subcutaneous tunnel next to the catheter, at the exit site, and within the abdominal wall. The presence of this epithelium increases the resistance to bacterial penetration of the tissue near the skin exit and peritoneal entry sites. The Dacron cuff provokes a local inflammatory response that progresses to form fibrous and granulation tissue within 1 month. This fibrous tissue serves to fix the catheter cuff in position and to prevent bacterial migration from the skin surface or from the peritoneal cavity past the cuff into the subcutaneous tunnel.

An extensive review of more than 17 trials comparing different catheter insertion techniques and catheter types was conducted.[46] This review showed no reduction in the incidence of peritonitis among catheter types or insertion techniques. Trials comparing single- versus double-cuffed catheters show no significant difference in the risk of peritonitis, exit site/tunnel infection, or catheter removal or replacement.[46]

After a PD catheter is placed, PD exchanges should be delayed for 2 to 4 weeks when appropriate. If the PD need is acute, the catheter can be used the day it is placed, but the method of dialysis is altered to prevent possible leakage around the catheter site. If PD is required the day of catheter placement, or before the 2-week waiting period, nephrologists usually prescribe smaller dwells and keep the patient in a supine position to prevent increased intra-abdominal pressure. If leakage occurs around the catheter site, the dialysis must be stopped, and the patient should go on temporary HD until the catheter site has completely healed.

Fluid Status

As with HD, patients with ESRD on PD can develop difficulties with hypervolemia causing hypertension and left ventricular hypertrophy over time. Several studies have shown that fluid status in PD patients is not better maintained compared with HD patients. Studies evaluating ECF volume in PD patients found that patients with significantly reduced residual kidney function had significantly elevated ECF volumes, even when using high-dextrose concentrations in their PD dwells to promote maximal ultrafiltration.[50] Peritoneal membrane characteristics and residual kidney function are important in the maintenance of fluid balance in PD patients.

Most attention has been devoted to peritoneal membrane characteristics as a culprit for excessive fluid status in PD patients. Ultrafiltration in PD is achieved by osmotic forces using dextrose concentrations in the PD fluid. The higher the dextrose concentration, the more free water is filtered from the extracellular compartment. Adequate clearance and ultrafiltration also is directly related to the permeability of the patient's peritoneal membrane. The chronic exposure to these dextrose peritoneal dialysate dwells, although required for fluid removal, can work against the patient.

Long-term exposure to glucose and glucose degradation products may have detrimental effects on the peritoneal membrane, with the gradual loss of ultrafiltration capacity.

Methods to improve fluid balance in PD patients include a sodium-restricted diet, fluid restriction, and the use of diuretics in patients with adequate residual renal function. Alternatives to glucose-containing solutions in PD dialysate, such as icodextrin, also need to be considered to save the peritoneal membrane from the effects of chronic exposure to glucose and to improve ultrafiltration. Improving fluid balance results in improved blood pressure control and cardiac status with reduction in left ventricular hypertrophy.

Electrolytes

Sodium

PD solution typically contains 132 mmol/L sodium. Most patients maintain normal serum sodium on PD. Patients who drink excessive amounts of water can get a dilutional hyponatremia. Conversely, with rapid ultrafiltration, hypernatremia may occur owing to the different convective forces of sodium on the semipermeable peritoneal membrane, resulting in more free water being ultrafiltrated compared with sodium (see Table 3-4). Current dietary restriction for sodium is slightly less stringent than that of HD. Although a 2-g restriction is preferred, 4 g of daily sodium intake is allowed (see Table 3-3).

Potassium

Standard PD solution contains no potassium. As in HD, potassium is removed during PD by diffusion and convection. Usually, only patients who are noncompliant in performing their dialysis exchanges have ongoing problems with hyperkalemia. Cases of hypokalemia are usually seen in patients undergoing continuous ambulatory PD with poor nutritional intake (see Table 3-4). PD patients usually do not require a potassium-restricted diet. In some cases of persistent hypokalemia, potassium supplementation my be required. This is reflected in current dietary recommendations of 3 to 4 g of daily potassium intake (see Table 3-3).

Calcium and Phosphorus

PD patients have the same difficulties with hyperphosphatemia as HD patients. The same treatments apply to both dialysis populations, including dietary recommendations (see Table 3-4). Patients taking calcium-containing phosphate binders may have hypercalcemia. In these particular patients, lower calcium–containing peritoneal dialysate can be used at 1.25 mmol/L in place of the normal 1.75 mmol/L of calcium in the dialysate.

Anemia

There is an increased incidence of iron deficiency, ranging from 40% to 90% of patients on PD. Lower intestine mucosal uptake and rates of iron transfer are present in PD patients. Usually these patients are able to retain only approximately 5% of oral iron therapy they are taking under ideal conditions. As anemia improves during iron therapy, there is a downregulation of iron mucosal uptake mechanisms that may be responsible for the dramatic reduction in iron retention found in these patients. In three separate studies, three fourths of PD patients known to respond inadequately to oral iron supplementation responded to intravenous iron therapy with improvement in hemoglobin, hematocrit, and iron parameters.[1,12] When iron is replete, there seem to be minimal differences in responsiveness to erythropoietic stimulating proteins.

Adequacy

A PD patient's treatment success depends on the functional and morphological integrity of the peritoneal membrane. NKF K/DOQI guidelines for PD adequacy include a weekly peritoneal and renal Kt/V of greater than 1.7 in both CAPD and CCPD. The functional integrity of the peritoneal membrane is investigated with kinetic modeling; this is done every 4 months and assesses the clearance of the peritoneal membrane and the clearance of the residual kidney function. The principal determinants of PD dose are the patient's body mass, residual kidney function, and peritoneal transport rates. Residual kidney function is important in maintaining PD adequacy and assisting in fluid removal. It contributes substantially to the PD adequacy, maintenance of fluid balance and kidney endocrine function, and a reduction in systemic inflammation. Several studies have confirmed the finding that there is a 12% survival advantage for every 5 L/wk/m^2 increase in residual kidney function.[50]

There are numerous techniques for measuring peritoneal transport, the most widely used being the peritoneal equilibration test for examining the morphological integrity of the membrane. This is usually performed 4 weeks after starting PD to define an appropriate dialysis schedule based on membrane properties. Long-term PD may lead to anatomical changes in the peritoneal tissues, including fibrosis, neoangiogenesis, vasculopathy, and peritoneal sclerosis. These anatomical changes cause alterations to the peritoneal membrane, which have an impact on a patient's dialysis and are reflective in changes of a peritoneal equilibration test.[6]

Complications

The most common and most dangerous complication involving PD is infection, specifically infection of the exit site of the peritoneal catheter, the tunnel of the peritoneal catheter, and peritonitis itself. Other important complications unique to PD include leaking around the catheter, bloody peritoneal effluent, abdominal pain *not* related to peritonitis, hyperlipidemia, new-onset or worsening diabetes, and chronic hypoalbuminemia.

Infection

Daily human error with PD technique can enhance infection risk. Peritonitis and exit site and tunnel infections remain the predominant complications of PD, ranging from abdominal pain and poor dialysis to permanent damage of the peritoneal membrane, loss of the catheter, sepsis, and death. Peritonitis remains a leading complication of PD. Although it can occur spontaneously, most cases are due to an initial exit site or catheter infection that eventually seeds the peritoneum.

The most common exit site pathogens are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[40] Because these organisms also lead to peritonitis, exit site infections must be

treated aggressively. The exit site should be cultured because other bacteria can be involved, and proper diagnosis should direct treatment. Gram-positive organisms are treated with oral penicillinase-resistant penicillin or a first-generation cephalosporin. In slow-to-resolve or severe *S. aureus* exit site infections, rifampin may be added, but it should never be used as monotherapy or where tuberculosis is endemic. *Pseudomonas aeruginosa* exit site infections often require prolonged therapy with two antibiotics. Oral quinolones are recommended as the first choice, with the second drug being intraperitoneally dosed ceftazidime.[40] Two weeks is the minimal treatment time for exit site infections. Any pain or erythema along the tunnel of the PD catheter should raise concerns for a possible tunnel infection. A bedside ultrasound examination along the tract of the tunnel with echogenic findings can lead one to presume likely tunnel infection.

PD patients presenting with cloudy effluent should be presumed to have peritonitis. Peritonitis should always be included in the differential diagnosis of a PD patient with abdominal pain, even if the effluent is clear, because a small percentage of patients with peritonitis present with such symptoms. Although patients with peritonitis often have severe pain, some episodes are associated with mild or even no pain. Peritonitis is confirmed by obtaining effluent cell count, differential, and culture. Although the Gram stain is often negative in the presence of peritonitis, this test should be performed because it may indicate the presence of yeast, allowing for prompt initiation of antifungal therapy and permitting timely arrangement of catheter removal. An effluent white blood cell count of greater than 100/μL, with at least 50% polymorphonuclear neutrophil cells,[40] indicates the presence of inflammation with peritonitis as the most likely cause.

Antibiotic therapy should be initiated as soon as cloudy effluent is seen, without waiting for the confirmatory cell count. Empirical antibiotics must cover gram-positive and gram-negative organisms. Intraperitoneal vancomycin or a cephalosporin (cefazolin) can be used for gram-positive coverage with a third-generation cephalosporin (ceftazidime, cefepime) or aminoglycoside for gram-negative coverage.[40] Aztreonam is an alternative to ceftazidime or cefepime for gram-negative coverage if aminoglycosides are not used and patients have a cephalosporin allergy. Antibiotic therapy should be adjusted when culture results are known. The antibiotics are given through the intraperitoneal route, and there are dosing schedules for once-daily extended dwells with intraperitoneal antibiotics versus antibiotics in each dwell. In intermittent dosing, the antibiotic-containing dialysis solution must be allowed to dwell for at least 6 hours to enable adequate absorption of the antibiotic into the systemic circulation. If there is no growth of the cultured peritoneal fluid by 3 days, a repeat cell count with differential should be obtained. If the repeat cell count indicates that the infection has not resolved, special culture techniques should be used for the isolation of potential unusual causes of peritonitis, including lipid-dependent yeast, *Mycobacterium, Legionella*, slow-growing bacteria, *Campylobacter*, fungi, *Ureaplasma, Mycoplasma*, and enteroviruses.

For gram-positive infections, vancomycin can be given intermittently with the next dosing based on serum trough levels drawn 72 hours after initial dose.[40] Repeat dosing is appropriate when serum vancomycin levels reach 15 μmol/L or less. Coagulase-negative *Staphylococcus* peritonitis, including *S. epidermidis*, is due primarily to touch contamination and is generally a mild form of peritonitis. This peritonitis responds readily to antibiotic therapy, but sometimes can lead to relapsing peritonitis as a result of biofilm involvement. In such circumstances, catheter replacement is advised. Most patients with *S. epidermidis* peritonitis have mild pain and can be managed on an outpatient basis. In programs with a high rate of methicillin resistance, vancomycin can be used as empirical therapy. Streptococcal and enterococcal peritonitis tend to be severe, causing considerable abdominal pain. They are best treated with intraperitoneal ampicillin.[40] Because enterococci are frequently derived from the gastrointestinal tract, intra-abdominal pathology must be considered, but touch contamination as a source is always possible. Peritonitis with enterococci or streptococci also may derive from infection of the exit site and tunnel, which should be carefully inspected.

S. aureus causes severe peritonitis. Although it may be due to touch contamination, it is often due to catheter infection. Catheter-related peritonitis is unlikely to respond to antibiotic therapy without catheter removal. After a rest period from PD (generally a minimum of 2 weeks), PD can be tried again. Polymicrobial peritonitis secondary to multiple gram-positive organisms is not only more common than polymicrobial gram-negative peritonitis but also has a much better prognosis than that due to enteric organisms. The source is most likely contamination or catheter infection.

Short-term use of aminoglycosides seems to be safe, inexpensive, and efficacious for gram-negative coverage. Oral quinolones are an acceptable alternative because they reach adequate bactericidal levels within the peritoneum, even with the cycler. Oral therapy is unsuitable for more severe cases of peritonitis. *P. aeruginosa* peritonitis is generally severe and often associated with catheter infection. If catheter infection is present or has preceded the peritonitis, catheter removal is necessary. Two antibiotics for 2 weeks of therapy should always be used to treat *P. aeruginosa* peritonitis. Single-organism gram-negative peritonitis (*Escherichia coli, Klebsiella, Proteus*) may be due to touch contamination, exit site infection, or transmural migration from constipation or colitis. Outcomes of these infections are worse than outcomes with gram-positive infections and are more often associated with catheter loss and death. If multiple enteric organisms are grown on culture, there is a possibility of intra-abdominal pathology, such as ischemic or perforated bowel, gangrenous cholecystitis, appendicitis, or diverticulitis. A surgical evaluation along with abdominal radiographs or computed tomography scan, or both, to rule out free air and identify intra-abdominal pathology is helpful. The minimal period of therapy for peritonitis is 2 weeks, and although not evidenced based, the common practice is to increase therapy to 3 weeks for more severe infections.[40]

Refractory peritonitis, defined as failure to respond to appropriate antibiotics within 5 days, should be managed by removal of the catheter to protect the peritoneal membrane for further use. Fungal peritonitis is serious, leading to death of the patient in approximately 25% or more of episodes. Some evidence suggests that prompt catheter removal poses less risk of death. Intraperitoneal use of amphotericin causes chemical peritonitis and pain, and intravenous administration leads to poor peritoneal penetration. Therapy with oral antifungals should be continued after catheter removal for an additional 10 days.[40] Mycobacteria are an infrequent cause of

peritonitis but can be difficult to diagnose, and the treatment requires multiple drugs. When culture is being considered, special attention should be paid to culture technique.

Catheter Leak

A catheter leak should be classified according to when the catheter was originally placed. An early leak is one that occurs within 30 days of catheter placement. A late catheter leak is more than 30 days from placement. A simple bedside test to determine if the clear exudate is dialysate is to place a urine dipstick against the fluid; a positive glucose on the urine dipstick is confirmatory. Early leaks usually are related to catheter placement and manifest as external leakage, either at the exit site or at a medial or parasternal surgical wound. Early leaks also can occur if the catheter site has not been given enough time to heal fully after implantation, and dwell size is creating enough intra-abdominal pressure to cause extravasation of PD fluid. Late leaks are most often related to a mechanical or surgical tear in the peritoneal membrane, presenting as internal leakage, which can be found in the pleural cavity, abdominal wall, and external genitalia.

Bloody Effluent

Hemoperitoneum is a benign condition of PD, with no significant long-term effects on patient survival, no predisposition to peritonitis, and no predisposition to ultrafiltration failure. Bloody effluent can occur at any time during a PD treatment, but usually disappears spontaneously. It is not associated with a specific disease, but nonperitoneal causes include retrograde menstruation and renal cyst hemorrhage. Treatment includes three 1.5% dextrose rapid exchanges with no dwell time or infusion of unwarmed dialysate, which induces peritoneal vasoconstriction.

Abdominal Pain Not Related to Peritonitis

Pain with PD with clear dialysate without an elevated cell count still must be evaluated thoroughly to ensure continued, adequate daily dialysis. Inquiring about fill and drain times can help discern if there is a mechanical problem with the catheter; this can be reviewed further with an abdominal film to look at the position of the catheter. An abdominal film also can diagnose other potential complications of PD, including free air and diaphragm perforation. Fibrin also can be a culprit for pain on fill or drain, and adding heparin to the dialysate bags can alleviate this problem. Pain on drain can be due to part of the membrane forming suction to the catheter. This can be remedied by decreasing dwell time or adding a tidal volume, which allows for an excess of dialysate in the abdominal cavity to keep the catheter free floating. Pancreatitis also should be considered, checkings amylase and lipase levels, because the calcium in the dialysate comes in direct contact with the lesser sac of the pancreas.

Hypoalbuminemia

Approximately 0.5 g of protein can be lost per each 1 L of dialysate drained during PD. This can account for 20 g of protein loss a day. Although the protein loss is predominantly albumin, 15% can be IgG. Higher peritoneal transport rates can cause increased albumin loss, and acute inflammation of the peritoneal membrane, as in peritonitis, creates a more permeable membrane and leads to higher protein losses. It is important to evaluate both peritoneal and renal losses of protein in a hypoalbuminemic patient.

Hyperglycemia and Hyperlipidemia

Glucose is a cheap, stable, and standard osmotic agent in peritoneal dialysate. With each CAPD exchange, 80% of dialysate glucose is absorbed across the peritoneal membrane, which can lead to metabolic derangements, in particular hyperglycemia. Peritoneal glucose absorption also may lead to abnormalities in the lipid profile and hyperinsulinemia. Increased glucose absorption also may lead to fatty liver infiltration. Hyperinsulinemia can result in persistently elevated plasma insulin levels, which are an independent risk factor for atherosclerosis. Supplemental insulin may be required for a diabetic patient undergoing PD. Regular insulin can be added to the dialysate, with specific amounts of insulin corresponding to the dextrose concentration within the PD dialysis solution. Serum blood glucose should be monitored closely. Compared with HD patients, PD patients have more difficulties with weight gain; 800 kcal/day can come from the dialysate dextrose alone. The elevated serum glucose levels from the dextrose dwells play a large part, along with the additional or increased need for exogenous insulin administration.

CONTINUOUS RENAL REPLACEMENT THERAPY

The major difference between intermittent and continuous therapies is the speed at which water and wastes are removed. Intermittent HD removes large amounts of water and wastes in a short time (usually over 2 to 4 hours), whereas continuous renal replacement therapy (CRRT) removes water and wastes at a slow and steady rate. Although intermittent dialysis allows patients with chronic renal failure to limit the amount of time that they are connected to a machine, the rapid removal of water and wastes during intermittent treatments may be poorly tolerated by hemodynamically unstable patients. The basic principles of conventional HD with slow, extended dialysis allowing gradual volume removal have resulted in the creation of CRRT.

Although initially developed for fluid management in patients with diuretic-resistant fluid overload, modifications of the original technique have provided a collection of related therapies designed to provide uninterrupted renal support to critically ill patients over a period of days. Hemofiltration, HD, and hemodiafiltration differ primarily in their mechanism of solute movement. In hemofiltration, solute removal occurs predominantly by convection; in HD, it occurs by diffusion; and in hemodiafiltration, it occurs by both convection and diffusion. Although achievable clearances of low-molecular-weight solutes are similar with hemofiltration and HD, the convective therapies provide higher clearances for solutes with molecular weights greater than 500 to 1000 D. It has been postulated that enhanced clearance of inflammatory mediators in this molecular weight range, particularly in patients with acute renal failure associated with sepsis, provides an added benefit to convective therapies. Although modulation of plasma levels of tumor necrosis factor-α and interleukin-6 can be achieved with CVVH, a corresponding clinical benefit has not yet been shown.[17,25]

CVVH is the removal of large amounts of water across the filter membrane for the purpose of clearing wastes. When large volumes of water are washed across the membrane, solutes are dragged along with the water (convection).

Hemofiltration is the removal of water over and above the surplus water removed during ultrafiltration. To prevent hypovolemia, any water removed during hemofiltration must be returned to the blood before it reaches the patient. This is called "replacement" fluid. Hemofiltration rates of 1 L/hr mean that 1 L of fluid is removed from the patient's blood and eliminated in the drainage fluid, and 1 L of replacement fluid is returned to the circuit before it reaches the patient. Hemofiltration rates are set by adjusting replacement rates. Any fluid removed during hemofiltration is given back to maintain a net neutral fluid balance. Replacement fluid must be sterile intravenous fluids with concentrations of electrolytes similar to plasma. Replacement fluids can be returned either before or after filter; this is referred to as predilution or postdilution sets. Predilution means that the replacement solution is returned to the blood before it reaches the filter, diluting the blood in the hollow fibers. Postdilution means that the replacement fluid is returned to the blood after the filter (but before the return side of the access catheter). Predilution dilutes the blood in the filter, reducing clotting. Postdilution concentrates the blood in the filter, enhancing clearance.

CVVHD is the infusion of dialysis fluid into the dialyzer. Solutes that are small enough to fit through the membrane of the dialysis filter move from an area of high concentration to low concentration (diffusion). The dialysate determines the solutes that will be removed. To remove solutes, the concentration in the dialysate should be made lower compared with the blood concentration. To increase solutes, such as electrolytes, in a patient, the concentration of solutes in the dialysate is higher than the blood. CVVHD is the removal of wastes by diffusion only, without the use of hemofiltration (replacement fluid). It can be administered with or without fluid removal from the patient.

Continuous venovenous hemodiafiltration is the use of dialysis and hemofiltration. Therapy includes the use of dialysate and replacement fluids and can be administered with or without fluid removal from the patient.[36]

Process

Although the machines may differ, the basics of the system setup remain relatively the same. For all modalities of CRRT, vascular access is the double-lumen HD catheter. In CVVH, blood is drawn out from the arterial port by a pump and is delivered to a dialyzer through tubing. Before the patient's blood reaches the dialyzer, there is a port for anticoagulation. When the blood reaches the dialyzer, a convective process occurs across a pressure gradient within the dialyzer. Ultrafiltrate is pulled off within the dialyzer through a separate pump, and this effluent is collected in a separate bag. When passed through the dialyzer, the blood is returned to the patient by another pump passing the blood back to the patient in the venous port. Replacement fluid is crucial to the circuit, mixing with the patient's blood either right before or after the dialyzer.

In CVVHD, the circuit is similar, with blood coming from the arterial port of the HD catheter by a pump, with anticoagulation added before the blood enters the dialyzer. Blood traverses the dialyzer by diffusion across a concentration gradient. Dialysate is pumped countercurrent to the blood when in the dialyzer, and ultrafiltrate is collected from the dialyzer through separate tubing into a separate effluent collection bag. When passed through the dialyzer, the patient's blood is returned by a pump through the venous port. Continuous venovenous hemodiafiltration is a combination of CVVH and CVVHD, using replacement fluid and dialysate along the same circuit. The current dialysis dose of replacement fluids in CVVH is 35 mL/kg/hr. Blood flow in CVVH runs between 100 and 300 mL/min. Replacement fluid runs between 1000 and 3000 mL/hr. Dialysate flow runs at 1000 mL/hr. Ultrafiltration varies depending on the clinical situation, ranging from none to 200 mL/hr.

Anticoagulation is needed in CRRT because the clotting cascades are activated when the blood touches the nonendothelial surfaces of the tubing and filter. CRRT can be run without anticoagulation, but filters last much longer if some form of anticoagulation is used. Advantages for longer filter life include reduced time off therapy. The main options for anticoagulation include heparin, citrate, or no anticoagulation. Regional anticoagulation of the filter can be achieved through the use of citrate.

Electrolyte Abnormalities

The convection in CRRT can predispose a patient to hypocalcemia, hypomagnesemia, hypophosphatemia, and metabolic acidosis with loss of bicarbonate. These conditions can be easily circumvented through the additional infusion of calcium and magnesium through a central line and addition of phosphorus and bicarbonate in the replacement fluids. To maintain normal serum electrolyte levels, dialysate fluid contains sodium, chloride, and magnesium levels that are equal to serum concentrations (removal of these electrolytes should occur only if the blood level exceeds normal serum concentrations). In renal failure, potassium is often high at the start of a treatment; we may begin dialysis with a low concentration of potassium in the dialysate. Because potassium is easily removed during dialysis, and continued dialysis is required to ensure removal of other wastes, such as urea and creatinine, potassium concentrations in the dialysate often require upward adjustment as the potassium level in the blood falls. Although in theory, potassium levels should not decrease to less than 4 mmol/L in the serum if the dialysate contains 4 mmol/L, many factors influence serum potassium levels in critical care. Insulin therapy and the use of sympathomimetic drugs promote the movement of potassium from the blood into the cells; this can reduce serum levels. Additionally, potassium loss through the gastrointestinal tract can increase the potential for hypokalemia. Low magnesium levels also suppress the serum potassium levels; magnesium deficits should be replaced as needed. Additionally, high hemofiltration rates can lead to additional potassium clearance. Potassium levels must be monitored closely and adjusted to maintain normal serum concentrations. In renal failure, serum bicarbonate levels are generally low; a source of bicarbonate is added to the dialysate to facilitate diffusion of bicarbonate into the blood.

Complications

Citrate Toxicity

Citrate is used as an anticoagulant during CVVH. Preventing clotting of the system is crucial to maintain adequate clearance and proper electrolyte balance. Citrate has been

shown to increase kidney filter half-life over normal saline.[34] It works as an anticoagulant by binding to calcium, an essential element to coagulation in the intrinsic pathway. Citrate is introduced into the system through the arterial line after the blood has left the patient and before it reaches the dialyzer. When in the dialyzer, it binds to calcium to prevent clotting within the membrane. Its efficacy is measured by comparing the ionized calcium in the blood before the filter (prefilter calcium) with the ionized calcium leaving the filter (postfilter calcium). A decrease in ionized postfilter calcium compared with prefilter calcium shows a trend toward adequate anticoagulation because it represents a binding up of citrate with ionized calcium, preventing the coagulation cascade. Understanding this process helps to interpret laboratory results during CRRT properly, specifically total and ionized serum calcium. Within the first 24 hours of starting citrate, there is an increase in the total calcium. This increase is to be expected because not only is circulating calcium bound to citrate, but also a separate infusion of calcium is infusing into the patient to ensure adequate levels of ionized calcium systemically. Calcium also can be bound by albumin, contributing to the total calcium. Problems arise when citrate levels become critically elevated in patients, causing acid-base and calcium disturbances.

The difference between citrate toxicity and citrate excess denotes not only changes in ionized calcium but also a serological change, which can be corrected and citrate continued (citrate excess) or cannot be corrected unless citrate is discontinued (citrate toxicity). In the instance of citrate toxicity, citrate is unable to be metabolized within the liver or skeletal muscle or both, leading to an acidosis and a decrease in ionized calcium with continued increase in total calcium. This condition is to be differentiated from citrate excess, in which citrate is able to be metabolized but, as citrate level increases, a profound metabolic alkalosis can occur. Although total calcium levels increase, the ionized calcium remains relatively stable. Appreciating these differences allows for appropriate decisions regarding citrate with CRRT. Patients exhibiting clear clinical signs of citrate toxicity need to have their citrate infusion stopped. Patients with citrate excess benefit from continuing CRRT, but with a lower rate of citrate infusion.

Access Issues

Because continuous modalities are considered temporizing measures for renal replacement therapy, the access for these therapies remains dialysis catheters. Even if a patient is a long-term HD patient and has a working fistula or graft, owing to the fact he or she would need to be cannulated for the entire duration of CRRT, the risk of needle infiltration or accidental misplacement can predispose the patient to thrombosis of the fistula or graft or possible exsanguination. A temporary dialysis catheter needs to be placed, along with another central line if CVVH is the chosen modality. These patients then have the same access issues as mentioned in the HD access section.

SUMMARY

Renal replacement therapy has had significant advances since its infancy in the early 1960s, but it remains an imperfect modality. The decision of what form of renal replacement to use must be based on the chronicity of the renal dysfunction and the patient's medical history, combined with the known outcomes of each type of modality. HD and PD differ from each other in their procedure and have risks and benefits unique to their technique.

Outcomes in fluid status, bone metabolism, anemia, cardiovascular disease, diabetes, nutrition, and overall morbidity and mortality have been compared between HD and PD. Patients on PD have improved stable fluid status compared with patients on HD, relative to the patient's residual renal function.[19,38] When residual renal function is lost, hypervolemia can worsen in PD patients, resulting in difficult-to-control hypertension. Related to bone metabolism, secondary hyperparathyroidism with elevated prevalence of bone lesions is more common in HD patients, whereas adynamic bone disease is much more frequent in PD patients.[9,47] Red blood cell survival is higher in PD patients, and PD has been found to be more effective in maintaining erythropoiesis compared with HD.[28,33,41]

Cardiovascular diseases are the main cause of death in dialysis, without a significant difference between the two modalities.[45] Although PD is associated with a higher atherogenic risk, after stratification by diabetic status and adjustment for differences in age, gender, and previous cardiovascular disease, Cox analysis showed no significant difference in the risk of developing cardiovascular disease between PD and HD.[30] Related to morbidity, hospitalizations between HD and PD patients are skewed toward PD, related in part to peritonitis. Findings from the USRDS 2003 Annual Report show that the number of admissions is similar but that the number of hospital days is higher by approximately 15% in PD patients.[10] The easily diffusible dextrose contained in PD dialysis solutions can cause new-onset hyperglycemia and cause worsening blood glucose levels in established diabetics. A large assessment of more than 890 diabetics treated with HD and PD found no difference in survival, although the survival curve indicated worse results in the PD population after 2 years.[32] Several reports have confirmed that nutritional indices are worse in PD patients compared with HD patients.[22,23,26] Nutritional status is influenced by dialysis dose and residual renal function.

There have been conflicting outcome studies regarding comparison of mortality between the two modalities. A comparative analysis of HD and PD survival controlled for the delivered dose of dialysis showed that when the dose of dialysis is the same between modalities, HD and PD have comparable 2-year survival rates, independent of diabetes, age, and history of cardiovascular disease.[24] In contrast to the previous study, a prospective cohort study published in 2005 showed the risk of death to be significantly higher among patients undergoing PD in the second year of follow-up.[21] Other studies have shown that in diabetics and nondiabetics, PD patients with chronic heart failure have a greater risk of death than HD patients.[45] Stratifying for diabetes, a study in 2004 found that diabetics older than 65 years had a similar risk of death in HD and PD, with younger patients, diabetic and nondiabetic, having a significantly lower risk of death on PD.[8]

Neither HD nor PD poses a greater long-term outcome after renal transplantation. Although early post-transplant PD patients have been observed to fare better with transplant function, long-term results of renal transplantation are no different in patients treated with either PD or HD.[44]

When a patient proceeds to dialysis, he or she must make necessary changes to his or her lifestyle to promote the best

outcome on dialysis. These changes include fluid restriction; a renal diet that includes moderation in potassium-containing, sodium-containing, and phosphorus-containing foods balanced with adequate protein intake; close monitoring and treatment of hypertension; and maintenance of dialysis access.

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Chapter 4

The Recipient of a Renal Transplant

Jeremy R. Chapman

THE PATIENT WITH CHRONIC KIDNEY DISEASE

Most patients with chronic kidney disease (CKD) in developed countries present to medical services at a stage when there is still time to plan their transition to end-stage renal failure treatment. Only a few take the opportunity to understand their disease, learn about their dialysis and transplant options, and then plan smooth adoption of one or another therapy. Insidious and asymptomatic decline in renal function perhaps accounts for the naive attitude that most people have when it comes to ignoring warnings. The physician's primary aim should be to educate and inform the patient and family, while treating the treatable components and retarding the progression of CKD. More educated and financially stable patients tend to be better prepared for the onset of end-stage kidney disease, whereas at the other extreme, poorly educated, frightened, or noncompliant patients tread a hazardous course to dialysis and often an earlier death.

Individuals who plan their treatment well and receive a living donor transplant preemptively before the requirement for dialysis tend to have the best outcomes.[17,63] Most transplant recipients worldwide do not have this optimal approach and are treated by hemodialysis or peritoneal dialysis for weeks, months, or years before the transplant operation. There are some benefits to pretransplant dialysis, especially if the patient is chronically debilitated by CKD, or if time is needed to enable a family member to understand the benefits of offering a kidney. The experience of dialysis also provides a window on the alternatives to a renal transplant, either strengthening or weakening an individual's resolve for the operation and the long-term consequences of immunosuppression. Each patient comes to make the decision about whether or not to opt for a renal transplant slightly differently, based on his or her precise medical, social, and family circumstances. This chapter presents the issues that the patient, the patient's family, and the community must consider in making the irreversible step of undergoing a renal transplant.

GENERAL CONCEPTS

Fitness for Transplant

The patient has, in principle, a simple question to consider: Will the quality and quantity of life be better after a transplant than on dialysis? For many individuals, the answer is clear and unequivocal—either because the alternative of long-term dialysis treatment is unavailable or unaffordable[41] or because transplantation is the obvious solution because the individual is young and otherwise fit. For some individuals, however, the answer is not clear because of the relative unavailability of organs for transplantation or because the individual has comorbid conditions that would be exacerbated by the operation or the ensuing immunosuppression. There is little information available to help individuals make the decision easily.

Quality of life is the most important issue for most individuals, and yet good studies comparing quality of life on dialysis and after transplantation have not been done. The clinician working in a transplant unit seldom identifies individuals with a lower quality of life after a successful transplant than they had or would have had on dialysis and finds it easy to advocate for transplantation. This simple perspective ignores the patient deaths and graft failures and ignores the individuals who struggle with the consequences

of the iatrogenic immunodeficiency state, with its attendant infections and malignancies. In the absence of information, transplant programs tend to substitute graft survival data for true quality-of-life data and use it as a surrogate, but objective, measure of the success that each individual might expect.[87]

The most secure data on which to base the decision to use dialysis or to receive a transplant are measured comparisons of quantity of life.[70,100] In a country without effective access to dialysis, the decision is obvious for all patients, except those with severe comorbid conditions that would prevent a safe operation. Transplantation is, for many individuals, the only alternative to a slow death from uremia. This statement assumes access to immunosuppressive drugs and lifelong specialist medical follow-up, either of which may be unavailable. The patient's and family's understanding of the commitment that a transplant requires is an important factor. Patient survival rates are substantially affected by compliance with follow-up, and that is substantially altered by the expectations of the patient. Transplantation has been promoted as a cure, when it is actually a complicated treatment requiring regular follow-up by specialists working in sophisticated medical centers and using expensive drugs that have been priced against the costs of a year of dialysis treatment. If the patient and family fail to understand the costs, level of follow-up, and compliance required of them, the published statistics of average survival likely would not apply to them.

Predicting the success rate after transplantation relies on characteristics of the recipient and the donor. Probably the most comprehensive studies comparing transplant recipients with patients remaining on dialysis come from the United States, where it has been possible to track the outcomes of all individuals entered onto the transplant waiting list and compare the individuals who underwent transplantation with those who remained on dialysis.[60,100] These studies show that transplantation carries the greater risk of death for the first 3 months or so, reversing after that time so that the risk of death is equal by 6 to 9 months, and thereafter favoring the transplant recipient (Table 4-1). Similar analyses show that a patient who is transplanted preemptively carries an advantage compared with a patient who has needed time on dialysis, and the longer the period of dialysis, the worse the outcome (Fig. 4-1).[17] These data apply to patients receiving an "average" donor graft, highlighting the fact that recipients of well-matched, young, living donor grafts fare substantially better.[69] The converse also is true: A marginal donor graft from an elderly deceased donor with hypertensive renal damage may not confer much survival advantage.[76] A dilemma exists for each patient on a deceased donor waiting list—whether to accept a worse quality graft early or to wait longer on dialysis for a better graft. Most patients take the first opportunity for a transplant instead of waiting in hope for a better one.

The patient's expectations from a successful transplant are to die in old age with a perfectly functioning kidney. The reality is that patients die much earlier than age-matched and sex-matched individuals without renal disease, and more than half lose their graft before they die.[14,68] From the patient's perspective, graft failure represents transplant failure, but from the community's perspective, premature death with a functioning graft represents wasted years of graft function that could have been applied to someone else.

Appropriateness for Transplant

There are two ways to examine the question of appropriateness for transplant, which can be considered from the view of the recipient or the donor. These views are not the same, and these perspectives may lead to different decisions about the appropriateness of a particular transplant. To illustrate the difference, consider an elderly father or mother in his or her late 60s without any comorbid conditions, deciding whether or not to accept a donation from a 30-year-old son or daughter. The son or daughter may consider it appropriate to offer a kidney to the parent, acknowledging the small but real immediate risks and possible, but unknown, long-term risks. The parent may consider it inappropriate to place his or her offspring at even the slightest risk to provide a benefit to the parent that would amount to only a few years of better quality of life. In the reverse situation, it may be considered appropriate for a 20-year-old recipient to undergo transplantation but not to accept a donor offer from an elderly parent because of the increased risk of donation by an elderly individual and because of the worse outcome predicted from an older kidney. Living donor transplantation provides the opportunity to address the individual circumstances of the donor and the recipient in great detail. It is the responsibility of the transplant unit to provide each individual with an independent medical advisor to ensure that the donor and the recipient can arrive at a considered decision.

In contrast to the living donor situation, the decisions on appropriateness to receive a transplant from a deceased

Table 4–1 **Survival Advantage in the United States during the 1990s Comparing Transplant Recipients with Patients on the Waiting List but Remaining on Dialysis**

Group	Relative Risk 18 Months after Transplant	*P* Value	Projected Years of Life without Transplant	Projected Years of Life with Transplant
All recipients of cadaver transplant	0.32	< .001	10	20
Age 0-19 yr	0.33	.03	26	39
Age 40-59 yr	0.33	< .001	11	22
Age 60-74 yr	0.39	< .001	6	10
Diabetes	0.27	< .001	8	19
Glomerulonephritis	0.39	< .001	11	18

From Bennett WM: The failed renal transplant: in or out? Semin Dial 18:188, 2005.

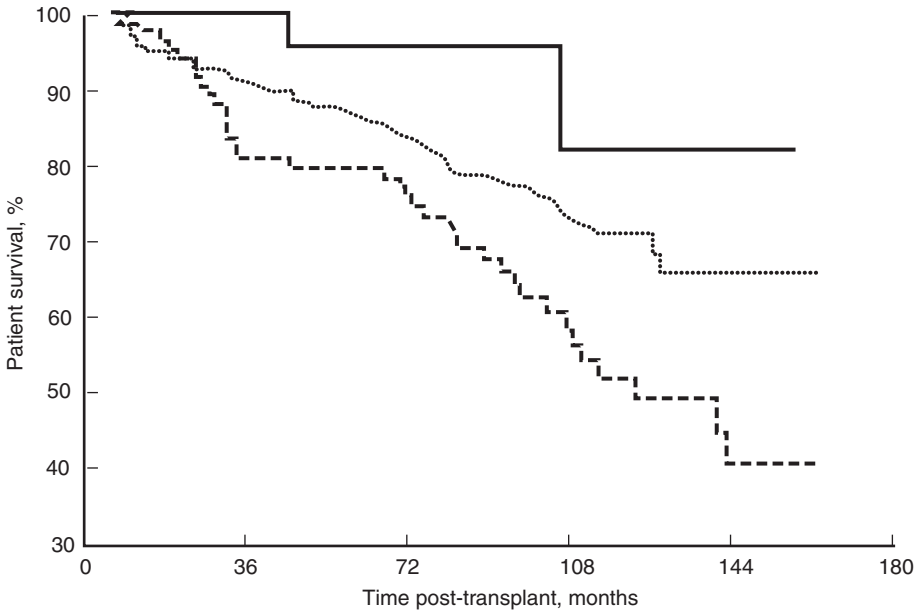

Figure 4–1 The role of preemptive transplantation in improving transplant survival. Kaplan-Meier plots of patient survival. Patients were divided into three groups according to the time they had spent on dialysis (any modality) before transplantation: no dialysis (—); 1 to 2 years (...); 3 or more years (---). Cox regression: P = .0003. (From Aalten J, Christiaans MH, de Fijter H, et al: The influence of obesity on short- and long-term graft and patient survival after renal transplantation. Transpl Int 19:901, 2006.)

donor organ have to be made largely in the absence of specific considerations and in advance of any offer. How many functioning years should the community expect from donation of a deceased donor kidney? For an elderly recipient, the decision to accept a transplant is, as described earlier, a reasonably simple equation comparing the prospects of life on dialysis and after a transplant.

For the individual, a likely benefit of 1 or 2 years of better quality life may be sufficient to sway the decision to accept a transplant. The community, as a whole, is faced with a different equation because of the undersupply of organs for transplantation in almost all countries. Should a kidney be allocated to a patient who has a life expectancy after transplantation of only 1 or 2 years, or should it be allocated only to someone with a life expectancy greater than the survival expectancy of the graft? As the mismatch between recipient and graft life expectancy increases, the community would be wasting functioning graft years and blighting the lives of individuals who could have used the organs better. If a community takes the view that only individuals with the best chance of maximizing the graft potential should be allocated a kidney, they would allocate only to young, unsensitized first-graft recipients without comorbid conditions; everyone else would be banned from receiving a deceased donor organ. All deceased donor allocation systems need to find balance between utility and fairness or equity. The elements of such systems include criteria for acceptance onto transplant waiting lists, including criteria such as age and medical fitness, and algorithms for allocation of particular organs, taking into account issues such as blood group, histocompatibility, crossmatching, waiting times, and donor and recipient ages.[55,66]

Most developed countries have well-organized computer algorithms determined by committees including medical and lay representatives, with audit of compliance. In allocation systems in which such algorithms are not applied, it is hard to see how either fairness or utility can be served and the appropriateness of organ allocation shown. The overall final impact of the multiple selection criteria can be seen in the Australian data (Fig. 4-2) in which the proportion of the dialysis population in each age cohort actually awaiting a deceased donor transplant is shown to diminish dramatically as age increases, whereas children are few in number and frequently transplanted with living donor kidneys.

COUNSELING

What the Patient Needs to Know

The transplant unit has the responsibility to provide each patient with advice based on his or her own medical conditions and education about the options for long-term treatment. The starting point for such education is comprehensive evaluation of the availability and financial cost of dialysis options. The physical and emotional well-being provided by dialysis therapy usually becomes abundantly clear to most patients through meeting other patients already on dialysis, through dialysis education programs, and finally through direct personal experience except in the few patients able to undergo preemptive transplantation.

The transplant unit needs to provide a comprehensive evaluation of each individual's medical risks if he or she were to undergo a renal transplant.[44] Much of the rest of this chapter details the medical assessment; a checklist is provided in Table 4-2. This list includes the issues that affect an individual patient's transplantability and the short-term and long-term factors that influence outcomes. In assessing the patient's suitability for a transplant operation, the physician focuses on the heart and lungs, and the surgeon focuses on the blood vessels and bladder. The surgeon needs to discuss the various complications and risks of the surgical procedure, whereas the physician discusses the drugs and long-term

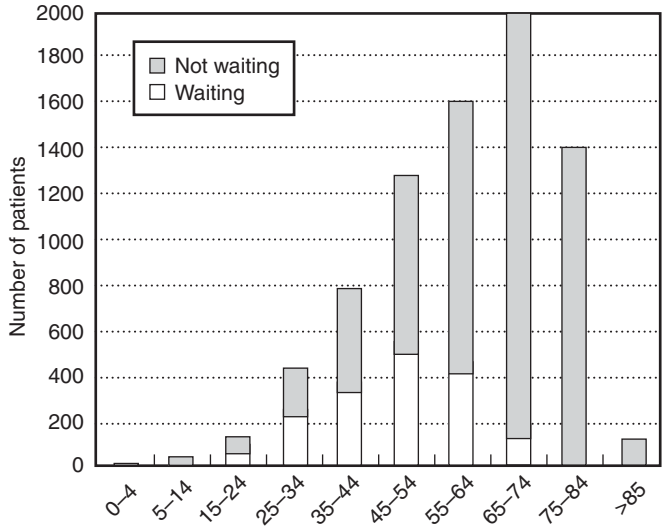

Figure 4–2 The proportion of patients on dialysis in each age group who were actively awaiting a cadaver donor transplant in Australia in 2000. (Data courtesy of ANZDATA.) Available at www.anzdata.org.au.

risks and follow-up protocols. Providing the patient with sufficient knowledge about organ allocation processes, the pros and cons of particular donor kidneys, and the financial costs that the patient will be expected to bear can be easily left out of a traditional medical consultation. Most established transplant programs have additional formal education sessions provided by a range of specialized coordinators, social workers, and pharmacists. The Internet also provides a wide range of good and bad information, which patients are sure to access extensively.[39,40] Guides to the good sources and warning against the bad sources of information also must be a part of the advice provided by the transplant program.

It is normal practice to seek written informed consent just before undergoing any surgical procedure, and all transplant operations are preceded by such a ritual signing of a legalistically phrased document. Somewhere among this scant and hastily signed documentation is the expectation that the individual has accepted the myriad risks of transplantation, ranging from transmission of serious disease from the donor to the side effects of every drug that recipient will be given. Many patients also are presented with an array of research protocols to sign up to, with patient information sheets of many pages of closely typed and densely constructed language designed to protect the researcher more than the patient. This documentation of "consent" often takes place under pressure of time and in the middle of the night, even sometimes via the telephone. It is hard to see how anything provided by the patient in the haste of the anesthetic workup, no matter what it is written on, can be argued to be informed consent. Legal opinions have been given that suggest that no reliance can be placed on a patient's decision taken under the pressure of an immediate pretransplant consent, unless backed by extensive prior education and information. In constructing education programs, it would be wise for the transplant unit to consider the traditional "operation consent form" a legally valueless document.

Table 4–2 **Checklist for Pretransplant Education of the Patient and Family**

1. Medical condition
   - General cardiorespiratory fitness for operation
   - Impact of obesity
   - Vascular system suitability for operation
   - Urological complications
   - Risks of recurrent renal disease
2. Fitness for lifelong immunosuppression
   - Infections
   - Malignancies, especially skin cancers
   - Cardiovascular risk factors
3. Histocompatibility and organ donor source impact on outcomes
4. Waiting times and allocations systems on the deceased organ donor waiting list
5. Availability and donor outcomes of living donor procedures
6. Financial costs and specific risks of the donor and transplant procedures, including disease transmission from the donor
7. Financial and adverse-event costs of prophylactic immunosuppressive and anti-infective drugs
8. Long-term follow-up protocol
9. Short-term and long-term risks of graft failure and death after transplantation
10. Consideration of acceptance of extended criteria donor organs
11. Patient-specific issues
    - Options for pancreas transplantation in diabetics
    - Options for liver-kidney transplantation in primary oxalosis

What the Potential Living Donor Needs to Know (see also Chapter 7)

A potential living donor usually needs to be provided information on the recipient outcomes and the donor operation with its attendant risks to decide on whether or not to proceed.

A donor who expects only a successful outcome of the donation for the recipient has a reasonable chance of being badly disappointed. It is essential that the best estimates of the risks of death and graft failure are clearly laid before the donor.[46,47] It also is important for most donors to understand the dialysis alternatives available to the patient and the deceased donor waiting lists times. In countries with substantial waiting list and long waiting times, living donation offers huge advantages that are not so clearly apparent in countries where waiting times are short. In countries with high deceased donor rates, the advantages of providing a better kidney with better long-term survival may be less obvious.

A living donor must provide fully informed consent to a surgeon with no conflicts of interest through his or her care of the potential recipient (see Chapters 7 and 38).[24] In addition, it is relevant for the donor to understand the blood group and histocompatibility match with the recipient and any concerns that there might be about the crossmatching data (see Chapter 10). A general overview of the risks that the recipient faces would help to ensure that the few procedures that end in disaster are not followed by endless recrimination and litigation. More importantly, a well-prepared donor is better able to face the future after a failed transplant or even death of the recipient.

What the Family Needs to Know

The families of pediatric patients are best regarded as if they were the patient with respect to the information and counseling that they require, although there are special considerations that young age brings to bear on the decision making in renal transplantation (see Chapter 35). The family of an adult patient is in a special situation compared with other areas of medicine because the family members represent a potential source of organ donation and cannot simply be thought of as interested onlookers and supporters for the patient. Transplant units vary in the way in which information is provided about family members' potential to donate a kidney; some units distribute information packs directly to all known family members, whereas others await specific approaches before providing information on living related donation. In countries with low deceased organ donation rates, the increasing attention being placed on living donation creates the atmosphere for routine dissemination of information to family members and friends.[86] Accurate provision of specific relevant information depends on the consent of the recipient to release private medical details. Asking the question, "Is there anyone in the family who would donate you a kidney?" elicits interesting insights into the dynamics of families with members with serious chronic illness. Some patients refuse to consider a discussion of their illness with their families, whereas others are glad that an independent individual is prepared to raise awareness in their family of the seriousness of their illness.

Lack of information is almost always the starting point for a breakdown in trust and communication among patients, their families, and their medical attendants. For this reason, it is important that even the most distant of families are aware of the possibility of a poor outcome from transplantation and the importance of compliance with medication and follow-up to the long-term success of the transplant.

SPECIFIC MEDICAL CONSIDERATIONS

Cardiac

The first consideration of any patient undergoing a 3- or 4-hour operation is the state of the patient's cardiac function. Dialysis patients and especially diabetic dialysis patients have high incidences of symptomatic and asymptomatic ischemic heart disease, and a careful evaluation of the heart is essential.[56] Evidence-based agreement on how to perform that assessment is lacking, so the assessment is highly dependent on local expertise and opinion.

All patients require a careful clinical history and examination, including an electrocardiogram and usually an echocardiogram to assess left ventricular function and a stressed myocardial perfusion study to exclude significant ischemic heart disease. Although CKD itself is the strongest risk factor for coronary artery disease, it also is important to assess obesity, family history, lipid profile, blood pressure, smoking history, and diabetes.[44] Attitudes toward smoking history vary among transplant units from refusal to transplant patients who continue to smoke to more liberal approaches.[18]

Some transplant programs require routine coronary angiography before acceptance onto a waiting list. A rationale exists for such an approach given the high levels of coronary disease uncovered by such a strategy.[27] The only randomized trial of surgical or medical intervention in this situation (diabetics with CKD) was so unequivocal about the value of intervention that the trial was halted, and the nonintervention arm was offered surgery or angioplasty.[60] The weaknesses of this study (it assessed only diabetics, and optimal medical therapy would not have been considered optimal more than 10 years on) and the lack of alternative randomized studies leave the field with uncertainties, but a clear view that diabetic patients need comprehensive cardiac evaluation.

An alternative strategy is to use a noninvasive test, such as a stress dopamine echocardiogram[91] or stress nuclear study,[16] as a screening method for asymptomatic and low-risk patients, reserving coronary angiography for patients with symptoms, significant risk factors, or a positive stress test. This strategy is not foolproof and relies on the negative predictive value of the screening test so that the occasional patient with ischemic heart disease would still be transplanted unknowingly and without the consideration of prior treatment of the cardiac disease.

Proceeding to transplantation in patients with normal left ventricular function and normal coronary vasculature is an easy decision. More complex issues surround deciding who to transplant despite their cardiac disease, which needs to be considered not only on its own merits but also because of the implications that it carries for widespread vascular disease.[44] There is no evidence-based answer to this question, and clinicians must rely on local opinion-based decisions, guided by some general principles, as follows.

Treatable coronary and valvular disease is almost always worth treating before transplantation rather than afterward because of the risks posed by the cardiac disease during the transplant procedure, and because of the risks that cardiac interventional procedures carry in the presence of immunosuppression and a functioning transplant.[59]

It is usually wiser to avoid transplantation if, despite treatment of coronary artery disease or valvular disease, or both, there remains a substantial risk of infarction of a large area of myocardium, or there is substantial left ventricular dysfunction. Cardiac disease is the largest cause of mortality in the dialysis and transplant populations, and there is little evidence that transplantation would beneficially alter the outcome of ischemic heart disease.[29] Less certainty exists with respect to congestive cardiac failure, where poorly dialyzed patients may recover significant function when uremia and chronic fluid overload are corrected by transplantation.[8] In patients with severe and irreversible cardiac dysfunction, the remaining consideration is the option of combined heart and kidney transplantation, available to limited numbers of young and otherwise healthy individuals treated in highly specialized centers.[36]

Vascular

An available artery for anastomosis of the transplant renal artery is absolutely required (see also Chapters 11 and 26). Atheromatous iliac arteries that have been ossified through years of CKD management must be assessed carefully by the surgeon planning to perform the transplant. Absence of intermittent claudication and presence of palpable femoral and pedal pulses may be sufficient to confirm transplantability. There are, however, many potential recipients with a high risk of severe vascular disease, where duplex ultrasound scanning of the femoral and carotid vessels would identify those who may have peripheral or cerebrovascular events either during or after transplantation.[73]

Selection of patients with known preexisting peripheral vascular disease must include a general assessment of their prognosis and specific assessment of the vascular supply needed for the transplant operation. The largest numbers of patients starting dialysis in most developed countries are elderly, obese, type 2 diabetics, and many have severe peripheral vascular disease.[95] Only a few such patients prove to be suitable for transplantation because of the combined effects of obesity and cardiac and vascular disease on their operative mortality and 3- to 5-year survival rates.[3] Two thirds of dialysis patients requiring lower limb amputations are dead within 2 years, implying that this group of patients has such a poor prognosis that they should not be accepted for transplantation.[26]

Symptomatic cerebrovascular disease presents a separate problem in selection for transplantation. A history of transient ischemic attacks should have promoted a search for a cardiac or carotid vascular cause, which if diagnosed and resolved or treated should not contraindicate subsequent transplantation.[21] The complication that warfarin anticoagulation of patients with atrial fibrillation provides the transplant unit usually can be overcome with a rapid anticoagulant reversal protocol and use of heparin in the post-transplant period before reinstituting warfarin anticoagulation. Warfarin therapy is not an absolute contraindication to acceptance for a deceased donor transplant. Completed stroke and severe carotid disease often place the patient in the same category, however, as patients with severe cardiac or peripheral vascular disease with respect to their general prognosis and the futility of transplantation. One group of patients that needs particular attention are those with adult polycystic kidney disease, especially if they have a personal or family history of cerebral aneurysm.[42] Evaluation of such high-risk patients requires cerebrovascular imaging, such as cerebral computed tomography (CT) angiography, to exclude berry aneurysms and specific neurosurgical advice, before considering transplantation.

Respiratory

Assessment of respiratory disease in the potential transplant candidate has two purposes: (1) to identify patients at risk from the anesthetic and (2) to identify patients who would be at risk of life-threatening infection in the long-term as the result of immunosuppression. The former is based around assessment of smoking and chronic obstructive airways disease and is no different from the assessment that must be made before any elective operation.[43] The latter is a more complex decision and remains largely subjective. The diseases of importance are bronchiectasis, tuberculosis, and prior fungal infections, all of which may become uncontrollable under the influence of immunosuppression. Formal evaluation of the degree of respiratory compromise and the frequency and severity of infective exacerbations determines the advisability of transplantation of a patient with bronchiectasis.

Active pulmonary tuberculosis must be identified from routine chest films and treated before consideration of transplantation.[4] Patients at high risk of reactivation of tuberculosis after transplantation include those from areas with high endemic rates.[90] History of exposure, calcified lesions seen on chest films or elsewhere, and a positive skin test to purified protein derivative all provide evidence of past exposure and risk of disease, but a negative purified protein derivative test cannot be relied on to exclude disease in anergic dialysis patients. Bacille Calmette-Guérin vaccination is not safe in transplant recipients[96]; in endemic areas, transplant units tend to advise high-risk patients to take a full treatment course for tuberculosis after transplantation, whereas in developed countries the practice, based on slender evidence,[4] is usually to add a prophylactic course of isoniazid for 6 months.[83]

Hepatic Disease

Hepatitis B

Most dialysis patients with past or current hepatitis B are identified through routine testing of serum for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core and surface antigens (see also Chapter 30). Many dialysis programs have a routine hepatitis vaccination policy to improve protection from cross-infection, even though vaccination is much more effective if administered before the need for dialysis.[45] Most patients being assessed for transplantation have been screened for prior exposure to hepatitis B.

Data from transplantation of chronically infected HBsAg-positive patients, predominantly gained in the 1980s and 1990s, show worse outcomes than for HBsAg-negative patients.[30] Knowledge of the status of the liver histology is important in predicting outcomes after renal transplantation, with poor medium-term to long-term results with preexisting chronic active hepatitis and with cirrhosis.[32] It is unclear if the poor outcomes would still be reflected in data from transplantation performed in the past few years, with

use of post-transplant lamivudine therapy.[79] Choice of immunosuppression after transplantation may influence the progression of hepatitis, with concern expressed about steroids, azathioprine, and cyclosporine reactivating hepatitis B in a chronic carrier.[20] Hepatitis B is not a contraindication to renal transplantation, but established cirrhosis raises the option of combined liver-kidney transplantation.

Hepatitis C

Hepatitis C represents a different challenge to transplant programs in different countries, with very high prevalence in some dialysis programs and in patients who were dialyzed and transfused in the 1980s (see also Chapter 30). A high proportion of patients with hepatitis C infection eventually develop significant liver disease.[53] Treatment of patients with hepatitis C infection is made complex, if not impossible, by renal failure and requirement for dialysis because peginterferon alfa-2b and ribavirin are poorly cleared in, and thus not tolerated by, dialysis patients.[23] Hepatitis C genotypes 2 and 3 are more responsive to therapy than genotype 1, and it is warranted to attempt to treat patients before the onset of dialysis, although the genotype may not affect post-transplant outcomes.[74] Assessment of patients for the transplant waiting list should include hepatitis C antibody routinely, and, if that test is positive, hepatitis C RNA testing and assessment of viral load and genotyping. Most units rely on liver histology to assess the severity of hepatitis in potential transplant recipients, with advanced disease providing a contraindication to renal transplantation.[32] Patients without significant liver disease survive better if transplanted than if they remain on dialysis[81] but do not fare as well as patients without hepatitis C. The shortage of donor organs raises the question of whether patients with hepatitis C, especially hepatitis C RNA positive with genotype 1, should accept a kidney from a donor who is positive for hepatitis C antibodies, and which would otherwise be discarded. There is an argument for such a strategy in this limited group of recipients because they already are currently infected with hepatitis C, and the potential additional risk of transmission of further virus is small. There is no suggestion that a patient who has never been infected or who has cleared virus (i.e., is hepatitis C antibody positive, but RNA negative) should risk reinfection from a hepatitis C antibody–positive donor because the infection rates are high.[82]

Other Liver Disease

Potential renal transplant recipients may have other types of significant liver disease, such as alcoholic liver disease, polycystic liver in association with polycystic kidney disease, or cholelithiasis. It is important and simple to assess liver function and appearance of the liver on ultrasound. Fatty infiltration of the liver is the most common finding of such screening protocols and may be associated with diabetes, but is not in itself a contraindication to transplantation. Severe liver disease, no matter what the cause, inhibits acceptance for renal transplantation of most patients. Opinions on the role of prophylactic cholecystectomy in dialysis patients with known gallstones are diverse, but larger studies do not support this approach.[35]

Infectious Disease

See Chapter 29.

Vaccination Strategies

General community protection from infectious disease in most countries results in routine childhood vaccination against measles, mumps, polio, rubella, diphtheria, tetanus, pertussis, *Haemophilus influenzae* b, and varicella zoster; it is hoped that papillomavirus will soon be added to this list. Pneumococcal and hepatitis B vaccination programs are becoming more widespread but are far from universally applied. It is especially important in pediatric practice to ensure that vaccination has not been forgotten among the problems of pediatric renal failure.[28] In adult practice, it also is important to understand each patient's vaccination history and to remedy deficiencies as soon as possible because the responses to vaccines are generally impaired in the dialysis population.[52]

Vaccination of patients after transplantation is dangerous with live vaccines or may fail with killed antigen vaccines because the medication used to prevent allograft rejection is well designed to suppress production of an antibody response to a viral antigen. Mycophenolate mofetil is especially capable of preventing antibody production after vaccination.[92] Live vaccines are contraindicated after transplantation, with the most common error being the use of chickenpox vaccination with attenuated virus leading to life-threatening disseminated virus infection in transplant recipients.

Human Immunodeficiency Virus

Transplantation of patients with human immunodeficiency virus (HIV) was contraindicated until the recent era of antiretroviral therapy. The dire consequences of immunosuppression in a patient who was infected with HIV were discovered during the 1980s in patients infected before transplantation or when the virus was unwittingly transmitted through organ donation.[88] In the past few years, a few centers have developed expertise in managing HIV-positive patients after transplantation and have acceptable results.[80] It is universal practice to test recipients and donors for antibodies to HIV, with the decision to transplant the positive recipient depending on the concomitant availability of highly active antiretroviral therapy and local expertise in the transplant center.

Other Viral Infections

Knowledge of a recipient's status with respect to all herpes viruses has become increasingly relevant with developing understanding of the impact of these viruses after transplantation. Prophylaxis for cytomegalovirus, which also protects recipients for human herpesvirus 6 and human herpesvirus 7, is usually based on knowledge of the donor and recipient cytomegalovirus serological status (see Chapter 29). Transplantation of an Epstein-Barr virus–positive organ into an Epstein-Barr virus–negative recipient carries an increased risk of active Epstein-Barr virus infection after transplantation and of development of post-transplant lymphoproliferative disease. There is some evidence that anticytomegalovirus viral prophylaxis may reduce this risk.[85] All patients should be tested for antibody status with respect to each of the herpes viruses.

Dental Infections

The traditional approach to evaluation of the transplant recipient includes ensuring adequate dental hygiene and

review of dentition before acceptance for transplantation. Gingival hypertrophy is a consequence of higher doses of cyclosporine, especially when combined with nifedipine, and infected dentition may cause problems after transplantation, but it would be an unusual candidate in whom the risk of transplantation outweighed the risk of continued dialysis therapy on the basis of the patient's dentition.

Miscellaneous Infections

Transplant programs must pay heed to the particular infectious risks that are endemic and prevalent in their geographical region to evaluate properly the post-transplant risks for their transplant recipients (see also Chapters 29 and 36). *Trypanosoma cruzi*, the causative organism of Chagas' disease, is prevalent in South and Central America. It may be transmitted by donation and reactivated by immunosuppression, requiring serology and blood polymerase chain reaction surveillance and early treatment.[58]

Syphilis, strongyloidiasis, and toxoplasmosis all have been reported as opportunistic reactivations after transplantation. In most areas of the world, transplant programs require a heightened awareness and lower threshold for suspicion of these diseases, rather than specific strategies for these uncommon problems. Testing for syphilis serology is still practiced by many programs, but is not seen as essential in recipients from most developed countries.

Malignancy

It is well known that there is an increased risk of malignancy after transplantation, which is assumed to be due to an effect of immunosuppression on normal mechanisms for control of neoplastic cells or the impact on viral oncogenesis (see also Chapters 32 and 33). This knowledge has been translated to a reluctance in transplant patients who have had a prior cancer, for fear that immunosuppression would allow recurrence that might otherwise not happen. More recent data have questioned this set of assumptions in two ways. First, the increased risk of some cancers also is seen in CKD patients on dialysis and after transplantation, and a few cancers also are increased in the 5 years preceding the onset of dialysis. Second, the major increase in risk is restricted to many types of cancer, such as skin and lip cancers, renal tract cancers, and cancers for which a viral cause is either established or suspected.[97] The implication for the potential transplant recipient is that cancers that are now understood to occur at the same or a modestly increased rate as in the normal population probably should be considered differently from cancers where the risk is increased substantially.

It has been standard advice not to transplant a patient within 2 to 5 years of diagnosis and definitive treatment of cancer, depending on which cancer is under consideration. It also is advised to consider careful screening for some cancers in patients on the transplant waiting list.[44] Such blanket rules, although easy to apply, do not take into consideration the variability of the biology of the different cancers and especially do not consider the individual risks of recurrence. Table 4-3 provides a list of cancer types that are known to be increased in dialysis and transplant patients and should be viewed with considerable caution in patients being assessed for transplantation. Melanoma is known to respond to T cell immunotherapy and has a substantially increased risk after transplantation. It is known to recur in normal individuals and to metastasize aggressively. Melanoma also has been observed to recur after transplantation with long disease-free intervals before transplantation and must be approached very conservatively. Breast cancer and prostate cancer are not increased in dialysis and transplant patients, but they have substantial metastatic potential. To avoid transplanting a patient who would die as a result of metastatic cancer soon after transplantation, it is prudent to advise a waiting period of at least 2 years, depending to a certain extent on the predicted risk of spread in any given individual.

Common cancers also occur commonly in dialysis and transplant patients. It is important not to shift the clinical emphasis from common cancers to rare cancers, such as Kaposi's sarcoma, just because these rare cancers occur with a greatly increased risk compared with the general population. Common cancers, in the Australian population, each occurring in more than 60 patients in a series of nearly 900 cancers occurring in almost 25,000 dialysis patients, were kidney, bladder, colon, lung, melanoma, breast, and prostate (see Table 4-3). At present, there are no specific guidelines for cancer screening in dialysis and transplant patients, so it would be reasonable to implement guidelines recommended in the general population—such as for cervical, breast, and bowel screening—in patients on the transplant waiting list.

Psychiatric Disease and Drug Dependency

Compliance after transplantation and the patient's responses to the psychological stresses of transplantation should be uppermost in the minds of clinical teams evaluating recipients. Noncompliance with medication and clinical follow-up are among the most distressing and devastating causes of loss of grafts. Prevention of this problem starts with understanding the patient before transplantation and responding to the different risks for noncompliance.[15] Most noncompliant patients do not have a psychiatric disorder, but many with a psychiatric disorder are at risk of poor compliance.

There are two dominant reasons for careful evaluation of the psychiatric state of the potential recipient: the individual's ability to understand and consent to the transplant procedure and the impact of psychiatric disease after transplantation. Formal psychological testing and psychiatric assessment may be required to evaluate an individual's capacity to provide properly informed consent (see also Chapter 38). Alcohol and drug abuse raise many practical, medical, ethical, and moral questions, which also have to be evaluated carefully in each individual. Abstinence from chemical dependency would be regarded as essential for acceptance to the transplant waiting list by most transplant programs, but it is difficult to ensure and monitor in practice.

Bone

Renal bone disease status and the degree of control of the calcium-phosphate product are important indicators of bone disease and vascular risk after transplantation. In children, the additional consideration of growth potential and the impact of uremia on the one hand and corticosteroids on the other are relevant considerations (see Chapter 35). Recent years have seen an explosion in available therapies for renal bone disease, and the exact status of hyperparathyroidism at the time of transplantation is less critical than it was in the past.[78] It is still relevant to optimize control of the features of

Table 4–3 **Suggested Disease-Free Time Intervals before Transplantation of Patients with Prior Cancers, Noting Increased Risk of Different Cancers before and during Dialysis and after Transplantation and Cancers That Are Most Commonly Seen**

Site (ICD 10 Codes)	Prediagnosis*	Diagnosis†	Transplantation‡	Duration before Considering Transplantation (Comments)§
Chronic Kidney Disease–Associated Cancer				
Kidney (C64)	↑	↑ C	↑	>2 yr
Renal pelvis (C65)	↑	↑	↑	>2 yr
Ureter (C66)	↑	↑	↑	>2 yr
Bladder (C67)	↑	↑ C	↑	>2 yr
Other urinary organs (C68)	↑	↑	↑	>2 yr
Multiple myeloma and malignant plasma cell neoplasms (C90)	↑	↑	↑	? >2 yr (bone marrow transplantation?)
Cancer Not Associated with Chronic Kidney Disease				
All cancers (C00-C96 excluding C44, C64-C68, C90)	↑	↑	↑	
Nonmelanoma skin	↑	↑ C	↑	(Local treatment)
Lip (C00)	↑	↑	↑	(Local treatment)
Tongue (C01-C02)		↑	↑	>2 yr
Mouth (C03-C06)			↑	>2 yr
Salivary gland (C07-C08)			↑	>2 yr
Esophagus (C15)			↑	>2 yr
Stomach (C16)			↑	>2 yr
Small intestine (C17)				>2 yr
Colon (C18)		C	↑	>2 yr
Rectum (C19-C20)				>2 yr
Anus (C21)			↑	>2 yr
Liver (C22)			↑	(Contraindicated without liver transplantation)
Gallbladder (C23-C24)			↑	>2 yr
Pancreas (C25)				>2 yr
Larynx (C32)				>2 yr
Trachea; bronchus and lung (C33-C34)		↑ C	↑	>2 yr
Melanoma (C43)		C	↑	>5 yr—assess risk of metastasis
Mesothelioma (C45)				>2 yr
Kaposi's sarcoma (C46)	↑	↑	↑	>2 yr—use TOR inhibitor immunosuppression
Connective and other soft tissue (C47-C49)			↑	>2 yr
Breast (C50)		C		>5 yr
Vulva (C51)			↑	>2 yr
Cervix uteri (C53)		↑	↑	>2 yr
Corpus uteri (C54)				>2 yr
Ovary (C56)				>2 yr
Penis (C60)			↑	>2 yr
Prostate (C61)		C		>2 yr
Testis (C62)				>2 yr
Eye (C69)			↑	>2 yr
Brain (C71)				>2 yr
Thyroid (C73)	↑	↑	↑	>2 yr
Hodgkin's disease (C81)			↑	>5 yr
Non-Hodgkin's lymphoma (C82-C85)			↑	>5 yr
Leukemia (C91-C95)			↑	>5 yr

*5-year period before start of dialysis.

†While on long-term dialysis therapy.

‡After transplantation.

§The period after apparent successful cure of the individual cancer when transplantation may be considered if investigations substantiate cure of the cancer. Note also comments for individual cancers. Recurrence of cancer has been recorded despite disease-free periods exceeding those suggested here. Each individual patient must be assessed individually, and these intervals may be too long or too short for individual circumstances.

↑, increased incidence compared with the age-matched and sex-matched general population. Cells left blank do not have a known increased risk.

C, common cancer in dialysis recipients.

Data from Vajdic CM, McDonald SP, McCredie MR, et al: Cancer incidence before and after kidney transplantation. JAMA 296:2823, 2006.

renal bone disease, with special attention to attempting to normalize the calcium-phosphate product to minimize osteoporosis and vascular calcification after transplantation.[73]

Gastrointestinal Tract

Perforation of a peptic ulcer has led to many transplant recipient deaths in the era of high corticosteroid use and before the routine introduction of H_2 receptor blockers after transplantation. The incidence of untreated *Helicobacter pylori*/peptic ulcer disease is now quite low, and many units use low-dose or no steroids combined with omeprazole or a similar proton-pump inhibitor to prevent peptic ulceration. Gastroesophageal reflux, malabsorption syndromes, celiac disease, diverticulosis, and cholelithiasis all may present issues for specific consideration in individual patients. It is difficult to justify routine screening for peptic ulcer disease or cholelithiasis, but there are proponents for both strategies.

Diabetes

Recipients with diabetes require special consideration, with different issues in patients with type 1 diabetes and type 2 diabetes. Transplantation rates of diabetic patients have fluctuated widely driven by the observed mortality rates, development and availability of simultaneous pancreas-kidney transplantation, and comorbid conditions experienced by many patients with type 2 diabetes.[13]

Type 1 Diabetes Mellitus

The primary decision for patients with type 1 diabetes is whether or not to seek a simultaneous kidney and pancreas transplant (see also Chapter 34). In countries where this expertise is available, the two options that provide the best patient survival are preemptive living related renal transplantation and simultaneous pancreas-kidney transplantation. Acceptance criteria for simultaneous pancreas-kidney transplantation usually include a stricter age cutoff than for kidney transplants and routine invasive cardiac investigation, ensuring that it is a realistic therapy for approximately half of the potential type 1 recipients with end-stage renal failure.[13] Selection of patients for simultaneous pancreas-kidney transplants is focused more on vascular and cardiac operative risks but is otherwise similar to selection for kidney transplantation. The procedure is more demanding on the surgeon and the patient; it takes longer; and it involves the additional risk of pancreas exocrine drainage into either the bladder or, more commonly, the bowel. Postoperative recovery takes longer because of the ileus induced by the bowel surgery, and immunosuppression is on the whole more intense than for a simple kidney transplant. Against these issues, the patient must set the benefits of good glucose control without exogenous insulin administration, reduced long-term complications of diabetes, and improved survival.[37] Detailed consideration of simultaneous pancreas-kidney transplantation is beyond the scope of this chapter, but it is a good solution for a proportion of patients with type 1 diabetes. The evolving role of islet transplantation is still such that consideration of islet transplantation before, after, or with a simultaneous kidney transplantation is subject to formal clinical trial conditions in only a few centers globally.

Type 2 Diabetes Mellitus

Transplantation of most patients with end-stage renal failure secondary to type 2 diabetes represents a challenge to surgical and medical expertise because the epidemic of type 2 diabetes that is sweeping the developed and developing world involves predominantly obese older patients with significant comorbid vascular disease. The disease is treacherous because the neuropathy that so often accompanies the nephropathy leads to underestimation of the severity of symptoms, especially ischemic heart disease, and to exacerbation of the clinical impact of comorbid peripheral vascular disease. Only a small proportion of type 2 diabetics are suitable for transplantation because of the impact of age, obesity, and these comorbid conditions. Routine evaluation of the diabetic potential transplant recipient usually exposes the issues that affect postoperative mortality and the medium-term to long-term success of renal transplantation; however, many units have specific policies in place for evaluation of potentially asymptomatic cardiac and vascular disease in these patients.

Renal Disease

The underlying renal diseases of patients on dialysis and patients accepted for transplant waiting lists are similar because few diseases prevent successful renal transplantation. The physical size of the kidneys in patients with adult polycystic kidney disease may prevent the operation. The threat of oxalate deposition in primary oxalosis and the presence of anti–glomerular basement membrane antibodies in Goodpasture's syndrome are sufficient to ensure immediate graft failure, but there are few other renal diagnoses that provide absolute contraindications to transplantation. The causes of renal failure in patients beginning dialysis and patients receiving a renal transplant are listed in Table 4-4. These data show the skewed distribution of proportions of each type of disease in the transplant population, especially noting the underrepresentation of type 2 diabetes.

Recurrent Renal Disease

See also Chapters 24 and 25.

GLOMERULONEPHRITIS

Recurrence of glomerulonephritis in the renal transplant is an issue that requires routine discussion with patients who

Table 4–4 **Causes of Renal Failure in Patients Starting Dialysis Therapy and Receiving a Renal Transplant in Australia**

Diagnosis	Dialysis Patients (%)	Transplant Recipients (%)
Glomerulonephritis	25	51
Analgesic nephropathy	2	3
Polycystic kidney	7	10
Reflux nephropathy	3	13
Hypertensive nephropathy	13	2
Diabetes mellitus	30	6.5
Miscellaneous	13	11
Unknown	7	3.5

Data courtesy of ANZDATA.

have a diagnosis of focal and segmental glomerular sclerosis, IgA nephropathy, and, to a lesser extent, other immune-mediated glomerular diseases. It is important to distinguish between the risk of recurrence and the risk of graft failure owing to recurrence, and although the risk is real, it is seldom sufficient to contraindicate transplantation. An analysis of the ANZDATA database showed, however, that recurrent disease is a significant cause of late graft loss, causing twice as many losses over a 10-year period as acute rejection, but half as many as episodes of chronic allograft nephropathy or death with a functioning graft.[9] There have been many attempts over the years to summarize the risks for different diseases,[9,44,49,64,84] and a further attempt is shown in Table 4-5, in which the risks of disease recurrence and graft failure are presented from general literature review.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Recurrence of primary focal segmental glomerulosclerosis is a difficult issue that must be addressed by transplant units. Risk factors for recurrence include young age of the recipient, duration of native disease from onset to development of end-stage renal failure, mesangial proliferative pathology, and the possibility that the risk is higher in related donor grafts.[67,93] There is a very high risk of recurrence in a second graft after loss of the first graft from focal segmental glomerulosclerosis, questioning the wisdom of retransplantation under those circumstances. The disease behaves as if there is a circulating plasma factor that causes the disease—from data identifying a putative factor and from partial response to intervention using plasma exchange.[34,62,93]

IgA NEPHROPATHY

IgA glomerulonephritis is a common disease in most countries, accounting for a relatively high proportion of end-stage renal failure. Recurrence rates are high, especially if sought using specific identification of IgA deposits in the glomeruli.[6] IgA nephropathy is one of the most common recurrent diseases, but it is generally slow to cause renal impairment and graft loss.[67] It is more common after living related donor grafts, but recurrence does not seem to have an impact on early and medium-term graft survival and should not restrain use of living donors.[10] Assessment of the family donor needs to include consideration of the possibility that IgA nephropathy may be a familial disease, however, and may affect the potential donor.

HENOCH-SCHÖNLEIN PURPURA

Henoch-Schönlein purpura is a predominantly pediatric disease with a high recurrence rate and graft loss in 10% or more.[64,71] It is uncertain whether there is increased risk in living related donor grafts, and it is common practice not to transplant during active clinical disease.

MEMBRANOUS NEPHROPATHY

Membranous glomerulonephritis may occur as either primary or recurrent disease after transplantation. It causes progressive renal impairment. Because it is untreatable, membranous glomerulonephritis leads to a significant chance of graft loss when recurrence is identified.[19,67]

MESANGIOCAPILLARY GLOMERULONEPHRITIS

Type I, type II, and type III mesangiocapillary glomerulonephritides are uncommon diseases with quite high recurrence rates after transplantation.[2,50] Type II mesangiocapillary glomerulonephritis has the highest risk of graft failure.

ANTI–GLOMERULAR BASEMENT MEMBRANE DISEASE

There is little recent experience of recurrence of Goodpasture's syndrome after transplantation because of the early and

Table 4–5 **Risks of Recurrence of Renal Disease after Transplantation and Risks of Graft Loss as a Result of Recurrence, Derived from Literature Review**

Disease	Risk of Recurrence (%)	10-Yr Risk of Graft Loss from Recurrence (%)
Glomerulonephritis		
Focal segmental sclerosis	20-30	8-15
IgA nephropathy	40-50	5-15
Henoch-Schönlein purpura	10-20	5-10
Mesangiocapillary type I	20-30	10-15
Mesangiocapillary type II	80-90	5-10
Membranous	10-20	10-25
Hemolytic-uremic syndrome	10-30	10-15
ANCA-positive vasculitis	10-15	5
Pauci-immune	10-20	5-10
Goodpasture's syndrome (antibody-positive)	100	80
Systemic lupus erythematosus	1	1
Metabolic and Other Diseases		
Diabetic nephropathy	100	Low
Amyloidosis	30	Low
Oxalosis	90-100	80
Cystinosis	0	0
Fabry's disease	100	0
Alport's syndrome*	3-4	2
Light chain nephropathy	10-25	10-30
Mixed essential cryoglobulinemia	50	40
Scleroderma	20	5-10

*The risk of de novo anti–glomerular basement membrane antibody–mediated Goodpasture's syndrome.

ANCA, antineutrophil cytoplasmic antibody.

convincing reports of recurrence in the presence of circulating antibody and advice to await clearance before transplantation.[12,99] It is standard clinical practice to ensure a negative anti–glomerular basement membrane antibody test before transplantation. Patients with Alport's syndrome have abnormal basement membrane antigens and may produce an immune response to the normal basement membrane of a transplanted kidney, resulting in the unusual appearance of allogeneic Goodpasture's syndrome in a small percentage of patients.[33,77]

RECURRENT VASCULITIS

Antineutrophil cytoplasmic antibodies have been discounted as a possible cause of recurrent crescentic glomerulonephritis in a pooled series of 127 patients in which recurrence was 17%, but in which there was no association with presence of antineutrophil cytoplasmic antibodies. Recurrence has been treated with cyclophosphamide, and some authors support the use of co-trimoxazole as a prophylactic agent.[75]

HEREDITARY DISEASE

Primary oxalosis has a high recurrence rate after transplantation and is now usually successfully treated by combined kidney-liver transplantation, correcting the metabolic abnormality simultaneously. The condition has been mimicked by self-administration of long-term high-dose vitamin C in a dialysis-dependent patient, leading to widespread secondary deposition of oxalate throughout the body giving the appearance of pseudogout.

Fabry's disease and cystinosis are inherited enzyme deficiencies that cause renal disease through accumulated glycosphingolipid and cystine. The former leads to recurrent disease in the transplant, but the latter only to extrarenal deposition of cystine. Both are, to a certain extent, treatable, and recurrent disease should be preventable with recombinant α-galactosidase A enzyme replacement and oral analogues of cysteamine.[54,61]

Tuberous sclerosis, although it does not lead to recurrent disease, warrants special consideration because of the high lifetime risk of developing renal cell carcinoma in the native kidneys. The risk of tumor can be managed by bilateral nephrectomy or through regular screening by CT.

Urogenital Tract Abnormalities

Bladder

Recognition of a patient with bladder dysfunction is important and sometimes obvious and sometimes subtle. Patients with the triad syndrome or other congenital obstructive uropathy, spina bifida, and diabetes have an easily recognizable risk of poor bladder function. This risk usually can be recognized on careful history taking and can be investigated appropriately with urodynamic studies. More subtle problems that may be encountered include asymptomatic prostatic enlargement in an anuric dialysis-dependent patient and the very small capacity bladder encountered in patients who have been anuric for many years (see Chapters 11 and 12). There is less tendency to provide patients with alternative bladder conduits now than there was 10 years ago because of the morbidity of the surgical procedures required and the long-term risks of carcinoma if a bladder reconstruction has been achieved using bowel. Self-catheterization has become an easier and safer option than major abdominal surgery for most patients with bladder dysfunction.

Reflux Nephropathy

Recurrent urinary tract infection and reflux nephropathy seldom lead to life-threatening septicemia before transplantation, but when the experience of an individual indicates otherwise, bilateral nephrectomy can be justified if antibiotic prophylaxis fails to ameliorate the risk. Recurrent urinary sepsis is much more common after transplantation despite prophylactic measures and may threaten the graft and the patient. Bilateral native nephrectomy becomes the lesser risk in a few patients after transplantation.

Polycystic Kidney Disease

The size of polycystic kidneys must be evaluated before transplant surgery, preferably by the surgeon who will be implanting the new kidney into the space that may be occupied by the native kidneys. CT can provide an excellent view of the anatomical challenge that will face the surgeon when the patient is horizontal on the operating table, but it underplays the space available for the transplant when the patient stands up. Unilateral nephrectomy may be needed between the onset of dialysis therapy and a renal transplant, precluding preemptive transplantation.

Coagulation Disorders

Hemorrhage during the transplant and coagulation of the graft or other vital vascular conduit after the operation require careful prediction and management (see also Chapter 26). Coagulation disorders and the risk of thrombosis are much more predictable today through screening tests (Table 4-6). Use of heparin starting soon after transplantation in patients identified as having a possible thrombotic tendency seems to reduce the risk of thrombosis.[51]

The risk of hemorrhage usually is easily identified from the medical history and from a careful review of the medication list. Iatrogenic hemorrhage is much more common than inherited disorders such as hemophilia, especially with the widespread use of anticoagulation for atrial fibrillation and after vascular stenting. Each transplant unit requires a protocol for the rapid reversal of anticoagulation,

Table 4–6 Screening for Risk of Thrombosis and Coagulation Disorders

Coagulation
Medical history of thromboses
Coagulation tests—prothrombin time, activated partial thromboplastin time, factor V Leiden, protein C, protein S, antithrombin III deficiency
Antiphospholipid antibodies
Complete blood count
Hemostasis
Medication history (warfarin, aspirin, clopidogrel, dipyridamole)
Medical history of bleeding
Medical history of liver disease
Coagulation tests—skin bleeding time, activated partial thromboplastin time, congenital factor deficiencies

usually involving small doses of vitamin K with fresh frozen plasma replacement.

Obesity

Increasing body mass index is associated with increased risks of death and complications of surgery and with new-onset post-transplant diabetes mellitus.[1] The depth of abdominal fat not only causes an increased complexity during the surgery but also leads to an increased risk of wound infection and poor wound healing. The cutoff body mass index values for acceptance and for the risks of post-transplant complications vary with the surgeon, the ethnic background of the patient, and the guidelines of the transplant program. A body mass index of 28 might not be seen as a problem in a white population but might represent significant obesity in an Asian population. There is little disagreement that obesity increases the risks of transplantation—quantified to be an 8% to 9% increased risk of death and graft loss by 5 years.[1] The problem that the physician and the patient face is the task of reducing weight before transplantation. In patients treated by peritoneal dialysis, it is especially hard to change the body habitus derived from the high carbohydrate intake from peritoneal dialysis fluids, such that a switch to hemodialysis may be the only option.

Psychosocial Factors

Smoking presents serious cardiovascular and pulmonary risks before, during, and after transplant surgery and is heavily discouraged by all programs.[7] The unanswered question remains whether or not it is appropriate to transplant patients who continue to smoke. There are many who would argue that it is inappropriate for the community to provide access to the scarce resource represented by a donated kidney if the patient continues self-harming behavior by smoking.

Recreational drug abuse is often a more covert, but equally important risk, factor for success after transplantation.[89] It is important to wean patients from drug dependency, testing compliance and assessing the possibility of recent hepatitis or HIV infection before activating them on the transplant waiting list. Psychiatric evaluation and treatment are often an essential component of preparation for transplantation in drug dependency but may be rejected or unsuccessful. Families may be harsh critics of such individuals and not offer living kidney donation, leaving transplant programs with the decision of whether or not it is appropriate to provide access to scarce community resources. Documented abstinence for 6 months and determination of likely compliance after transplantation provide a nonjudgmental approach to resolving this dilemma but are in themselves complex assessments.

Alcohol dependency leads to challenges similar to those presented by other recreational chemicals. Alcoholism may be well hidden and necessitates an enquiring and suspicious clinical evaluation to detect reliably. When detected, the impact of alcohol on the liver needs to be evaluated, as does the psychological state of the patient. Compliance and reliability for follow-up after transplantation are important factors that influence patient and graft outcomes.

Mental illness requires formal evaluation and treatment, with the additional facet of determination of the ability of the patient to understand and consent to renal transplantation. In a small cluster of patients, renal failure results from chronic lithium toxicity used in the treatment of bipolar depression. Additionally, patients with a variety of psychiatric diseases are not immune from developing renal failure. There is no substitute for an independent psychiatric evaluation of fitness to consent and ensure optimal preoperative and postoperative psychiatric treatment.

Sensitization and Transfusion Status

Blood transfusion was a measurable factor in the success of a transplant during the 1970s and early 1980s. Graft survival was enhanced by 10% when using azathioprine and prednisolone immunosuppression, and it was routine practice to ensure pretransplant transfusion.[94] Transfusion was always a double-edged sword because it is also associated with the development of antibodies to HLA antigens, which limit the available donors because of positive crossmatches.

The other powerful influences on sensitization to HLA antigens are pregnancy and previous transplantation.[98] There is still debate about the relative roles of inherited and noninherited maternal and paternal haplotypes in regulation of the immune response after transplantation,[11] but that debate has had little or no practical impact on clinical decision making. Good knowledge of the antibody status and the patient's HLA type provides a prognostic guide to the likely availability of deceased and living donors and to the likely immune responses after transplantation. See Chapter 10, which discusses this issue in depth.

Previous Transplantation

One or more previous renal transplants provide visible and invisible barriers to the next transplant, both of which need to be considered carefully. There is need to focus on the physical aspects of retransplantation and the immunology.

Retransplantation is less successful than the first transplant procedure, especially if the first graft is lost within 3 months because of acute rejection.[57] Careful assessment of immunological reactivity and selection of the second donor has removed this disincentive in patients who lost their first graft chronically. Although the proportion of patients losing their first graft acutely has decreased dramatically in the past 10 years, the total number of individuals with chronic graft loss is increasing, presenting a significant challenge to the fair allocation of deceased donor kidneys.[22] The decision to accept retransplantation is one that the patient is in a better position to make because he or she has been educated in the hard school of reality. The clinician's decision to offer transplantation is sometimes harder. Should a patient who has lost the first graft because of noncompliance with medication be offered the chance to destroy another priceless donation the same way? Would the older, wiser, and now experienced individual be a model of compliance the second time around? Assessment of the medical suitability for transplantation needs to be just as rigorous the second time as it was the first time, noting especially that infective, malignant, and cardiovascular diseases all are more common in the transplanted patient than the dialysis patient.

Opinion and practices vary with respect to the management of a failed graft.[5,25] Transplant nephrectomy is

a reasonably low-risk procedure that removes an ongoing source of foreign antigenic stimulation and allows for discontinuation of immunosuppression without risk of incurring a rejection response. Nephrectomy is always done in cases of early acute graft failure from whatever cause, but it may not be required in many chronically failed grafts. A proportion of long-term failed grafts still undergoes a significant rejection process when immunosuppressants are reduced and stopped, leading to swelling and tenderness of the kidney and general symptoms of malaise and lethargy. The unanswered question is whether or not there is good reason to remove the grafts that are quiescent despite removal of immunosuppression, other than to make a second transplant physically possible at the same site. Some data suggest that antibody development is enhanced if a nonimmunosuppressed graft is left in situ, whereas other data identify the graft as a sink for antibody binding, which, when removed, exposes the circulating antibody.[48] Other opinions note that if the patient has developed antibodies to the graft antigens, those HLA antigens need to be avoided anyway, whether or not the graft is still in situ.[31]

PREPARATION FOR TRANSPLANTATION

Joining and Remaining on Deceased Donor Waiting List

Most of this chapter has defined the issues of importance for assessment, selection, and preparation of candidates for the transplant waiting lists. Table 4-7 lists issues that should be considered in every patient. Acceptance should lead to histocompatibility testing and entry on the transplant waiting list. The care with which the initial evaluation is usually performed is not often replicated in repeated reassessment in the subsequent years. Depending on the waiting list allocation strategy, it may be many years before a kidney is allocated, with the local record being 33 years and 4 months on the waiting list before transplantation. Re-evaluation of the physical fitness of patients on the transplant waiting list is an essential component of safe transplantation, but one of the more difficult to achieve. Annual reconsideration of suitability for the transplant waiting list is a reasonable precaution against calling unsuitable patients in for operation with the attendant delays and disappointments.

Compliance with the needs of the transplant waiting list and, in particular, providing a current blood sample for crossmatching may sort out willing and motivated patients from noncompliant patients. Most programs maintain serum screening protocols to identify patients who are sensitized to predict the chances of receiving a transplant and to better evaluate donor T and B cell crossmatch results obtained after working hours (see Chapter 10).

Maintaining a current record of clinical events and relevant serology for infectious disease (especially HIV, hepatitis B, and hepatitis C) should be the province of the dialysis unit responsible for the patient's treatment. Ensuring that all these data are available to the transplant program in the middle of the night is challenging and likely to fail without a good information system. In the final analysis, there is little alternative but to ensure that the individuals who are managing the patient on a daily basis are always contacted when a kidney offer is made.

Table 4–7 Screening Tests That Should Be Routinely Considered before a Live Donor Transplant or Acceptance onto a Transplant Waiting List

General history and physical examination
Diagnosis of cause of renal disease, with specific tests as required
Virus exposure
HIV antibody—HIV 1 and HIV 2
Hepatitis B—HBsAg, HBcAb, HBsAb
Hepatitis C—HCVAb (HCV RNA if HCVAb positive)
Cytomegalovirus—CMV IgG
Herpesvirus—herpes simplex IgG, herpes varicella zoster IgG, HHV 6 and HHV 7 IgG
Epstein-Barr virus—EBV IgG
Other infectious disease
In endemic areas
Purified protein derivative skin testing
Trypanosoma cruzi serology
Coccidioides serology
Strongyloides serology
West Nile virus serology
HTLV I and II serology
HHV 8 serology
Toxoplasma screening
Syphilis screening
Chest x-ray with follow-up tests as required if abnormal
Other disease
Electrocardiogram
Echocardiogram/stress cardiac test—with follow-up tests as required if abnormal
Abdominal ultrasound (kidneys, gallbladder, liver, spleen)
Vascular duplex ultrasound (femoral/carotid)

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBcAb, hepatitis B core antibody; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HCVAb, hepatitis C virus antibody; HHV, human herpesvirus; HIV, human immunodeficiency virus; HTLV, human T leukocyte virus.

Undergoing Elective Living Donor Transplantation

The assessment of the recipient of a living donor graft is, in contrast to the deceased donor recipient, a more orderly and planned affair (see Chapter 7). Despite this fact, the focus is often more on the suitability of the donor and less on the recipient. Ensuring that the donor and recipient are assessed by different nephrologists and different surgeons brings suitable attention back to the recipient. Provided that there is good communication between the two teams, it is possible to manage the interface between donor and recipient issues smoothly and effectively. It is just as important for the donor to understand the risks of a poor outcome in the recipient as it is for the recipient to understand them. A donor unaware of the possibility of recurrent disease in a patient with focal segmental glomerulosclerosis would reasonably ask why he or she was not informed before the donation. The risk of death at operation for a particular recipient may be acceptable to the recipient, but not to the donor, who may be unprepared for the possibility that the transplant procedure could lead directly to the death of the recipient. The opposite situation also may occur. A donor may undertake risky behavior, such as intravenous drug abuse or unprotected high-risk intercourse, which the recipient may know more about than either the donor's medical team or the recipient's

medical team. Understanding the level and nature of the risk is paramount for the recipient. Table 4-7 lists issues that should be attended to before living donor transplants.

Undergoing Deceased Donor Transplantation

A transplant team receives the news that a kidney is available for a particular patient only a few hours before the operation must be performed. The allocation often takes place in the middle of the night, and the news is passed through a transplant coordinator and junior medical officer. The patient and family are not in contact with the individuals who have assessed them and who care for their dialysis. The questions and uncertainties that they harbor are carried away in a rush of investigations, including a chest radiograph, an electrocardiogram, routine blood tests, bowel preparation, shower, anesthetic evaluation, immunosuppressive medication, and perhaps preoperative hemodialysis. The pressure to reduce cold ischemia time for the kidney and to meet the deadlines and timetables of operating suites tends to overshadow the needs for discussion and informed consent. This situation emphasizes the need for full education and information during the workup for acceptance onto the transplant waiting list.

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Chapter 5

Access for Renal Replacement Therapy

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The steadily increasing acceptance rate for renal replacement therapy[42,96] and prolonged patient survival on dialysis[149] mean that provision and maintenance of reliable access for peritoneal and hemodialysis, suitable for long-term use, presents a considerable challenge. The workload involved traditionally has been the responsibility of the transplant surgeon, but in more recent years vascular surgeons and interventional radiologists have become more interested in vascular access surgery.

Patients requiring vascular access are becoming progressively more elderly and often have numerous comorbidities and atheromatous or calcified vessels. There has been a concomitant increase in the number of patients with inadequate forearm veins, removing the simplest option of creating an arteriovenous fistula (AVF) at the wrist. This situation has required the judicious use of long-term central venous catheters; upper arm fistulas, including the brachiobasilic operation; and prosthetic grafts in the arms and the legs. Simultaneously, there have been improvements in fistula surveillance to diagnose failing AVFs and grafts and an increasing role for interventional radiology.

VASCULAR ACCESS CATHETERS

Temporary Vascular Access

Approximately 40% of patients with end-stage renal failure present acutely and require some form of temporary vascular access. Table 5-1 lists the indications for venous catheter insertion. The vessel of choice is the internal jugular vein. The subclavian approach gained popularity in the 1960s and early 1970s, providing convenient positioning and allowing patient ambulation, in contrast to the femoral approach of nontunneled catheters. Subclavian vein cannulation is associated with an incidence of 42% to 50% stenosis, however, as detected by venography (Fig. 5-1).[77,124,137] The risk of stenosis is greater when the procedure is difficult or is complicated by hematoma formation. The point at which the subclavian vein runs between the first rib and the clavicle is the most common site of injury, and stenosis is more common on the left and when multiple catheterizations have been performed.

Schwab and colleagues[124] evaluated 47 patients with fistula dysfunction using upper arm venography. Subclavian vein stenosis was documented in 12 patients, all of whom had undergone previous subclavian vein catheterization. This study highlights two further important points. First, subclavian stenosis may be clinically asymptomatic until an AVF is fashioned in the ipsilateral arm. Second, central venous stenosis accounts for 40% of venous stenoses associated with AVFs. It is vital that long-term access options are preserved at all costs when providing acute vascular access for hemodialysis. For this reason, subclavian vein catheterization is now avoided except as a last resort.

Adoption of the internal jugular site, along with the use of soft Silastic catheters and short catheterization periods, has been effective in reducing the venous complications of temporary hemodialysis access.[23,25] The disadvantage of jugular catheters is that they remain visible when clothed, and some patients find this unacceptable. The preferred site for insertion is the right internal jugular vein because this provides the most direct route to the superior vena cava (SVC) and right atrium and is associated with greater patency rates and fewer complications than other sites,[29] including the left internal jugular vein.[79] Catheters introduced through the left internal jugular vein have a tendency to abut the caval wall and are not as successful as catheters placed on the right.

The internal jugular vein is approached easily through a transverse incision centered over the lower part of the sternocleidomastoid muscle. The tip of the catheter should be placed at the junction of the SVC and the right atrium.

Table 5–1 Indications for Tunneled Hemodialysis Catheters

Maturation of autogenous AVF
Maturation of CAPD
Patients waiting for living related transplantation
Dialysis bridge after failed previous vascular access/CAPD to allow planning and imaging for long-term access
Permanent access—all other sites exhausted, severe cardiac dysfunction, vascular arterial disease

AVF, arteriovenous fistula; CAPD, continuous ambulatory peritoneal dialysis.

When the catheter tip is placed in the right atrium itself, there is the danger of a thrombus developing around the tip. Because internal jugular "permcaths" can function for many months or years, their insertion allows for a period of careful investigation and planning for long-term definitive vascular access. In selected subgroups, in which all other routes have been exhausted, tunneled catheters can be placed in the external iliac vein, the femoral vein,[146] or the inferior vena cava via a transhepatic[109] or translumbar approach.[82]

Types of Tunneled Catheters

Modern cuffed, tunneled hemodialysis catheters, made of silicone or polyurethane, have many advantages over noncuffed, nontunneled catheters. Silicone is thermoset, and the catheter is soft and flexible at room temperature, whereas polyurethane is thermoplastic and softens at body temperature. Either way, endothelial damage and thrombogenicity are reduced.[77] Incorporation of the cuff into surrounding tissue and the formation of a subcutaneous tunnel provide physical barriers to infection.[137] Other features of catheter design, such as larger lumens and the separation of inlet and outlet ports, maximize flow and reduce recirculation. Designs vary among different types of catheters; common catheters in use are dual-lumen catheters (Permacath, Quinton Instrument Co, Bothell, Wash; Vascath, Bard, Salt Lake City, Utah), although twin single-lumen catheters also are used (Tesio, Medcomp, Harleysville, Penn). A prospective, randomized study of these three types of hemodialysis catheter in 64 patients compared mean blood flow, reliability (defined as the percentage of treatments performed at a median blood flow of ≥350 mL/min), and recirculation.[9] The mean blood flows were 384 mL/min (Permacath), 396 mL/min (Tesio), and 320.4 mL/min (Vascath). Permacath and Tesio catheters had significantly higher blood flows and reliability than Vascath catheters ($P < .005$), whereas recirculation rates were comparable (3.7% to 4%). Although there were clear differences among these catheters, all three catheters proved inferior to the control AVF group, necessitating longer dialysis times.

Despite modifications in catheter design, thrombosis and catheter-related sepsis remain the major complications limiting long-term use.[51,52,114,124] Catheter-related bacteremia rates for nontunneled, noncuffed hemodialysis catheters range from 0.16 to 0.86 per 100 days,[4] whereas the rates for tunneled, cuffed catheters range from 0.016 to 0.29 per 100 days.[92,112] Andrivet and coworkers[6] showed a reduction in catheter-related sepsis with cuffed, tunneled catheters in immunocompromised patients, although this failed to reach statistical significance, whereas Timsit and associates[137] showed a significant reduction in catheter-related sepsis in patients who received a tunneled internal jugular catheter after admission to the intensive care unit ($P < .02$).

Catheter Insertion Techniques

Tunneled catheters traditionally have been inserted by surgeons in the operating room, using a cutdown method. Interventional radiologists and nephrologists are increasingly inserting them, however, by percutaneous Seldinger techniques in the radiology department or treatment rooms. Results from percutaneous techniques are encouraging and seem comparable to surgical insertion.[138] The use of ultrasound to guide percutaneous cannulation significantly increases first-time successful cannulation rates.[87]

Whichever technique is used, the catheter should be placed under fluoroscopic control[41] with its tip in the SVC. Deitel and McIntyre[33] reported a malposition rate of 29% in the absence of radiological guidance. Some authors report improved patency with the tip in the atrium.[128] Atrial placement also minimizes recirculation[71] and catheter migration associated with changes in posture. Atrial perforation and catheter-induced arrhythmias have been reported, but are less likely to occur with softer silicone tunneled catheters. Overall, the risk of thrombosis outweighs the benefits of atrial placement, however. Table 5-2 lists complications of insertion.

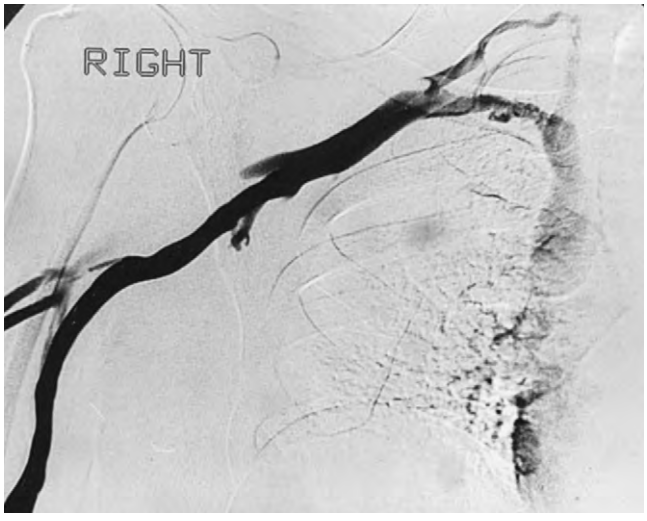

Figure 5–1 Venogram shows central venous stenosis at the junction of the right subclavian and right brachiocephalic veins.

Table 5–2 Complications of Catheter Insertion

Arterial puncture
Bleeding
Pneumothorax
Hemothorax
Hemomediastinum
Atrial perforation
Air embolus
Arrhythmias
Primary failure

Venous Catheters for Long-Term Use

Some patients who require hemodialysis do not have any suitable arm or leg vessels, and in this situation a long-term central venous catheter is placed. The main problem with long-term central venous catheters is thrombosis. This situation may respond to thrombolysis using streptokinase or recombinant tissue plasminogen activator, but the catheter also may need to be replaced. This procedure usually can be done over a guidewire placed through the nonfunctioning catheter. Long-term anticoagulation with warfarin should be considered in patients who are maintained purely on a central venous catheter.

Complications of Hemodialysis Catheters

Catheter Dysfunction

Catheter dysfunction is defined as failure to attain and maintain an extracorporeal blood flow sufficient to perform hemodialysis, without significantly lengthening the dialysis treatment. Adequate extracorporeal flow is estimated to be 200 to 300 mL/min (Table 5-3). Inadequate flow accounts for 17% to 33% of catheter removals.[109,126] Early catheter dysfunction in the postoperative period is due to technical errors in catheter placement. Common problems include kinking of the catheter in the subcutaneous tunnel and malpositioning. Later dysfunction is due to catheter thrombosis, fibrin sheath formation, or central vein thrombosis.[48,93] Other causes of catheter dysfunction are catheter migration or vascular underfilling.[67]

The reported incidence of catheter lumen thrombosis is 46% and accounts for most catheter dysfunction.[71,138] Intracatheter instillation of a fibrinolytic agent such as urokinase (5000 IU/mL) is the primary management of catheter dysfunction. In 70% to 90% of cases, 1 to 2 mL fills the internal lumen of the catheter and results in successful restoration of function.[53,138] If this treatment fails, higher doses of 40,000 IU/mL for 6 hours may be attempted.[57] Shrivastava and associates[127] salvaged 21 catheters in 24 patients using mechanical clearance with a guidewire in whom urokinase instillation had previously failed. If the fibrinolysis infusion fails and catheter migration or patient dehydration has been excluded, the presence of mural thrombus in the SVC or the presence of a fibrin sheath should be suspected. Injection of contrast material through the catheter ports under fluoroscopic screening (a "permacathogram") may show features that suggest a fibrin sheath (a persistent filling defect at the catheter tip or reflux of the contrast material retrogradely along the sheath).[32]

Table 5–3 **Definitions of Catheter Function**

Patency	Length of time that catheter provides adequate extracorporeal flow for effective hemodialysis (in practice >300 mL/min)
Primary patency	Cumulative catheter patency until first therapeutic intervention required to maintain patency
Secondary patency	Cumulative patency from catheter placement to failure regardless of number of therapeutic interventions required to maintain patency

Fibrin sheaths account for 13% to 57% of catheter dysfunction,[133] but are a ubiquitous response to indwelling catheters because they are present in 100% of patients with central catheters at postmortem.[63]

Treatment of fibrin sheaths is by prolonged infusion of fibrinolytic agents (6 hours), mechanical stripping by means of a snare inserted via the femoral vein, or exchange of a catheter over a guidewire.[53,57,122] There is no clear indication which of these therapies is superior. Fibrin stripping is reportedly successful at restoring function in 92% to 98% of cases with acceptable primary patency (28% at 6 months).[53] Other published short-term results of this procedure are poor, however, with catheter dysfunction returning in most patients by the fifth dialysis session after initial stripping.[53,59] Alternatively, catheter exchange over a guidewire or guidewire-assisted manipulation of the catheter tip can be attempted.

Angle and coworkers[7] reviewed their experience of 340 dysfunctioning tunneled hemodialysis catheters referred to a single institution's interventional radiology department. Failure rates were higher after catheter exchange or catheter tip manipulation compared with fibrin stripping or infusion of thrombolytic agent.

Central Vein Thrombosis

Mural thrombus in the SVC can be detected on transesophageal echocardiogram in 30% of patients with central catheters and is often asymptomatic, although it can manifest with arm and facial edema. Magnetic resonance or conventional venography can identify central vein thrombosis. Treatment with infusion of a fibrinolytic agent produces good results, but angioplasty and stenting may be required for organized thrombus.[29] More recently, attention has focused on the prevention of thrombotic complications from these catheters. Trials of anticoagulant therapy in patients with end-stage renal failure are awaited, and low catheter thrombosis rates have been reported in patients on low-dose aspirin and warfarin therapy.[114] In a randomized controlled trial of low-dose warfarin (1 mg/day) in cancer patients with central catheters for chemotherapy, subtherapeutic anticoagulation reduced thrombotic complications from 38% to 10%.[14]

Infection

Catheter infection is responsible for the failure of 6% to 28% of catheters[4,114,124] and represents a major cause of catheter-associated morbidity and mortality. The causative organism is usually a coagulase-negative *Staphylococcus*, although *Staphylococcus aureus*, gram-negative bacilli, and *Pseudomonas* also are common.[77] Infection occurs most commonly by migration of (skin) organisms along the external surface of the catheter from the exit-site wound or via the lumen of the catheter.[41] The organisms are embedded in a biofilm layer that confers protection from antibiotic therapy, and there is a link between the number of organisms retrieved by culture from a catheter surface and the risk of systemic sepsis. Infection occurs when the organisms on the catheter exceed a certain quantitative threshold.[109] Reported rates of exit-site infections and catheter-related systemic sepsis range from 6 to 45 and 2 to 18 per 100 patient days, respectively.[4,73,77,114,124]

In most cases (90%),[4,114] exit-site infections respond to oral antibiotics without necessitating catheter removal.

Topical antibiotics should be used for minor infections; parenteral antibiotics are indicated if there is a discharge from the tunnel or exit site, and there are no signs of systemic sepsis or positive blood cultures. If the infection fails to resolve using these measures, the catheter should be removed, and a new one should be inserted through a different track. Systemic sepsis or bacteremia is associated with a much higher rate of catheter removal, with conservative measures successful in treating the infection in only 20% to 25%.[4,114] Catheter-associated sepsis has considerable morbidity, with the potential for severe complications such as osteomyelitis, septic arthritis, septic discitis, endocarditis, and death. Rapid catheter removal is recommended in unstable patients with bacteremia, or in stable patients remaining symptomatic 36 hours after achieving bactericidal serum levels of antibiotic. The National Kidney Foundation–Kidney Disease Outcomes and Quality Initiatives recommended that parenteral antibiotics be continued for 3 weeks in such cases,[64] although the evidence to support this recommendation is sparse. Preventive strategies aimed at reducing the rates of catheter infection include the handling of dialysis catheters only by specially trained staff, the use of dry gauze dressings at the exit site, and (possibly) the use of antibiotic-coated catheters. Although antibiotic-coated catheters reduce the incidence of line sepsis in intensive care unit patients,[109] the antibiotic tends to be washed off with time and may not be beneficial in catheters used for intermediate-term or long-term dialysis. Other recommendations, such as the routine application of topical antibiotic to the exit site, are unproved and may encourage colonization with fungi or multiresistant organisms.

FISTULAS AND SYNTHETIC GRAFTS

Autogenous vein AVFs are the vascular access of choice in patients requiring long-term hemodialysis because they are associated with good long-term patency and low complication rates.[17,39] These advantages are offset by high primary failure rates (11% to 30%)[73,116] and the time taken for maturation (at least 6 weeks for radiocephalic fistulas).[94] Arteriovenous grafts (synthetic materials) display low rates of primary failure[123] and can be used 2 weeks after formation for hemodialysis. They are associated with inferior patency and higher complication rates, however, leading to increased overall morbidity and escalating hospital costs in the long term.[58]

Historical Development of Vascular Access Surgery

In 1960, Quinton, Dillard, and Scribner described the external arteriovenous shunt (Fig. 5-2) and opened the era of repeated hemodialysis for end-stage renal disease. The technique had many disadvantages, however. External shunts were inconvenient for the patient, were prone to infection and thrombosis, and often required many revision procedures. The introduction of the Brescia-Cimino internal radiocephalic AVF in 1966 was the next major advance and solved many of the problems associated with external shunts. This operation has been so successful that it remains the first-choice procedure for dialysis vascular access. The number of patients with inadequate forearm veins or previously failed radiocephalic AVFs increased, and the 1970s saw the introduction of various elbow AVFs. Prosthetic graft fistulas for dialysis followed; these have been favored more in the United States than in Great Britain, Europe, and Australasia.

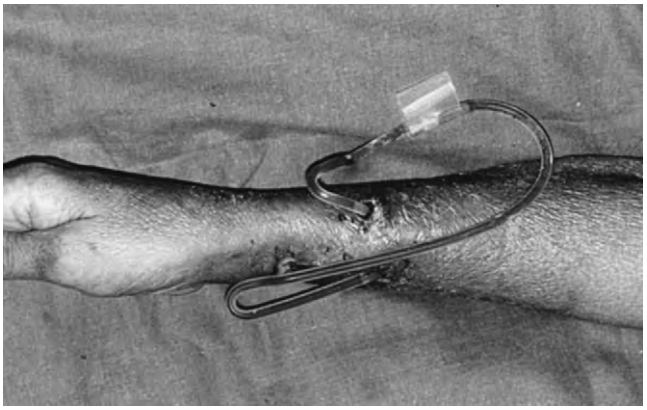

Figure 5–2 Scribner shunt.

Planning Vascular Access

Patients with end-stage renal failure are living longer and may need to be maintained on dialysis for decades. Careful planning is a key feature of vascular access surgery, and this includes protecting the venous system of chronic renal failure patients. The veins of the forearm and antecubital fossa must not be used for phlebotomy or for intravenous catheterization. These procedures should be confined, as far as possible, to the veins on the dorsum of the hand. Most medical and nursing staff members working in renal units understand this issue, but staff members working outside the renal unit may be less well informed. The importance of preserving the arm veins should be emphasized to all dialysis patients so that they are in a position to take some responsibility for their own veins. The central veins are equally important, and every effort must be made to avoid direct catheterization of the subclavian vein. Temporary vascular access catheters placed through this route may be complicated by subclavian stenosis, which can preclude subsequent ipsilateral access operations.

A few general rules relate to the planning of vascular access. Arm vessels should be used in preference to the legs, and the nondominant arm should be used first. The latter rule is especially important in patients who needle their own fistula for home hemodialysis. Distal sites should be used first because this allows the greatest possible length of vascular conduit and preserves proximal sites for the future. When possible, the AVFs should be created preemptively. This approach requires careful judgment and the use of a reciprocal creatinine plot to estimate when individual patients will reach end-stage disease.

The Brescia-Cimino radiocephalic fistula is the first choice for long-term hemodialysis access. If a radiocephalic AVF fails in the longer term, it usually is because of the development of intimal hyperplasia at or near the anastomosis. In some cases, the patient still has usable forearm veins that can be anastomosed to the radial artery at a more proximal site. In patients with unsuitable forearm veins or failed wrist fistulas in both arms, the next step should be

formation of a brachiocephalic fistula. If this is impossible, a brachiobasilic AVF using superficially transposed vein should be considered. Prosthetic AVF grafts are most commonly created using expanded polytetrafluoroethylene (PTFE), but should not be used until all the native arm veins, including both basilic veins, have been used or deemed to be unsuitable. Patients with failed secondary and tertiary access procedures may be suitable for long-term management using tunneled, cuffed central venous catheters.

Many patients who need vascular access surgery develop central venous stenoses or occlusions, including lesions in the SVC. These patients present some of the most challenging vascular access problems. Interventional radiology is an important adjunct to management because some central venous lesions can be treated successfully by percutaneous transluminal angioplasty with or without stenting. Only when all potential upper limb procedures fail should the legs be considered for vascular access using a prosthetic graft AVF.

Requirements of Arteriovenous Fistulas for Hemodialysis

The veins of the arm can be catheterized easily and repeatedly, but their blood flow is too low to support hemodialysis. The creation of an AVF produces an arterialized venous channel, which yields the combined advantages of large diameter and high blood flow. The ideal AVF has the following features:

1. A blood flow of at least 300 mL/min
2. A large diameter, which facilitates venipuncture
3. Sufficient length to allow two dialysis needles to be inserted
4. Creation by a simple and quick operation, preferably under local anesthesia
5. A good long-term patency rate

A small anastomosis between the radial artery and cephalic vein leads to arterialization of much of the venous system of the arm, and this has profound hemodynamic consequences. Immediately after creation of a radiocephalic AVF, the radial artery blood flow increases tenfold to 200 to 400 mL/min. The flow rate increases further over the next 2 to 4 weeks, after which it plateaus. The underlying mechanism is a loss of the downstream resistance in the arterioles and capillary bed. To achieve these dramatic flows, the artery and the vein must dilate. Although failure of maturation of a radiocephalic AVF may be due to an inadequate vein, it also can be due to an atheromatous or frankly calcified artery that is unable to dilate. The vessels of diabetic patients often fall into this category.

Preoperative Assessment

The three requirements for a successful AVF in the arm are the following:

1. A good arterial inflow
2. A suitable superficial vein
3. Patent axillary and subclavian veins

The first two of these requirements usually are assessed clinically, but the third requires radiological investigation. The radial and ulnar arteries can be palpated at the wrist to assess the pulse volume and to identify overt atheromatous disease, such as calcification. Allen's test usually is described as a method of establishing the patency of the palmar arches, but it also can provide a subjective assessment of the arterial inflow to the hand and define dominance of the radial or ulnar artery. The veins of the forearm can be assessed by simple inspection and palpation after placing a tourniquet around the upper arm. The cephalic vein is most often used for AVF creation, and for success it needs to be patent from the wrist to the antecubital fossa and have a diameter of at least 3 mm.[116] The patency of the vein can be established easily by lightly percussing it at the wrist and feeling for a transmitted wave at the elbow. A good-caliber cephalic vein at the wrist may divide quickly into many small branches in the forearm, and this relatively common anatomical variation may preclude successful AVF formation.

Assessment of the patency of the major venous drainage of the upper limb is particularly important. The only overt clinical signs of stenosis or occlusion in the axillary and subclavian venous system are prominent collateral veins around the shoulder and chest and associated arm swelling. Most venous stenoses and some occlusions are clinically silent, and the venous drainage of the arm can be assessed properly only by performing a duplex ultrasound scan or a contrast venogram. Logistical and financial constraints usually dictate that these investigations are performed selectively, but it is wise to perform some form of imaging in any patient who has previously had an ipsilateral temporary subclavian vascular catheter.

Anesthesia

Patients with end-stage renal failure commonly have comorbid conditions, such as cardiovascular disease and diabetes, and may be a poor risk for general anesthesia. Many vascular access procedures can be performed using a local anesthetic. For simple operations such as wrist fistulas, local infiltration of 1% lidocaine usually suffices. The addition of epinephrine is helpful in reducing the oozing that commonly occurs in renal patients as a result of qualitative platelet dysfunction. Regional anesthesia can be achieved by local anesthetic blocks, and these techniques are ideal for more extensive operations, such as elbow AVFs and forearm prosthetic loop grafts. These methods also block the sympathetic nervous system, which has the advantage of inhibiting vasospasm. An alternative for more extensive operations is to use simple local anesthetic infiltration and to ask the anesthesiologist to supplement this with boluses of a short-acting sedative, such as propofol.

Surgical Technique

Vascular access surgery requires adhering to the basic principles of vascular anastomosis. The vessels are anastomosed using a fine, continuous, nonabsorbable, monofilament suture with eversion of the edges to ensure a smooth transition between the two intimal surfaces. There must be no tension between the anastomosed vessels, and the sutures must pick up all layers of the arterial wall to avoid the creation of a subintimal flap. Because suture placement is so crucial, optical magnification using surgical telescopes is an advantage, and good-quality microvascular instruments should be available.

Autogenous Arteriovenous Fistulas

Wrist Arteriovenous Fistulas

The radiocephalic AVF is the first-choice procedure in vascular access surgery. This operation is straightforward and can be performed under local anesthesia as an outpatient procedure. It has a low complication rate and excellent long-term patency rates. The original operation described by Brescia and colleagues in 1966 was a side-to-side, radial artery–to–cephalic vein AVF formed close to the wrist joint (Figs. 5-3 and 5-4). The main variant, a side of artery–to–end of vein fistula, is preferred by many surgeons because there is a lower incidence of venous hypertension in the hand (Fig. 5-5). Radiocephalic AVFs have a primary patency rate of approximately 80% at 2 years.[39,116]

A radiocephalic fistula can be fashioned in the anatomical snuffbox. This is a greater technical challenge because the vessels are of smaller diameter, but the advantage of this site is that it maximizes the length of cephalic vein available for venipuncture. If the cephalic vein or radial artery at the wrist is found to be unsuitable or thrombosed, an AVF can be fashioned by anastomosing the basilic vein to the ulnar artery. The awkward medial position of the basilic vein can make venipuncture difficult, and this operation should not be performed after a failed radiocephalic fistula because there is a theoretical risk of ischemia to the hand.

The surgical technique for formation of radiocephalic fistulas follows. The radial artery and cephalic vein are exposed at the wrist using an S-shaped, longitudinal or transverse incision depending on how close together the vessels are and the surgeon's preference. Hemostasis is achieved using diathermy, which should be in bipolar mode if the patient is awake. The lateral skin flap is elevated to expose the cephalic vein, which is mobilized over a distance of approximately 3 cm, preserving the sensory dorsal branch of the radial nerve. The radial artery is sought just lateral to the flexor carpi radialis tendon and exposed by dividing the transverse fibers of the deep fascia of the forearm over the pulse. Only 2 cm of artery needs to be mobilized by ligating and dividing any small branches. The cephalic vein and radial artery may be anastomosed in a side-to-side or an end-to-side arrangement. We prefer the end-to-side arrangement in which the spatulated end of the divided cephalic vein is anastomosed to the side of the radial artery using a continuous monofilament 7-0 vascular suture.

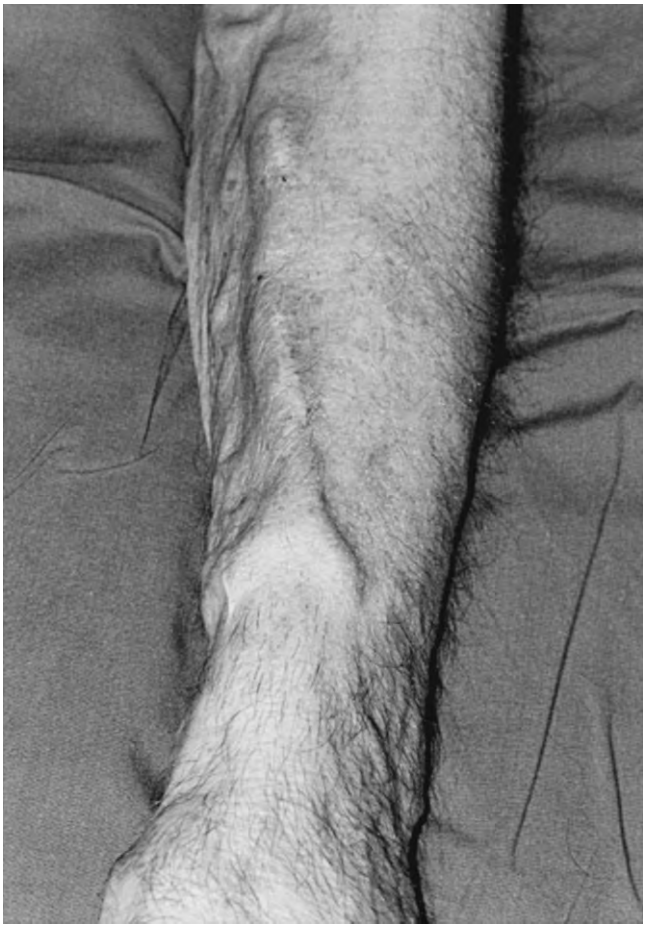

Figure 5–4 An established Brescia-Cimino radiocephalic arteriovenous fistula.

Figure 5–3 Operative photograph of a completed side-to-side radiocephalic arteriovenous fistula.

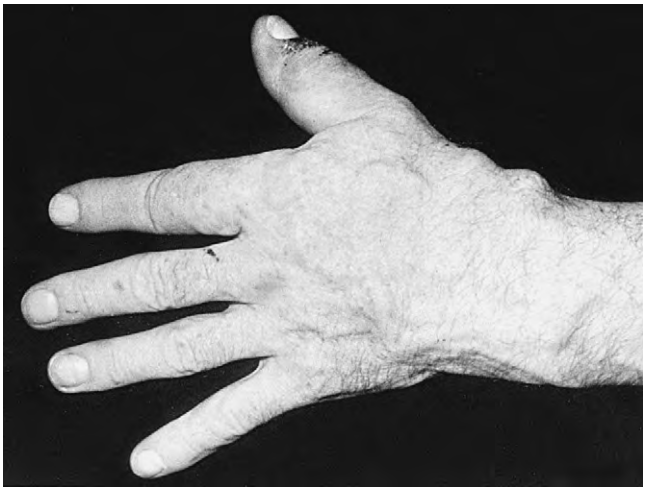

Figure 5–5 Venous hypertension in the thumb secondary to a side-to-side radiocephalic arteriovenous fistula.

After controlling the artery with Silastic vessel slings or miniature vascular clamps, a short arteriotomy is made. The length of the arteriotomy depends on the diameter of the cephalic vein and radial artery, but usually is in the region of 5 mm. Systemic anticoagulation with heparin is unnecessary and unwise in renal patients, but the proximal and distal radial artery can be filled locally with heparinized saline solution. For a side-to-side anastomosis, the vessels are mobilized sufficiently to allow them to be held together over a distance of 2 cm, and the cephalic vein is not divided. Both vessels are opened by equal-length longitudinal incisions, and the side-to-side anastomosis is completed using a fine monofilament suture.

At clamp release, the flow should be high enough to produce an obvious thrill in the cephalic vein. The causes of an absent thrill include systemic hypotension, adventitial bands that kink the venous runoff, and technical errors in constructing the anastomosis. It also is possible that the radial artery or the cephalic vein, or both, are too small to support a high flow. If there is any doubt, the anterior wall suture line should be taken down to look for an intimal flap or other technical error.

Elbow Fistulas

Many patients referred for vascular access have either inadequate forearm veins or a previously failed wrist fistula. The principal choice for secondary vascular access in this situation is between an upper arm AVF using autogenous vessels or a prosthetic graft AVF. Autogenous elbow fistulas have proved to be more popular in European countries than in the United States, where prosthetic grafts have tended to be favored for secondary access.

Brachiocephalic Arteriovenous Fistulas

Brachiocephalic AVFs probably are the best option when it is impossible to form a wrist fistula in either arm. The direct brachiocephalic AVF was first described by Cascardo and colleagues in 1970.[26] The operation is straightforward to perform and can be done under local anesthesia. The side of artery–to–end of vein configuration is preferred. A side-to-side anastomosis may be possible if the cephalic vein and brachial artery are close enough but, as with radiocephalic fistulas, this arrangement may give rise to venous hypertension in the hand. The main disadvantage of brachiocephalic fistulas is that they produce a relatively short length of arterialized vein. The procedure may be unsuitable for the arms of obese patients.

Several technical variations of the brachiocephalic fistula are possible, depending on the venous anatomy in the antecubital fossa. When present, the median cubital vein may be anastomosed directly to the brachial artery, and this technique has the advantage of arterializing the cephalic and the basilic venous systems. Alternatively, the deep perforating branch of the median cubital vein can be anastomosed to the brachial artery. The long-term results of brachiocephalic AVFs are good, with secondary cumulative patency rates approximately 80% at 3 years[13] and 70% at 4 years.[38] Elbow fistulas can have high flow rates, and hemodynamic complications, such as steal syndrome and high-output cardiac failure, occur more commonly in these fistulas than in wrist fistulas. To avoid these complications, the length of the brachial arteriotomy should be limited to a maximum of 75% of the diameter of the artery.

Brachiobasilic Arteriovenous Fistulas with Vein Transposition

Only a short section of the basilic vein at the elbow is superficial, and most of the vein is protected under the deep fascia. The subfascial position of the basilic vein means that it is protected from venipuncture, and this usually ensures that it is of good quality and diameter. If the basilic vein is left in this anatomical position and anastomosed to the brachial artery, only a short length of vein is available for venipuncture. A much longer conduit can be created by dissecting the basilic vein from its bed and transposing it into a more convenient subcutaneous position down the middle of the upper arm. The operation is best performed under general anesthesia because of the extensive incision required. This incision runs along the median aspect of the arm from the antecubital fossa to the axilla, but staged incisions with short skin bridges also can be used. The medial cutaneous nerve of the forearm usually is closely applied to the basilic vein and needs to be preserved carefully during dissection. The vein is transposed into its new position using a subcutaneous tunneling device and anastomosed end of vein to side of brachial artery (Figs. 5-6 and 5-7).

The operation may be performed as a tertiary procedure after failed wrist and brachiocephalic fistulas, but it also is particularly useful as a primary procedure in small children. The formation of the brachiobasilic AVF does not compromise the arm for future prosthetic grafting. This operation probably has been underused.

The 1- and 2-year functional patency rates are approximately 50% to 80%.[20,38,95] The disadvantage of the brachiobasilic fistula lies in the extensive incision required. Postoperative analgesia can be improved by administering boluses of a long-acting local anesthetic such as bupivacaine via an epidural catheter placed directly into the axillary sheath at the time of surgery.[21]

Graft Arteriovenous Fistulas

In Europe and Australasia, graft AVFs are reserved for patients with previous multiple access failures. In contrast, many centers in the United States favor prosthetic grafts as primary or secondary access procedures. Graft AVFs can be

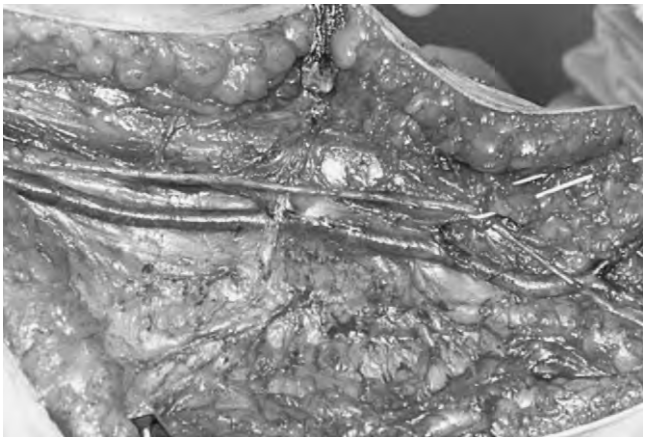

Figure 5–6 Operative photograph of the extensive incision required to dissect the left basilic vein free from its subfascial bed. The medial cutaneous nerve of the forearm runs parallel to the basilic vein.

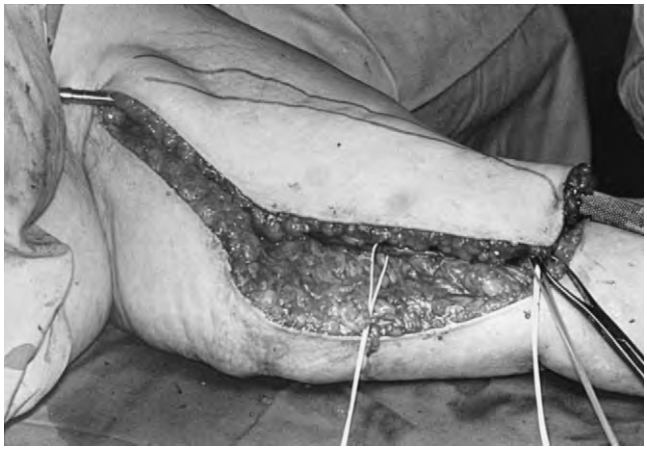

Figure 5–7 Operative photograph shows the use of a Kelly-Wick tunneling device to create the transposition tunnel of a left brachiobasilic arteriovenous fistula.

constructed under local or regional anesthesia with a short inpatient stay. The graft material is tunneled subcutaneously between a suitable artery and vein and anastomosed to these using an end-to-side technique. The choice of graft material is between biological materials, such as autogenous long saphenous vein, and prosthetic materials, of which expanded PTFE is preferred.

The use of autogenous saphenous vein is attractive in principle, but has been limited by the variable quality of this vein. This variable quality has been reflected by the poor patency rates of 20% to 66% at 2 years.[75,90] The extensive dissection of the long saphenous vein can be performed only under general anesthesia, and the procedure is not cost-effective because the patient needs to be hospitalized for several days owing to the leg incision. In view of these disadvantages, the long saphenous vein is used rarely in vascular access surgery. Other biological materials, including human umbilical vein and bovine carotid artery, have not been found to be successful. Although these materials handle well, they are associated with high incidences of infection, rupture, and false aneurysm formation, and overall patency rates are poor.[47,70] More recently, a bovine ureteric graft, which is chemically treated to remove the urothelium and leave what is essentially a collagen tube, has been introduced into clinical practice (Cryolife, Hampshire, United Kingdom). Early results from the Oxford group show encouraging patency rates and an acceptable complication profile,[44] but longer term follow-up is required to define the role of this new graft.

Expanded PTFE is the most popular prosthetic graft material in access surgery. It is easy to handle, of predictably high quality, and available in a wide range of sizes. The expected 2-year graft patency rate using expanded PTFE is approximately 60%. The disadvantages of expanded PTFE are its expense and higher complication rates than those seen with autogenous AVF. A further disadvantage of PTFE is the need to wait 6 weeks before venipuncture because the PTFE wall is not self-sealing, and perigraft fibrosis must develop before the graft can be punctured safely. Of particular concern is the overall infection rate of 11% to 35%[115] compared with 2% to 3% for autogenous fistulas.[89] Thrombosis and intimal hyperplasia at the venous anastomosis are more common with prosthetic graft materials than autogenous vessels. Newer graft materials, such as stretch PTFE, have improved compliance and elastic recoil, and these properties may reduce the incidence of intimal hyperplasia and permit earlier venipuncture. It remains to be seen whether or not such innovations will translate into better long-term graft patency rates.

Many variations of prosthetic interposition grafts have been used, including loop, straight, or J-shaped configurations. Forearm graft AVFs can take their arterial inflow from the brachial or radial artery, and any of the antecubital fossa veins can be used for the outflow. The most popular configuration is a loop graft between the brachial artery and the basilic vein. This configuration is favored by many surgeons because its full length facilitates the rotation of needle sites, reducing the risk of thrombosis, infection, and false aneurysm formation. In the absence of suitable veins in the antecubital fossa, a graft AVF can be placed between the brachial artery and the axillary vein. These vessels can be exposed through two short incisions, and the operation can be performed under local anesthesia. This technique seems to be an effective tertiary or quaternary vascular access procedure with 1-year primary and secondary patency rates of approximately 70% and 90%, respectively. A brachiojugular graft fistula may be performed in patients with exhausted arm and axillary veins.[111] Interposition loop grafts can be placed in the leg with anastomosis to the common femoral artery and vein in the groin. In this site, there is a particular susceptibility to infection, especially in diabetic patients. An alternative method that avoids the groin involves exposure of the superficial femoral artery and femoral vein in the midthigh where they run in the adductor canal. This is a clean area and allows a graft to be positioned in a loop configuration in the lower thigh.

Fistula Maturation and Venipuncture

The venous outflow of an autogenous fistula becomes arterialized over several weeks, developing a degree of dilation and thickening of the vessel wall. The time when a new AVF can first be punctured varies and requires some judgment. Hematoma and early thrombosis are potential complications if a fistula is punctured before it has matured sufficiently. A conservative approach is to leave all fistulas for 6 weeks, but in patients with good-quality veins, a new AVF can be punctured successfully after only 2 weeks. The development of a fistula may be aided by exercising the arm, possibly with a tourniquet in place; the rationale is that increased arm blood flow improves fistula maturation, but there are no studies of the effectiveness of such a policy.

In the longer term, persistent venipuncture in exactly the same spot can weaken the vessel wall and may lead to false aneurysm formation. In the same way, the skin over a prosthetic graft may be eroded, leaving the graft exposed and infected. Rotation of the venipuncture site is advisable in autogenous and prosthetic AVFs. This advice often is ignored, and there may be considerable pressure from the patient to use the same venipuncture sites repeatedly because the skin here becomes numb with time, and venipuncture becomes more comfortable.

Complications of Arteriovenous Fistulas and Graft Formation

Hemorrhage

If hemorrhage occurs in the first 24 hours postoperatively, it is usually due to a technical error with the anastomosis or a slipped ligature. Generalized oozing resulting in hematoma formation is more common and is related to the functional platelet disorder associated with uremia.[117] The synthetic vasopressin analogue, desmopressin acetate (DDAVP), can be used as a specific prophylactic measure in uremic patients who have additional risk factors for bleeding and in whom extensive surgery is planned. DDAVP releases stored factor VIII from the endothelium into the circulation and restores the bleeding time to normal by promoting platelet adhesion and aggregation.[88] The desired effect on the bleeding time is short-acting with a return to the pretreatment value after 8 hours.

Late hemorrhage from an AVF can occur after venipuncture or as a complication of aneurysm formation and infection. In the emergency situation, this hemorrhage can be controlled by firm pressure over the bleeding point, but surgical exploration is usually required.

Thrombosis

Thrombosis may occur in the first 24 hours postoperatively. Although thrombosis may result from preoperative overdialysis leading to dehydration or intraoperative hypotension, a technical error should be suspected. Immediate re-exploration is indicated because it may be possible to salvage the situation by thrombectomy using a Fogarty catheter and subsequent refashioning of the anastomosis.

Thrombosis is the most common cause of AVF failure in the long term. In this situation, thrombosis is usually due to an underlying stenosis that develops gradually. The type of access and the site of thrombosis are important determinants of outcome. If a radiocephalic or brachiocephalic AVF thromboses in a localized manner at or close to the anastomosis, the runoff usually remains patent because it has many natural tributaries that maintain some venous flow. This situation can be remedied by refashioning the arteriovenous anastomosis at a more proximal site. In contrast, when a brachiobasilic AVF thromboses, it is usual for the whole vein to clot by propagation of thrombus. This clotting is a direct result of the fact that all the tributaries of the venous outflow will have been ligated during the creation of this type of AVF. The only hope of salvage in this situation is to perform an immediate thrombectomy before the clot organizes. AVFs also can thrombose at venipuncture sites as a result of poor technique leading to hematoma formation or undue postcatheterization compression to control bleeding.

Interventional radiological techniques are being used increasingly in the treatment of thrombosed vascular access conduits. Pulse-spray catheters, which originally were designed for thrombolysis in patients with peripheral vascular disease, can be used with equal success in patients with thrombosed vascular access.[16] The catheter is introduced into the clotted segment, and the thrombus is dissolved by intralesional spray infusion of agents such as streptokinase, urokinase, or recombinant tissue plasminogen activator. Percutaneous access thrombectomy also has been performed using balloon catheters.[12,139] After successful thrombolysis or thrombectomy, any underlying stenotic lesion can be identified by angiography, then treated by percutaneous transluminal angioplasty with or without endoluminal stenting. The immediate success rate of this type of procedure may be 90%.[105,142] Restenosis is frequent, however, and may require repeated angioplasty.

Surgical thrombectomy is the alternative treatment, and many new technologies have been introduced. The standard surgical thrombectomy technique uses a conventional Fogarty balloon catheter. Although this technique is effective in removing soft new thrombus, it is less successful when older, adherent clot is present. Two other catheters, the adherent clot catheter and the graft thrombectomy catheter, are more effective in removing densely adherent thrombotic material. Angioscopy, which allows direct visualization of luminal surfaces and the identification of retained thrombus, also may prove helpful in treating the thrombosed vascular access conduit.

Infection

Although vascular access procedures are essentially clean operations, patients with end-stage renal failure are more susceptible to infection for many reasons. Uremia is associated with a reduction in the chemotactic, phagocytic, and bactericidal actions of neutrophils and defects in T cell–mediated and B cell–mediated immune responses.[56] Patients with renal disease have a 70% incidence of nasal, throat, and skin colonization by *S. aureus* compared with 15% of the general population.[151] Prophylactic antibiotics are essential when prosthetic materials such as expanded PTFE are being used to create a graft AVF. Staphylococci found in renal patients have a high resistance to flucloxacillin, and the best choice of antibiotic is vancomycin.

Wound infection after a vascular access procedure must be treated seriously because there is a risk of massive secondary hemorrhage. The patient must be hospitalized until the situation has resolved completely. Relatively minor infections manifesting with erythema and swelling can be managed by elevation and intravenous antibiotic therapy. If frank pus is present, the wound should be explored under general anesthesia, drained of all pus, and thoroughly irrigated with saline or antibiotic solution. If the vascular anastomosis is directly infected, the risk of serious secondary hemorrhage is high, and the safest course of action is to ligate the fistula. Early infection associated with prosthetic graft material presents a particularly serious management problem. Superficial cellulitis can be treated by high-dose intravenous antibiotics, but for purulent infections, the prosthetic graft must be removed.

Aneurysm Formation

False and true aneurysms (Fig. 5-8) may occur in vascular access conduits. False aneurysms occur most commonly at venipuncture sites that have been overused. The incidence is 10% for PTFE grafts compared with 2% for autogenous AVFs.[152] Treatment is by resection and restoration of the AVF by direct end-to-end anastomosis or by the placement of a short PTFE bridge graft. True aneurysmal dilation of autogenous arterialized veins is common. In many cases, no action is required, but if the overlying skin becomes thin, and evidence of progressive expansion exists, corrective surgery is indicated. Localized aneurysms can be resected and continuity restored by

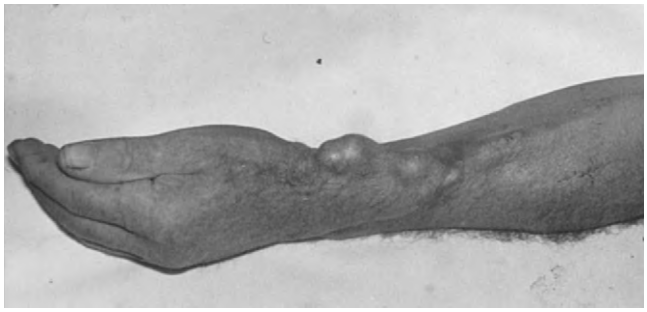

Figure 5–8 True aneurysm of a left radiocephalic arteriovenous fistula.

direct end-to-end anastomosis. Alternatively, if the whole length of an arterialized segment of vein becomes aneurysmal, the AVF may have to be ligated.

Vascular Steal Syndromes

A vascular steal is diagnosed when there is hypoperfusion of the limb (usually the arm) distal to the arteriovenous anastomosis. This hypoperfusion occurs most commonly after procedures involving the brachial artery and in patients with generalized arteriosclerosis and diabetes. The patient complains of a cold, weakened hand, and there may be pain and paresthesias. The incidence of this complication can be reduced by careful attention to detail during the formation of AVFs. Total fistula flow and steal can be limited by reducing the anastomotic length to 75% or less of the proximal arterial diameter; in most patients, this translates into an arteriotomy length of approximately 5 mm. Steal syndromes also can be caused by preexisting arterial lesions and must be investigated by an angiographic study from the aortic arch to the digital vessels. This study may show a distal stenosis that may be amenable to treatment by percutaneous transluminal angioplasty.

Mild steal syndromes can be expected to improve spontaneously over weeks, but in more severe cases further surgery is required to limit the fistula flow. This limitation of flow can be achieved by a crescent-shaped plication suture placed in the vein or graft just beyond the anastomosis.[118] An elegant, albeit more complicated, alternative is to ligate the artery distal to the AVF anastomosis, then to perform a saphenous vein bypass from the proximal artery to a point beyond the ligature.[121] If these interventions are unsuccessful, and in cases in which there are clinical signs of severe hand ischemia, the fistula should be ligated.

Arteriovenous Fistula Surveillance

The aim of fistula surveillance is to detect stenotic lesions before frank thrombosis occurs and to allow treatment of the failing fistula rather than the failed fistula.[125] Methods of surveillance include regular clinical examination, monitoring of venous pressures during dialysis, and measurement of urea recirculation.[125] The failing fistula is characterized by increasing venous pressures and poor flow, sometimes accompanied by a decrease in the palpable thrill or audible bruit. Color flow Doppler examination is another alternative for the detection of intimal hyperplasia; flow rates of less than 500 mL/min should arouse suspicion of venous or graft stenosis.[132] Venous stenoses that are detected by surveillance can be confirmed by contrast fistulography and treated by procedures such as percutaneous angioplasty,[11] endoluminal stent placement,[11] or surgical revision.[40] The establishment of fistula surveillance programs has been shown to reduce the incidence of vascular access thrombosis significantly and to improve long-term patency rates.[98,125]

PERITONEAL DIALYSIS

In the United Kingdom, approximately half of dialysis patients use peritoneal dialysis (PD),[44] compared with 9% in the United States.[148] Most patients favor continuous ambulatory PD. The less common type is continuous cyclic PD. The concept behind PD is straightforward. The peritoneum, with a total surface area 2 m^2, is composed of endothelium, interstitium, and mesothelium, and can act as an efficient semipermeable membrane.[35] Infusing a hypertonic dialysate fluid into the peritoneal cavity allows ultrafiltration of solutes and electrolytes.

Peritoneal Dialysis Delivery Systems and Catheters

PD is a closed loop system comprising dialysate fluid, a delivery system, and an indwelling peritoneal catheter (Fig. 5-9). Fluid is infused under gravity from a reservoir of dialysate. Luer-Lok or rotating safe lock devices have been devised to connect the

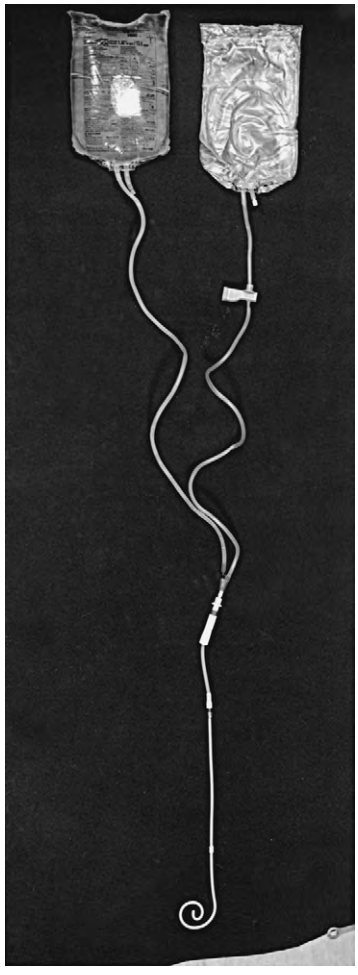

Figure 5–9 Peritoneal dialysis delivery system and catheter. A Y delivery system. The dialysate reservoir (left bag) and collecting bag (right bag) with a titanium connector to the curled, single-cuffed Tenckhoff catheter.

dialysate with the delivery system for ease of connection and sterility. The Italian Y delivery systems[19] are the most common. The single branch of the Y is connected to the indwelling peritoneal catheter via an inert titanium connector, and the upper two branches are connected to the dialysis reservoir and an empty bag. This configuration allows complete drainage of any contaminating dialysate fluid before infusion of sterile, fresh fluid through the indwelling delivery catheter. Several randomized controlled trials have shown the superiority of various Y systems in reducing the incidence of infective complications over conventional PD systems.[28,86]

Catheter Selection

PD catheters should be soft, flexible, atraumatic, radiopaque, and relatively inert. Several different types of catheter are available (Fig. 5-10), but the Tenckhoff catheter is the most popular.[136] The original Silastic Tenckhoff design was a straight, 5-mm external diameter tube, with two Dacron cuffs[136] and a perforated intraperitoneal segment. Many variations of the Tenckhoff device exist, including catheters with single Dacron cuffs and curled intraperitoneal ends. Curled catheters exhibit lower rates of catheter migration than the straight variety.[85]

Catheter Insertion

Not all patients are suitable for PD. Severe peritoneal adhesions, inflammatory bowel disease, and previous sclerosing peritonitis are absolute contraindications. Obesity, advanced age, abdominal hernias, stomas, and chronic obstructive pulmonary disease are relative contraindications. Severe colonic diverticular disease may increase the translocation of gut organisms, and there is a strong association between diverticular disease and gram-negative PD peritonitis. Although PD can be performed in patients with abdominal wall stomas, there is a predisposition to infection. Abdominal wall hernias (Fig. 5-11) may enlarge in patients receiving continuous ambulatory PD and should be repaired if possible at or around the time of catheter insertion. Table 5-4 lists the relative and absolute contraindications to PD catheter insertion.

A variety of techniques for catheter insertion have been described, including open surgical (direct vision), percutaneous (blind), peritoneoscopic, and laparoscopic. Open and closed techniques can be performed with local or general anesthesia, the choice of which may be dictated by comorbidity and fitness for anesthetic.

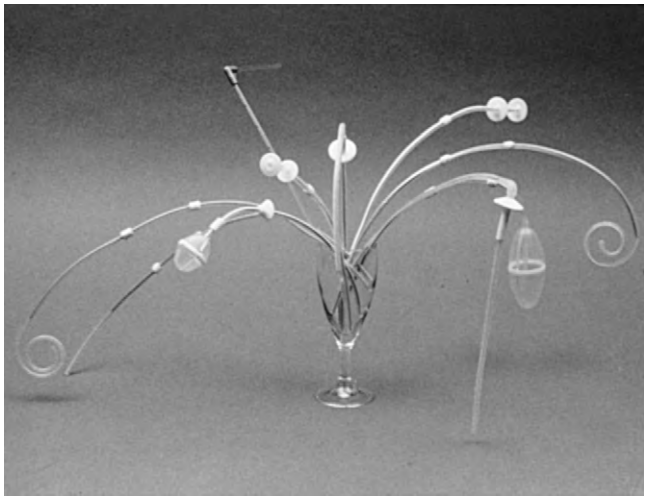

Figure 5–10 Peritoneal dialysis catheters.

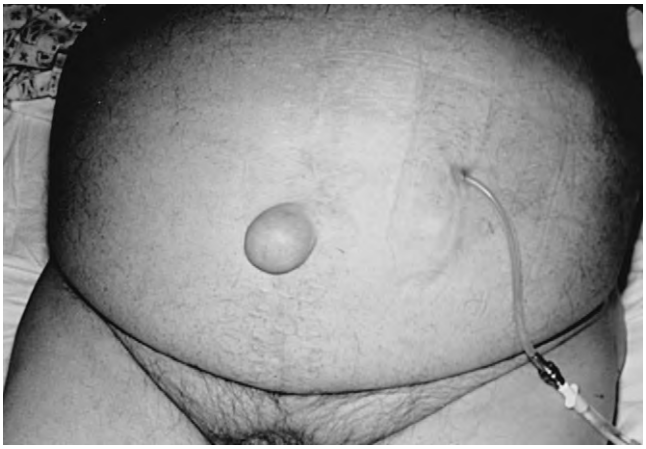

Figure 5–11 Reducible umbilical hernia protruding because of increased intra-abdominal pressure from the infusion of peritoneal dialysis fluid.

In the open technique, the catheter is introduced through a small vertical infraumbilical incision placed in the midline or laterally, with the preperitoneal cuff positioned in the rectus abdominis muscle. Before positioning, the catheter should be flushed and immersed in sterile saline because wet cuffs stimulate more rapid ingrowth compared with dry, air-containing cuffs. A small incision is made in the peritoneum, and the tube is inserted using blunt forceps, or a metal stylet placed through the catheter lumen. The tube tip must be placed in the rectovesical pouch in men and the rectovaginal pouch in women (Fig. 5-12). The peritoneum is closed with an absorbable suture around the cuff to create a watertight seal, and the linea alba or rectus sheath is closed using a continuous nonabsorbable suture. The extraperitoneal segment of the catheter is tunneled subcutaneously and brought out at a conveniently placed lateral exit site. At the end of the procedure, the catheter should be flushed to ensure free inward and outward flow of dialysate fluid.

The percutaneous technique of PD tube insertion requires a dilator introduced over a guidewire to develop a track into the peritoneal cavity.[103] This track allows the introduction of a sheath through which the PD tube is inserted. This technique can be performed at the bedside and has equivalent outcomes to open surgical tube insertion.[46,103] In the peritoneoscopic technique, the PD tube also is introduced through a single infraumbilical stab incision, but a 2.2-mm telescope is introduced first to inspect the peritoneal cavity.[2,46] The laparoscopic method of insertion is a similar approach that requires a 10-mm trocar for insertion of the camera and usually two further 5-mm ports for the instruments used to manipulate the PD tube into the pelvis.[5,18]

Complications Associated with Peritoneal Dialysis Catheters

Bleeding

Bloody fluid is a common finding, occurring in 30% of patients for the first few catheter exchanges after insertion.

Table 5–4 **Contraindications to Peritoneal Dialysis**

Absolute Contraindications	Relative Contraindications
Encapsulating peritoneal sclerosis	Severe obesity
Inflammatory bowel disease	Severe peritoneal adhesions
Large irreparable abdominal wall hernias	Large hernias of anterior abdominal wall
	Abdominal wall stomas
	Chronic obstructive pulmonary disease
	Psychosocial factors likely to result in poor compliance
	Physical disability
	Learning disability

The bleeding most often arises from small vessels on the surface of the peritoneum at the point of catheter entry and usually stops within 24 hours.

Pain

The first attempts at dialysate infusion can produce discomfort. This pain is more common with straight Tenckhoff catheters when infusion pressure is greatest. With coiled catheters, pain is less likely because dialysate flows through the side perforations. The pain is most often temporary and resolves within a few weeks. Slower infusion rates and incomplete drainage alleviate these short-term symptoms.[141]

Cuff Extrusion

The most important factor for cuff extrusion is the depth at which the subcutaneous cuff is implanted; at least 2 cm below the skin is required. Tension on the extraperitoneal portion of the catheter, such as during bag exchange, can bring a poorly implanted subcutaneous cuff to the surface. Reimplantation is required.

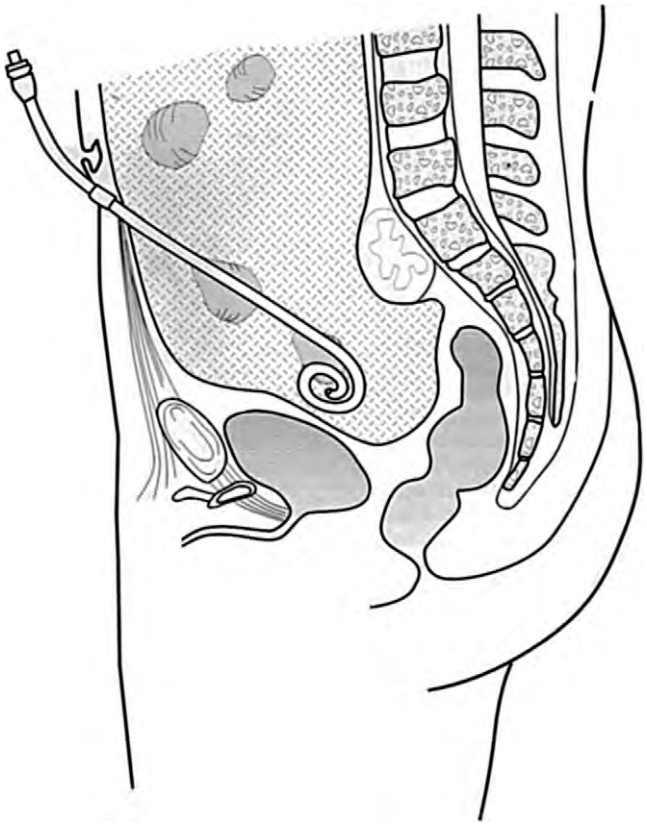

Figure 5–12 Correct anatomical positioning of the peritoneal dialysis catheter in the pelvis.

Catheter Obstruction

Catheter obstruction is usually due to outflow obstruction (Table 5-5). Obstruction may be extrinsic or related to catheter positioning. Clotted blood may collect in the distal portion of the catheter shortly after surgery; this can be treated effectively with a per-catheter infusion of heparin, urokinase, or streptokinase.

Extrinsic compression resulting in obstruction can be caused by bladder distention or an impacted sigmoid colon.[131] Although these causes should be ruled out, they are uncommon causes of obstruction compared with omental wrapping (Fig. 5-13). Intra-abdominal adhesions also can obstruct outflow. In vulnerable patients, this potential problem can be avoided by laparoscopy and adhesiolysis before catheter insertion. Techniques for repositioning or catheter replacement are discussed subsequently.

Catheter Tip Migration

Twardowski[140] stated that the incidence of catheter migration is 20%, but that only 20% of migrated catheters obstruct. Some obstructions resolve spontaneously, but most require intervention to allow repositioning.[78] Treatment of tip migration includes stiff wire manipulation,[68,69] fluoroscopically guided repositioning,[36,66] or (as a last resort) surgical repositioning. Manipulation with a Fogarty catheter inserted into the PD tube has been shown to be successful in repositioning a migrated catheter in 70% of cases.[45]

Pericatheter Leak

Any variable that predisposes to poor wound healing (e.g., steroids, obesity, malnutrition) may culminate in pericatheter leakage. Choice of surgical technique may determine leak rates; leaks are said to be more common with midline catheter insertion compared with lateral insertion through the rectus muscle,[37] but this is not our experience. Pericatheter leakage allows fluid extravasation around the catheter or accumulation in the lower abdominal wall. Leakage rates of 7% to 24% have been described.[49]

Some investigators suggest leak localization with computed tomography combined with peritoneal contrast enhancement[141] or magnetic resonance peritoneography.[8,113] When an early postoperative leak develops, dialysate exchange should be stopped for 2 to 4 weeks, necessitating a

Table 5–5 **Causes of Catheter Obstruction**

Cause	Treatment
Extrinsic	
Omental wrap	Catheter repositioning
Impacted sigmoid colon	Enemas
Urinary retention	Urethral catheter
Adhesions	Adhesiolysis
Luminal	
Blood clot/fibrin	Catheter flush; heparin in dialysate fluid; urokinase/streptokinase
Omentum	Flush or omentectomy
Bowel	Catheter repositioning
Catheter Position	
Malposition or kinking	Reposition by open/laparoscopic surgery or radiologically

temporary vascular catheter for hemodialysis. Late-onset leaks usually require catheter replacement. Pericatheter leaks associated with herniation should be treated by catheter removal and hernia repair. Only after allowing for adequate healing (e.g., 2 to 3 months) should further catheter insertion be attempted.

Hernias

The increased intra-abdominal pressures after infusion of dialysis fluid can enlarge preexisting hernias, so it is best to repair them before catheter insertion, although repair can be performed at the time of, or after, insertion if necessary.

The reported prevalence of de novo abdominal hernias in PD patients is 2.5% to 25%.[99,101,147] One study showed 32% of all hernias occur at the site of catheter insertion, 18% occur in the inguinal region, 27% are epigastric or umbilical, and 23% occur at the site of previous incisions.[101] Herniation into the thoracic cavity also has been reported.[65] The pressure of dialysate fluid can produce recanalization of a patent processus vaginalis, which manifests as scrotal or labial edema, shortly after full dialysate exchange regimens are begun.[72] Surgical ligation is necessary, with a postoperative regimen of low-volume, high-frequency dialysate exchanges until healing has occurred[1] or temporary conversion to hemodialysis.[99]

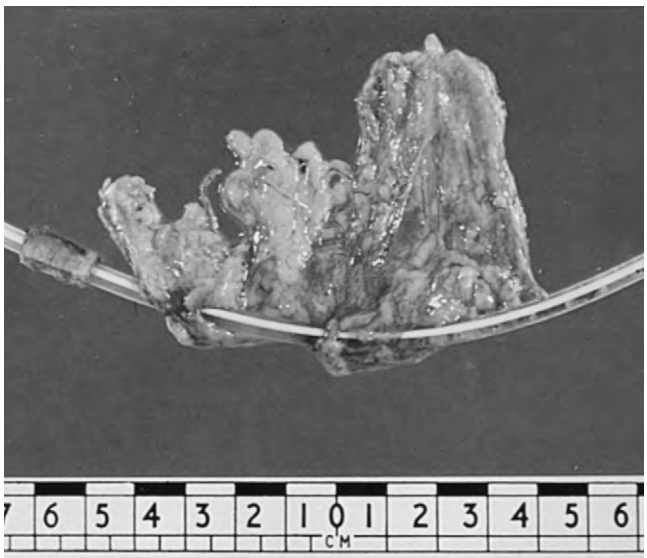

Figure 5–13 Catheter obstruction caused by omental wrapping.

The repair of hernias that develop while a patient is being treated with dialysis is controversial. Ideally, the repair should avoid a breach of the peritoneal membrane. Use of polypropylene prosthetic mesh in incisional hernia repair, attached to the deep fascia of the abdominal aponeurosis without opening the peritoneum, allows immediate use of continuous ambulatory PD.[55]

Some surgeons withhold PD for many weeks after inguinal herniorrhaphy,[106] fearing fluid leak or hernia recurrence. PD can safely be resumed immediately, however, with a modified (high-frequency, low-volume) exchange regimen[91] in the postoperative period.

Exit-Site and Tunnel Infections

As lone entities, exit-site and tunnel infections pose little risk, but the possibility of developing PD peritonitis demands careful attention to these infections; PD peritonitis occurs in approximately 12% of cases of exit-site or tunnel infections.[34,108] A positive culture at the exit site does not indicate an exit-site infection; it merely represents colonization and is not an indication for treatment. Vychytil and colleagues[145] suggested, however, that diabetic or immunosuppressed patients should be treated for a single positive culture indicating exit-site colonization by (or nasal carriage of) *S. aureus*. In all other patients, treatment should be instigated only if there are two or more positive cultures. Rates of exit-site infections range from 0.05 to 1.02 episodes per patient per year.[85,108,143] Table 5-6 lists common microorganisms that cause exit-site infections. *S. aureus* is the most common microorganism, and nasal carriage results in a fourfold increased risk of exit-site infection.[84]

Table 5-7 summarizes suggested management strategies for exit-site problems and infections. Erythema alone with no discharge should be treated with topical chlorhexidine, mupirocin, or hydrogen peroxide. In these circumstances, ultrasonographic examination of the subcutaneous catheter tract can be useful to exclude tunnel infection because this cannot always be ascertained clinically.[61] Purulent exit-site

Table 5–6 Microorganisms Causing Exit-Site Infections

Rights were not granted to include this table in electronic media. Please refer to the printed publication.

From Luzar MA, Brown CB, Balf D, et al: Exit-site care and exit-site infection in continuous ambulatory peritoneal dialysis (CAPD): results of a randomized multicenter trial. Perit Dial Int 10:25-29, 1990.

infections should be swabbed for culture and Gram stained, in addition to culture of the peritoneal dialysate. Generally, for gram-positive microorganisms, a cephalosporin or flucloxacillin is indicated while awaiting the results of culture sensitivities. Alternatively, intravenous or per-catheter vancomycin can be used with careful monitoring of levels. If there is no improvement after 1 week, rifampicin should be added, and if infection persists, the tunnel should be explored and the cuff shaved, avoiding interruption of dialysis. Alternatively, the catheter can be removed,[50] although some authors advocate partial reimplantation of the catheter, with removal of the infected portion and connection of the remaining section to a new, divided catheter.[30]

For gram-negative organisms, per-catheter gentamicin or oral ciprofloxacin or both can be used. Persistent gram-negative infections may require catheter removal because infection is usually due to a deep tunnel infection, with a risk of peritonitis. In the absence of peritonitis, simultaneous catheter insertion can be considered with a contralateral exit site. The usual duration of treatment with oral antibiotics should be a maximum of 14 days, to avoid fungal infection.[83,84] When they do occur, fungal infections require catheter removal and systemic antifungal treatment, such as fluconazole.[50] It is unlikely that a *Pseudomonas* exit-site infection would be eradicated by antimicrobial therapy; early catheter removal is required.

Peritoneal Dialysis Peritonitis

Peritonitis is the most significant complication of PD and is the second most common cause of mortality in patients undergoing PD.[27,110] Incidence ranges from 0.5 to 1.4 episodes per patient per year,[10] with about 60% of patients developing PD peritonitis in the first year.[134] At least a quarter culminate in catheter failure. The most common portal of entry for infection is the exit and tunnel site. The first indications of peritonitis are generalized abdominal pain and tenderness in the presence of a cloudy effluent containing greater than 100×10^6/L white blood cells. Cell counts of 50 to 100×10^6/L may result in a cloudy effluent,[74] and for this reason the polymorphonuclear neutrophil cell percentage may be a more useful indicator of infection (>85% polymorphonuclear neutrophil cells is suggestive).[43]

The causative organisms in PD peritonitis generally differ from the organisms causing "surgical" peritonitis. In the latter case, infections are usually polymicrobial consisting of anaerobic and aerobic bacteria. In contrast, a single microorganism, usually a skin-colonizing, gram-positive bacterium, is the common cause of PD peritonitis; *S. aureus, Staphylococcus epidermidis,* and *Streptococcus* species account for 60% to 80% of cases. The categories of microorganisms commonly cultured in the effluent are listed in Table 5-6. Coagulase-negative staphylococci constitute 30% to 40%, and streptococci constitute 10% to 15%. Yeasts, such as *Candida*, are the most common cause of fungal peritonitis, entering the peritoneal cavity via the catheter or commonly from the vagina.[120]

Dialysate samples for microbiological examination should be taken from the first cloudy bag, providing culture with antibiotic sensitivities. Cultures can be negative, however, in half of patients, even when there are signs of PD peritonitis.[144] In a nonsurgical, PD peritonitic patient, clinical signs are usually mild. Along with abdominal pain, signs include pyrexia (35% to 65%), nausea and vomiting (30%), and diarrhea (10%)[144]; bowel sounds are often present. Other investigations include abdominal and chest radiographs to check for the catheter position and air under the diaphragm, although a pneumoperitoneum is not attributable to gastrointestinal perforation in patients with PD.[24]

There have been reports of nonbacterial, nonspecific eosinophilic peritonitis in PD patients.[129] Prognosis is usually excellent with resolution within days,[52] although it can lead to encapsulating sclerosing peritonitis in recurrent cases.[97]

Early surgical assessment and regular review are necessary for distinguishing PD peritonitis from a surgical cause. Antibiotics and peritoneal flushes form the mainstay of treatment. In mild cases, patients do not require hospital admission, unless they are systemically unwell. Because dialysate

Table 5–7 Treatment of Exit-Site Problems and Infections

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From Gokal R, Ash SR, Helfrich GB, et al: Peritoneal catheters and exit-site practices: toward optimum peritoneal access. Perit Dial Int 13:29-39, 1993.

cultures are not available in the first instance, antibiotic coverage should be broad spectrum. Blood cultures are rarely of value in mild cases.[144] Management of so-called sterile peritonitis, in which no microorganisms are cultured, is controversial. Sterile peritonitis may represent inadequate sampling, or may be a result of indiscriminate use of antibiotics.

Where antimicrobial therapy is required, we administer intraperitoneal gentamicin (10 mg/2-L bag q.d.s.) and vancomycin (50 mg/2-L bag q.d.s.) for 10 days. In cases of recurrent peritonitis, it is prudent to use a urokinase flush on days 5 and 7 of antibiotic cover[107] because the focus of infection may have been walled off by protective fibrin deposits. Intraperitoneal *Pseudomonas* infection is difficult to treat.[31] Patients should receive gentamicin, 15 mg added to each 2-L dialysis bag, in combination with oral ciprofloxacin, 750 mg, twice daily; however, as with exit-site infections, the catheter almost always needs to be removed.

Mycobacterium infection is a particular problem in at-risk cohorts, but all patients with end-stage renal failure are generally at risk because of impaired cell-mediated immunity. Diagnosis can be difficult, but should be suspected in the presence of persistently elevated mononuclear cell counts combined with negative cultures. Acid-fast bacilli smears of the dialysate fluid may be negative in 90% of cases, but formal cultures are likely to be positive in most cases.[80]

Treatment consists of long-term antituberculous drugs; a suggested regimen is isoniazid, rifampicin, pyrazinamide, and ofloxacin for 9 to 15 months.[81] Catheter removal is usually undertaken,[80] although it is not considered necessary for cure. Mortality attributable to tubercular peritonitis is approximately 15%, and much of this may be due to treatment delay.[135]

Fungal infection complicating PD requires a different approach because some antifungal treatments, such as amphotericin and ketoconazole, cannot be administered directly into the peritoneal cavity. *Candida* has a tendency to adhere to the catheter making eradication difficult. Recommended treatment is flucytosine, 1 g orally, and intraperitoneal fluconazole, 150 mg daily. If there is no improvement within 48 to 72 hours, the catheter should be removed, and antifungal treatment should be continued. Fungal peritonitis has a mortality of 15%.[27]

Encapsulating Peritoneal Sclerosis

Encapsulating peritoneal sclerosis is a rare, but potentially life-threatening complication of PD. Reported prevalence rates are three to four cases per 1000 PD patient-years.[60,150] Reported mortality rates are 43% to 75%.[3,100,119] Patients present with abdominal pain, a decline in net ultrafiltration, ascites, bloody effluent, or bowel obstruction. Malnutrition and death are common with encapsulating peritoneal sclerosis.

Recurrent episodes of peritonitis result in loss of the mesothelial layer of the peritoneal cavity causing extensive fibrinogenesis, hyalinization, loculated ascites, and eventual encapsulation of the peritoneal cavity. Radiological contrast studies show delayed transit or intestinal obstruction.[22] Abdominal ultrasonography may show loculated ascites containing echogenic fibrin strands. Calcification also may be present, and the bowel wall is thickened[62] with tethering of the small bowel.[130] Computed tomography shows these features in greater detail.[76]

Renal Transplant Issues with Peritoneal Dialysis

When a PD patient receives a successful renal transplant, the PD catheter can be removed, but the timing of removal requires careful judgment. In most cases, transplant recipients have the catheter removed during the first 2 to 3 months post-transplant. Earlier removal is an alternative when good early allograft function is expected, such as after a live donor transplant. In contrast, the PD catheter must be protected in patients who need to continue dialysis in the post-transplant period because of delayed graft function. Every effort must be made not to breach the peritoneum at the time of transplant because this may lead to a PD leak and the need for temporary hemodialysis. There also is a theoretical risk of serious peritransplant infection if PD fluid leaks into the transplant bed.

Active PD peritonitis is an absolute contraindication to transplantation, but it may be safe to proceed with the operation if the patient has had several days of treatment with intraperitoneal antibiotics, and the bags are clear. Previous studies have highlighted the risks of infection after renal transplantation. The incidence of peritonitis can be 35%,[102] but it is not usually life-threatening. Management strategies should include antibiotics and peritoneal lavage, and in resistant cases there should be a low threshold for catheter removal and conversion to hemodialysis. Patients receiving PD at the time of transplantation have significantly higher general infection rates compared with patients receiving hemodialysis.[104] This risk seems to be reduced when patients convert to hemodialysis just before transplantation.[104]

Studies comparing differences in graft survival rates between patients receiving PD or hemodialysis are contradictory. Some report no difference,[51] whereas others have found improved graft survival in hemodialysis patients.[54] It also has been suggested that rejection rates are 50% higher in PD patients.[51] A more recent study involving more than 9000 renal transplant recipients showed no difference between PD or hemodialysis in terms of acute rejection rates.[15]

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Chapter 6

Brain Death and Donor Management

Kenneth E. Wood

The most immediate and practical solution to the current organ donor crisis is the maximal use and optimal management of the existing potential organ donor pool. This approach provides the greatest opportunity to enhance the conversion of potential donors to actual donors and similarly maximize the yield and quality of the organs procured from each donor. Organ donor management is fundamentally a standardized process that occurs in the following sequence: (1) surveillance to identify patients with severe neurological injury likely to progress to brain death or eventuate in withdrawal of support, establishing candidacy for donation after cardiac death; (2) declaration of brain death using standardized methodology and a standard protocol for withdrawal and declaration in the cases of donation after cardiac death; (3) a uniform request for consent; and (4) optimal medical management of the potential donor.

With the more recently recognized immunological continuum between the donor and the recipient, optimal medical management mandates continued intensity of support from declaration to procurement. This continued support requires a focus shift away from cerebral-protective strategies to maintaining and optimizing organs for transplantation against the background of the physiology of brain death. Given the possibility of procuring multiple organs per donor for multiple recipients, medical management of the potential donor is analogous to providing critical care to multiple patients simultaneously. This management period is crucial for several reasons, as follows:

1. It ensures donor somatic survival so that procurement can be undertaken.
2. Hemodynamic stabilization and mitigation of repetitive ischemia-reperfusion injury maintain the organs to be procured in optimal condition.
3. With the increasing recognition of an immunological continuum between the donor and the recipient, optimal management of the donor can have an impact on the short-term and long-term graft function and the quality of life of the recipient.

Similar to the care of any critically ill patient, a collaborative multidisciplinary approach that integrates the skill sets of critical care physicians, nurses, respiratory therapists, and the organ procurement coordinator is essential. This chapter provides an overview of the potential organ donor management process in the intensive care unit. Emphasis is placed on the physiology and declaration of brain death and medical management, focusing on the cardiopulmonary system given its crucial role in optimizing all organ systems.

BRAIN DEATH: HISTORICAL PERSPECTIVE AND STANDARD

The contemporary approach to the understanding and the declaration of brain death originates from the report of 23 cases of a new type of coma called "le coma dépasse" in 1959 by Mollaret and Goulon.[47] This comprehensive clinical and electroencephalographic description of irreversible coma effectively defined brain death and the consequent physiological abnormalities. The widespread availability of mechanical ventilation in conjunction with the developing field of transplantation intersected in 1967, when Christian Barnard transplanted the heart of a brain-dead, mechanically ventilated patient into a cardiomyopathy patient. This operation precipitated enormous controversy related to the neurological criteria for death and galvanized the movement to codify brain death criteria[48] and define death by either cardiac or neurological criteria.

In 1968, an ad hoc committee at the Harvard Medical School, consisting of representatives of the Law School; Graduate School; Divinity School; School of Public Health; and physicians from Anesthesiology, Neurology, and Neurosurgery sought to "define irreversible coma as a new criterion for death." After excluding hypothermia and central nervous system depressants, and using a whole brain definition,

the committee proposed that brain death required unreceptivity and unresponsivity, no movements or breathing for at least 1 hour with total absence of respiratory effort when disconnected from mechanical ventilation for 3 minutes, no brainstem reflexes, and a flat electroencephalogram (EEG) for a minimum of 10 minutes.[1] They suggested all tests be repeated and document no change at least 24 hours later. Subsequent commentary from the committee in 1969 suggested that the EEG was not essential to the diagnosis but could provide valuable supporting data.[4] In 1971, the Minnesota Criteria further established the time periods for apnea and observation and, for the first time, attempted to define irreversible damage to the brainstem[46] as the "point of no return" that needed to be established "beyond reasonable doubt." The United Kingdom first established brain death criteria in 1976[10] and subsequently defined brain death as brainstem death.[9]

Cardiac and neurological death in the United States was equated in 1981 when the report of the Medical Consultants on the Diagnosis of Death to the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research produced "Guidelines for the Determination of Death."[68] Death was defined by either irreversible cessation of circulatory and respiratory functions or irreversible cessation of all functions of the entire brain, including the brainstem. Brain death required the following: (1) cessation defined by deep coma with cerebral unreceptivity and unresponsivity and absence of brainstem functions, including an apnea test with failure to respond to a partial pressure of arterial carbon dioxide ($PaCO_2$) of 60 mm Hg, and (2) irreversibility defined by coma whose cause is established and sufficient to account for the loss of brain functions, exclusion of the possibility of recovery of any brain function, and persistence of the cessation of all brain functions for an appropriate period of observation or trial of therapy, or both.

The committee recommended a 6-hour period of observation documented by clinical examination and a confirmatory EEG; a 12-hour period in the absence of a confirmatory study; and a 24-hour period for anoxic brain injury, in which the extent of the damage is more difficult to ascertain. Confirmation of clinical findings by electroencephalography was deemed desirable when objective documentation was needed to substantiate the clinical findings. Similarly, the committee addressed the issue of drug and metabolic intoxication and recommended that death not be declared until the intoxicant is metabolized or intracranial circulation is tested and found to have ceased. In the case of hypothermia, the committee believed there were insufficient data to know whether tests of absent or diminished circulation are confirmatory.

Although advisory, the President's Commission guidelines effectively established the criteria used for the declaration of brain death. The American Academy of Neurology developed practice parameters reflecting an evidenced-based literature approach in 1993. These parameters comprehensively reviewed clinical testing of brainstem function, observations compatible with the diagnosis of brain death and confirmatory testing. For practical purposes, these practice parameters have become the standard approach throughout the United States.[91]

Although brain death has been accepted in most countries throughout the world, there is substantial variability in the criteria used; the requirement for confirmatory tests, interval between examinations, and number of physicians or nonphysicians required are the main areas of inconsistency. Readers should consult their local statutes to ensure a clear understanding of and compliance with the legal requirements in their state, jurisdiction, or country. Insofar as the elements related to the clinical examination are the most consistent, these are reviewed, and various available confirmatory options are discussed. A full description of brainstem death and its diagnosis is available in the last edition of this book.[56a]

CLINICAL EXAMINATION

Prerequisites, Confounding Conditions, and Exclusions

A clinical diagnosis of brain death requires that certain prerequisites have been met, reversible or confounding conditions have been excluded, and the cause of coma has been established before undertaking a comprehensive examination. Figure 6-1 illustrates a generalized approach to the process. The cause of coma should be obvious and evident. The diagnosis of brain death in patients with coma of undetermined etiology is problematic. Contemporary practice mandates that some form of neuroimaging, usually computed tomography (CT), be undertaken and be consistent with a neurological catastrophe. Most brain-dead patients have CT evidence of herniation, cerebral edema, or large hemispheric lesions. CT findings consistent with brain death should not eliminate the assessment of confounding conditions. A normal CT scan, which occasionally can be seen after cardiac arrest or meningitis, should raise doubt regarding the diagnosis of brain death.

Table 6-1 lists key conditions and their characteristics that must be excluded in the evaluation of brain death. A diagnosis of brain death cannot be made reliably when the core temperature is 32°C or less. A core temperature of 28°C to 32°C is associated with decreased levels of consciousness and pupillary dilation, and a core temperature less than 28°C reportedly aborts brainstem reflexes.[13] This lack of brainstem reflexes effectively precludes assessment of the key portion of the examination needed to establish the diagnosis of brain death. In this circumstance, hypothermia should be aggressively managed, and examination for brain death should proceed only when the core temperature is greater than 32°C and preferably normal between 36°C and 37.5°C. The use of confirmatory studies to establish the diagnosis of brain death in hypothermic patients is controversial and should be avoided.

Coma of undefined etiology necessitates consideration of poisoning or drug intoxication. Barbiturates or tricyclics can mimic brain death by producing coma and abolishing brainstem reflexes. Preserved pupillary reactivity is present in many drug intoxications and is especially helpful in differentiating this condition from brain death. Barbiturate intoxication may abolish pupillary reactivity, however, and mydriasis may be present after intoxication with tricyclic antidepressants, antihistamines, stimulants, and sympathomimetics. The presence of trace drug metabolites can complicate the diagnosis of brain death significantly. In this circumstance, the following approach has been advocated:

1. Administer specific antidotes, such as naloxone or flumazenil.
2. Proceed with a brain death evaluation when screening tests reveal drug levels that are less than therapeutic levels, or the alcohol level is less than the legal driving level.

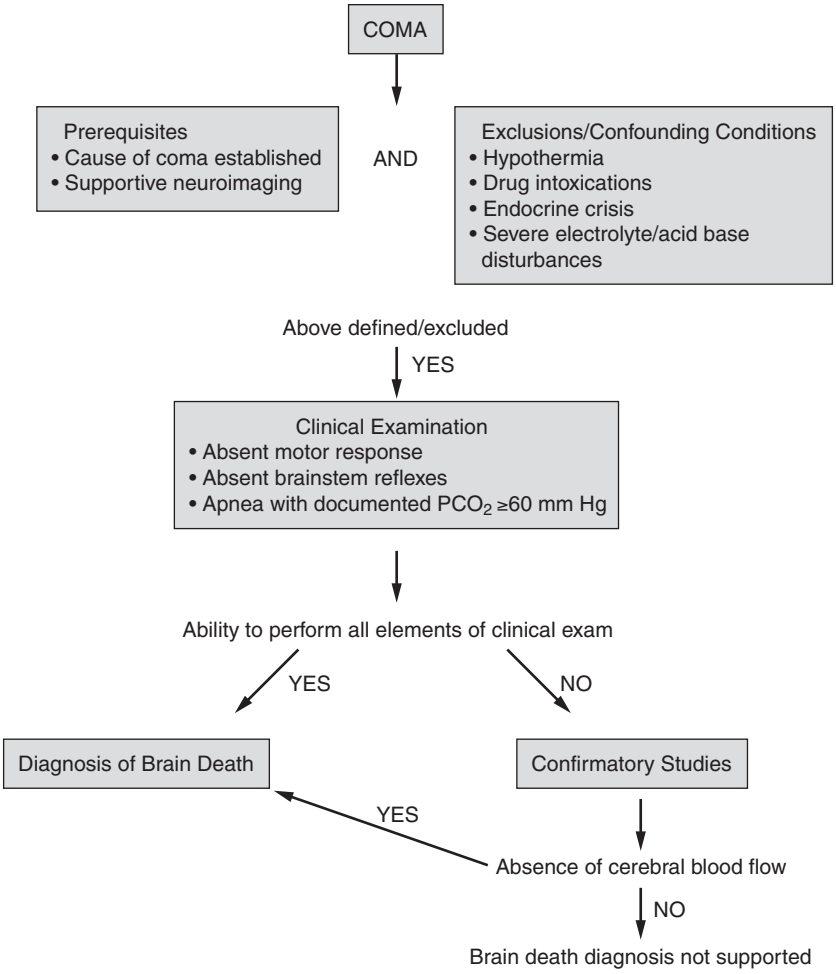

Figure 6–1 General approach to the diagnosis of brain death.

Table 6–1 **Confounding Conditions and Exclusions in the Diagnosis of Brain Death**

Hypothermia
Diagnosis of brain death requires core temperature >32°C
Absence of brainstem reflexes when core temperature <28°C
Drug Intoxications
Barbiturates
Tricyclics
Alcohols
Narcotics
Benzodiazepines
Antipsychotics
Antiepileptics
Antihistamines
Acute Metabolic Endocrine Derangements
Electrolyte, acid-base derangements
Uremia
Hepatic coma
Hypoglycemia
Hypothyroid
Neurological Diseases
Persistent vegetative state
Locked-in syndrome
Akinetic mutism

3. Observe the patient for at least four times the elimination half-life when the drug cannot be quantified.
4. Observe the patient for 48 hours, assessing brainstem function and motor responses in circumstances where suspicion for drug intoxication is high but unknown.

Continued absence of responsiveness and brainstem function necessitates a confirmatory study in this circumstance.[92]

The therapeutic use of barbiturate coma in patients with severe brain injury and intractable intracranial pressure (ICP) elevation can mimic brain death and make the diagnosis of brain death challenging. One approach would be to proceed with a confirmatory study to document the absence of cerebral blood flow and declare brain death. In a study of 36 patients who met clinical and electroencephalograhic criteria for the diagnosis of brain death except for the presence of significant serum levels of barbiturates, demonstration of absent cerebral blood flow with transcranial Doppler and ^{99m}Tc-HMPAO flow scans decreased the period between presumptive and definitive diagnosis of brain death. In the group waiting for the metabolic clearance of the drug, the interval between presumptive and definitive diagnosis of brain death was 34 hours compared with 17 hours for ^{99m}Tc-HMPAO scan and 5 hours for transcranial Doppler; this represented a decrease of 49% and 85%, respectively.[40] Alternatively, it has been suggested that the clinical diagnosis

of brain death is a sacrosanct principle, and that replacement of a comprehensive neurological examination by a technical study in patients to be evaluated for brain death should be considered unacceptable.[93]

Metabolic abnormalities defined in Table 6-1 should be corrected before establishing the diagnosis of brain death. Frequently, brain-dead patients exhibit hypernatremia consequent to diabetes insipidus or hyperglycemia. Levels greater than 160 mEq/L should be corrected before assessment for brain death. Occasionally, patients in a persistent vegetative state or a locked-in syndrome may be mistaken for brain dead. The latter may be attributable to an initial neurological event or reflective of Guillain-Barré syndrome, persistent neuromuscular blockade, or end-stage amyotrophic lateral sclerosis. A thorough and comprehensive neurological examination should exclude these processes.

Coma

Patients in coma reflective of brain death completely lack responsiveness assessed by examination of eye and motor responses to painful stimuli, such as pressure on the nailbed or supraorbital nerve. Occasionally, motor responses of spinal origin can occur spontaneously during apnea testing or hypotension. These responses are brief and episodic and frequently do not persist with repetitive testing. Neuromuscular blockade can produce sustained neuromuscular weakness. Bedside testing using a peripheral nerve stimulator with a train-of-four stimulus should result in four thumb twitches to ensure residual paralytic agents are not contributing to unresponsiveness in patients previously receiving these agents.

Absence of Brainstem Reflexes

Pupils

Pupillary response to bright light evaluates cranial nerves II and III and should be absent in both eyes. Most pupils in brain death are nonreactive and midposition. Round, oval, irregular, or dilated pupils are compatible with brain death, however, provided that they are *not* reactive. Although many drugs can affect pupil size, the response to light should be preserved. Neuromuscular blockade should not affect pupillary reactivity; atropine in conventional doses likewise should not affect reactivity.

Ocular Movements

Any ocular movements, including nystagmus, should be absent in response to head turning or caloric testing, which evaluates cranial nerves III, VI, and VIII. The oculocephalic reflex, or doll's eye reflex, which consists of vigorous rotation of the head from midposition to 90 degrees, should be undertaken only after cervical spine stability is ensured. In non–brain-dead patients, the eyes deviate to the opposite side of the turning, which is termed the presence of doll's eyes. In brain-dead patients, the eyes do not move and retain their orientation, which is termed the absence of doll's eyes. Caloric testing is complementary to the preceding and necessary when the assessment of the oculocephalic reflex cannot be assessed by head turning because of cervical spine injury. Caloric testing is undertaken after inspection visualizes the tympanic membrane and with the head at 30 degrees. Approximately 50 mL of ice water is injected through a small suction catheter directly into the ear canal. In non–brain-dead patients, there is slow deviation to the cold caloric stimulus. In brain-dead patients, there should be no response. One minute of observation and 5 minutes between right and left stimulations are required. Aminoglycosides, sedatives, tricyclic antidepressants, anticholinergics, and antiepileptic medications can minimize or abort the caloric response.

Facial Sensation and Facial Motor Response

The corneal reflex and response to pressure on the supraorbital nerve evaluates cranial nerves V and VII and should be absent in brain-dead patients. A blink response to corneal stimulation with a throat swab represents brainstem function and is inconsistent with brain death. Pressure on the supraorbital nerve or any painful stimulus should not provoke grimacing in brain-dead patients.

Pharyngeal and Tracheal Reflexes

Stimulation of the posterior pharynx with a tongue blade (gag reflex) and bronchial suctioning evaluates cranial nerves IX and X and should produce no response, such as gagging or coughing, in brain-dead patients.

Apnea Testing

After prerequisites have been fulfilled, confounding conditions have been excluded, and absent responses to the preceding brainstem stimulation have been documented, it is appropriate to proceed with apnea testing. Before initiating the apnea test, it is often prudent to assess whether the patient is breathing above the set ventilator rate because this indicates respiratory activity and brainstem function and obviates the need to assess brainstem function or perform an apnea test. Loss of brainstem function precipitates a loss of control of breathing and resultant apnea. The chemoreceptors of the respiratory center in the brainstem are evaluated when maximally stimulated by the elevated Pa_{CO_2} that occurs with apnea. Failure to respond to accepted thresholds of Pa_{CO_2} signifies loss of brainstem function and is consistent with brain death. Before performing an apnea test, it is recommended that the following be addressed[91]:

1. Core temperature should be 36.5°C or greater because lower temperatures may decrease metabolism and carbon dioxide production and shift the oxyhemoglobin dissociation curve to the right, impairing oxygen release.
2. Systolic blood pressure should be 90 mm Hg or greater because lower levels preclude the clinical diagnosis of brain death.
3. Preoxygenation with 100% oxygen for 10 minutes should be applied because the development of desaturation mandates cessation of the test.
4. Eucapnia with a Pa_{CO_2} of 40 mm Hg should be present before the test.

After the preceding have been addressed, the patient is disconnected from the ventilator, with apneic oxygenation provided by a catheter placed at the carina delivering 100% fraction of inspired oxygen (F_{IO_2}) at 6 L/min, and the patient is carefully observed for respiratory activity. Any spontaneous respiratory activity, which usually occurs at the beginning of the test, necessitates reconnection to the ventilator and implies preserved brainstem function. The increase

in $PaCO_2$ is biphasic, with the greatest increase in the first minute and overall $PaCO_2$ increases of 3 to 6 mm Hg per minute.[15] The acknowledged $PaCO_2$ threshold of 60 mm Hg for maximal brainstem respiratory stimulation should be achieved after 8 minutes in apneic patients. Provided that the patient maintains hemodynamic stability and reasonable oxygen saturations, an arterial blood gas measurement should be obtained first, followed immediately by reconnection of the patient to the ventilator at the previous setting. $PaCO_2$ of 60 mm Hg or greater, representing failure of the brainstem to respond to maximal stimulation, is consistent with brain death. $PaCO_2$ of less than 60 mm Hg in the absence of observed respiratory efforts probably signifies reduced carbon dioxide production, necessitating a repeat test of 10 minutes' duration.

The presence of hemodynamic instability, desaturation, or cardiac arrhythmias mandates immediate cessation of an apnea test. In this circumstance, an arterial blood gas measurement should be drawn at the first sign of instability, and the patient should be reconnected immediately to the ventilator. Similar to the preceding, $PaCO_2$ of 60 mm Hg or greater is consistent with brain death, whereas lower levels are indeterminate. In the latter circumstance, the apnea test may be repeated after stabilization. Inability to achieve stability necessitates a confirmatory study. An option to an absolute threshold value of $PaCO_2$ of 60 mm Hg is a 20 mm Hg increase in $PaCO_2$ above the patient's baseline $PaCO_2$. Insofar as it is frequently difficult to define a patient's baseline $PaCO_2$, proceeding directly to a confirmatory test should be strongly considered in patients with suspected baseline $PaCO_2$ elevations.

Provided that appropriate precautions are undertaken, the apnea test generally can be performed safely. In a large series reporting a 25% incidence of complications consisting of hypotension or arrhythmias, or both, 48% of patients began the apnea test with unfavorable conditions; 39% of these patients, compared with 15% of the 52% with favorable preconditions, developed complications. In descending order of frequency, failure to preoxygenate, failure to correct electrolyte and acid-base abnormalities and preexisting cardiac arrhythmias, inotropic drug use, and hypotension were predisposing characteristics of patients developing complications. Preoxygenation was identified as the crucial factor to prevent complications. Cardiac arrest occurred in one patient.[28]

Confirmatory Studies

Unless required by law, a confirmatory study is not mandatory, but it is needed for patients in whom specific components of clinical testing cannot be reliably evaluated. Table 6-2 shows generally available confirmatory studies. All studies except the EEG are predicated on the principle that the absence of cerebral blood flow is consistent with the diagnosis of brain death. As the name implies, these are confirmatory studies and should be undertaken only as required or needed in conjunction with a comprehensive neurological examination and not used in place of a neurological examination unless absolutely necessary.

The diagnosis of brain death should require not only a precise neurological examination but also precise documentation of the findings. More recent retrospective reviews of brain death declarations revealed incomplete documentation.

Table 6–2 **Confirmatory Studies**

Cerebral Angiography
Contrast agent injected under high pressure into anterior and posterior circulations
Absence of cerebral filling at carotid and vertebral entrance into skull
Potential for contrast-induced nephrotoxicity
Rarely performed
Cerebral Scintigraphy (Technetium ^{99m}Tc-HMPAO)
Can be performed at bedside in brief time
Good correlation with conventional angiography
Isotope Angiography
Albumin labeled with technetium 99m
Can be performed at bedside
Delayed filling of sagittal and transverse sinuses
Posterior cerebral circulation not visualized
Transcranial Doppler Ultrasound
Middle cerebral artery through temporal bone above zygomatic arch and vertebral or basilar arteries through suboccipital transcranial windows bilaterally
Lack of transcranial Doppler signals should not be interpreted as confirmatory because 10% of patients may not have temporal windows
May not be diagnostic with intratentorial lesions
Electroencephalogram
No electrical activity for 30 minutes
Complex technical requirements

In this series, the clinical tests most likely to be documented were tests of pupillary (86%) and gag (78%) reflexes. Motor responses were commented on in only 66%, and corneal reflexes were tested in only 57% of cases.[88] In this era in which failure to document presumes failure to perform, it is crucial to ensure that appropriately performed tests are documented. Figure 6-2 is a representative example of a tool that can be implemented to standardize the documentation of brain death.

Donation after Cardiac Death

A prospective donor's death is determined by neurological criteria in the case of donation after brain death or by cardiopulmonary criteria in the case of donation after cardiac death. Both require demonstration of cessation and irreversibility. In the case of donation after cardiac death, cessation of function is defined by a clinical examination documenting absence of responsiveness, heart sounds, pulse, and respiratory efforts. In contrast to the routine pronouncement of general hospital patients using only the preceding criteria, the donation process necessitates confirmation of the physical findings by electrocardiography and an arterial catheter tracing to ensure the patient is dead.[5,61] Equating pulseless electrical activity with asystole because neither results in blood flow is controversial, and individual practitioners should review their hospital donation after cardiac death policy for guidance. Donation after cardiac death irreversibility is defined by cessation for an appropriate period of observation, and death occurs when circulatory and respiratory functions do not resume spontaneously. The Institute of Medicine recommended a 5-minute interval from asystole to the declaration of

DOCUMENTATION OF BRAIN DEATH

	YES	NO
• Prerequisites		
• Core temperature ≥32˚C	☐	☐
• Neuroimaging consistent with diagnosis of brain death	☐	☐
• Cause of coma defined	☐	☐
• Assessment of exclusions and/or confounding conditions		
• Drug intoxications excluded	☐	☐
• Residual paralysis excluded	☐	☐
• Severe metabolic disturbances excluded	☐	☐
• Severe endocrine disturbances excluded	☐	☐
• Clinical examination		
• Absence of motor response to painful stimuli	☐	☐
• Absence of pupillary response to light	☐	☐
• Absence of corneal reflex	☐	☐
• Absence of oculocephalic reflex (head turning and/or caloric if cervical spine injury)	☐	☐
• Absence of gag reflex	☐	☐
• Absence of cough in response to tracheal suctioning	☐	☐
• Apnea test with no respiratory efforts and $PaCO_2$ ≥60 mmHg	☐	☐

A clinical diagnosis of brain death requires an affirmative "yes" answer to all of the above.
A confirmatory study is not mandatory but is required for patients in whom specific components of clinical testing cannot be reliably tested.

• Confirmatory studies		
• Absence of cerebral blood flow on Technetium flow scan	☐	☐
OR		
•Absence of flow on transcranial Doppler	☐	☐
OR		
• Absence of activity on EEG	☐	☐
• Brain death pronounced and OPO notified	☐	☐

Resident MD _____
Staff MD _____
Time _____

Figure 6–2 Documentation of brain death.

death in donation after cardiac death cases.[61] The Society of Critical Care Medicine recommended that at least 2 minutes of observation is required, and more than 5 minutes is not recommended.[16]

PHYSIOLOGY OF BRAIN DEATH

The impact of brain death on the donor graft function was first appreciated in the early 1980s when it was recognized that hearts retrieved from healthy anesthetized baboons functioned immediately on transplantation, yet hearts retrieved from brain-dead donors frequently manifest delayed function. Given the similar retrieval and storage techniques, the differences in post-transplantation function seemed to be attributable to the brain death process.[11] Contemporarily, this is best exemplified in kidney transplantation because non–HLA-matched living donor kidney transplants almost uniformly do better than HLA-matched deceased donor transplants. These observations suggest that the brain death process is not static, and the transplanted graft is not biologically inert.[84]

An immunological continuum between the donor and the recipient has been proposed to explain the influence of brain death on donor organ quality and outcome after transplantation.[24,67] In this model, brain death and other associated ischemia-reperfusion events that can occur with the trauma preceding brain death, procurement, cold storage, and transplantation can induce nonimmunological injuries that are important risk factors for short-term and long-term graft function. This model proposes that brain death induces an intense inflammatory response, and that the graft is inflamed and primed to initiate and amplify recipient responsiveness. Initial and long-term results of deceased donor organ transplantation have been reported to correlate with donor demographics and the cause of the brain injury.[7] Consequently, an implicit understanding of the physiology of brain death not only is crucial to maintaining donor somatic survival and optimizing organ function but also provides a framework to develop strategies that would attenuate this brain death–induced inflammatory response, which potentially may have an impact on early and late graft rejection.

In contrast to animal models of brain death in which the process is well choreographed in a controlled setting, an implicit understanding of human brain death is challenging for multiple reasons, as follows[62]:

1. The time of actual brain death may be different from the certification time, and significant pathophysiological changes may occur during this time.
2. The pathophysiological changes depend on the rapidity of the progression of the brain injury resulting in herniation.
3. Treatment of brain dead donors may result in pathophysiological changes independent of the brain death.
4. No human model will ever be available.

Consequently, an understanding of brain death physiology and its implications must be inferred from animal models and observations in human case series.

Figure 6-3 depicts the distribution and pathophysiological correlation of the rostral-caudal progression of cerebral-spinal ischemia termed coning that eventuates in herniation and brain death. Figure 6-4 shows a magnetic resonance image obtained at brain death compared with a normal magnetic resonance image. Initial cerebral ischemia results in vagal activation and bradycardia decreasing cardiac output and blood pressure. Caudal progression of ischemia to the level of the pons produces superimposed sympathetic stimulation resulting in Cushing's reflex of bradycardia and systemic hypertension. Ischemia at the medullary level begins to inactivate the brainstem, eliminating vagal stimulation and leaving only unopposed sympathetic stimulation.

Termed the autonomic surge and characterized by a hyperdynamic state with tachycardia and frequently extreme hypertension, this condition represents an attempt to maintain a cerebral perfusion pressure gradient against an elevated ICP. The magnitude of the autonomic surge seems to be related to the rapidity of increase in ICP. In animal models, an explosive increase in ICP is associated with profound levels of catecholamines and systemic hypertension, whereas a slow, gradual increase as can be seen after cardiac arrest may not provoke such an exaggerated response. Coincident ischemia at the hypothalamic and pituitary levels produces thermoregulatory dysfunction and the basis for endocrine abnormalities. Herniation produces spinal cord ischemia resulting in sympathetic deactivation characterized by a decreased heart rate, low cardiac output, and vasodilation.[82] Somatic death inevitably occurs within hours to days in the absence of aggressive hemodynamic and hormonal support. Prolonged somatic survival for a mean duration of 23 days has been reported in circumstances where brain death was not acknowledged, and hemodynamic and hormonal support was instituted.[95] Histopathological examination of patients after clinical declaration of brain death reveals necrosis and liquefaction of brain tissue.[6]

The devastating physiological instability and metabolic derangements that may precede the actual herniation process[55] and the above-described brain death process often conspire to produce profound levels of donor instability. The autonomic surge to maintain cerebral perfusion pressure engenders dramatic increases in myocardial work, producing physiological, histological, and electrocardiographic evidence of left ventricular dysfunction.[49] Catecholamines increase cytosol calcium, activating cellular enzymatic pathways, and disrupting adenosine triphosphate (ATP) generation, which compromises myocardial energy production. Activation of xanthine oxidase generates free radicals, impairing organ function further.[50] Autonomic surge–induced vasoconstriction may jeopardize peripheral organ blood flow, and postherniation sympathetic deinnervation with attendant vasodilation may follow. This hemodynamic sequence creates the potential for ischemia-reperfusion injury and the associated inflammatory response. Against this background, there is speculation that hypothalamic-pituitary destruction produces an endocrinopathy of brain death that is

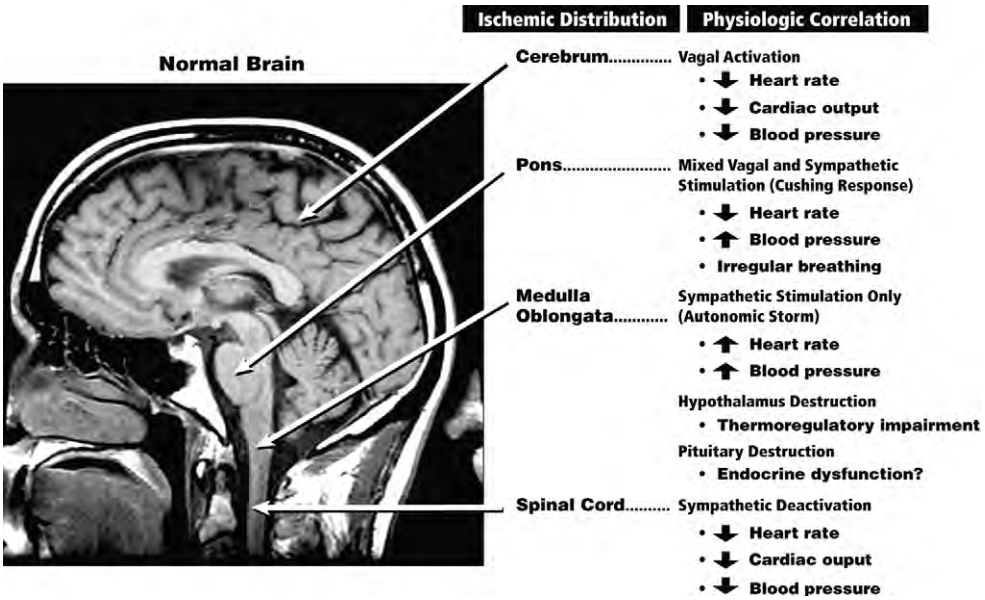

Figure 6–3 The distribution and pathophysiological correlation of the rostral-caudal progression of cerebral-spinal ischemia termed *coning,* which eventuates in herniation and brain death. (Courtesy of Kenneth E. Wood, DO.)

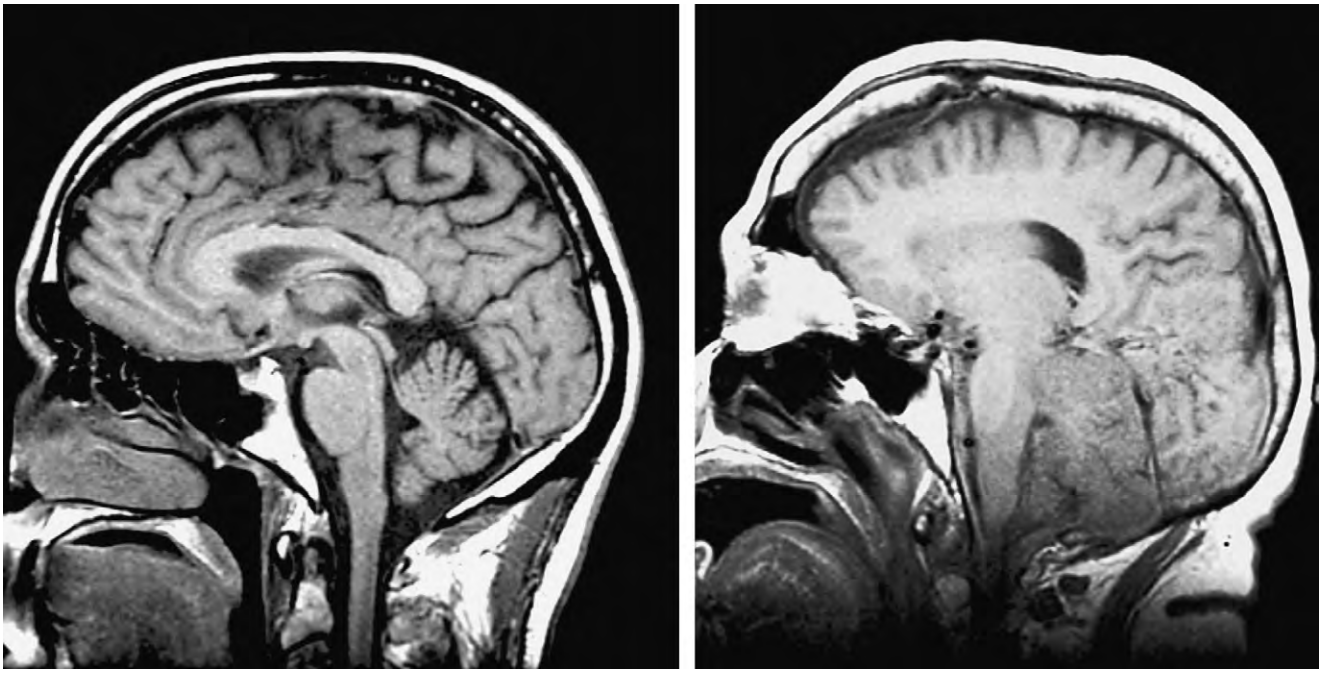

Figure 6–4 Normal magnetic resonance image of the brain (*left*) compared with magnetic resonance image obtained at brain death (*right*). Progressive cerebral-spinal ischemia coning. (Courtesy of Kenneth E. Wood, DO.)

dominated by thyroid and cortisol depletion. The absence of these hormones is proposed to mediate cellular dysfunction and metabolic abnormalities and contribute further to hemodynamic instability.

Ischemia-reperfusion injury may occur as a consequence of the precipitating traumatic event and resuscitation, the brain death process, the removal of the organ, cold storage, and transplantation. Ischemia-reperfusion represents a complex series of molecular and cellular events that produce substantial organ injury. In the case of brain death, autonomic surge–induced vasoconstrictive ischemia is followed by vasodilation and reperfusion with oxygen-rich blood. The latter produces highly reactive oxygen radicals that can directly exert their cytotoxic effects or initiate a cascade of additional molecules with detrimental effects. Damage consequent to oxygen radicals is widespread and characterized by inhibition of ion transmembrane transport, ATP store depletion, disturbances in arachidonic acid metabolism, peroxidation of membrane lipids, and desaturation of proteins, compromising cellular function. Activation of the vascular endothelium and circulating leukocytes along with triggering of the adhesion molecule and cytokine cascade similarly contribute to cellular damage. Endothelial cell swelling compromises vascular space, and the production of chemotactic factors for leukocytes can obstruct the microcirculation further. Release of various lymphocyte-derived and macrophage-derived cytokines may increase the immunogenicity of the donor organs. Tilney and coworkers[84] proposed that ischemia-reperfusion events produce an early insult that precipitates a series of inflammatory events that include the expression of major histocompatibility antigens. This increased immunogenicity of the graft is proposed to amplify the continuum between antigen-independent and antigen-dependent events, which may explain the apparent associations between delayed graft function, acute rejection, and compromised long-term renal graft function.

Brain death–induced hypothalamic-pituitary axis disruption may contribute to donor instability and graft dysfunction. Appreciable disparity exists, however, regarding the functional status of the hypothalamic-pituitary axis between animal and human studies. Low levels of circulating thyroid hormone are proposed to compromise cellular mitochondrial function and impair the use of metabolic substrate, resulting in diminished ATP production.[11,52,53] The transition from aerobic to anaerobic metabolism has correlated with organ dysfunction and hemodynamic instability. Dramatic improvement in cardiovascular stability, abolition of anaerobic metabolism, normalization of acid-base status and electrocardiograms, and improved organ suitability for transplantation have been reported with the use of exogenous thyroid hormone supplementation.[52,53] Several studies in humans have failed, however, to establish the presence of endocrine dysfunction[29,31,65]; correlate hemodynamic instability, inotropic requirements, or lactate levels with hormonal levels[31,65]; or show improvement with supplementation of exogenous hormones.[26,69] Despite the apparent benefits of hormonal supplementation seen in a large retrospective analysis,[72] the standard use of this treatment is controversial. Prospective randomized trials are needed to establish efficacy and practice guidelines.

MEDICAL MANAGEMENT OF A POTENTIAL ORGAN DONOR

General

The causative events precipitating brain death in conjunction with the physiology of brain death often conspire to produce an unstable donor. In addition to the systemic effects of polytrauma, isolated brain injury before brain death is reported to affect the cardiac and neuroendocrine systems. Subarachnoid hemorrhage is associated with

electrocardiographic changes, troponin release, and reduced left ventricular ejection fraction. An apex-sparing pattern of left ventricular dysfunction and diastolic dysfunction are reported to contribute to pulmonary edema in subarachnoid hemorrhage independent of brain death.[3,36,98] Similarly, neuroendocrine dysfunction after traumatic brain injury has been described and is attributed to direct injury to the hypothalamic-pituitary axis, effects of catecholamines and cytokines, or systemic infection and inflammation. The estimated incidence of hormonal reduction is adrenal, 15%; thyroid, 5% to 15%; and vasopressin, 3% to 37%.[64,77] The preceding events and their physiological correlates produce a period between brain death and procurement that is characterized by instability that is directly proportional to the interval between declaration and procurement.[55] It has been estimated that 10% to 20% of potential donors progress from brain death to somatic death during this time.[41] This period after brain death requires a continued intensity of support; however, the focus is shifted away from cerebral protective strategies to approaches designed to optimize the donor organs for transplantation. In effect, this support should be viewed as providing simultaneous critical care to the organs of the multiple recipients. This support facilitates donor somatic survival so that procurement can be undertaken, maintains the organs to be procured in their best condition, and potentially can affect the recipient's quality of life. Standardized guidelines and algorithms focusing on hemodynamic stabilization have proved beneficial in this setting.[34,39,89]

Benefits of these approaches have included recovery of organs initially deemed unsuitable, salvage of unstable donors, and increasing the number of organs procured and transplanted with good outcomes. A trial employing a standardized donor management protocol increased the number of organs procured by 10.3% per 100 donors and the organs transplanted by 11.3% per 100 donors compared with conventional management.[71] The greatest benefit was noted in pancreas, heart, and lung transplantation. All organs benefit from optimal hemodynamic management, which is best illustrated by the increased percentage of kidneys procured with better renal recipient graft function when the heart and the kidneys are jointly procured compared with procurement of the kidneys alone.[71] A review of variables during the care of donors that can influence the outcomes of kidney transplantation concluded that increasing urine output to more than 100 mL/hr at least during the hour before procurement and returning the serum creatinine to admission baseline are the two factors that can be altered during donor management.[63] Insofar as these reflect optimal hemodynamic management, the rest of this chapter is focused on cardiovascular and hemodynamic management.

Echocardiographic and Stability Assessment

Figure 6-5 shows an algorithmic approach to achieving donor hemodynamic stability. While continuing full intensive support, all potential donors should undergo transthoracic echocardiography (TTE) to identify structural abnormalities that might exclude procurement and to define the left ventricular ejection fraction. TTE was first recognized as a potential screening tool for cardiac donors in 1988, when in the absence of TTE, 29% of donor hearts would have been excluded on clinical criteria such as chest trauma, sustained hypotension, prolonged catecholamine use, or cardiac arrest. TTE identified hearts that could be procured and successfully transplanted despite clinical factors previously thought to preclude their use.[25]

Currently, echocardiographic evidence of left ventricular dysfunction is responsible for 28% of hearts that are not transplanted and is the most significant predictor of nonuse with an odds ratio of 1.48 per 5% decrease in ejection fraction.[97] Consequently, it has been suggested that efforts to improve yield should focus on the prevention or reversal of left ventricular dysfunction.[97] Abnormalities of left ventricular function are common with brain injury and brain death.[3,86,96,98] In a study of brain-dead patients, echocardiographic evidence of systolic dysfunction was present in 42%, which was not predicted by electrocardiogram or clinical history. Apical left ventricular function was frequently preserved despite regional abnormalities. This apical sparing is proposed to represent the relative absence of sympathetic nerve terminals and diminished norepinephrine content in this area minimizing damage during the catecholamine surge associated with brain death. There was no reported histopathological correlation with the areas of echocardiographic abnormality postmortem.[14]

Hemodynamic Support

In potential donors in whom the recommended cardiovascular thresholds are not achieved or in whom the ejection fraction is less than 45%, consideration should be given to placement of a pulmonary artery catheter (PAC). Although the use of the PAC is controversial in general,[80] several studies have used the PAC to improve the management of potential organ donors, which resulted in increased rates of recovery and optimized organ function.[34,60,89] As shown in Figure 6-5, the PAC can be used to assess left and right heart filling pressures, define cardiac hydraulic pump function, guide vasoactive medications, and adjudicate the fluid balance between competing organ systems.

Hemodynamic instability is common in 80% of donors and may be sustained in 20% of donors despite vasoactive support.[90] Hypotension is more common in volume-depleted donors treated with vasopressors and donors with diabetes insipidus not receiving antidiuretic hormone.[17] Ongoing hypotension jeopardizes organ function and creates more potential for ischemia-reperfusion injury, donor cardiac arrest, and donor loss.[62] An expeditious approach to diagnosis and treatment of hypotension is imperative. Figure 6-6 presents a three-compartmental model of the circulation that can be used to define the physiological abnormality in any hemodynamically unstable patient. This model conceptualizes the circulatory system as having three compartments: volume in a venous capacitance reservoir, two hydraulic pumps linked in series, and a vascular impedance bed into which the common pump empties. All three compartments usually are affected in potential organ donors.

Volume Resuscitation

In the period immediately after brain death, most donors tend to be intravascularly fluid depleted, which is multifactorial in origin. Although inadequate volume resuscitation from the original trauma or third spacing secondary to the inflammatory response potentially contributes, management of elevated ICP by intentional hypovolemia is

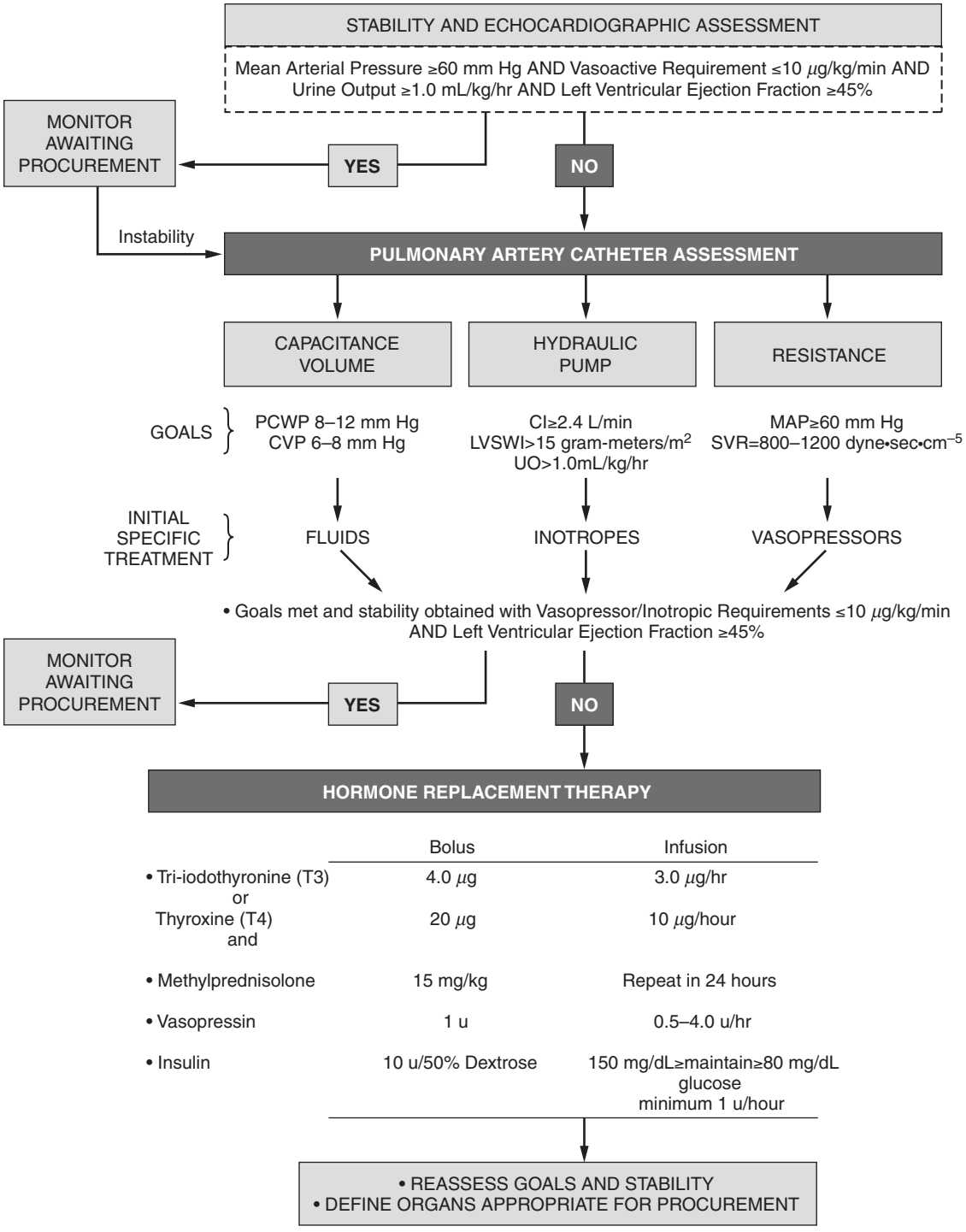

Figure 6–5 Algorithmic approach to achieving donor hemodynamic stability. CI, cardiac index; CVP, central venous pressure; LVSWI, left ventricular stroke work index; MAP, mean arterial pressure; PCWP, pulmonary capillary wedge pressure; SVR, systemic vascular resistance; UO, urine output.

usually responsible. Mannitol, diuretics, and fluid restriction to minimize ICP and preserve cerebral perfusion pressure gradients in conjunction with diabetes insipidus, hyperglycemia-induced osmotic diuresis, and hypothermic cold diuresis tend to deplete the intravascular volume. This depletion is compounded by the loss of vasomotor tone after brain death, resulting in venous and arterial dilation. Initial volume resuscitation should use a balanced salt solution (Ringer's lactate or normal saline) to achieve adequate intravascular volume and packed red blood cells to achieve a hematocrit of 30% to ensure adequate oxygen delivery. Subsequent fluid management strategies need to consider the following:

1. Diabetes insipidus predisposes to hypernatremia. Continued use of normal saline with inadequate control of diabetes insipidus can produce levels of hypernatremia that are associated with impaired liver function in the recipient.[85]

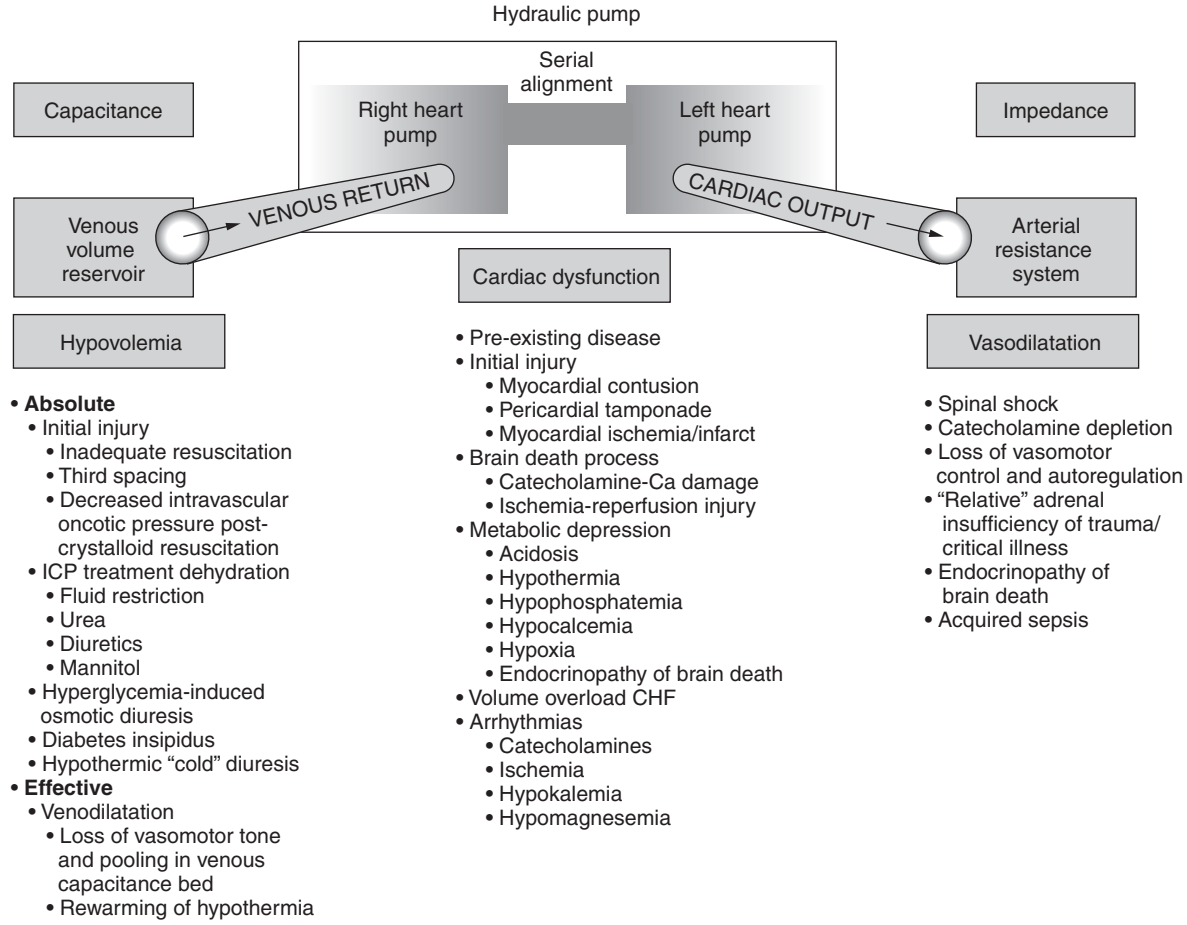

Figure 6–6 Three-compartmental model of the circulation that can be used to define the physiological abnormality in any hemodynamically unstable patient. CHF, congestive heart failure; ICP, intracranial pressure. (Courtesy of Kenneth E. Wood, DO.)

2. Overzealous volume resuscitation with a significantly positive fluid balance is associated with progressive pulmonary dysfunction and loss of donor lungs.[70] An increase in hydrostatic pressure coupled with brain death–induced pulmonary capillary permeability changes is thought to precipitate pulmonary edema.[2]
3. Infusion of substantial amounts of dextrose solution (5% dextrose in water) to treat diabetes insipidus–induced hypernatremia can precipitate hyperglycemia with its attendant problems of worsening osmotic diuresis, electrolyte abnormalities, and the newly recognized inflammatory consequences of blood glucose elevations.[12]

Consequently, fluid management requires vigilant monitoring and ongoing adjustments, with the goals of ensuring adequate intravascular volume for organ perfusion, a serum sodium level of 145 mmol/L or less, and a blood glucose level less than 110 mg/dL. The last goal may require an insulin infusion, which may have the additional benefits of immunomodulation.[12] The appropriate choice of a crystalloid or colloid for donor fluid management is controversial; colloid would seem to be advantageous in an intravascularly depleted, extravascularly edematous patient and was used in a successful management strategy that enhanced lung recovery dramatically.[20] Hydroxyethyl starch has been reported to precipitate injury to renal tubular epithelial cells, possibly impairing early renal graft function, and probably should be avoided.[8] All fluids should be warmed to minimize the risk of hypothermia.

Antagonistic strategies for fluid replacement frequently complicate donor management, pitting lung procurement teams against abdominal organ procurement teams. The former advocate a minimally positive fluid balance because increased lung volume jeopardizes the critical oxygenation ratio (Pa_{O_2}/F_{IO_2}), can worsen the chest radiograph, and is associated with reduced rates of lung procurement.[70] The latter promote aggressive volume repletion to facilitate maintenance of kidney function and urine output, which has been shown to improve renal function in the recipient.[63] Adjudicating these competing interests is necessary for continued optimal management. When the lungs are unsuitable (i.e., massive aspiration, gunshot, significant contusion—all with significantly impaired gas exchange), a more liberal fluid strategy is appropriate, provided that oxygenation is preserved. In the setting of ideal or marginal lungs, invasive monitoring, preferably with a PAC, would be recommended. Brain death–induced left ventricular abnormalities may distort the left ventricular pressure-volume relationship, and the pulmonary capillary wedge pressure may be higher than the central venous pressure. In a controlled trial of donors with a central venous pressure of 6 mm Hg or less, a targeted

central venous pressure end point of 8 to 10 mm Hg was associated with worsening oxygenation—hence the goals in Figure 6-5.[57] Ongoing fluid requirements may be best guided by a PAC with measurements of flow to maintain urine output and pulmonary capillary wedge pressure to minimize lung edema.

Vasoactive Support

The use of vasoactive support is frequently necessary when hemodynamic instability or echocardiographic abnormalities persist despite adequate volume resuscitation. As shown in Figure 6-6, the differential diagnosis of hypotension is complex. Firm recommendations regarding vasoactive support are controversial and compromised by the absence of randomized controlled trials. Many recommendations are derived from retrospective cases series, which may have been compromised by insufficient focus on assessment of the adequacy of volume resuscitation; this is illustrated by studies suggesting adverse[44,76,87,90,94] and beneficial[17,27,43,79] effects of vasopressors. With more recent studies showing either a limited effect or no association between vasopressor requirements of the donor and recipient outcomes,[17,35,78] however, there is evolving consensus that high vasoactive requirements do not preclude successful donation.

After brain death, cardiac dysfunction and vasodilation are usually coincident processes. Ideally, the physiological lesions should be localized, and specific therapy should be initiated: dobutamine or β adrenergics for primary cardiac dysfunction and targeting of α-adrenergic receptors to treat vasodilation. Most donors can be managed successfully with judicious volume resuscitation and low-dose vasoactive agents (5 to 10 μg/kg/min),[39] with management goals depicted in Figure 6-5. Traditionally, dopamine has been the first-line vasoactive agent because it possesses inotropic and vasoconstrictive properties. More recently, vasopressin has been advocated as the vasopressor of choice along with serial lactate levels to monitor perfusion.[81] The specific timing or best combination of vasopressors is unknown at present. Schnuelle and associates[78] reported that combinations of vasoactive agents (dopamine, dobutamine, norepinephrine) were associated with few rejection episodes and a better 4-year survival for renal transplantation. This benefit was attributed to the immunomodulating effects of catecholamines, which have been shown to inhibit the upregulation of adhesion molecules and may diminish brain death–associated inflammation.[78,83] The beneficial effects of catecholamine seen with kidneys did not benefit heart or liver recipients, leaving the best vasopressor or combination uncertain at this time.

Hormonal Replacement

Traditionally, hormonal replacement therapy was reserved for donors with persistent hemodynamic instability despite volume resuscitation/vasoactive support and echocardiographic evidence of a continued low ejection fraction. A large retrospective analysis of brain-dead donors found significant benefits, however, in all donors receiving a methylprednisolone bolus, infusions of vasopressin, and either triiodothyronine or thyroxine. In the 701 donors receiving hormonal resuscitation, the number of organs procured (4.2 organs per donor age 40 years and 3.1 organs per donor age >40 years) was significantly greater than in 10,292 donors who did not receive hormonal resuscitation (3.8 organs per donor age 40 years and 2.5 organs per donor age > 40 years). Hormonal replacement therapy resulted in a 22.5% increase in the number of organs from hormonally resuscitated donors with the following significant increases in the probabilities of an organ being transplanted from a donor: kidney 7.3%, heart 4.7%, liver 4.9%, lung 2.8%, and pancreas 6%. Extrapolation of these results to the donor population at large would translate to an annual increase of 2053 transplantable organs.[72] Insofar as this was not a randomized, controlled trial, it is important to recognize that the hormonally resuscitated group was younger, had fewer deaths related to cerebrovascular accidents, had less diabetes and hypertension, and had a lower creatinine level. Based on such retrospective analyses and other reports,[73] the use of hormonal replacement has been advocated for use in all donors, not just donors with hemodynamic instability.

In early studies, exogenous hormonal supplementation led to dramatic reversals of cardiac dysfunction and acid-base disturbances and were thought to mediate the transition from anaerobic back to aerobic metabolism.[53] More recent studies have been unable to document abnormal hormone levels, however, and speculate that the previous interpretations are more consistent with the inflammatory response of critical illness and brain death.[38] Although the levels of evidence varied, a review of thyroid hormone administration in donor care concluded that no studies supported the routine administration of thyroid hormone for all donors.[66] Of the 10 studies reviewed, 4 supported the use of thyroid hormones,[51,53,56,75] whereas 4 did not offer support.[26,32,58,69] The data related to hormonal resuscitation seem controversial, and individual transplant organizations need to develop an individualized approach to using hormone replacement as routine, as rescue therapy, or only in selected indications.

Disturbances of cardiac rhythm are frequent in brain-dead donors and predominantly occur during the catecholamine surge with brain herniation, consequent to the initiation of vasoactive support or as the terminal event in the 48- to 72-hour period after brain death has occurred. These cardiac dysrhythmias, which are thought to follow the catecholamine surge–induced conduction system necrosis, are frequently resistant to antiarrhythmic therapy. Similarly, acid-base and electrolyte abnormalities are thought to contribute and predispose to the development of these dysrhythmias. After correcting the various electrolyte or acid-base abnormalities, standard antiarrhythmic therapy for ventricular rhythm disturbances (lidocaine or amiodarone) or supraventricular dysrhythmias (amiodarone) should be considered similar to any other rhythm management in a critically ill patient. With brainstem vagus nerve disruption, the bradyarrhythmias that are frequently seen do not respond to atropine, and isoproterenol or epinephrine is needed. Realizing that a small percentage of organ donors sustain cardiac arrest during maintenance, it is crucial to institute full advanced cardiac life support because it has been shown that the recovery of cardiac function of the potential donor can result in successful transplantation.[17]

Respiratory Management

Similar to the previously defined cardiac management approach, an understanding of the pathophysiology of

donor lung injury is useful in facilitating a more optimal management strategy. The pathophysiology of donor lung injury is complicated by multiple factors. Frequently, there is an unknown history of occupational lung injury, infectious disease, or tobacco use. The causative brain death event is commonly associated with aspiration, pulmonary contusion, and the effects of resuscitation for shock patients. Mechanical ventilation is associated with multiple pulmonary problems, including the newly recognized barotrauma/volutrauma, oxygen toxicity, and the development of nosocomial pneumonia.

These events are superimposed on the brain death–induced neurogenic pulmonary edema related to the sympathetic surge that occurs with herniation. Intense catecholamine constriction increases systemic vascular resistance and decreases cardiac output, with a resultant increase in left atrial pressures. Coincidentally, the sympathetic surge increases venous tone, facilitating venous return and increasing pulmonary artery pressures, resulting in a substantial degree of circulating blood volume in the pulmonary capillary bed. The combination of augmented venous return and an increased left atrial pressure precipitates a transient massive increase in hydrostatic pressure with structural damage to the capillary endothelium.[54] Sympathetic alterations of the pulmonary capillary permeability also are thought to contribute to neurogenic pulmonary edema.[2]

More recently, it has been recognized that brain death results in an intense inflammatory response in the lung secondary to elevated levels of circulating cytokines. Tumor necrosis factor-α and interleukin-1 are thought to activate endothelial cells to express adhesion molecules and mediate the production of interleukin-8. This neutrophil activator subsequently binds to endothelial cells and facilitates the migration of inflammatory cells and mediators into the interstitium and alveolar spaces, releasing reactive oxygen species and proteolytic enzymes. The extent to which this intense inflammatory response is present in the donor lung and correlates with recipient outcome is best exemplified by reports defining the inflammatory response of brain death via open lung biopsy and bronchoalveolar lavage. In a report of patients with nontraumatic causes of brain death, Fisher and colleagues[18] showed that there was a dramatic increase in the neutrophil concentration and interleukin-8 signal in biopsy specimens and lavage. This increase strongly suggested that there was an acute systemic inflammatory response to brain death that resulted in the release of proinflammatory mediators from the brain causing injury to the lung before transplant. The same authors performed a subsequent study correlating the extent of inflammation in the donor with recipient outcome.[19] The degree of neutrophil infiltration and interleukin-8 expression in the donor correlated with a degree of impairment in graft oxygenation, the development of severe early graft dysfunction, and early recipient mortality. This study emphasizes further that there is a preexisting subclinical inflammatory response in the lung after brain injury in addition to the multiple other phenomena that can affect pulmonary function.[19]

The respiratory management of the brain-dead donor can be dichotomized into two patient groups: patients with severe lung injury that would preclude lung use and patients with either ideal or marginal lungs. The ventilator management of the former patients is similar to the ventilator management generically used in the intensive care unit for either diffuse or focal lung injury. The goals are to maintain adequate tissue levels of oxygenation and to ensure that mean airway pressures do not impair venous return, which can jeopardize cardiac output and flow to the various organs. Recognizing that the lungs will not be used in transplantation, a higher mean airway pressure or tidal volume may be accepted to facilitate better oxygenation. In the latter circumstance of an ideal or marginal lung, there is significant potential for deterioration in the ideal lung, and the capacity exists to manage a marginal lung that can be used for transplantation.

Table 6-3 outlines a generic approach to the respiratory management of potential organ donors. The goals of respiratory management should be to ensure continued stability and suitability of the ideal lung and to apply intensive care respiratory management to facilitate the use of marginal lungs. It has been reported that implementing a standardized approach to the management of respiratory function in a potential donor has resulted in the procurement and successful transplantation of lungs that were initially deemed unsuitable. Current challenges include developing indices to quantify and qualify the degree of lung injury, identify reversible causes of lung dysfunction, and define interventions to modify the unacceptable lungs successfully. This approach to maximizing lung transplantation is best exemplified in the report by Gabbay and colleagues[22] of applying intense donor pulmonary management to marginal lung donors, which resulted in significant improvement and successful transplantation with outcomes indistinguishable from ideal lungs. The management approach consisted of mechanical ventilation with positive end-expiratory pressure recruitment maneuvers, chest physical therapy, attention to fluid balance, antibiotic administration, and bronchoscopy.[22]

The traditional approach to mechanical ventilation for potential lung donors has been a high tidal volume (10 to 15 mL/kg) and the initial use of positive end-expiratory pressure. The combination of improved oxygenation and

Table 6–3 Respiratory Management

Goals of Mechanical Ventilation
Fraction of inspired oxygen 0.40
Partial pressure of arterial oxygen >100 mm Hg or oxygen saturation >95%
Partial pressure of arterial carbon dioxide 35-40 mm Hg
Arterial pH 7.35-7.45
Tidal volume 8-10 mL/kg of predicted body weight
Positive end-expiratory pressure 5 cm H_2O
Static airway pressure <35 cm H_2O
Bronchoscopy
Evaluate anatomy
Assess for foreign body and assist in removal
Define and locate aspirated material, secretions, or apparent infection
Clearance of Secretions
Pulmonary hygiene
Prevent atelectasis with use of suction, percussion, postural drainage, and lung expansion techniques
Fluid Management
Central venous pressure 6-8 mm Hg
Pulmonary capillary wedge pressure 8-12 mm Hg
Anti-infective Therapy
Use of antibiotic agents based on Gram strain of aspirated secretions

improved lung appearance on chest radiography probably reflects hyperinflation. The recognition that lung injury and inflammation may occur as a result of the use of mechanical ventilation consequent to volutrauma and shear injury to the lung has defined a strategy for alveolar recruitment that seeks to minimize ventilator-associated lung injury. Positive end-expiratory pressure should be applied judiciously, and end-expiratory plateau pressures should be limited to less than 30 to 35 cm H_2O. The high minute ventilation and accompanying low carbon dioxide frequently used to treat elevated ICP can be normalized further, preventing volutrauma to the potential donor lung. Oxygen toxicity should be minimized by using the lowest level of F_{IO_2} to achieve arterial saturations of 90% or greater. Bronchoscopy should be performed in all potential organ donors who are candidates for lung transplantation to inspect the anatomy, remove foreign bodies, and assist in the assessment of donors with abnormal gas exchange and unilateral lung disease. In this circumstance, bronchoscopy and chest radiography can facilitate the evaluation to allow for use of the contralateral lung.

Atelectasis and pulmonary edema consequent to aggressive fluid resuscitation are probably the two most correctable causes of hypoxia that preclude the use of lungs for transplantation. Strategies targeted at ventilator management focused on lung expansion, early bronchoscopy, frequent suctioning and pulmonary toilet, and judicious volume resuscitation have been reported to increase the rate of lung procurement.[20,22] Adjudication of the competing fluid requirements may require the placement of a PAC as previously defined. Pulmonary capillary wedge pressure of 8 to 10 mm Hg with a central venous pressure of 6 to 8 mm Hg should minimize the accumulation of extravascular lung water. In patients who are edematous with high filling pressures, diuretics may be necessary. Large doses of corticosteroids (methylprednisolone, 15 mg/kg), which are part of the hormonal resuscitation protocol, have been shown to stabilize lung function and facilitate the procurement of lungs that were previously defined as unsuitable.[21] In addition to diuretics, albuterol has been shown in animal studies to facilitate the clearance of pulmonary edema and should be considered in patients with pulmonary edema.[74] Similar to the echocardiographic evaluation of cardiac donors, no procurement decision should be made based on the initial lung evaluation, and suitability should be defined only after therapeutic attempts to optimize the pulmonary status have been exhausted.

Renal Management

Kidneys are extremely susceptible to injury in the potential organ donor for multiple reasons. The initial trauma and hypovolemia with hypotension are associated with a significant incidence of acute kidney injury that has been estimated to occur in 31% of severe trauma cases.[45] Other associations with trauma include elevated intra-abdominal pressure related to an abdominal compartment syndrome impairing renal blood flow, acute crush injuries with rhabdomyolysis, and the use of contrast agents for vascular imaging in trauma patients. Although a more recent study suggested no long-term difference in the recipients of donors who received contrast agents, this study did not address the risk of increased delayed graft function.[30] Brain death and the associated catecholamine surge and inflammatory response can contribute to the renal dysfunction in the potential organ donor.

The variables during the care of adult donors that can influence the outcomes of renal transplantation have been reviewed by Powner.[63] Factors that were most amenable to intensive care unit management included increasing urine output to greater than 100 mL/hr at least during the hour before explantation and returning the creatinine level to match the original serum concentration when the patient was admitted. Although there seems to be benefit in maintaining a urine output of at least 100 mL/hr, there does not seem to be any benefit of an extremely high urine output (>300 mL/hr).[42] Similarly, it has been reported that an improving serum creatinine level of less than 2 mg/dL seems to exert a favorable effect on renal graft function in recipients.[42] Other studies have shown that a serum creatinine level that exceeded 1.5 mg/dL just before explantation shortened the time to graft failure.[59] Elevated serum creatinine in donors was associated with worse renal function 2 or 3 years after transplantation.[37] Ensuring the adequacy of intravascular volume and maintaining appropriate perfusion pressures and flow to the kidneys are the most important donor management factors that govern the success of renal transplantation, and these can be influenced by intensive care unit management.

Supportive Care

Potential organ donors warrant the same level of aggressive intensive care management that is provided to other patients in a critical care unit. This care involves frequent and ongoing assessments of hemodynamic status, respiratory function, and metabolic parameters. Diabetes insipidus is common in potential organ donors secondary to the absence of vasopressin after pituitary destruction during the herniation process. Complications of diabetes insipidus include intravascular volume depletion, hyperosmolality, and electrolyte abnormalities. Diabetes insipidus frequently needs to be differentiated from mannitol-induced, diuretic-induced, or hyperglycemia-induced osmotic diuresis. Table 6-4

Table 6–4 **Evaluation of Polyuria in a Potential Organ Donor**

	Diabetes Insipidus	Mannitol	Hyperglycemia
Serum sodium	≥150 mEq/L	≥150 mEq/L	≥150 mEq/L
Serum osmolarity	≥300 mOsm	≥300 mOsm	≥300 mOsm
Serum osmolar gap	Normal	>10-15 mOsm	>10-15 mOsm
Urine output	≥300 mL/hr	≥200 mL/hr	≥200 mL/hr
Urine sodium	<10 mEq/L	50-70 mEq/L	50-70 mEq/L
Urine osmolarity	<200 mOsm/L	≥300 mOsm/L	≥300 mOsm/L
Urine specific gravity	≤1.010	≥1.020	≥1.020
Urine glucose	Absent	Absent	Present

presents an overview of the differential diagnosis and associated laboratory values of polyuric patients. In most instances, it is appropriate to match the urine output milliliter for milliliter with 5% dextrose in water and monitor the blood glucose closely. When the urine output exceeds 250 mL/hr, it is necessary to give either arginine vasopressin or desmopressin acetate (DDAVP). The former has an antidiuretic effect in addition to vasoconstrictive properties, whereas the latter is purely an antidiuretic. Although DDAVP may be given subcutaneously, intramuscularly, or intranasally, the intravenous route is recommended for a potential organ donor. Judicious monitoring of urine output, serum sodium, and hemodynamic parameters is necessary when using DDAVP. Frequently, the use of 5% dextrose in water for the treatment of hypernatremia, catecholamines, glucose-containing solutions, and corticosteroids produces hyperglycemia in a potential donor. Hyperglycemia has increasingly been recognized as a contributor to inflammation and an impaired immune response. It is similarly likely that these mechanisms are applicable to a potential organ donor. Blood glucose should be controlled with an insulin infusion when necessary.

The combination of brain injury with the release of thromboplastin, ongoing hemorrhage, transfusions, hypothermia, acidosis, and the dilution of coagulation factors frequently conspires to produce coagulopathy in a potential organ donor. Similar to the approach used in other critically ill patients, packed red blood cells and fresh frozen plasma should be administed to achieve a hematocrit of approximately 30% and control of coagulation parameters. Goals should include an international normalized ratio less than 2 and platelet count greater than 80,000/mm^3. Thermoregulation is frequently impaired in potential organ donors. The combination of hypothalamic-pituitary destruction and peripheral paralysis impairs the ability to shiver or vasoconstrict. Impaired thermoregulation is compounded by the use of unwarmed fluids and blood products. Hypothermia can exaggerate the previously described coagulopathy and predispose to cardiac rhythm disturbances and cardiac dysfunction. The donor should have a core temperature of at least 35°C with active and passive rewarming as necessary.

SUMMARY

A standardized approach to declaration of death and management of potential organ donors ensures that the greatest number of organs can be recovered in the best possible condition to provide optimal outcome for recipients. The management of a potential organ donor influences the medical management of seven solid-organ recipients and requires the same level of vigilance and attentiveness provided to other critically ill patients.

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Chapter 7

Medical Evaluation of the Living Donor

Dicken S. C. Ko • Francis L. Delmonico

It has been more than half a century since the first successful renal transplant was performed with the donation of a kidney from one identical twin to another who had end-stage renal disease.[56-58] The presence in most normal individuals of two kidneys—each with a physiological reserve capable of providing four to five times the minimal required function—has led to the acceptance of renal transplantation using living related or unrelated volunteers as organ sources.[22,86] The success of live kidney donor transplantation has been so overwhelming that in 2000 to 2004 in the United States, the number of live donor kidney transplants surpassed that of cadaver donors.[63] In many parts of the world where cadaver donor kidney transplants are still limited, live donor kidneys are the most common source of organs for transplantation.[47] The advantages of live donor kidney transplantation include earlier transplantation and excellent long-term survival.

Despite reduced morbidity and mortality of the donation procedure, it is important to discern patients who are not candidates for donation because it might have an impact on their immediate and future health.[2,7,54,60,61,83] Although predicting future health is imprecise at best, clinical experience suggests that there are reasons why it is not medically advisable to donate an organ. However, singular abnormalities, such as well-controlled hypertension or obesity, are no longer considered absolute contraindications to organ donation today.[1,17] Live kidney donors present unique ethical, legal, and social implications[82,91,92] that must be addressed carefully to protect the health and rights of the donor.

JUSTIFICATION FOR LIVE KIDNEY DONATION

Among the many reasons that may be cited for the continued use of living related donors, the most important has been the more favorable results that can be achieved with a physiologically perfect kidney that also is biologically matched. The morbidity and mortality after cadaver donor transplantation were so great until the early 1980s that many dialysis patients were hesitant to consider transplantation unless a related donor was available.[36] With the introduction of calcineurin inhibitors, monoclonal and polyclonal antibody immunosuppression, and other new immunosuppressive drugs into clinical regimens, the historical gap in graft survival between living related and cadaver donor renal transplantation narrowed considerably. This change led some groups to conclude that living related donor renal transplantation might no longer be justified.[84] Living related donor grafts still have a 10% to 12% better survival rate at 1 year and a significantly higher probability of function thereafter.[13] Almost all transplant units continue to recommend live donor renal transplant if suitable individuals volunteer.[18,23]

Because the improved results using familial donors were believed to be directly related to the degree of histocompatibility between donor and recipient, living unrelated donors were historically not thought to provide any biological advantage over cadaver donors. The experience of using living unrelated kidneys in transplantation has shown, however, that such organs have a graft survival profile that approaches that of related donors and is even better than that of parental donors.[37,85] Between 1988 and 1996, the number of living unrelated donor transplants in the United States increased from 4.1% to 14.2% of living donors.[29] Later Organ Procurement and Transplantation Network (OPTN)/United Network for Organ Sharing (UNOS) reports further confirm a trend to use living unrelated donors, which account for 22.6% of the living donor pool.[40]

Even with the current widespread application of calcineurin inhibitors and monoclonal and polyclonal antibody immunosuppression, there is a persisting biological advantage of living donor kidneys (living related donor or living unrelated donor) over cadaver donor allografts. Although short-term graft survival after transplantation from both donor sources is excellent, the 5-year success rate of greater than 80% attained using living donor kidneys exceeds any reported cadaver donor results by 10% to 15%.

Another justification for using living donors is that the timing of the operation can be planned, limiting waiting time on dialysis. This aspect is relevant for socioeconomic reasons. Because successful transplantation allows more

complete patient rehabilitation,[27,28,53] this approach proves to be approximately one third as expensive as long-term dialysis.[43] Of greater importance is the ability to perform the transplant when the recipient is in optimal medical condition. This ability is particularly pertinent for diabetic patients, whose condition may deteriorate rapidly on dialysis. Finally, there is the risk that the patient may develop antibody to HLA antigens (see Chapter 10) during prolonged dialysis, especially if intermittent blood transfusions are required. As a result of such allosensitization, a negative crossmatch donor kidney becomes increasingly difficult and sometimes impossible to find even with today's emergence of desensitization protocols.

The final reason for the continued expansion of living donor transplantation is the insufficient supply of cadaver donor organs required to fulfill the needs of renal failure patients awaiting transplantation.[16] Because the results of kidney transplantation have improved dramatically, increasing numbers of patients are being placed on waiting lists. The supply of kidneys has increased minimally, however.[69] Figure 7-1 shows a flow chart outlining the projected need for donor organs, assuming no net yearly increase in numbers of patients on dialysis. For each 1 million population, approximately 75 to 80 renal transplants would have to be performed annually to keep pace with the more than 100 new patients diagnosed with end-stage renal disease and previous transplant recipients whose allografts eventually fail. Even in areas with outstanding cadaver donor retrieval rates or with less stringent criteria for donor selection, the number of potential recipients greatly exceeds the supply of donor kidneys. A steadily growing population of patients is being maintained on dialysis in most areas of the world.

Despite these compelling reasons for using living donors, the procedure could not be justified if unacceptable morbidity or mortality were to be incurred by the donor. Generally, a specific medical treatment is selected on the basis of a balance in favor of its beneficial effects versus the potential adverse effects. The concept of removal of an organ for transplantation is unique among major surgical procedures in that it exposes the healthy donor to the risks of surgery solely for the benefit of another individual. This concept has been evaluated carefully not only by the medical profession but also by the courts and by life insurance carriers. Some courts have ruled in favor of donation, even by a minor, on the grounds that the donor not only would benefit psychologically and spiritually from the act of charity but also might be psychologically harmed if prevented from donating, at little risk, when the life of a close relative is at stake (see Chapter 39).

With the extension of minimally invasive techniques to living kidney donation, the potential adverse impact of the operation has become less significant. The major advantages to the donor are decreased morbidity of the surgery and quicker return to normal daily activities, including earlier return to work. The worldwide results for laparoscopically removed kidneys are now comparable to the results achieved after transplantation of organs procured through the classic open incision.[10,11,21,39,78,90]

INITIAL DONATION PROCESS

The decision to donate a kidney for the benefit of another individual is one that is arrived at voluntarily by the donor, without coercion and without financial remuneration. The process begins with education when the patients and their family and friends learn about the health care risks of end-stage renal failure. The clearest and most consistent message that patients and family members receive is that the best form of long-term therapy for end-stage renal disease is kidney transplantation. The sources of donor organs are discussed in detail, with particular attention given to the different classifications within live and cadaver donors.

The obligation of the transplant health professional to the potential live donor, independent of the recipient's health condition, is to ensure that the donor fully and undeniably understands the immediate and long-term risks and benefits of organ donation. Because of the unique nature of live donor kidney transplantation, in which there is defined health benefit for the recipient, and the donor bears only the burden of being subjected to a medically unwarranted surgery

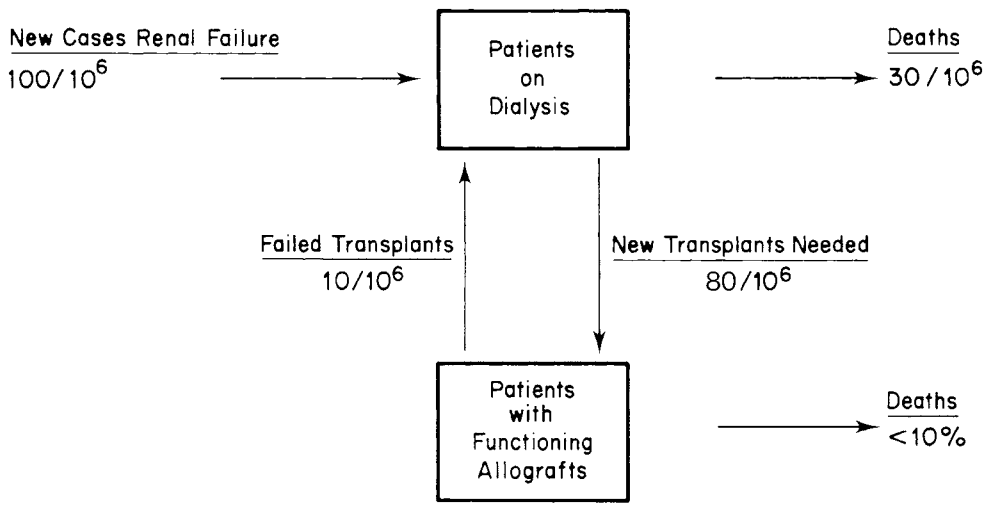


Figure 7–1 Flow chart depicts the annual renal transplant rate required to maintain a stable dialysis population. An estimated total of 75 to 80 transplants per 1 million population is projected if previous failed allografts and new cases of renal failure are included.

without immediate or long-term health improvements, it is important to address "conflict of interest" in management of the recipient and the donor by one team.

From the outset, the donor evaluation must be done by an independent health care team that can provide unbiased medical decision making and advice. Some centers have internists and surgeons evaluate these donors independently and arrive at a decision as to whether they are fit for the donation process, whereas other centers have devised parallel processes in which such evaluations and discussions about donation and transplantation are presented by a team to the family and friends. Approaches to the potential live donor usually include a comprehensive medical team that consists of primary care physicians, nephrologists, psychiatrists, transplant surgeons, coordinators, social workers, and financial coordinators. Only by this process of education and informed consent can a donor truly determine whether to proceed or not with donation. In return, this process allows medical professionals ample opportunities to arrive at a judgment of whether the donor is suitable to donate even if the donor already has committed to doing so.

As more allocation protocols develop to help end-stage renal disease patients obtain organ transplants, we face even more complexities that make the education and evaluation challenging. If a donor-recipient pair is blood group incompatible, the individuals might go on a separate regional list, where they might have opportunities to exchange donors and recipients with other incompatible pairs. The concept of increasing live donor transplantation in this manner benefits both pairs. Since the emergence of this concept in the early 2000s, some regions of the United States and other countries participate in paired-exchange programs that administrate the matching of ABO-incompatible pairs.

Immunological sensitization of a recipient toward potential donors and the process of desensitization also have created more challenges for a potential live donor renal transplant. Many centers have adopted new immunosuppressive strategies, such as plasmapheresis of recipients, to permit long-term allograft survival. However, the cost of more extensive immunosuppressive therapy, coupled with an increased incidence of antibody-mediated allograft rejection, and a more complicated postoperative course have made some donor-recipient pairs reconsider their options and risks during the evaluation process.

The results of ABO-incompatible renal transplants in Japan have been encouraging and prompted some centers to adopt such programs as an alternative for the recipient to have access to kidney transplantation. Such highly specialized programs, which subject the donor kidney to a higher risk of immunological loss and the recipient to more intense immunosuppressive therapy, must be considered carefully. These risks must be disclosed not only to recipients but also to donors in the evaluation process so that they, too, have an opportunity to evaluate the risk of their donated kidney being rejected.

RISKS TO THE DONOR

Because of the unusually careful follow-up of thousands of renal donors, in addition to the extensive information available from other unilaterally nephrectomized cases,[61] the long-term risks of living kidney donation can be assessed precisely.[44] Table 7-1 lists the most common complications observed. Survival studies indicate that the 5-year life expectancy of a unilaterally nephrectomized 35-year-old male donor is 99.1% compared with a 99.3% normal 5-year life expectancy; this has been compared with the risk incurred in driving a car 16 miles every working day. The quality of life after kidney donation has been reported in 979 patients who had donated a kidney for transplantation.[44] Most of the responders had an excellent quality of life.[59,60] Multivariate analysis of individuals who did not respond favorably identified the following two factors for a negative psychosocial outcome: relatives other than first degree and recipients who died within 1 year of transplantation. In an updated survey of major life insurance companies, it was found that 100% now accept applications from kidney donors after nephrectomy, assuming the remaining renal function is normal.[44,80,81] Of these companies, 94% do not consider the otherwise healthy donor to be at increased risk for shortened survival or medical problems; only 2% indicated they would raise the premium for such an individual.

Despite studies of extensive renal ablation in rats, which have shown that glomerular hyperfiltration in the remaining kidney tissue can produce progressive sclerosis and deterioration in renal function,[9] such data correlation in humans has not been found. Concerns had been raised that healthy human donors might develop hypertension and renal dysfunction years after unilateral nephrectomy. However, 20-year follow-up studies of hundreds of living donors have

Table 7–1 Complications of Living Donors

Procedure Complications	Incidence (>2000 Cases) (%)
Aortogram	
Prolonged discomfort	<1
Femoral thrombosis or aneurysm	<1
Intraoperative	
Splenic laceration	<1
Pancreatic injury, pseudocyst	<1
Nephrectomy Wound	
Prolonged discomfort	3.2
Infection	2.1
Hernia	<1
Hematoma	<1
Pulmonary	
Atelectasis	13.5
Pneumothorax or pneumomediastinum	3.2
Pneumonitis or pleural effusion	4.3
Urinary Tract	
Infection	4.5
Retention	3
Acute tubular necrosis	<1
Late proteinuria	3
Other	
Prolonged ileus	5.2
Thrombophlebitis with or without pulmonary embolus	1.9
Peripheral nerve palsy	1.1
Hepatic dysfunction (late)	<1
Acute depression	<1
Hypertension (late)	15*

*Similar to general population.[50]

been unable to identify any convincing evidence of long-term functional abnormalities associated with unilateral nephrectomy.[2,3,6,60,61]

A 2002 survey of 234 UNOS-listed kidney transplant programs, to which 171 responded, sought to determine current living donor mortality after donor nephrectomies (open nephrectomy, hand-assisted laparoscopic nephrectomy, and non–hand-assisted laparoscopic nephrectomy). It was discovered that between January 1, 1999 and July 1, 2001, these centers performed 10,828 living donor nephrectomies: 52.3% open nephrectomy, 20.7% hand-assisted laparoscopic nephrectomy, and 27% non–hand-assisted laparoscopic nephrectomy. Two donors (0.02%) died as a result of surgical complications, and one was in a persistent vegetative state (all after laparoscopic nephrectomy).[54] The current estimated mortality of donor nephrectomy is 1 in 3000 donors. The potential donor must understand that the risk of mortality is *not* zero as they weigh their decision to undergo the surgery.

What is the likelihood of renal disease occurring in a donor after kidney donation? One of the most comprehensive reviews of this was the OPTN database in the United States to determine the number of renal waitlist candidates who previously had been living donors.[24] The living renal donors in the OPTN database were cross-referenced against the renal waitlist. Fifty-six previous living donors were identified as having been subsequently listed for cadaver donor kidney transplantation (43 have received transplants, 36 currently have functioning grafts, 1 died after transplantation, and 2 candidates died while waiting). The numbers reported underestimate the actual number of living donors with compromised renal function or actual renal failure because they include only patients listed for a kidney transplant. Patients who are not candidates because of concomitant illness or a variety of other factors are omitted by this analysis. To determine risk factors for postdonation renal failure, long-term living donor follow-up data are needed.

Despite these risk considerations, living donors continue to represent a significant proportion of the total donor pool. The percentage of transplanted kidneys obtained from this source varies, accounting for nearly all renal transplants in areas where cadaver donor transplantation is unavailable, but for less than 5% in other areas. In the United States, approximately 50% of transplanted kidneys are currently obtained from living donors,[63] and in 2005 the number of living donor kidneys transplanted in the United States exceeded the number of cadaver donor kidney transplants for the first time.

PSYCHOLOGICAL ASPECTS OF DONOR SELECTION

The current trend of increased worldwide use of live donor kidneys for transplantation points out the special nature of this form of medical care. First, we must do no harm according to the Hippocratic Oath. However, Hippocrates also alluded to the fact that the art (of medicine) is long, and judgment is difficult. In the setting of live organ donation, an evaluation of the psychological and ethical aspects is a crucial part of the comprehensive medical review of the donor.[20]

Throughout the evaluation, at least some unavoidable family pressure to donate must exist despite the physician's attempts to ensure that the final decision is voluntary, reasoned, and based on full awareness of relevant information. Scrutiny of the decision process of familial donors has revealed that most donors make an immediate decision when first contacted, and this decision precedes the acquisition of further scientific data required for truly *informed consent*. Long-term follow-up of familial donors indicates that they continue to believe they made a correct and an informed decision and would do it again if the opportunity were available.[79] These observations do not diminish the responsibilities of the renal transplant team to supply all relevant medical facts to the potential donor, but the nature of this decision process is complex.

SELECTION OF A POTENTIAL LIVING DONOR

In April 2004, an international consortium of more than 100 leading kidney transplant physicians and surgeons from 40 countries met in Amsterdam to discuss the standard of care for live donors. In conjunction with the World Health Organization, this forum proposed a set of standard recommendations that the World Health Organization could assist to implement worldwide (Table 7-2). A position paper was published in *Transplantation* in 2005 that describes the standards for patients who are potential altruistic kidney donors. Although these recommendations are derived from the best evidence-based medicine available today, they are still only a set of guidelines, not mandatory regulations.[17,26]

Table 7-3 lists considerations for routine evaluation of the potential living kidney donor. Table 7-4 lists screening questions for nondirected altruistic donation. The basic evaluations are the standard history and physical examination and hematological and biochemical profiles. Potential donors remaining after the initial screening process are evaluated meticulously and repeatedly to confirm excellent general health and bilateral renal function. Typical evaluations performed[17,26,45,46] are listed in Table 7-3. Many of the studies are directed toward detection of unsuspected extrarenal pathology. This medical evaluation may reveal significant but treatable problems of which the donor was unaware.

This intensive medical evaluation may exclude the volunteer as a donor, but also is designed to lead to an uncomplicated operative procedure and postnephrectomy recovery period for selected donors. The final studies are concerned with the quality of renal function and the clarification of any anatomical abnormalities in either kidney. It must be determined that the nondonated kidney is normal. This determination is especially relevant when the renal failure in the potential donor's relative has resulted from causes that may be hereditary (e.g., diabetes, polycystic disease, or hypertension).

Family History of Inheritable Diseases

In the case of diabetes, further evaluation, including glucose or cortisone-glucose tolerance tests, may be undertaken to identify any subclinical evidence of diabetes. Because of the hereditary nature of polycystic renal disease, cadaver donors often are needed for these patients. If a familial donor is considered, selection may be limited to relatives older than age 30 years, in whom latent polycystic disease can be ruled out by ultrasonography or computed tomography (CT). Genetic

Table 7–2 **Amsterdam Forum Guidelines: Donor Evaluation**

Before donation, the live kidney donor must receive a complete medical and psychosocial evaluation, receive appropriate informed consent, and be capable of understanding the information presented in that process to make a voluntary decision.
All donors should have standard tests performed to ensure donor safety.

Hypertension

Patients with BP >140/90 mm Hg by ABPM are generally not acceptable as donors.
BP should preferably be measured by ABPM, particularly among older donors (>50 years old) and donors with high office BP readings.
Some patients with easily controlled hypertension who meet other defined criteria (e.g., >50 years of age, GFR >80 mL/min, and urinary albumin excretion <30 mg/day) may represent a low-risk group for development of kidney disease after donation and may be acceptable as kidney donors.
Donors with hypertension should be regularly followed by a physician.

Obesity

Patients with body mass index >35 kg/m^2 should be discouraged from donating, especially when other comorbid conditions are present.
Obese patients should be encouraged to lose weight before kidney donation and should be advised not to donate if they have other associated comorbid conditions.
Obese patients should be informed of acute and long-term risks, especially when other comorbid conditions are present.
Healthy lifestyle education should be available to all living donors.

Dyslipidemia

Dyslipidemia should be included along with other risk factors in donor risk assessment, but dyslipidemia alone does not exclude kidney donation.

Acceptable Donor Renal Function

All potential kidney donors should have GFR estimated.
Creatinine-based methods may be used to estimate the GFR; however, creatinine clearance (as calculated from 24-hour urine collections) may underestimate or overestimate GFR in patients with normal or near-normal renal function.
Calculated GFR values (MDRD [Modification of Diet in Renal Disease study] and Cockcroft-Gault) are not standardized in this population and may overestimate GFR.
GFR <80 mL/min or 2 SD below normal (based on age, gender, and body surface area corrected to 1.73/m^2) generally precludes donation.

Urinalysis for Protein

A 24-hour urine protein >300 mg is a contraindication to donation.
Microalbuminuria determination may be a more reliable marker of renal disease, but its value as an international standard of evaluation for kidney donors has not been determined.

Urinalysis for Blood

Patients with persistent microscopic hematuria should not be considered for kidney donation unless urine cytology and a complete urologic workup are performed.
If urological malignancy and stone disease are excluded, a kidney biopsy may be indicated to rule out glomerular pathology such as IgA nephropathy.

Diabetes

Individuals with a history of diabetes or fasting blood glucose ≥126 mg/dL (≥7 mmol/L) on at least two occasions (or 2-hour glucose with oral glucose tolerance test ≥200 mg/dL (≥11.1 mmol/L) should not donate.

Stone Disease

An asymptomatic potential donor with history of a single stone may be suitable for kidney donation if
- No hypercalcuria, hyperuricemia, or metabolic acidosis
- No cystinuria or hyperoxaluria
- No urinary tract infection
- Multiple stones or nephrocalcinosis are not evident on CT

An asymptomatic potential donor with a current single stone may be suitable if
- The donor meets the criteria shown previously for single stone formers and current stone <1.5 cm, or potentially removable during the transplant

Stone formers who should not donate are those with
- Nephrocalcinosis on x-ray or bilateral stone disease
- Stone types with high recurrence rates and are difficult to prevent (see text)

Malignancy

Prior history of the following malignancies usually excludes live kidney donation
- Melanoma, testicular cancer, renal cell carcinoma, choriocarcinoma, hematological malignancy, bronchial cancer, breast cancer, and monoclonal gammopathy

Prior history of malignancy may be acceptable for donation only if
- Prior treatment of the malignancy does not decrease renal reserve or place the donor at increased risk for end-stage renal disease
- Prior treatment of malignancy does not increase the operative risk of nephrectomy

A prior history of malignancy usually excludes live kidney donation, but may be acceptable if
- Specific cancer is curable, and potential transmission of cancer can reasonably be excluded

Urinary Tract Infections

Donor urine should be sterile before donation; asymptomatic bacteriuria should be treated before donation.
Pyuria or hematuria at the proposed time of donation is a contraindication to donation.
Unexplained hematuria or pyuria necessitates evaluation for adenovirus, tuberculosis, and cancer; urinary tuberculosis and cancer are contraindications to donation.

Table continued on following page

Table 7–2 **Amsterdam Forum Guidelines: Donor Evaluation—cont'd**

Live Unrelated Donors

Current available data suggest no restriction of live kidney donation based on the absence of an HLA match.
An unrelated donor transplant is equally successful to the outcome achieved by a genetically related family member, such as a parent, child, or sibling, who is not HLA identical to the recipient.

Determination of Cardiovascular Risk

Clinical predictors of an increased perioperative cardiovascular risk (for noncardiac surgery) by the American College of Cardiology/American Heart Association standards fall into three categories: major, intermediate, minor

- All major predictors—unstable coronary syndromes, decompensated heart failure, significant arrhythmias, and severe valvular disease—are contraindications to live kidney donation
- Most intermediate predictors—mild angina, previous myocardial infarction, compensated or prior heart failure, and diabetes mellitus—are contraindications to donation
- Minor predictors—older age, abnormal ECG, rhythm other than sinus, low cardiac functional capacity, history of stroke, and uncontrolled hypertension—warrant individual consideration

Assessment of Pulmonary Issues

Careful history and physical examination are the most important parts of assessing risk.
Routine preoperative pulmonary function testing is not warranted for potential live kidney donors, unless there is an associated risk factor, such as chronic lung disease.
Increased risk of postoperative pulmonary complication is associated with FEV_1 <70% or FVC <70% of predicted, or a ratio of FEV_1/FVC <65%.

Smoking Cessation and Alcohol Abstinence

Smoking cessation at least 4 wk before donation is advised based on recommendations for patients undergoing elective surgical procedures.
Alcohol should be avoided for a minimum of 4 wk to decrease the known risk of postoperative morbidity.
Cessation of alcohol abuse defined by DSM-III (*Diagnostic and Statistical Manual of Mental Disorders, Third Edition*): 60 g of alcohol/day sustained over >6 months

ABPM, ambulatory blood pressure monitoring; BP, blood pressure; CT, computed tomography; ECG, electrocardiogram; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GFR, glomerular filtration rate; HLA, human leukocyte antigen.

tests for the *PKD1* gene and various mutations are available to determine the existence of such genetic predisposition for the development of autosomal dominant polycystic kidney disease.

Hypertension

In the last 25 years, several studies have identified an increased incidence of hypertension in first-degree relatives of patients with renal failure. Potential donors from this pool should be screened carefully for hypertension.[41] The exact risks to this group have not been accurately quantified.[38] Patients with a resting blood pressure greater than 140/90 mm Hg generally are unacceptable as kidney donors.[64,87]

Potentially acceptable individuals for live kidney donation may have a history of mild hypertension that is easily controlled, but their glomerular filtration rate (GFR) should be greater than 80 mL/min and their urinary albumin levels less than 30 mg/day. Older patients (>50 years old) also can be acceptable if these criteria are met. Patients with mild hypertension who elected and are accepted for kidney donation should have close medical follow-up to ensure that their health status remains excellent. Despite these relaxations in acceptability of an individual who has mild hypertension for live kidney donation, the fact remains that the long-term risks are still not entirely delineated.

Obesity

Society faces increasing health-related problems stemming from cultural excesses and increasingly sedentary lifestyles. Obesity is defined as a body mass index greater than 30 kg/m^2; morbid obesity is defined as a body mass index greater than 35 kg/m^2. Obesity is associated with or causally related to comorbidities such as hypertension, cardiovascular diseases, hyperlipidemia, diabetes, and nonalcoholic fatty liver disease. Analogous to hypertension, the long-term risks of obesity and kidney donation are not well understood. In the short-term, the renal function after donation is similar to function in individuals who are not obese.[70] Although we always encourage weight loss and exercise programs for patients, the effectiveness and sustainability of such initiatives are often suboptimal. The fact that we encourage a patient to lose weight before kidney donation does not translate to a long-term adherence to their dietary and exercise regimen. The changes that occur for an individual to lose weight before consideration for kidney donation have to reflect changes in their established lifestyle.

Nephrolithiasis

The incidence in the general population of nephrolithiasis is affected by multifaceted extrinsic and intrinsic factors because the disease varies according to geography, age, anatomical site, climate, water intake, diet, occupation, and genetics.[42,51,65,72-74] In 2005, long-term studies at the Mayo Clinic in Rochester, Minnesota, updated their initial 1979 study to evaluate the longitudinal epidemiology of stone disease in the general population.[51] The age-adjusted incidence of new-onset symptomatic stone disease for men was 105 (± 16.8) per 100,000 per year. For women, the corresponding rate was 68.4 (± 12.3) per 100,000 per year. A prospective

Table 7–3 Routine Screening for Potential Living Kidney Donors

Urinalysis
Dipstick for protein, blood, and glucose
Microscopy, culture, and sensitivity
Measurement of protein excretion rate
Assessment of renal function
Estimation/measurement of glomerular filtration rate
Blood tests
Hematological profile
Complete blood count
Hemoglobinopathy (where indicated)
Coagulation screen (PT and APTT)
G6PD deficiency (where indicated)
Biochemical profile
Creatinine, urea, and electrolytes
Liver tests
Urate
Fasting plasma glucose
Bone profile
Glucose tolerance test (if fasting plasma glucose >6-7 mmol/L)
Blood lipids
Thyroid function tests (if indicated)
Pregnancy test (if indicated)
Prostate-specific antigen (if indicated)
Virology and infection screen
Hepatitis B and C
Toxoplasma
Syphilis
HIV and HTLV 1/2
Malaria (where indicated)
Cytomegalovirus
Trypanosoma cruzi (where indicated)
Epstein-Barr virus
Schistosomiasis (where indicated)
HHV8 and HSV (where indicated)
Strongyloides (where indicated)
Typhoid (where indicated)
Brucellosis (where indicated)
Cardiorespiratory system
Chest x-ray
Electrocardiogram
Stress test
Echocardiography (where indicated)
Assessment of renal anatomy*

*Appropriate imaging investigations should allow confirmation of the presence of two kidneys of normal size and enable abnormalities of the collecting system and calcification or stone disease in the renal tract to be detected. They also must delineate the anatomy of the renal vasculature.

APTT, activated partial thromboplastin time; G6PD, glucose-6-phosphate dehydrogenase; HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; HHV, human herpesvirus; HSV, herpes simplex virus; PT, prothrombin time.

Table 7–4 Initial Screening Interview for Nondirected Donation

Medical/Personal History
How old are you?
Are you healthy and physically fit?
Do you have a history of cancer, heart disease, diabetes, kidney disease, or high blood pressure?
Do you take medications?
Have you undergone any previous operations?
Is there a history of kidney disease in your family?
Do you receive disability benefits for any reason?*
Do you live alone; are you married?
Where do you live?†
Knowledge about Nondirected Donation
How did you learn or hear about organ donation?
Do you understand that donating a kidney is not like donating blood?
Are you aware that the risks of donating a kidney include the possibility of dying?
Do you understand that there are risks to the recipient (i.e., that the kidney may be rejected)?
Do you understand that you cannot be paid money for being a donor?
Are you aware that several months may be necessary to determine your suitability as a donor by required clinical and psychological testing?
Do you understand that you cannot select your recipient, and that he or she will be from the list of patients who are already waiting?
Donor-Related Questions
Why do you wish to donate a kidney?
Have you told a member of your family that you wish to be a kidney donor?
Have you and your family considered the burdens associated with donation that could include out-of-pocket expenses for travel, physician appointments, and time out of work?
Is there a specific time frame to have your donor surgery performed?
Would somebody be available to assist you at home during your recovery from surgery?

*This does not rule out a donor a priori; the donor should be asked to elaborate.

†This affects costs and convenience associated with donation.

cohort study in southern Italy of more than 500 men who had no stones at study onset and were followed for 8 years found an overall 10.3% incidence of stone disease.[12] One can extrapolate correctly that the absence of nephrolithiasis at the time of donor evaluation does not imply the patient would not have symptomatic stone disease in the future.

When assessing the risks of kidney donation in the setting of a history of nephrolithiasis, the risks and risk factors for stone formation in that individual have to be delineated. Individuals who have more than one stone or who have metabolically active stone formation are not candidates for kidney donation. Candidates who have a genetic predisposition to stone disease, such as cystinuria or hyperoxaluria, also are unsuitable. Any metabolic abnormalities of hypercalciuria, hyperuricemia, and recurrent urinary tract infections pose higher risks for recurrent stone formation.[17] The true incidence of the risks and complications of recurring stone disease after live kidney donation is unknown and is likely to remain unknown because historically donors have been chosen who are otherwise healthy, non–stone formers.

As a general guideline, stone formers who should be declined as potential donors are individuals who have bilaterality of stone disease or have nephrocalcinosis on radiographic evaluation. In addition, patients contemplating live kidney donation who have stone types with etiological factors that predispose to recurrence should be ruled out. Although the guidelines have indicated that patients who have stone recurrence while on therapy should not be potential donors, some would argue that the need for metabolic therapy in stone disease already suggests either intrinsic or extrinsic factors to promote stone formation; these patients should not donate a kidney.[17]

Malignancy

The risk of malignancy increases with age. It is imperative especially that older donors be screened to exclude malignancy.[66-68] We have detected early breast, lung, and renal cell carcinomas in asymptomatic potential donors. All of these patients are alive without evidence of disease after appropriate treatment for the cancer.

Generally, potential donors with malignancies such as melanoma, renal cell carcinoma, transitional cell carcinomas, hepatocellular carcinomas, hematological malignancies, and lung cancers are precluded from kidney donation. If an individual has been treated for a low-grade curable tumor and has sustained disease-free survival constituting a cure, he or she can be evaluated to be a kidney donor.[17] The risks of transmission are not zero, however, and the discussion of risks and benefits must occur with the potential donor and recipient.

Infectious Disease

The transmissibility of disease is highly scrutinized because many forms of infectious disease may be benign in a nonimmunocompromised host. In a transplant recipient undergoing an immunosuppressive regimen, these disease entities might be detrimental, however, and sometimes can be fatal.

Human immunodeficiency virus (HIV) detected in an individual by antibodies should be confirmed by a neutralization test and a Western blot analysis. If such confirmations are obtained, the donor is ruled out for live kidney donation.[32,33] Hepatitis B virus (HBV) and hepatitis C virus (HCV) are of greater concern because the prevalence of both diseases is enormous worldwide. The transmissibility of hepatitis virus is well documented.[34,48] Individuals who are being evaluated undergo enzyme-linked immunosorbent assays to detect the presence of HBV and HCV antibodies. If the enzyme-linked immunosorbent assays are negative, the individuals are deemed suitable serologically for kidney donation. If these serological tests are positive, confirmatory RNA or DNA polymerase chain reaction quantitative assays for HCV and HBV can ascertain further the presence or absence of the virus. If the kidney recipient is positive for HCV, potential introduction of different genotypes of HCV can offset benefits that the new allograft might confer.[48] With HBV immunization over the past 2 decades, there are many recipients who have developed HBV surface antibodies potentially to protect the host against the virus. Organs from a donor who is either HBV surface antibody positive or HBV core antibody positive can be used successfully in an immunized recipient with remote risk of subsequent infection.[55]

Many other serological screens are commonly performed as part of the donor evaluation, including cytomegalovirus (CMV) screening. Because CMV is so common in the general population, with greater than 85% of the census population likely to have been exposed to CMV, prior infection of the donor is not of consequence most of the time. Nevertheless, the basis of such testing is to determine how best to use prophylactic antiviral agents in the recipients to prevent new infection after the exposure. Serological CMV-positive donors are not precluded from donation to a CMV-negative recipient. The agents for viral prophylaxis might be more intensive during the initial transplant and might extend for a long time to prevent acute infection with CMV in the recipient (see Chapter 29).[31,88,89]

There are regions throughout the world in which there are endemic infectious diseases that have geographical preponderances. Tuberculosis and strongyloidiasis in Asia, Chagas' disease in Central and South America, and schistosomiasis and malaria in Africa are examples of infectious diseases that have risks of transmission at the time of transplantation. It is important for potential donors to be screened for these disease entities in countries where they are endemic. In addition, travelers who have spent considerable time in those regions and are being considered as donors should have screening to exclude the presence of occult exposure.

Reports of rare infections that have had deleterious outcomes in the recipients have been published.[30,49] These publications heighten awareness that immunocompromised hosts respond differently and unpredictably to infectious agents. In a competent host, virus and other agents of disease might not be as virulent, but under heavy immunosuppression, especially with induction therapies, they can cause significant host insult. The fact that these opportunistic infections are rare can delay the diagnosis and treatment. Even if diagnosis is readily achieved, the lack of specific therapies can result in host morbidity and mortality.

ABO Grouping

Incompatibility of ABO between donor and recipient typically has resulted in irreversible rejection so that selection of major blood group compatibility usually is practiced. More recent reports have changed this paradigm, however, as the availability of intensive immunosuppression may have overcome some of these obstacles (see Chapter 22). Even without current ABO-incompatible protocols, historically, some groups have reported successful results after transplantation of blood group A_2 kidneys into group O recipients.[62] Approximately 20% of blood group A individuals are subtyped as A_2. The highly successful transplantation of A_2 kidneys into group O recipients has been explained by the low expression of A determinants in A_2 kidneys compared with A_1 kidneys. A_1 kidneys have been transplanted successfully into O recipients after elimination of ABO isoagglutinins by plasmapheresis and occasional splenectomy of the recipient. (See Chapter 22 for further discussion.)

HLA Typing

If several medically suitable relatives are available, the decision for donation can be made on the basis of histocompatibility testing—an HLA-identical sibling being the ideal choice (see Chapter 10). Not long ago, the decision to proceed with transplantation from a genetically unrelated living donor had been a difficult one to make because it was presumed that living unrelated donor allografts would have survival comparable to that of cadaver donor organs. It was initially proposed that living unrelated donor kidneys should be chosen only in exceptional cases.[8] As noted earlier, however, it is now clear that living unrelated donor kidneys provide significant physiological and consequently long-term survival advantages and are being accepted with increasing frequency. Nevertheless, results with HLA-mismatched living donors are generally superior to even well-matched cadaver donors, suggesting that the quality of the kidney and short preservation time outweigh the benefit of matching. Most centers continue to require that a stable emotional relationship

between donor and recipient exist and that donation for monetary compensation not be allowed.[14,15] In practice, living unrelated donor transplantation occurs most frequently in husband and wife pairs; some spouses have expressed the belief that they have "a right to donate."[82]

Age

Selection also may be determined on the basis of age (avoiding elderly volunteers or minors if possible) or on less objective factors, such as the special social obligations of a particular family member. In different countries, there are special legal constraints for donation by minors. If the only suitable donor has not attained the age of majority, it is necessary in the United States to present the medical facts to a court of law so that the necessity and advisability of donation by a minor can be scrutinized.

An analysis of the UNOS database has indicated that kidneys from live minors were transplanted more frequently to adult than to pediatric recipients. Only 12% of all recipients were identical twins. The report also concluded that the use of a minor donor provided no better long-term outcome than that expected from an adult donor. The resulting recommendation is that live organ donation from a minor should be considered only when there is no other living donor available, and all other opportunities for transplantation have been exhausted.[19] The longer the life expectancy after altruistic kidney donation, the more variables influence outcomes that simply are impossible to predict. Donation from minors is rightfully discouraged.

Normal Renal Function

Although there are many modalities to attempt to quantify renal function, the multifactorial nature of the tests may either overestimate or underestimate the true physiological function of the kidneys.[4] Individuals who are being evaluated for kidney donation should have "normal" renal function as determined by GFR. The GFR can be age dependent, however; it decreases as individuals age.[25,35,52]

Commonly, GFR is estimated by creatinine-based methods calculated from 24-hour urine collections.[75,76] Calculated methodologies, such as MDRD (Modification of Diet in Renal Disease study) and Cockcroft-Gault, are not standardized in normal individuals and can overestimate the GFR. Although isotopic estimation of GFR can be carried out to delineate the renal function further when GFR estimations are borderline, many centers do not do these routinely because of the additional costs and complexities associated with the study.[77]

The current guidelines define unacceptable renal function for donation as GFR less than 80 mL/min or 2 SD below normal when taking into consideration age, gender, and body surface area corrections.[5,45,46] Recipients of a kidney outside of these guidelines have a higher relative risk of graft loss.

Radiological Evaluation of a Living Donor

The optimal study for evaluation of kidney donors has become a subject of debate as newer, more sensitive multiplanar simulation technologies have become available using CT and magnetic resonance imaging (MRI). The ability to visualize data obtained with CT or MRI in a three-dimensional laboratory, carefully reconstructing the images isolating arteries, veins, or parenchymal structures, has immensely assisted surgical planning, particularly with laparoscopic donor nephrectomies (Fig. 7-2).

We retrospectively assessed the sensitivity and specificity of three-dimensional 16-section CT in the evaluation of vessels, pelvicaliceal system, and ureters in living renal donors, with surgical findings as the reference standard. Forty-six renal donors (18 men, 28 women; mean age 42 years) were examined with 16-section CT. Two blinded reviewers independently studied renal vascular and urographic anatomy of each donor CT scan by first using three-dimensional images alone, then transverse images alone, and finally transverse and three-dimensional data set. For three-dimensional images, transverse images, and transverse in conjunction with three-dimensional data sets, the respective sensitivity and specificity of CT in evaluation of accessory arteries and venous anomalies approached 98% for both compared with findings at surgery. We concluded that for focused assessment of renal vascular and urographic anatomy, review of three-dimensional data set alone provides high sensitivity and specificity with regard to findings seen at surgery.[71]

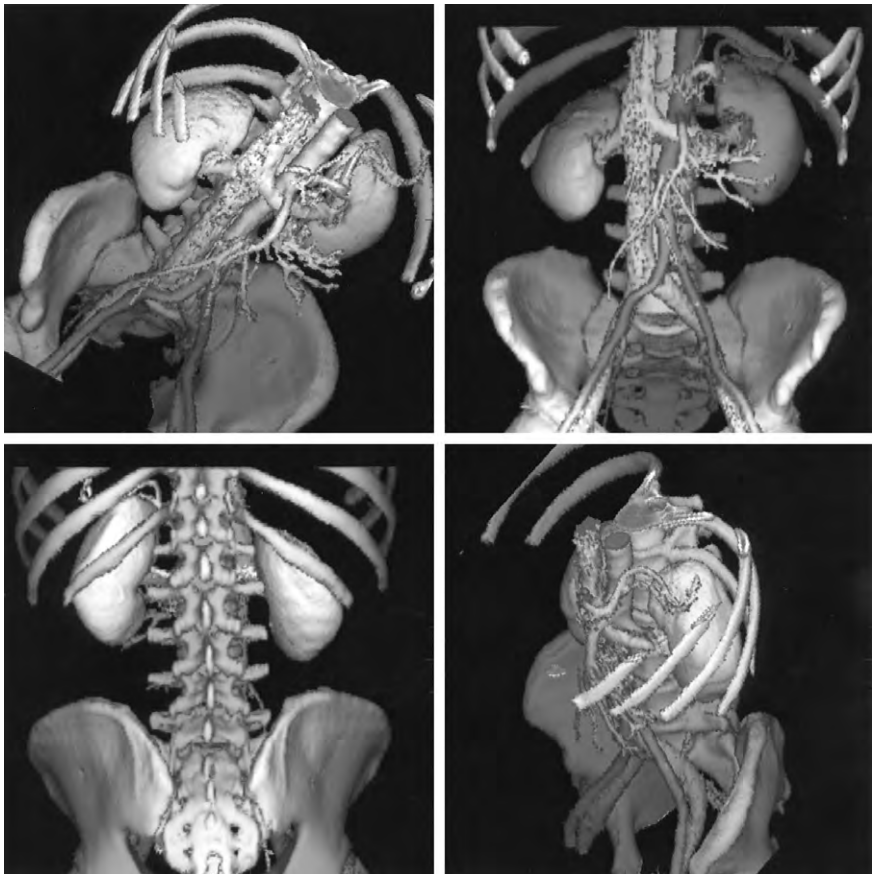
Despite the increasing availability of these noninvasive modalities and their lower risk potential for kidney donor patients, there are centers where these modalities are unavailable, and the traditional aortogram and selective renal angiography might be appropriate. In addition, the aortogram may be the only study that leads to the decision against accepting a particular donor, for example, when unilateral fibromuscular dysplasia is shown. CT or MRI with three-dimensional reconstruction has not been able to identify the more subtle forms of this lesion reliably. Alternatively, some groups traditionally have recommended digital subtraction angiography, which can be accomplished through peripheral venipuncture, avoiding some of the costs and morbidity of the aortogram. Although it is technically reasonable to transplant a kidney with multiple arteries, a kidney with a single artery is preferable. When either kidney is shown to be satisfactory, the left is usually chosen because the longer renal vein contributes to the technical ease of the nephrectomy and subsequent transplant.

SUMMARY

The success of organ transplantation and the shortage of suitable cadaver donor organs worldwide have shifted the paradigm of reluctance to use living donors for transplantation to widespread general acceptance. Of particular concern in this development are the expansion of acceptable criteria for donation and the interplay of physician-patient relationships that rightfully must be addressed before organ donation. Numerous worldwide consensus conferences in recent years have addressed these concerns. These sessions have resulted in proposed guidelines that are generally accepted by practicing communities.

These guidelines and consensus statements can help clinicians understand the nature of how best to proceed with evaluating a potential kidney donor. There are still significant uncertainties, however, regarding the use of donors with isolated medical abnormalities. As we develop a greater understanding and a better ability to predict outcomes, guidelines may be adjusted to reflect those observations and changes.

Figure 7–2 Three-dimensional reconstructed images of renal CT angiography. **A**, Images reconstructed with bone structures. Top images, anterior view; bottom images, posterior/oblique views. **B**, Images reconstructed without bone structures. Top images, posterior/oblique views showing early bifurcation of right renal artery; bottom images, anterior view showing orientation of left renal vein and superior mesenteric artery. (See color plate.)



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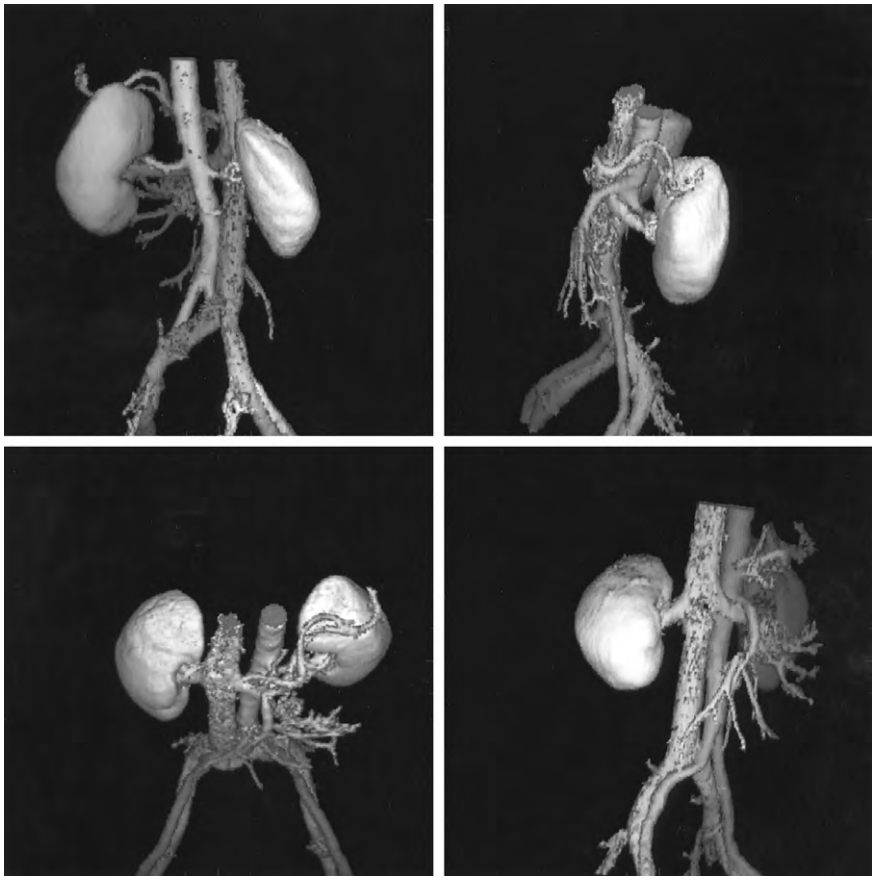

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Chapter 8
Donor Nephrectomy

Chapter 8A
Open Nephrectomy

A. Benedict Cosimi • Dicken S. C. Ko

Living Donor

Donor Nephrectomy
Postoperative Care and Complications
Open versus Laparoscopic Nephrectomy

Cadaver Donor

Removal of Kidneys Alone
Removal of Kidneys with Other Organs

LIVING DONOR

Donor Nephrectomy

The technical details of donor nephrectomy vary among different centers—some favor an anterior transperitoneal approach, whereas others favor the loin approach. Many centers have embarked on laparoscopic living donor nephrectomy as the standard for anatomically suitable living related donors and living unrelated donors (see Chapter 8B). If an open approach is to be used, the procedure described herein and in Figure 8-1 is our preferred technique. We emphasize the principles of (1) adequate exposure; (2) careful handling of the tissues, especially during periarterial dissection to limit vascular spasm; (3) preservation of adequate perihilar and periureteral fat to ensure adequate vascularity to limit the possibility of subsequent ureteral necrosis; and (4) maintenance of active diuresis, which makes prompt post-transplantation function more likely.

After induction of general endotracheal anesthesia, the donor is placed in the lateral position with the table flexed to extend the presenting flank (Fig. 8-1A). The incision is made anterior to and extending to or, if necessary, over the 11th or 12th rib. The latissimus dorsi muscle posteriorly and the external oblique muscle anteriorly are divided. This step exposes the periosteum and permits the subperiosteal removal of the rib (Fig. 8-1B) if necessary for adequate exposure. In nonobese patients, removal of the rib generally is not required, which results in less postoperative discomfort. The internal oblique and transverse abdominis muscles are divided with the underlying transversalis fascia to enter the retroperitoneal space. Care is taken to avoid entering the pleural or peritoneal cavities (Fig. 8-1C). The paranephric fat and Gerota's fascia, lying in the central part of the wound, are entered. The presenting surface of the kidney is dissected free of the underlying perinephric fat. No dissection is done in the renal hilus to protect the blood supply to the ureter. The renal vein is dissected to its junction with the vena cava, the adrenal and gonadal tributaries being ligated and divided (Fig. 8-1D). The renal artery is skeletonized at its origin from the aorta after lifting the kidney from its bed and rotating it anteriorly (Fig. 8-1E). The ureter is freed, with its investing vessels and fat, down to or below the pelvic brim, then transected. The kidney is now mobilized except for its vascular connections. A brisk diuresis should be evident from the cut ureter, mannitol and furosemide plus adequate crystalloid solutions having been infused during dissection of the kidney.

If the transperitoneal approach is used (which has been the practice, e.g., in Oxford), a transverse incision is made beneath the costal margin on the side of the kidney to be removed. On the left side, the spleen, pancreas, and splenic flexure of the colon are mobilized and retracted to the right to expose the kidney, renal vessels, aorta, and ureter. On the right side, the duodenum and hepatic flexure of the colon are mobilized and reflected to the left to expose the kidney, inferior vena cava, renal vessels, and ureter. Dissection then proceeds much as outlined previously.

When urinary output from the skeletonized kidney is ensured, the renal artery and vein are clamped and divided, leaving a sufficient cuff of the retained donor vessel to allow secure repair. Although some surgeons prefer to anticoagulate the donor systemically before clamping the vessels, most omit this step and simply perfuse the excised kidney with a chilled, heparinized electrolyte solution. Increasing the osmolarity of the perfusate with mannitol is believed by some surgeons to protect the kidney further from ischemic damage. The use of more complex and more expensive preservation solutions (see Chapter 9) is not required for living donor kidneys, which typically are reimplanted with only a brief cold ischemic interval. The wound is closed without drains, and the patient is returned to the recovery room, where a chest radiograph is obtained to exclude the possibility of pneumothorax.

Postoperative Care and Complications

Available evidence indicates that patients undergoing clean contaminated procedures, such as unilateral nephrectomy, can benefit from prophylactic antibiotics.[19] We routinely administer a first-generation cephalosporin for 24 hours, beginning 1 hour before surgery. Nasogastric tubes usually are not required. Bladder catheters, if present, usually are removed in the immediate postoperative period. Graded resumption of oral alimentation is necessary because

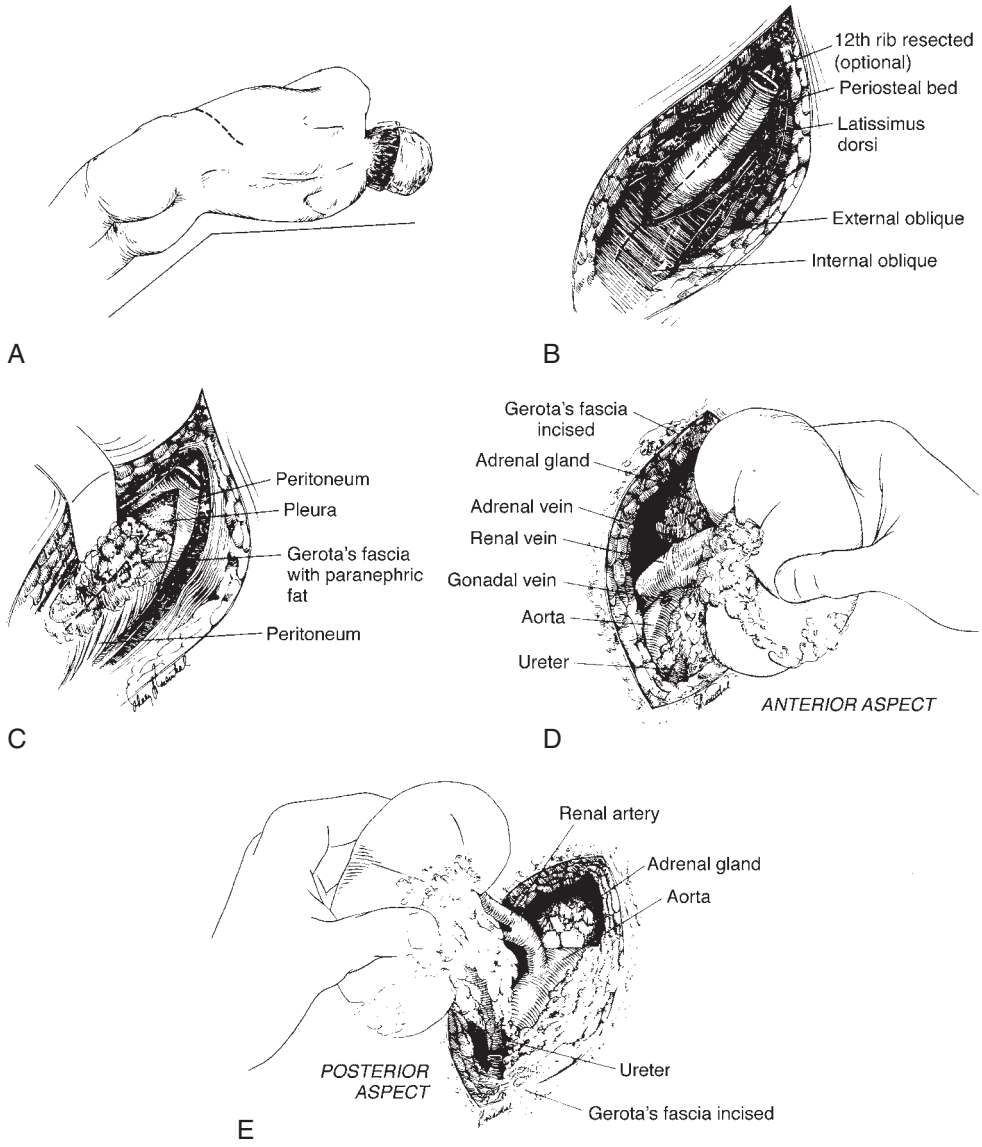

Figure 8–1 Living donor nephrectomy. **A-C**, In this patient, the kidney is approached through the bed of the 12th rib. Care is taken to avoid entering the pleural or peritoneal cavities. **D**, The renal vein is dissected to the vena cava, ligating and dividing the gonadal and adrenal branches. **E**, The renal artery is approached by lifting the kidney anteriorly. Gentle dissection continues down to the aorta. The ureter is divided at or below the pelvic brim, carefully preserving the periureteral vascular supply.

these patients may exhibit a more prolonged ileus than might be anticipated after retroperitoneal surgery. This ileus may be the result of the extensive periaortic dissection and consequent autonomic nerve disruption. Nevertheless, most of these patients are ready for discharge from the hospital in 3 to 4 days and for return to employment by 3 to 4 weeks if unusually strenuous physical labor is not involved. Urine culture, renal function tests, and a complete blood count are obtained and reassessed before discharge. The patient has follow-up evaluations at increasing intervals.

The perioperative mortality rate for kidney donors is estimated to be 0.03%.[9] At least 20 deaths have been reported after living donor allograft donation over 40 years.[8] Other complications of the renal donor procedure are generally minimal and easily remedied.[3,6,7,18] The current overall complication rate is approximately 2%.[2] Table 8-1 lists commonly observed problems and their approximate incidence, compiled from published reports and from our own experience with more than 1000 patients.

Occasionally, a complication occurs during the preoperative evaluation, most likely related to the aortogram, which is now being used less frequently. Such complications include localized hematoma formation, femoral artery thrombosis or false aneurysm formation at the arterial puncture site or, more rarely, reaction to the radiographic dye, such as an allergic response or acute tubular necrosis. Most complications occur in the perioperative period, with atelectasis, urinary retention or infection, wound problems, and prolonged bowel dysfunction accounting for most complications. These conditions typically are resolved by the time the patient is discharged from the hospital. One of the most dangerous complications is thrombophlebitis with possible life-threatening pulmonary embolus. In a worldwide experience estimated to be greater than 100,000 donor operations, the most frequent cause of the approximately 20 known deaths was pulmonary embolus. Single fatal cases of hepatitis, myocardial infarction, and depression leading to alcoholism and death in an automobile accident have been reported.

Table 8–1 **Complications in Living Donors**

Procedure Complications	Incidence (>2000 Cases) (%)
Aortogram	
Prolonged discomfort	<1
Femoral thrombosis or aneurysm	<1
Intraoperative	
Splenic laceration	<1
Pancreatic injury, pseudocyst	<1
Nephrectomy Wound	
Prolonged discomfort	3.2
Infection	2.1
Hernia	<1
Hematoma	<1
Pulmonary	
Atelectasis	13.5
Pneumothorax or pneumomediastinum	3.2
Pneumonitis or pleural effusion	4.3
Urinary Tract	
Infection	4.5
Retention	3
Acute tubular necrosis	<1
Late proteinuria	3
Other	
Prolonged ileus	5.2
Thrombophlebitis with or without pulmonary embolus	1.9
Peripheral nerve palsy	1.1
Hepatic dysfunction (late)	<1
Acute depression	<1
Hypertension (late)	15*

*Similar to general population.

Longer term morbidity should be minimal. Endogenous creatinine clearance rates rapidly approach 70% to 80% of the preoperative level, and reports of late renal failure have been extremely rare. An important factor is the exclusion during the selection process of pathology or potential pathology in the donors. As part of a continuing study at Massachusetts General Hospital of the long-term impact of kidney transplantation on patients and family members, 70 adults who had donated a kidney to a close relative between 1963 and 1975 have been studied for the perceptions of the effect of that donation on their lives. No long-term medical problems related to the nephrectomy were identified. As mentioned earlier, in describing how they had come to donate a kidney, more than 50% reportedly made their decision to donate instantaneously and believed that as a result of donation their relationship with the recipient had been strengthened. Approximately one third of the donors reported a positive change in their outlook on life, often citing increased appreciation of their own health. In cases in which the allograft failed, many donors reported initially a sense of anger and frustration. Ultimately, however, they claimed a sense of worthwhile accomplishment and belief that they would pursue the same course again. Representing a wide variety of occupations, the donors agreed almost universally that their earning capacity and ability to carry out their occupational responsibilities were not adversely affected by the donation. The immediate and long-term morbidity of nephrectomy is sufficiently low to make the risk acceptable for fully informed, genetically or emotionally related donors for patients with chronic renal disease.

Open versus Laparoscopic Nephrectomy

Reasons for recommending open rather than laparoscopic donor nephrectomy include (1) lack of surgical expertise with laparoscopic nephrectomy, (2) lack of resources for laparoscopic nephrectomy, (3) previous abdominal surgery in the donor in which the laparoscopic technique is unlikely to be feasible, and (4) need for greater donor vessel length in cases in which the blood vessel anatomy is marginally acceptable. Multiple studies comparing open versus laparoscopic donor nephrectomy have been reported. Studies also have compared minimal open incision and laparoscopic, hand-assisted versus straight laparoscopic, and hand-assisted versus open techniques. The open method was the standard method before the development of laparoscopic nephrectomy and is safe and always the default procedure when other techniques are not feasible.[14] Quality-of-life studies suggest that patients' return to work is slower, narcotic use is higher, but patient satisfaction is equivalent with laparoscopic nephrectomy.[1,10,11,16] In a Swiss study, open nephrectomy was accepted with equal satisfaction as the laparoscopic method and did not deter donation.[5]

DECEASED DONOR

The most commonly practiced procurement technology today continues to be retrieval of viable organs for transplantation from brain-dead patients who are maintained in stable physiological balance by artificial support. This approach gives rise to the term heart-beating cadaver donor. These donors are brought to the operating room where organ procurement is undertaken under semielective conditions while using the usual sterile precautions of any aseptic surgical procedure. The donor may require large volumes of intravenous fluids to restore blood volume, which typically has been severely depleted by premortem attempts to decrease brain swelling and achieve neurological resuscitation. Diuretics, mannitol, and vasopressors are administered as needed to promote diuresis during the nephrectomy procedure. Some groups systemically heparinize the donor and administer vasoactive agents, such as phenoxybenzamine or phentolamine, to combat vasospasm in the kidneys. Other donor pretreatment modalities, such as possible immunomodulating measures, are seldom used, and are not discussed here.

In situations in which the criteria for brain death have been fulfilled, but the concept of heart-beating donation has not been accepted, or in which there is irreversible brain injury, but not fulfilling the criteria of brain death, respiratory support is discontinued in the operating room (termed donation after cardiac death [DCD]). After cardiac function ceases, the donor is declared dead, and the surgical procedure is expeditiously undertaken.[12] The kidneys must be removed and chilled more rapidly than in the heart-beating donation procedure to minimize ischemic damage to the retrieved organs. The goal is to limit the warm ischemic period, whenever possible, to less than 30 minutes.

In an effort to increase further the number of kidneys available for transplantation, interest also has been revived in the possible procurement of organs from donors who are

dead on arrival or who die after unsuccessful cardiorespiratory resuscitation (uncontrolled non–heart-beating donation). Several studies have confirmed that significant numbers of patients die in emergency departments or intensive care units without brain death being declared.[4] Presumably, suitable allografts could be salvaged from such potential donors if reliable methods could be identified to control the ischemic damage that occurs shortly after death. Current approaches include combined in situ kidney flushing and core body cooling by femoral artery and peritoneal catheters placed at the bedside immediately after cardiac arrest.[12,15] The non–heart-beating donor can be transported to the operating room for bilateral nephrectomy.

Removal of Kidneys Alone

If only the kidneys are to be removed, bilateral nephrectomy is accomplished through a long midline incision. The objective is to take both kidneys with the full length of the renal artery and vein, preferably on aortic and vena caval cuffs. This approach limits the possibility of injuring accessory vessels, which are present in 12% to 15% of normal kidneys. The technique we prefer entails en bloc removal of both kidneys with an intact segment of aorta and inferior vena cava to allow early in situ cooling of the kidneys. This approach reduces the time required for the nephrectomies because the fine dissection necessary for identification and isolation of the artery and vein can be performed after the kidneys are removed. With this technique, the risk of damaging accessory vessels is essentially eliminated. Continuous perfusion of the kidneys, if this preservation technique is used, usually can be provided via the aorta, avoiding direct renal artery cannulation and the possibility of intimal injury. Multiple arteries can be left on a cuff of aorta, giving the transplant surgeon the option of using a single Carrel patch anastomosis for a simpler reimplantation procedure.

On entering the donor's abdomen, rapid exploration excludes the presence of unsuspected sepsis, neoplasia, or other important pathology. The small bowel and mesentery are retracted to the right, and the posterior parietal peritoneum is incised over the great vessels and through the ligament of Treitz. The peritoneal incision is extended around the ascending colon so that the bowel can be retracted upward and to the left (Fig. 8-2A). The duodenum and pancreas are retracted superiorly. The proximal aorta is freed to above the celiac axis, dividing and ligating the superior mesenteric artery (Fig. 8-2B).

Tapes or large silk sutures are passed around the distal aorta and vena cava just above the iliac bifurcations. Because only the kidneys are being removed, the proximal aorta also

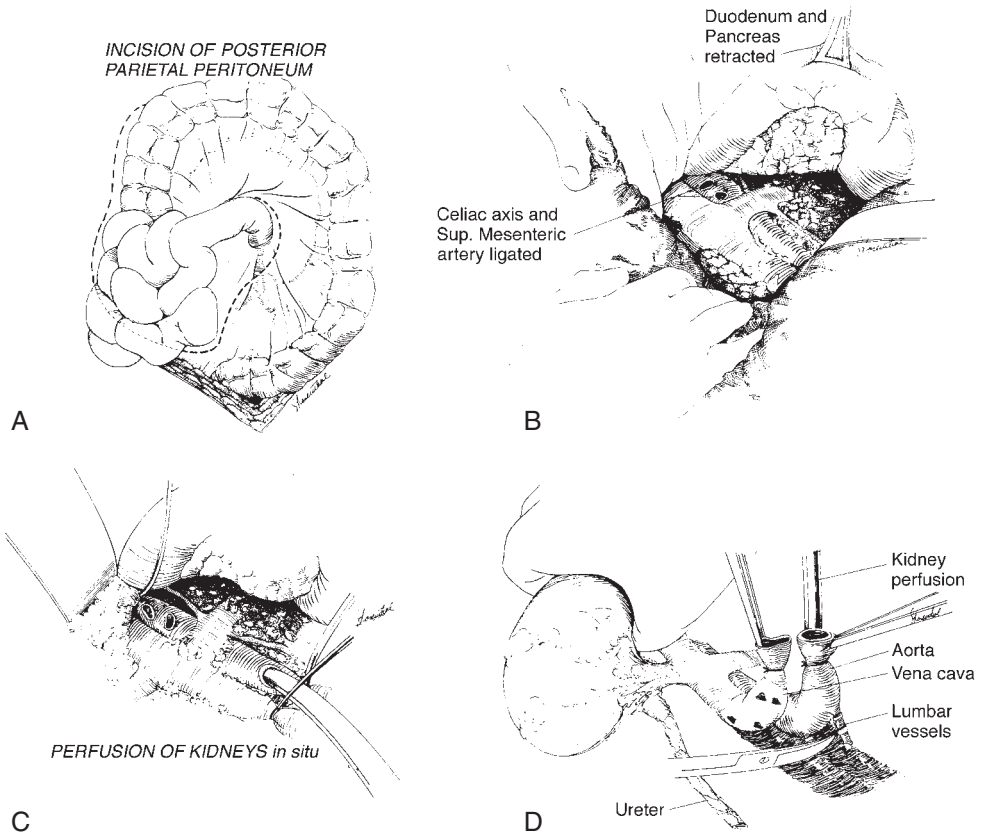

Figure 8–2 Cadaver donor nephrectomy without other organ retrieval. **A**, After widely opening and exploring the peritoneal cavity, the small bowel is retracted to expose the posterior parietal peritoneum, which is incised. This allows retraction of the bowel superiorly and to the left. **B**, The duodenum and pancreas are retracted superiorly to obtain exposure of the proximal aorta and vena cava. The superior mesenteric and celiac trunks are ligated and divided several centimeters above the level of the left renal vein crossing the aorta. **C**, After ligation of the proximal and distal aorta and the distal vena cava, perfusion of the kidneys is begun through the intravenous tubing that has been introduced into the distal aorta. **D**, Isolation of the kidneys and ureters has been completed (left kidney not shown). The distal aorta and vena cava are transected, and the lumbar vessels posteriorly are clamped and divided, allowing removal of the entire block of tissue while cold perfusion continues.

is encircled, enabling isolation of the renal circulation. After achieving proximal aortic, distal aortic, and distal caval occlusion, preservation of the kidneys in situ is begun by perfusion with chilled University of Wisconsin solution, Euro-Collins solution, or Ringer's lactated solution containing mannitol (18 g/L) and heparin (20,000 U/L) infused through sterile intravenous tubing that has been placed directly into the aorta. The perfusate is allowed to return to the donor circulation via the proximal vena cava (Fig. 8-2C). The kidneys are generally cool and pale after rapid infusion of 500 to 600 mL of perfusate, but the perfusion is continued at a slower rate throughout the remainder of the procedure.

The final mobilization of the kidneys is undertaken within the plane of Gerota's fascia in a more leisurely manner. Care is taken to free and section the ureters as far down toward the bladder as possible and to avoid dissection within the renal hilus. The distal aorta and vena cava are divided, and the entire block is lifted anteriorly to expose the lumbar vessels posteriorly (Fig. 8-2D). When the proximal aorta and vena cava have been divided, the block consisting of both kidneys and ureters, aorta, and inferior vena cava can be lifted out of the abdomen and placed immediately into a basin of cooled perfusion solution. A more complete dissection and assessment of the anatomy of the renal vessels can be undertaken. Before closure of the abdominal incision, specimens of donor lymph nodes and spleen are removed for subsequent histocompatibility and other immunological studies.

Removal of Kidneys with Other Organs

The more typical situation involves multiple organ procurement from the same donor. Acceptable donors for heart, liver, or pancreas transplantation are younger (generally <70 years old) and hemodynamically more stable than some donors from whom kidneys alone can be retrieved. Kidneys suitable for transplantation can be salvaged from a donor after cardiac function has ceased, whereas multiple organ procurement is seldom accomplished from a non–heart-beating donor cadaver.

The successful undertaking of multiple organ recovery requires careful coordination among three surgical teams to ensure that there is no compromise in viability of any transplantable organ. It is crucial to have anesthesia support to monitor and maintain cardiovascular integrity of the donor during the extensive dissection, which may take 1 to 3 hours. Although the details differ, depending on the combination of organs to be removed, certain common principles prevail, including wide exposure, dissection of each organ to its vascular connection while the heart is still beating, placement of catheters for in situ cooling, and removal of organs while perfusion continues, usually in the order of heart, lungs, liver, kidneys, and pancreas.[17]

The organs are exposed through a midline incision extending from the suprasternal notch to the pubis (Fig. 8-3A). If the heart is to be retrieved, it is usually partially mobilized as the first maneuver so that it can be removed quickly at any later stage should vascular instability occur during the dissection of the other organs. The preparatory steps for cardiectomy require opening of the pericardium, mobilization of the superior vena cava, and separation of the aorta from the pulmonary artery. Dissection is undertaken to mobilize the liver or pancreas, or both. If the pancreas is not to be used, the splenic and superior mesenteric arteries may be ligated or divided, or both (Fig. 8-3B). The common bile duct is transected, and the gallbladder is incised and flushed with cold saline to prevent biliary autolysis. The portal vein is dissected to the confluence of the splenic and superior mesenteric veins where a catheter can be placed into the splenic vein for subsequent rapid portal perfusion (see Fig. 8-3B). Alternatively, the inferior mesenteric vein is used for the placement of the portal vein catheter. Isolation of the liver is completed by mobilizing the vena cava posteriorly.

If the pancreas is to be transplanted, the spleen is mobilized, the short gastric vessels are divided, and the spleen and pancreas are retracted to the right (Fig. 8-3C). The body and tail of the pancreas are carefully dissected free. Although now used infrequently, this mobilization can terminate at the junction of the splenic and superior mesenteric veins, where the pancreas can be transected for segmental transplantation. More commonly, the entire pancreas and a segment of duodenum can be mobilized for pancreaticoduodenal transplantation.

The kidneys and major abdominal vessels are exposed next by retracting the ascending colon and small bowel to the left and lifting the mobilized duodenum anteriorly (Fig. 8-3D). The kidneys are elevated from the retroperitoneum, and the distal aorta and vena cava are completely freed. The donor is given heparin and mannitol, after which a perfusion catheter is placed in the aorta, and a venous drainage catheter is placed in the vena cava (Fig. 8-3E).

Initial organ cooling usually is begun via the previously placed portal vein catheter (see Fig. 8-3B). When the donor core temperature decreases to about 30°C, or if hemodynamic instability occurs, the aorta is cross-clamped at the diaphragm, and the aortic flush is begun for rapid cooling of the abdominal organs. Precise coordination among the retrieval teams is required at this critical stage. Cardioplegic infusion into the ascending aorta is begun, and cardiectomy and pneumonectomy are performed first. The liver is removed next. Finally, the remaining mobilization of the kidneys is undertaken. Care is taken to free and section the ureters as far down toward the bladder as possible and to avoid dissection within the renal hilus. The distal aorta and vena cava are divided, and the entire block is lifted anteriorly to expose the lumbar vessels posteriorly. These vessels are divided after being doubly clamped with vascular clips (Fig. 8-3F). When these vessels are controlled, the block consisting of both kidneys and ureters, aorta, and inferior vena cava can be lifted out of the abdomen and placed immediately into a basin of cooled perfusion solution. A more complete dissection and assessment of the detailed anatomy of the renal vessels can then be undertaken.

In donors from whom whole pancreaticoduodenal procurement is included, we advise removing this organ block after the kidneys have been taken from the field to avoid possible contamination from the transected duodenum. Although it was previously believed that total removal of the pancreas is anatomically incompatible with simultaneous retrieval of the liver, most groups currently procure both organs from the same donor routinely and use vascular grafts for pancreatic rearterialization in the recipients.[20] Before closure of the abdominal incision, specimens of donor lymph nodes and spleen are removed for subsequent

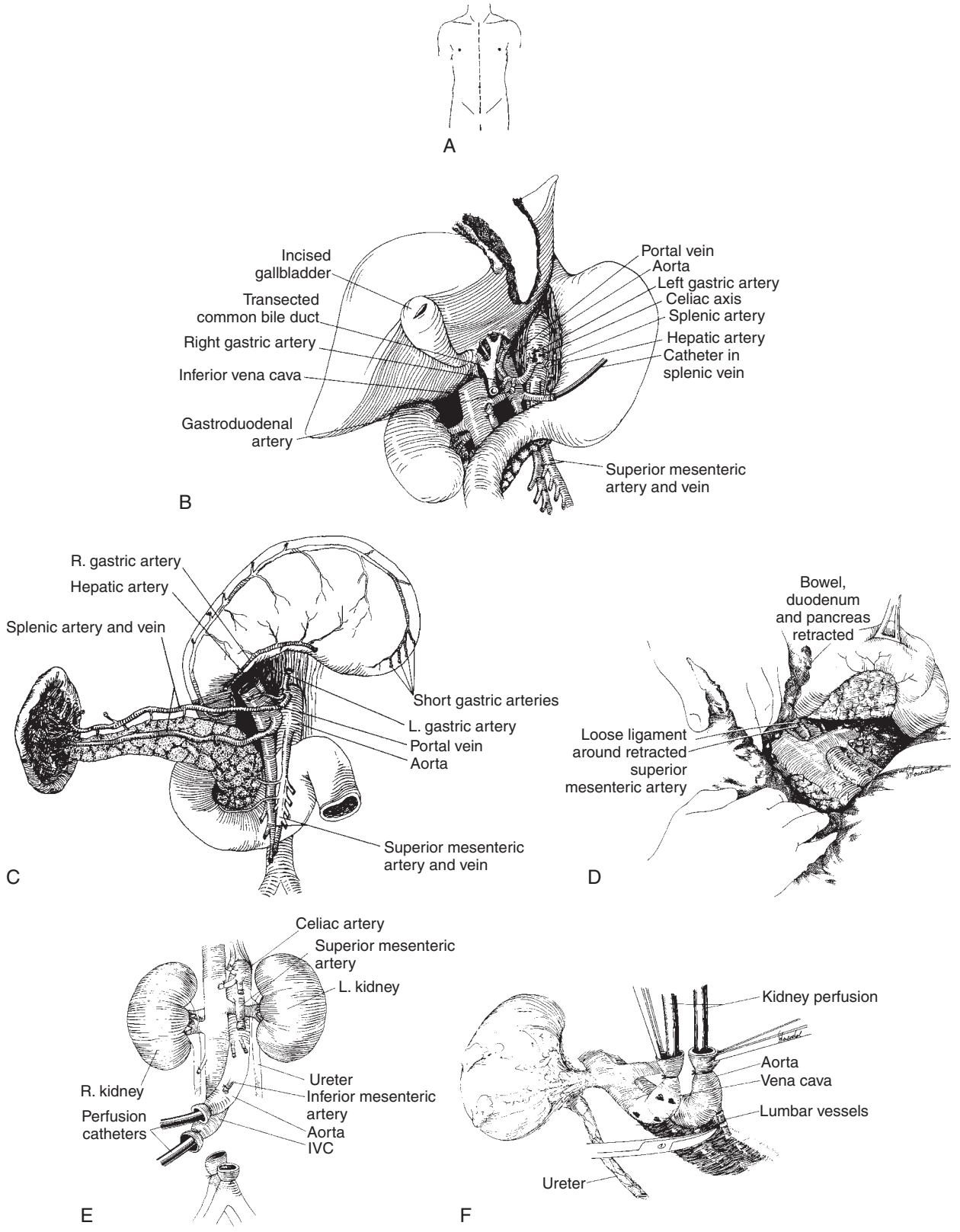

Figure 8–3 Cadaver donor multiple organ retrieval. **A**, The chest and abdominal cavities are entered through a long midline incision. After general evaluation of the organs to be procured and initial mobilization of the heart, the liver dissection is completed. **B**, The splenic vein is catheterized for portal perfusion. The gastroduodenal and splenic arteries are divided if the pancreas is not to be used. **C**, For pancreas retrieval, dissection is begun from the left, retracting the spleen and pancreas to the right, carefully preserving the splenic artery and vein. For simplicity, the superior mesenteric vessels are depicted as separate from the pancreas, but they remain closely adherent to the posterior pancreas. **D**, Returning to the right side, the duodenum and pancreas are retracted exposing the superior mesenteric artery. **E**, Mobilization of the kidneys and ureters from the retroperitoneum is completed, and the distal vena cava and aorta are catheterized. For illustrative purposes, the bowel, which remains attached via the mesenteric vessels, is not shown in this figure. **F**, After cooling and removal of the heart and liver, the kidneys are removed by lifting the entire tissue block (left kidney not shown) anteriorly, while clamping and dividing the lumbar vessels posteriorly. IVC, inferior vena cava.

histocompatibility and other immunological studies. Most groups have concluded that the immediate and long-term functional results observed in transplanted kidneys obtained from multiple organ donors are comparable to those obtained from procedures involving donor nephrectomy alone.[13]

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Chapter 8B
Laparoscopic Live Donor Nephrectomy

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RATIONALE

Despite numerous attempts to promote increased organ donation after death, the disparity between graft supply and demand has continued to broaden. As a result, waiting times for kidney transplantation have increased significantly in recent years. Although live donor renal transplantation has offered numerous recipient outcome advantages compared with deceased donor transplantation, significant disincentives to live donation, including the magnitude of the donor nephrectomy operation, limited the expansion of this source of organs for transplantation. The morbidity and long convalescent period associated with a flank incision represented significant barriers to some individuals interested in donation. Before the development of the laparoscopic technique, a survey of a cohort of patients uncovered common fears about participation in the donor process, which included lost wages owing to prolonged convalescence, job security, inability to tend to other responsibilities such as child care, fear of postoperative pain, and unease about the cosmetic results of the operation.[17,45] Laparoscopic live donor nephrectomy was developed with the intent to limit these deterrents to live kidney donation by reducing the impact of the operation on the donor's life.

The procedure's history dates back to the early 1990s. Clayman and colleagues[7] performed the first successful laparoscopic nephrectomy for renal disease in 1991 and showed that minimally invasive techniques resulted in less pain, shorter hospitalization, and a reduced duration of convalescence compared with the open flank approach. Within 3 years, a laparoscopic renal procurement technique was developed in a large animal model.[12] This was followed by the first human live donor laparoscopic nephrectomy, which was performed by a Johns Hopkins team led by Ratner and Kavoussi in 1995.[43] The donor was discharged from the hospital on the first postoperative day and returned to full activity 2 weeks after the procedure. The recipient had immediate graft function. We later showed that the application of

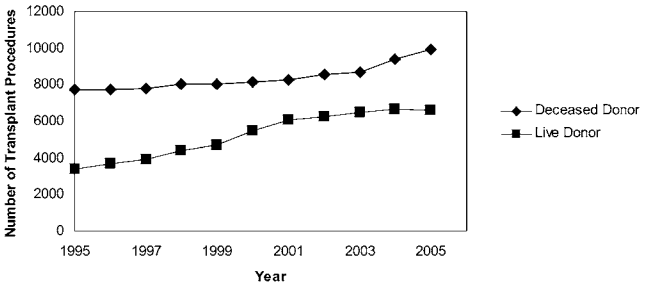

Figure 8–4 Donor nephrectomy procedures in the United States by donor type, 1995–2005. Data from UNOS/OPTN as of January 5, 2007. Following the development of the laparoscopic donor procedure, there has been a substantial increase in the number of renal transplant procedures that have taken place in the United States through an expansion in donation that has largely been attributable to the increase in kidneys from live donors.

minimally invasive techniques resulted in less pain, decreased duration of hospitalization, and a shorter convalescent period compared with the open flank approach.[46,47,52]

Since the development of the laparoscopic donor procedure, there has been a nearly 50% increase in the number of renal transplant procedures that have been performed in the United States through an expansion in donation that has largely been attributable to the increase in kidneys from live donors (Fig. 8-4). Laparoscopic procurement of donor kidneys has become widely adopted and is now becoming the standard technique for live donor procurement in most countries. At our institution, the annual number of live donor renal transplant procedures has increased more than threefold since the introduction of laparoscopic live donor nephrectomy. In a study conducted soon after the routine introduction of the laparoscopic technique at our institution, approximately 20% of donors reported that they would not have donated an organ if the laparoscopic procedure had not been available. Two thirds of donors state that the availability of the laparoscopic operation had a major influence on their decision to donate.[45] The application of minimally invasive techniques to the donor nephrectomy procedure undoubtedly raised consciousness about the gratuitous nature of the flank incision and rib resection and was probably responsible for the transition to the minilaparotomy approach for groups that were disinclined to the laparoscopic approach.

An additional unexpected phenomenon associated with the greater ease of donation resulting from the laparoscopic procedure has been the presentation of numerous individuals with an interest in donation without an intended recipient, so-called nondirected live donation. More than 300 such procedures have been performed in the United States since the 1990s. The availability of a safe operation that uses minimally invasive techniques also may have enabled the advent of innovative protocols to provide transplant solutions to high-risk patients with incompatible live donors, including preconditioning for ABO incompatibility and a positive crossmatch, and kidney paired donation.[35,36,38]

PREOPERATIVE EVALUATION

All live kidney donors receive thorough preoperative surgical, nephrological, and psychological evaluations in accordance with the clinical practice guidelines established by the American Society for Transplantation and the Consensus Statement on the Live Organ Donor.[1,19] As with the open procurement procedure, preoperative consideration of anatomy and functional status of the donor kidneys is crucial. The use of preoperative imaging permits investigation of size, function, and anatomy of the potential donor kidneys to facilitate planning of the safest approach to procurement. We find it useful to obtain three-dimensional spiral computed tomography (CT) scans with intravenous contrast administration.[59] In addition to providing definition of the arterial anatomy, three-dimensional spiral CT provides excellent depictions of the venous anatomy. It provides superb definition of the renal parenchyma and a urography phase for evaluation of outflow. We use this technique in lieu of conventional angiography.[20] Magnetic resonance angiography is an alternative that has been used successfully for preoperative imaging at other centers.

Careful preoperative consideration must be given to the side of the nephrectomy procedure. An important consideration during the planning of this operation is the technical challenge associated with the recipient procedure after laparoscopic procurement of a right donor kidney. Use of the endoscopic GIA stapling device to divide the anatomically shorter right renal vein generally results in the loss of approximately 1 to 1.5 cm of length.[42] This may result in a short renal vein that increases the complexity of the recipient operation and has been associated with an increased risk of venous thrombosis in early series.[32] Because it is no longer acceptable practice to use a non–tissue affixing ligation technique, short right renal vessels are unavoidable. Our practice has been preferentially to use the left kidney, unless it is clearly more advantageous for the donor to retain this kidney because of functional or anatomical considerations. Multiple left renal arteries or anomalous left renal venous vasculature (e.g., circumaortic or retroaortic renal veins) have not been contraindications to the use of the left kidney in our experience.[32]

The contraindications to laparoscopic donor nephrectomy are the same as those established for open nephrectomy. Because the laparoscopic donor operation is performed through a transperitoneal approach, previous abdominal surgery may increase the complexity of the procedure but is rarely a reason for open conversion.[62]

The laparoscopic donor nephrectomy is a technically challenging operation and generally requires more operative time than the open technique. Early in our series, we were unable to identify any preoperative demographic, radiological, or anatomical parameters that accurately predicted operative difficulty.[49] The difficulty with any given procedure seems to be related to mobility of the mesentery, the quality of the retroperitoneal tissue, and laparoscopic working space, none of which can be quantified by noninvasive imaging before surgery. The hand-assisted approach provides tactile feedback to the operator and may be more attractive to surgeons who have limited advanced laparoscopic training. It also has been shown in several series to reduce operative time.[58,70-72]

INTRAOPERATIVE MANAGEMENT

The anesthetic management of patients who undergo a laparoscopic donor nephrectomy is of paramount importance, and good communication between the anesthesiologist and surgeon is essential to a good outcome for the donor

and the recipient. To obtain sufficient laparoscopic working space, the patient must be kept completely relaxed, and nitrous oxide anesthesia should be avoided. In our experience, patient-controlled analgesia should be limited to the night of surgery, and patients should be converted to oral analgesics when clear liquids are introduced on the first postoperative day. Prolonged use of intravenous patient-controlled analgesia can lead to ileus, oral intolerance, and constipation. The greatest postoperative pain manifests on the first evening, and this may be related to peritoneal stretching associated with the pneumoperitoneum. Some additional analgesic benefit leading to postoperative narcotic minimization may be obtained by injecting extraction and trocar sites with 0.25% bupivacaine. Some groups have been successful in limiting hospital stay to 1 day through the use of propofol-based anesthesia supplemented with desflurane to limit postoperative nausea.[26]

The effect of the pneumoperitoneum on renal blood flow dynamics dramatically changes the intraoperative fluid management from that which is used in the open donor operation. Experimental data suggest that the effects of this relative hypoperfusion can be ameliorated by fluid loading.[30] It is not unusual for laparoscopic donors to receive 6 to 8 L of crystalloid during the procedure. We monitor the adequacy of intravascular volume expansion by the turgor of the renal vein. A collapsed renal vein signals the need for more liberal use of intravenous fluids. A brisk diuresis is stimulated throughout the procedure by two 12.5-g bolus administrations of mannitol. Just before removing the kidney, the donor may be given 20 to 40 mg of intravenous furosemide and 3000 U of heparin. When the kidney has been removed, 25 mg of protamine may be given to reverse the anticoagulative effects of heparin.

LAPAROSCOPIC DONOR NEPHRECTOMY OPERATIVE PROCEDURE

The patient is placed in a modified lateral decubitus position with the hips rotated back and the arms extended above the head (Fig. 8-5). The table is flexed to expand the area between the costal margin and the pelvic brim. A 12-mm Hg pneumoperitoneum is established through a Veress needle inserted either above the umbilicus or into the left iliac fossa along the lateral rectus border. A Veress needle placed in the infraumbilical fold may track along the urachus and lead to extraperitoneal insufflation. Right subcostal Veress or Hassan trocar placement may be preferable for patients with previous abdominal surgery to avoid bowel injury. There is a risk of inadvertent liver injury from the right subcostal Veress needle placement, and this should be reserved for surgeons who have experience with this technique. The initial operative trocar placement consists of two 12-mm ports and one 5-mm port that are placed (1) in the midline just above the umbilicus, (2) at the level of the umbilicus or slightly below along the lateral border of the rectus sheath, and (3) in the midline two to three fingerbreadths below the xiphoid process. In patients who have not undergone previous surgery, the first port is placed using an optical access device.[65] The videoendoscope is placed in the umbilical port. The epigastric and lateral ports are the operative ports.

For left-sided procedures, the descending and sigmoid colon are taken down by dividing the lateral attachments with DeBakey graspers and curved scissors (Fig. 8-6). The colon is reflected medially, which exposes Gerota's fascia (Fig. 8-7). Care must be taken not to buttonhole the mesocolon. Much of the dissection can be accomplished bluntly by sweeping the tissue medially and developing a natural plane between the mesocolon and Gerota's fascia.

At this point in the operation, we often make a 4- to 5-cm Pfannenstiel incision just above the pubis and carry it down to the level of the fascia. A 12-mm trocar is placed at the midline in the center of the Pfannenstiel incision to pierce the fascia and peritoneum. The trocar is removed and replaced by an EndoCatch (U.S. Surgical Corporation, Norwalk, Conn) device, which is used during the remainder of the operation to provide medial retraction of the colon.

For right-sided procedures, the cecum is mobilized and reflected medially. The liver is lifted away from the upper pole of the kidney using a laparoscopic retractor placed through a fourth flank port. The right-sided nephrectomy is technically challenging and has unique problems that are discussed later.

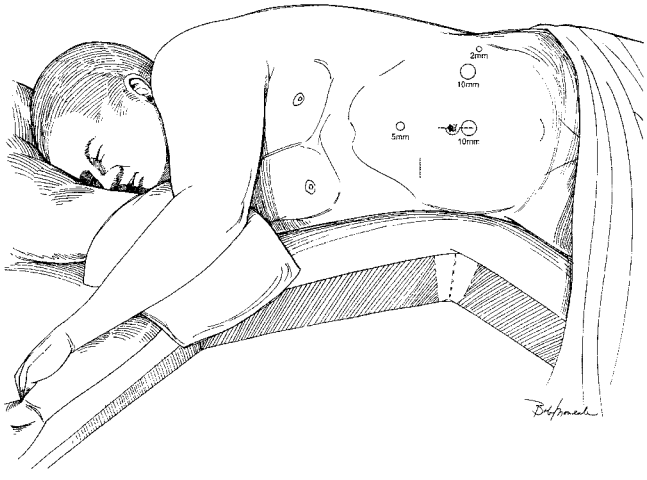

Figure 8–5 The patient is placed in a lateral decubitus position, and three laparoscopic ports are introduced.

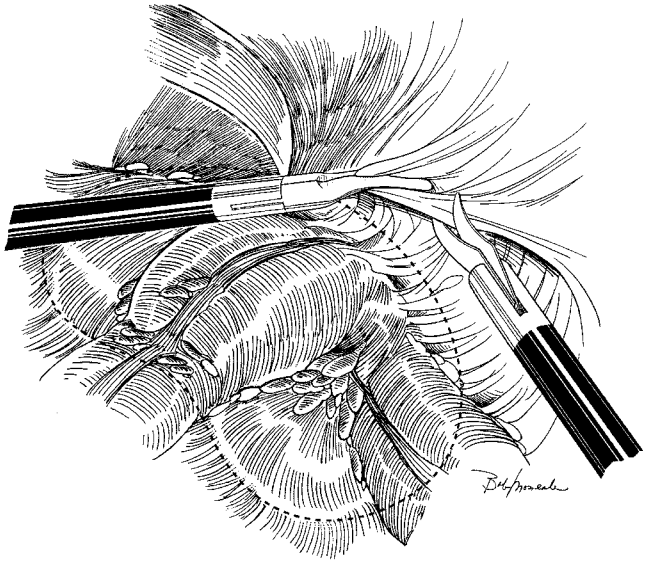

Figure 8–6 The lateral attachments to the colon are taken down.

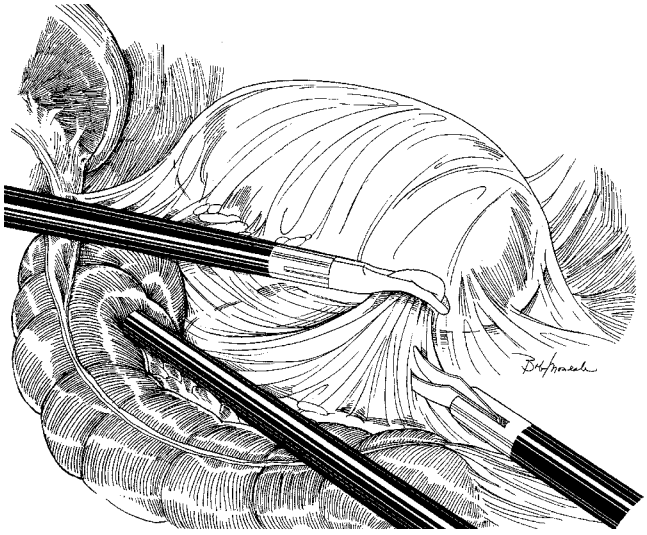

Figure 8–7 Gerota's fascia is separated from the mesocolon.

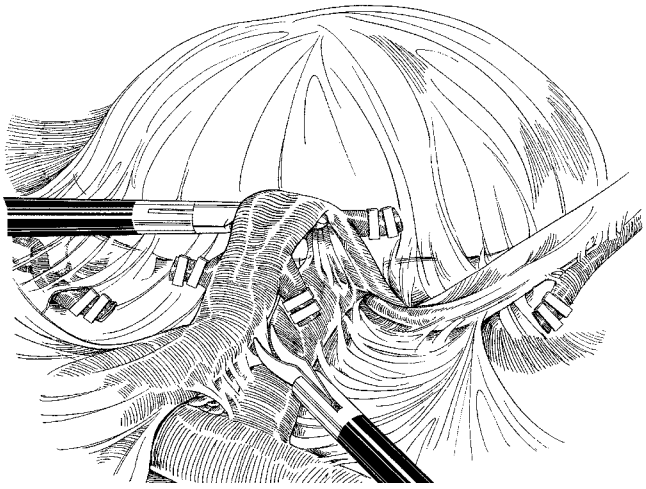

Figure 8–9 Posterior to the vein lies the renal artery, which is freed back to the level of the aorta.

At this point, the renal vein is exposed by tracing the gonadal vein in a cephalad direction or bluntly sweeping the perinephric tissue several centimeters medial to the renal hilum (Fig. 8-8). The renal vein is cleared completely of investing tissue, and the gonadal, lumbar, and adrenal branches are clipped and cut. The renal artery lies posterior to the vein and can be exposed by elevating the lower pole of the kidney with the DeBakey grasper. Dissection should be conducted medial to the gonadal vein to avoid devascularization of the ureter in the hilar region. The artery is separated from the surrounding nervous plexus and isolated to the level of the aorta. The plane of dissection is carried along the cephalad border of the vein between the adrenal gland and the upper pole of the kidney. Vascularized tissue in this region is divided between clips, and the upper pole is shelled out of the envelope of Gerota's fascia. The posterior attachments are lysed by elevating the upper pole and teasing the adherent tissue away.

The DeBakey grasper is inserted medial and posterior to the bundle of tissue that contains the ureter (Fig. 8-8). The gonadal vein, ureter, and mesoureter are separated from the psoas muscle and dissected free from a point below the lower pole of the kidney to the pelvic inlet. At the juncture where the ureter descends into the pelvis, the individual structures of the bundle are isolated, clipped, and cut. Mobilizing the ureter and gonadal vein deep into the pelvis to increase ureter length has been associated with lateral scrotal paresthesias in our experience and should be avoided.[21] The lateral attachments of the mesoureter are cauterized and cut in a caudad-to-cephalad direction. The plane of dissection is carried along the lateral surface of the kidney, completely mobilizing the kidney except for the renal pedicle.

The camera is moved to the lateral port, and a vascular endoscopic GIA stapler is used to divide the renal artery followed by the vein (Fig. 8-9). Before dividing the pedicle, mannitol, furosemide, and heparin are administered. The recipient procedure is facilitated by attempting to optimize the length of each of these vessels on the procured graft. The Endocatch bag that has been placed as a retractor is deployed. The kidney is placed in the bag and is removed by incising the fascia and peritoneum longitudinally through the Pfannenstiel incision.[44]

The fascia is closed with absorbable sutures. The 12-mm port sites are closed with figure-of-eight absorbable sutures

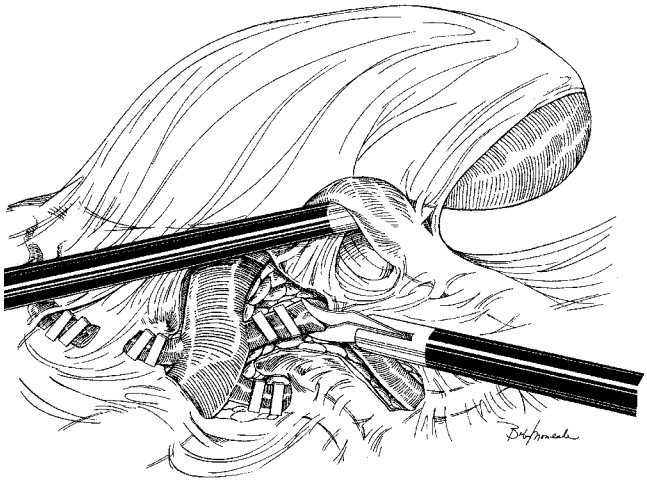

Figure 8–8 The renal vein is exposed, and the adrenal gonadal and lumbar branches are divided.

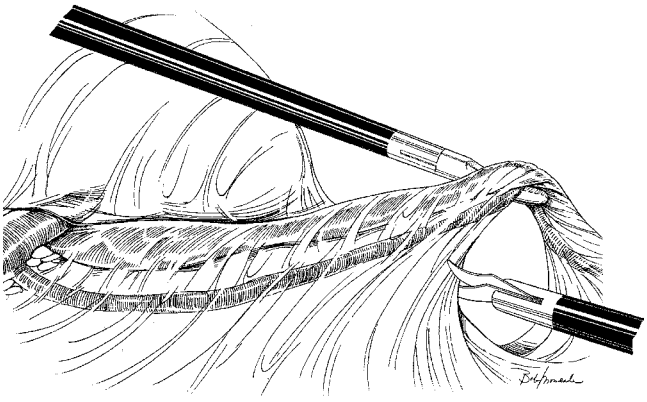

Figure 8–10 The mesoureter is isolated from the lower pole to the pelvic inlet, where it is divided.

aided by the Carter-Thomas instrument. After re-establishment of pneumoperitoneum, the abdomen is inspected for bleeding. The empty Endocatch tube can be reinserted through a gap left in the Pfannenstiel incision to retract the colon while the retroperitoneum is being inspected.

VARIATIONS IN TECHNIQUE FOR RIGHT LAPAROSCOPIC DONOR NEPHRECTOMY

Variations on the left-sided technique are recommended to preserve maximal renal vein length in cases in which it is necessary to procure a kidney from the right side. Port placement is a mirror image of that used for the left-sided procedure, although an additional flank port may be required to insert a fan blade for hepatic retraction. Alternatively, a locking grasper can be used to grab tissue on the right side wall to retract the liver superiorly.

Numerous modifications can be made to achieve maximal length of the renal vessels. The first involves division of the renal vessels in a plane parallel to the inferior vena cava by introducing the endoscopic GIA device into the right lower quadrant port, rather than the infraumbilical port. This modification permits the surgeon to achieve alignment parallel to the inferior vena cava to transect the renal vein in a nonangled plane at its origin.

A second adjunct technique involves making a 5- to 6-cm transverse incision in the right upper quadrant at a point overlying the confluence of the right renal vein and the inferior vena cava. This incision facilitates open placement of a side-biting vascular clamp across the inferior vena cava at the level of the renal vein after the laparoscopic dissection of the kidney is complete.[44] The incision is used for open division of the renal vessels maintaining a generous length of renal vein. The kidney is delivered through this right upper quadrant incision. The vena cava is oversewn on top of the clamp under direct vision.

A third modification is to introduce a hand port at the level of the 11th rib near the junction of the lateral edge of the rectus sheath and the oblique muscles. This modification allows the kidney to be lifted on its pedicle under stretch to divide the vein directly with the endoscopic GIA device, or for a laparoscopic side-biting clamp to be placed through a suprapubic port to divide the renal vein flush with the vena cava and close the vascular defect. If the renal vein is found to be too short after removal of the kidney, back-table reconstruction of the vein can be performed using a panel graft of recipient saphenous vein, but this is rarely necessary.[32]

HAND-ASSISTED LAPAROSCOPIC TECHNIQUE

Initially described in 1998 by Wolf and colleagues,[72] the hand-assisted laparoscopic donor nephrectomy procedure incorporates a hand port, typically placed through a 7- to 8-cm periumbilical midline incision, to provide access to the kidney for manipulation and manual removal through the hand-port site. The hand-assisted variation of the laparoscopic nephrectomy was developed to give surgeons greater tactile feedback and to facilitate the definition of the tissue planes to allow transplant centers that lack advanced laparoscopic expertise to perform the operation safely. Perhaps the most significant advantage is the technical ease with which removal of the kidney occurs by simple inversion of the sleeve, obviating the need to manipulate the kidney into the Endocatch bag and reducing warm ischemic time. To date, however, there has not been a definitive demonstration that the relatively small reduction in warm ischemic time associated with hand port–assisted removal of the kidney improves postoperative outcomes for the recipient compared with the completely laparoscopic approach.[5,54,60] Donor outcomes using the hand-assisted technique compare favorably with the open procedure, and early recipient graft function is comparable to that seen with the purely laparoscopic procurement technique.[3,71]

We prefer the completely laparoscopic technique because the requirement of a slightly larger incision placed more cephalad in the midline and the additional 12-mm port in the subcostal region may eliminate some of the advantages associated with the laparoscopic technique in terms of reduced postoperative pain. The Pfannenstiel incision has proved to be favorable in terms of low morbidity and good cosmesis.

Patient positioning and preparation is essentially unchanged with the hand-assisted technique. The pneumosleeve flange is placed below or at the level of the umbilicus, centered on an incision large enough to permit the smooth insertion of the surgeon's hand. Three 12-mm ports are placed in the following positions: (1) lateral to the midpoint between the umbilicus and the anterior superior iliac spine, (2) four to five fingerbreadths below the xiphoid process in the midline, and (3) along the lateral rectus border in the subcostal region. The operating surgeon's left hand is placed in the pneumosleeve, and the camera is inserted through the supraumbilical port. The surgeon's hand replaces the DeBakey instrument, but otherwise the processes and order of the subsequent stages of the procedure are unchanged.

The camera is moved to the subcostal port when the vascular pedicle is divided. At this point, the surgeon usually switches to the right hand for retraction. The endovascular stapler is introduced through the supraumbilical port.

DONOR SAFETY

The encounter with a live organ donor represents a unique interaction in health care. With no expectations for personal gain in health, these individuals are subjected to an invasive and potentially harmful surgical procedure. To justify the participation in a nontherapeutic procedure, the potential risks associated with the operation must be minimized. The safety of donors is of paramount importance.

Numerous published reports that investigated the issue of donor safety after the laparoscopic donor nephrectomy procedure have emerged. Systematic reviews of the literature comparing the laparoscopic and open donor nephrectomy techniques suggest that the laparoscopic procedure has a complication rate that is similar to the open procedure.[13,34,66] These reviews identified overall donor complication rates that ranged between 0% and 30% for the laparoscopic approach, and 0% and 38% for the open approach in studies that were published between 1997 and 2003. Donor complication rates have been comparable in retrospective analyses that were published by select centers in the early era of the procedure[9,10,17,29,39,46] and in analyses that have been published as the procedure has become more widely accepted

and practiced through the present,* although case definitions differ widely across studies.

Matas and colleagues[33] conducted a national survey of U.S. transplant centers to compare early donor complications between laparoscopic approaches, including hand-assisted and purely laparoscopic approaches, and open techniques.[33] The study included 5660 open donor, 2239 hand-assisted donor, and 2929 purely laparoscopic procedures in contemporaneous patients who underwent operation between 1999 and 2001. Complications that did not require reoperation were observed in 0.3%, 1%, and 0.8% of the open, hand-assisted, and purely laparoscopic groups. Reoperation was performed in 0.4%, 1%, and 0.9% of the respective groups. Although both of these comparisons differed in a statistically significant fashion ($P = .02$ and $P = .001$), the differences in rates are small and remain low relative to other complex surgical procedures. The authors identified two donor deaths (one from pulmonary embolus and one from an unreported cause in the hand-assisted group; donor mortality rate 0.02%) and one patient in a persistent vegetative state following complications caused by intraoperative hemorrhage (purely laparoscopic group). No deaths were observed for patients who underwent donation by the open technique.

A series of prospective, randomized clinical trials of open versus laparoscopic donor nephrectomy have been completed, which included secondary donor safety end points.[23,40,55] Kok and colleagues[23] compared a mini-incision open donor nephrectomy approach ($n = 50$) with the purely laparoscopic technique ($n = 50$) and found similar rates of intraoperative and postoperative donor complications between the two groups. Intraoperative complications occurred in 12% of donors who underwent laparoscopic procurement and consisted of bleeding, colonic injury, bladder injury, and splenic injury. All intraoperative complications that occurred in the open nephrectomy group were hemorrhagic in nature ($P = .23$). Both groups were followed for 1 year postoperatively, and similar rates of postoperative complications were observed. In each group, 6% of the donors encountered postoperative complications which consisted of wound infection at the kidney extraction site and blood transfusion requirement in the laparoscopic group, and urinary tract infection, pulmonary infiltrate, and infected retroperitoneal hematoma in the open group ($P > .05$).

Simforoosh and colleagues[55] conducted a prospective, randomized clinical trial of open donor nephrectomy ($n = 100$) and purely laparoscopic donor nephrectomy ($n = 100$). Although no statistical analysis was performed to compare complications between the two groups, different patterns of complications became apparent at the conclusion of their study. Intraoperative complications occurred in 4% of laparoscopic donors and 18% of open donors. In the laparoscopic group, these included splenic laceration, bowel serosal injury, and cardiac arrhythmia. All complications consisted of pneumothoraces in the group that underwent open procurement. Postoperative complications occurred in 17% of patients in the laparoscopic group and 9% of patients in the open group. The most frequently encountered postoperative complications among patients in the laparoscopic group were ileus, hemorrhage, and scrotal swelling. For patients in the open donor nephrectomy group, the most frequent postoperative complications included ileus and urinary tract infection.

Oyen and coworkers[40] found a greater proportion of postoperative complications necessitating reoperation among patients who underwent laparoscopic donation compared with patients who underwent open donation in a prospective, randomized clinical trial that included 122 donors.[40] Although no reoperations were encountered in the open donor nephrectomy group, five patients (8%) required reoperation after laparoscopic procurement. These reoperations were the result of port site bleeding, retained sponge, jejunal perforations, and postoperative hernias.

Donor safety is an extremely important issue in live donor renal transplantation. Clinicians have achieved a greatly improved understanding of the morbidity associated with procurement of kidneys from live donors. Although these rates vary widely depending on the study design and case definitions that are used, most studies show that the laparoscopic technique for renal allograft procurement is a safe approach and comparable to the traditionally used open donor nephrectomy method. In contrast, understanding of long-term morbidity associated with donor nephrectomy is limited, and few countries perform long-term surveillance of this population of patients. Longer term studies are warranted to better delineate morbidity associated with laparoscopic live donation.

ADVANTAGES OF THE LAPAROSCOPIC APPROACH FOR THE DONOR

The laparoscopic donor nephrectomy procedure was initially developed in response to data collected from prospective donors about perceived disincentives to live donation.[17] Common fears among potential kidney donors include financial concerns owing to lost wages, loss of job security, inability to tend to other responsibilities such as child care, fear of postoperative pain, and unease about the cosmetic results of the operation. Many of these potential deterrents to otherwise willing donors have been addressed by the laparoscopic procedure. We conducted a retrospective comparison of functional recuperative parameters between donors who underwent contemporaneous open ($n = 37$) and laparoscopic ($n = 25$) nephrectomy procedures early in our series (1995 and 1996).[45-47] We found that length of hospital stay, time to return to normal activities, and time to return to work all favored the laparoscopic technique. Mean duration of hospitalization was reduced by 2.5 days (2.9 ± 1 day versus 5.5 ± 1.2 days; $P < .001$). The time necessary to return to normal activities was reduced by more than 2.5 weeks (1.8 ± 1.5 weeks versus 4.5 ± 0.5 weeks; $P < .001$). Donors who underwent laparoscopic nephrectomy were able to return to work on average 3 weeks earlier than donors who underwent open procurement (3.2 ± 2.1 weeks versus 6.2 ± 3.2 weeks; $P < .001$). Numerous studies have since corroborated these findings.†

A principal factor associated with improved functional outcomes after surgery is the reduction in pain associated with the laparoscopic approach. We showed that parenteral narcotic requirements were significantly reduced for patients who underwent laparoscopic compared with open donor nephrectomy.[46] Patients who underwent an open

*References 2, 6, 8, 11, 14, 15, 18, 22, 24, 25, 27, 28, 31, 37, 41, 50, 51, 53, 57, 63, 64, 68, 69.

†References 10, 14, 16, 25, 28, 29, 39, 50, 53, 61, 67, 68, 70, 71.

flank incision required a mean of 124 ± 88 mg morphine sulfate equivalents compared with 34 ± 34 mg morphine sulfate equivalents in patients who underwent laparoscopic procurement (73% reduction). Flowers and associates[10] showed that the duration of parenteral narcotic requirements was reduced by more than 24 hours for patients who underwent laparoscopic compared with open donor nephrectomy, and this finding has been observed in several subsequent investigations.[28,39,68]

Kok and colleagues[23] administered the SF-36 and MFI-20 (Multidimensional Fatigue Inventory-20) questionnaires to randomized clinical trial patients who were being compared across open nephrectomy, mini-incision nephrectomy, and purely laparoscopic nephrectomy to determine patient-reported assessments of health-related quality of life after surgery. Patients who underwent laparoscopic procurement were found to have improved quality-of-life scores for the domains of physical function, general health, vitality, social functioning, emotional well-being, and mental health compared with the donors who underwent open nephrectomy. The laparoscopic group reported significantly reduced physical fatigue as measured by the MFI-20 questionnaire.

RECIPIENT OUTCOMES

Live donor renal allotransplantation provides the recipient with a graft that promptly functions postoperatively and is associated with more durable graft function compared with grafts from deceased donors. Laparoscopic procurement techniques have not been detrimental to these excellent outcomes expected from the live donor graft. From a technical standpoint, adequate lengths of renal artery, renal vein, and ureter can be achieved using a purely laparoscopic approach. In an analysis of our series of more than 500 purely laparoscopic cases, left-sided renal artery, vein, and ureter lengths were found to be 3.1 ± 0.8 cm, 4 ± 1.1 cm, and 11.4 ± 2.3 cm, respectively.[56] The lengths of right-sided structures were comparable with the exception of the right renal vein, which was approximately 1 cm shorter on average (right renal artery, 3.4 ± 1.3 cm; right renal vein, 2.7 ± 1.2 cm; right ureter, 11.6 ± 2.3 cm). Early in our series, the short length of the renal vein was likely associated with two cases of venous thrombosis.[32] Other groups have identified a similar association early in their series.[4] We have since attempted to use the left kidney, unless there is a compelling reason for the donor to retain this kidney. If the right vein is short after the kidney is completely mobilized, we make a transverse rectus-splitting incision and apply a Satinsky clamp to the inferior vena cava, which generally allows for an additional 1 to 1.5 cm of renal vein length. The vena cava is oversewn through the extraction incision. Alternatively, we perform a hand-assisted technique and are able to garner increased venous length through better lateral retraction of the kidney.

Grafts procured by the laparoscopic approach function promptly after surgery. Despite slightly longer warm ischemia times compared with the open (and hand-assisted) approaches, there has been no compelling evidence to suggest that the rate of delayed graft function is increased in recipients of laparoscopically procured kidneys. Delayed graft function occurs in approximately 5% to 10% of recipients. The rate of recovery of renal function, as measured by a decline in the serum creatinine level, has been shown to be comparable for grafts procured using laparoscopic and open techniques.[37,48] Two prospective randomized clinical trials failed to show a difference in renal allograft function at early and later time points.[23,55] These two studies compared renal function within a range of time that extended between 1 day and 1 year after the transplant procedure. Mean renal function at 1 year was excellent in open and laparoscopic groups across both trials (Kok and colleagues:[23] open donor nephrectomy, 114 μmol/L versus laparoscopic donor nephrectomy, 107 μmol/L; Simforoosh and colleagues:[55] open donor nephrectomy, 1.3 mg/dL versus laparoscopic donor nephrectomy, 1.3 mg/dL).

Laparoscopically procured kidneys have durable function that is comparable to grafts that are procured using an open approach. We analyzed more than 28,000 primary live donor renal allograft recipients who were reported to the Organ Procurement and Transplantation Network (OPTN)/United Network for Organ Sharing (UNOS) registry between 2000 and 2005 to compare graft and patient survival by procurement technique. Kaplan-Meier estimates of overall graft survival are shown in Figure 8-11. Graft outcome through 5 years after transplantation has been virtually identical between these two procurement approaches in the United States (P = .59). One-year graft survival estimates in the open and laparoscopic groups were 94.5% and 95.1%. Five-year allograft survival figures were 79.6% and 78.8%.

Estimates of patient survival are shown in Figure 8-12. Comparable mortality rates have been observed between these groups over the last 5 years in the United States (P = .61). One-year estimates of patient survival in the open and laparoscopic groups were 97.6% and 97.8%. Estimates at 5 years after transplantation were 89.2% and 89.1%.

The laparoscopic approach to live donor renal allograft procurement has removed many of the most common disincentives to live kidney donation and promoted an expansion of many live donor programs. The procedure can be undertaken safely, and the graft can be transplanted using standard techniques without modifying expectations of excellent short-term and long-term outcomes for the recipient.

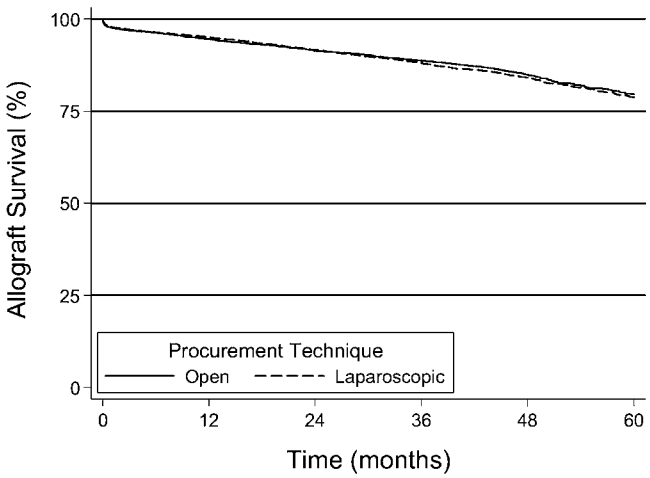

Figure 8–11 Kaplan-Meier estimates of graft survival following transplantation in recipients of primary live donor renal allografts, by procurement type. There was no difference in graft survival between the procurement technique groups (p=0.59).

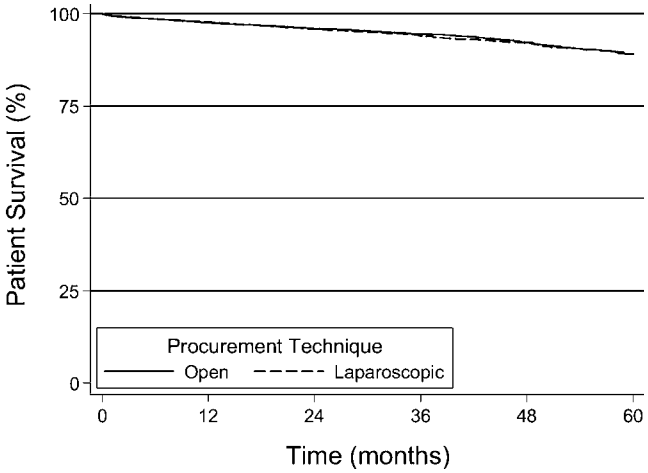

Figure 8–12 Kaplan-Meier estimates of patient survival following transplantation in recipients of primary live donor renal allografts, by procurement type. There was no difference in mortality between the procurement type groups (p=0.61).

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Chapter 9
Renal Preservation

Henri G. D. Leuvenink • Rutger J. Ploeg

Effective and successful kidney transplantation depends on a sequence of events. These events can be described as the "transplantation cascade" depicted in Figure 9-1. During this cascade, renal quality is jeopardized by biological and technical moments of danger. Because of the unique character of transplantation when a functioning kidney is retrieved from a human body, transferred to another human body, and connected to the vasculature and blood supply of the recipient, the kidney is exposed to a series of extremely nonphysiological insults.

At the time of retrieval of the organ from the donor, blood supply is interrupted, which results in ischemia. To ensure function after transplantation, this period of ischemia needs to be as short as possible because warm ischemic damage is extremely detrimental to the kidney. During the warm ischemic period, the kidney is devoid of blood, oxygen, and nutrients, while metabolism continues at full strength. Reducing metabolic activity is crucial to prevent organ damage beyond repair. An easy and convenient way is to cool the kidney. Most commonly used nowadays is the static cold storage technique, which includes an initial flush and washout of the kidney with subsequent storage in a preservation solution at 0°C to 4°C. The use of cold storage preservation provides time for tissue typing and crossmatching, allocation and transportation of the organ to the recipient center, and preparation of the recipient.

The implantation phase is another critical period. Apart from technical surgical issues, a second period of warm or semiwarm ischemia occurs. During this phase, vascular anastomoses need to be prepared before blood flow can be reconstituted. Intuitively, one may think that the most dangerous period for the graft should have passed after restoration of blood flow into the transplanted kidney. The supply of warm well-oxygenated blood should lead to an increase of metabolism resulting in a proper functioning graft. The reperfusion phase is not devoid of side effects, however. During the reperfusion phase, the preexisting damage occurring in the donor kidney as a result of brain death, cold preservation, and warm ischemia at the time of implantation becomes apparent, reflecting the viability of the donor kidney. The reintroduction of oxygen leads to enhanced formation of free radical oxygen species. Misbalances in intracellular and extracellular ion concentrations and edema need to be counteracted quickly to limit further damage. Preservation solutions are designed to counteract cold ischemia–induced changes in the graft. This chapter reviews the current use of preservation solutions and methods and discusses many innovations.

CURRENT USE OF PRESERVATION SOLUTIONS

Renal transplantation has become a standard therapy for end-stage renal failure. Owing to standardized techniques, better immunosuppression, and more experience of how to cope with post-transplant complications, the outcome of renal transplantation has improved. As a result, indications for transplantation have broadened—resulting in long recipient waitlists. Despite many major efforts to increase the donor pool of deceased heart-beating, brain-dead (DBD) donors, the addition of living donor programs, and the exploration of deceased cardiac death (DCD) donors, the persisting donor shortage remains a key problem in renal transplantation.

Regardless of the donor source, all kidneys to be transplanted need to be preserved during the time between retrieval and implantation. At present, static cold storage is the preferred organ preservation method, which includes a rapid vascular flush and washout with removal of blood, rapid cooling of the organ, and equilibration between the cold storage solution and tissue.[106,170]

With high patient survival and improved graft survival rates, despite the sometimes relatively long periods of preservation, renal transplantation seems to be a safe and reproducible therapy. This statement may imply that there is no room for improvement, but several authors have

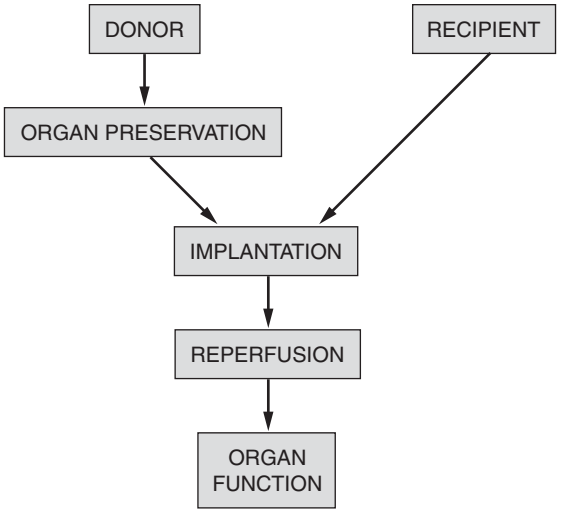

Figure 9–1 The transplantation cascade.

documented that the duration of preservation is one of the factors influencing outcome, especially with cold ischemia times greater than 24 hours.

The qualifications and criteria for kidney donation have changed. In the 1980s, the average deceased donor was a healthy young individual who was involved in an accident leading to cerebral trauma and brain death, whereas nowadays most brain-dead donors are middle-aged individuals who died as a result of a cerebral hemorrhage. Between 1988 and 2005, the United Network of Organ Sharing (UNOS) reported a 170% increase in deceased donors older than 50 years of age (Fig. 9-2A).[46,60]

The increased proportion of older donors is not accompanied by a similar number of renal transplants (Fig. 9-2B). The percentage of nontransplantable kidneys in the United States is increasing with age. In addition to the lower percentage of kidneys transplanted from older donors, graft survival is lower with older donor kidneys.[160] Also, marginal and DCD organs become damaged from additional warm ischemic injury. After kidney transplantation, these kidneys have higher primary graft nonfunction and delayed graft function (DGF), with rates of 4% to 9% and 22% to

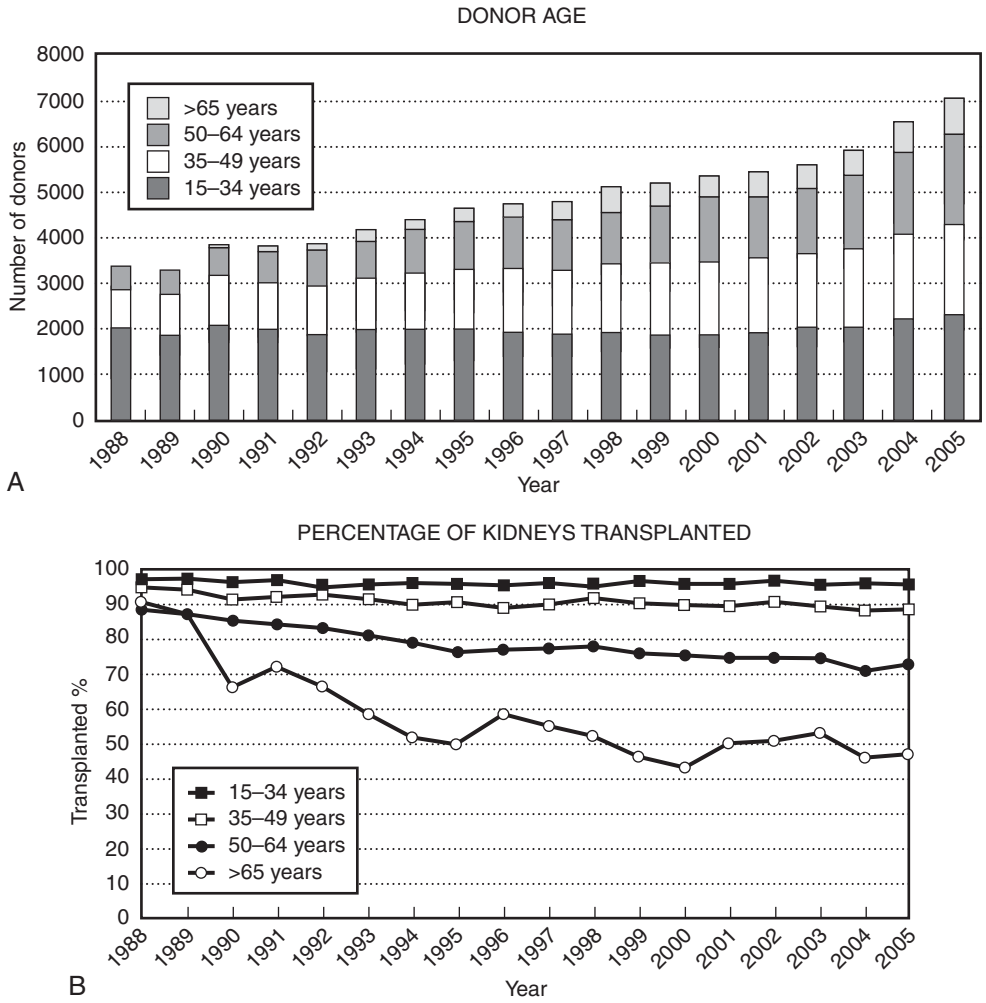

Figure 9–2 **A**, Donor age of retrieved kidneys. **B**, Influence of age on percentage of renal transplants. (Data from Organ Procurement and Transplantation Network.)

84% compared with 1% to 2% and 7% to 25%, respectively, with heart-beating donors.[7,112,136]

Maintaining organ viability during preservation has become an important prerequisite for successful outcome after transplantation. Currently, most centers use static cold storage to preserve organs. This preservation method was developed, however, in an era with younger and better quality donor organs.[20] Despite the aforementioned considerations, preservation of DBD kidneys for less than 24 hours generally results in adequate function and graft survival, whereas preservation times and methods seem to be more critical in the outcome of DCD kidneys, which are associated with inferior graft survival.

As illustrated in Figure 9-3A, in the UNOS region, most kidneys derived from deceased donors have been preserved in University of Wisconsin solution. In recent years, the use of histidine-tryptophan-ketoglutarate (HTK) solution has increased probably partly as a result of the inclusion of DCD donors. In Europe, HTK and University of Wisconsin solutions are both used in DBD donors. Also in the Eurotransplant region, the use of HTK solution is increasing because almost all DCD donor organs are flushed with and stored in HTK solution owing to the lower cost at high volumes (Fig. 9-3B).

PRINCIPLES OF COLD STORAGE PRESERVATION

Removal of the kidney from the circulatory system leads to disruption of the blood supply. The absence of oxygen delivery to the cells rapidly leads to major metabolic problems. Suppression of metabolism is essential to prolong the time of ischemia the kidney can sustain. Reducing the core temperature of the kidney to less than 4°C results in a reduction of metabolism to 5% to 8% in most cells and diminishes enzyme activity.[151] In 1963, Calne and colleagues[43] showed that simple cooling of kidneys in ice water preserved function of kidneys for 12 hours—the temperature effect. With a preservation solution, however, cold ischemia times can be significantly prolonged, and preservation quality can be improved—the solution effect.[109] Despite the beneficial concept of hypothermia, it causes several unwanted side effects in the preserved organ, such as cell swelling, acidosis, and production of radical oxygen species on reperfusion (Fig. 9-4).

Cell Swelling

Histological alterations in cellular structures observed during preservation are cell swelling and formation of protruding pockets.[90] The mechanism underlying these

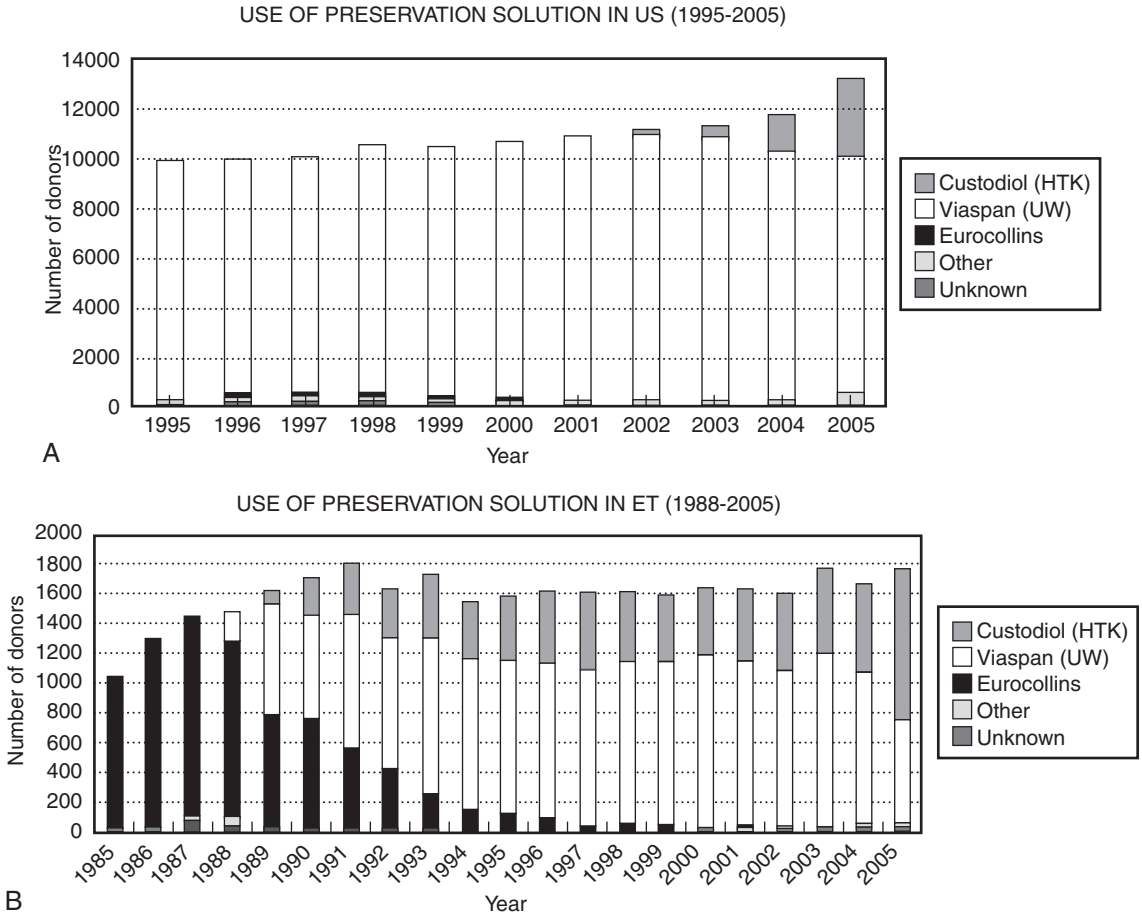

Figure 9–3 **A**, Current use of preservation solutions in the United States in cadaver donors. **B**, Use of cold storage preservation solution in the Eurotransplant (ET) region in cadaver donors. (**A,** Data from Organ Procurement and Transplantation Network; **B,** data from Eurotransplant International Foundation.)

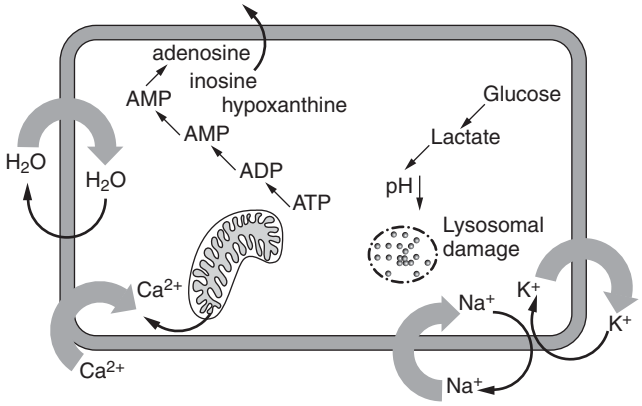

Figure 9–4 Negative effects of cold ischemia are breakdown of adenosine triphosphate (ATP), acidosis, release of lysosomal enzymes, increased Na^+ and Ca^{2+} entrance in the cell, and subsequent cell swelling. ADP, adenosine diphosphate; AMP, adenosine monophosphate.

structural changes is due to impaired activity of Na^+,K^+-ATPase. As a result, sodium is no longer excreted and passively enters the cell attracted by the negative charge of cytoplasmic proteins; this creates a hyperosmolar intracellular environment and subsequently an influx of water. To re-establish the disturbed Donnan's equilibrium of the membrane and to prevent cell swelling, impermeants and colloids are added to preservation solutions. Effective impermeants are saccharides and nonsaccharide anions. Molecular size and effectiveness of saccharides are positively related to prevention of cell swelling, with larger saccharides being most effective.[74,156,175]

Energy and Acidosis

Owing to the nature of aerobic metabolism, the absence of oxygen results in a rapid decrease in intracellular adenosine triphosphate (ATP) levels. Even at a dramatically reduced metabolic rate, at 0°C to 4°C, cellular ATP content is rapidly depleted. Within 4 hours, nearly 95% of ATP has disappeared with a shift to adenosine monophosphate as the predominant nucleotide. During cold storage, anaerobic metabolism of 1 mol of glucose yields only 2 mol of ATP versus a maximum of 38 mol in aerobic glycolysis. Two lactic acid molecules are formed leading to acidosis.[72,106]

The contribution of acidosis to ischemic injury is pH dependent. Severe acidosis activates phospholipases and proteases causing lysosomal damage and eventually cell death.[32] Mild acidosis (pH 6.9 to 7.0) has been suggested to have a protective effect, however, by inhibiting phosphofructokinase as the rate-limiting step in glycolysis.[32,82] Adequate control of pH is an important function of preservation solutions.

Reactive Oxygen Species

Reactive oxygen species (ROS) are generated by several processes in ischemic and postischemic reperfused organs.[110] An extensively studied generator of ROS is xanthine oxidase, which simultaneously produces hydrogen peroxide (H_2O_2) and the superoxide anion (O_2^-).[102,141] The subsequent reduction of H_2O_2, catalyzed by iron, leads to hydroxyl radical formation (OH). ROS reacts rapidly with other molecules, which results in severe damage to lipids, nucleic acids, and proteins during reperfusion.[42,99]

In addition to xanthine oxidase, which, in contrast to rodents, in human renal transplantation may be of minor importance because it is not abundant in humans,[150] several other sources of ROS are important, especially during the reperfusion phase. Infiltration of leukocytes into the graft after reperfusion results in production of mainly superoxides (the respiratory burst). Mitochondrial malfunctioning resulting from partial reduction of the respiratory chain is an important contributor to ROS formation after reperfusion. The formation of ROS has long been considered to contribute to cellular injury during the reperfusion phase, but not during cold preservation.[42,99] During cold ischemia, cellular metabolism and enzymatic activity are very low. Some reports suggest, however, that oxygen radicals are formed during reperfusion and during cold preservation.[137,138] Because free radical–mediated injury during preservation is strongly correlated with immediate and long-term kidney function,[99] preservation solutions should aim to counteract ROS-mediated injury.

Calcium

During normal circumstances, a large difference in free calcium concentration exists between the intracellular and extracellular space fluid. This difference is maintained by active transport of Ca^{2+} by several ATP-demanding processes, including Ca^{2+}-ATPase and Na^+-Ca^{2+} exchanger.[24] During cold preservation, cellular ATP concentrations are low leading to increased intracellular Ca^{2+}. Accumulation of Ca^{2+} in the cold leads to activation of calcium-dependent processes, such as calpain activation and protein kinase C signaling. Calpain activation leads to loss of cell structure by breakdown of the cytoskeleton spectrin.[71] During cold storage, calpain activity has been shown to be increased in cold stored hepatocytes and increased further during rewarming.[94]

Enzymes

Intracellular proteases are involved in the breakdown of proteins during preservation most likely because of the absence of oxygen. Also, matrix metalloproteinases may be activated during cold preservation leading to detachment of endothelial cells from the underlying matrix. This phenomenon has been predominantly studied in the liver, but also occurs in renal preservation.[161,162] To reduce this detrimental effect by blocking matrix metalloproteinases (especially matrix metalloproteinases 2 and 9), the addition of the often disputed colloid hydroxyethyl starch (HES) in University of Wisconsin solution has been shown to play an important role.[162] Another relevant family of enzymes activated during cold preservation are apoptosis-related caspases.[62]

COMPOSITION OF CLINICALLY USED SOLUTIONS

With the introduction of the first static cold storage solution by Collins in 1969,[53] prolonged preservation of kidneys became clinically feasible. The original Collins solution

Table 9–1 **Composition of Major Cold Storage Preservation Solutions**

	EC[9]	UW[176]	HTK[39]
Colloids (g/L)			
HES (g/L)	—	50	—
Impermeants (mM)			
Glucose	195	—	—
Histidine	—	—	198
Mannitol	—	—	38
Lactobionate	—	100	—
Raffinose	—	30	—
Buffers (mM)			
K_2HPO_4	15	—	—
KH_2PO_4	42.5	25	—
$NaHCO_3$	10	—	—
Histidine	—	—	198
Electrolytes (mM)			
Sodium	10	25	15
Potassium	115	120	9
Chloride	—	20	32
Calcium	—	—	0.0015
Magnesium	—	—	4
Magnesium sulfate	—	5	—
ROS scavengers (mM)			
Glutathione	—	3	—
Allopurinol	—	1	—
Tryptophan	—	—	2
Additives (mM)			
Adenosine	—	5	—
Ketoglutarate	—	—	1

EC, EuroCollins; HES, hydroxyethyl starch; HTK, histidine-tryptophan-ketoglutarate; ROS, reactive oxygen species; UW, University of Wisconsin cold storage solution.

was modified by the Eurotransplant Foundation in 1976, eliminating magnesium.[9] EuroCollins solution was a simple and cheap, intracellular-like preservation solution (Table 9-1). Phosphate was used for pH buffering, and glucose served as the osmotic agent. Since the introduction of the University of Wisconsin Cold Storage Solution (UW-CSS), most centers have been using this solution (see Fig. 9-3A). A randomized clinical trial comparing EuroCollins with UW-CSS showed that DGF was significantly lower in the UW-CSS group (23% versus 33%). Also, 1-year graft survival was found to be significantly higher in the UW-CSS group.[127] As a result of this study, EuroCollins was no longer the preferred solution for kidney preservation in Europe (see Fig. 9-3B).

University of Wisconsin Solution

Continuous and systematic research by Belzer and Southard in the 1980s led to the development of the University of Wisconsin solution and its clinical introduction in 1987. Metabolic inert substrates such as lactobionate and raffinose served as osmotic agents (see Table 9-1). HES was used as a colloid. Scavengers (glutathione, allopurinol) and an ATP precursor (adenosine) were added to the solution. To date, UW-CSS is considered the "gold standard" preservation solution for kidney, liver, pancreas, and small bowel.[31,45,58,63,67,91,127]

Histidine-Tryptophan-Ketoglutarate Solution

HTK solution was initially introduced as a cardioplegic solution in open heart surgery by Bretschneider in the 1970s.[39] The solution consists of a very potent buffer, histidine, combined with two amino acids (see Table 9-1). Tryptophan serves as membrane stabilizer and antioxidant, whereas ketoglutarate acts as substrate for anaerobic metabolism during preservation. HTK solution has a low viscosity, and to achieve complete tissue equilibration according to Bretschneider, high volumes (approximately 15 L) have to be rinsed through the organs at low flow rates. A multicenter randomized prospective trial comparing UW-CSS versus HTK solution in kidney preservation showed equal results in terms of the incidence of DGF (33% versus 33%).[58] For prolonged cold storage times with HTK solution (>24 hours), few data are available. One single-center study reported a significantly higher incidence of DGF of 50% for HTK solution–preserved kidneys versus 24% for UW-CSS–preserved kidneys when cold ischemia time was greater than 24 hours.[135] The opposite was reported in a more recent study, with a DGF rate of 16% after HTK solution preservation versus 56% after UW-CSS preservation.[1] Direct comparison of these conflicting findings is impossible because of a different definition of DGF in both studies.

Colloids and Impermeants

Glucose is a monosaccharide and was used in early cold storage solutions (e.g., EuroCollins). Because glucose is able to pass the cell membrane, it is a source for ATP and lactate in an anaerobic environment, reducing its impermeant effectiveness.[115] Mannitol is a slightly larger monosaccharide, but is not metabolizable and does not pass the cell membrane easily. It is added for its beneficial effect as a radical oxygen scavenger to HTK solution. The largest and most effective saccharide is raffinose, which is used in UW-CSS.

Nonsaccharide impermeants, such as gluconate, citrate, and lactobionate, limit cell swelling by electrochemical forces. Effectiveness of these anions is determined by molecular size and charge. UW-CSS and Celsior solution, which is a newer solution based on the University of Wisconsin concept, contain lactobionate.

Colloids are macromolecules that do not pass the cellular membrane. Colloids originally were added to hypothermic machine preservation solutions to prevent tissue edema owing to hydrostatic pressure. Belzer and Southard used diafiltrated HES in UW-CSS because they originally aimed at developing one solution suitable for cold storage and hypothermic machine perfusion (HMP). The feasibility of HES as a colloid in UW-CSS has been extensively debated. HES prevents interstitial edema and has a beneficial effect on matrix metalloproteinases, but it increases viscosity.[127,170] Analyzing the effect of HES on red blood cells, several authors have shown an increased red blood cell aggregability when large-molecular-weight HES is present.[113,170] This effect could partially explain the slower washout of blood and initially patchy reperfusion of organs when UW-CSS is used.[76]

The HES controversy initiated a search for other colloids (e.g., dextran and polyethylene glycol [PEG]).[22,26,44] In this respect, UW-PEG–preserved livers have shown lower transaminase levels, higher bile flow, and higher urea

synthesis after transplantation.[114] Several experimental and clinical studies have now confirmed the efficacy of PEG not only for liver, but also for kidney, pancreas, small bowel, and heart preservation.[21,88,179,183]

In contrast to UW-CSS, HTK and EuroCollins solutions do not contain a colloid. In a prospective study with short cold ischemia times, both solutions showed equal efficacy compared with UW-CSS for preserving kidney and liver grafts.[124] With prolongation of preservation times, the presence of a colloid does seem to be important to maintain organ viability.[152]

Electrolyte Composition

During the pioneering years of organ preservation, a high potassium-to-low sodium ratio of the solution (intracellular type) was assumed necessary to prevent cell swelling. It was hypothesized that because of the inactivity of Na^+,K^+-ATPase during hypothermia, an intracellular sodium-to-potassium ratio in the extracellular fluid compartment would prevent sodium and chloride from entering the cell.[13] Balancing extracellular sodium ions and intracellular protein anions creates the Donnan equilibrium, which prevents edema formation.[74] Intracellular-type solutions such as UW-CSS were long considered to be pivotal for preservation of cell viability.[13,54] More recent work has suggested, however, equal or better results of extracellular-type solutions with a low potassium-to-high sodium ratio.[23,79,149,157,163,177] As a result of the lower potassium content, washout of blood during organ procurement is facilitated because no potassium-induced vasospasm occurs.[107,149] In liver preservation, it has been suggested that HTK solution could be advantageous owing to its low potassium concentration. The need to flush the organ before reperfusion would be limited. Although patient numbers were relatively small and cold ischemia times short (<10 hours), two studies using HTK solution in liver preservation concluded equality of HTK solution and UW-CSS for short-term preservation.[63,128]

Reactive Oxygen Species Scavengers

In UW-CSS, the compounds allopurinol and reduced glutathione (GSH) were included to prevent formation of ROS. Allopurinol inhibits xanthine oxidase, which improves kidney preservation, whereas liver or pancreas preservation is almost unaffected.[30]

GSH is a tripeptide that has free radical trapping properties. This important antioxidant is oxidized to glutathione disulfide together with converting peroxides. Experimental studies have shown the importance of GSH in an isolated perfused rabbit liver model. In the absence of GSH, more lactate dehydrogenase was released into the perfusate.[89] Subsequent studies have shown that GSH is especially important in long-term liver preservation.[33]

In HTK solution, tryptophan protects the organs against ROS-mediated damage. Tryptophan acts as an antioxidant through its oxidative metabolites in the kynurenine pathway, such as 5-hydroxytryptophan.[50,66] In a cultured rat hepatocyte experiment, the amount of thiobarbituric acid reactive substances as a marker for ROS-mediated injury was measured. After 24 hours of preservation, thiobarbituric acid reactive substances were significantly higher in HTK solution–preserved hepatocytes compared with UW-CSS–preserved hepatocytes, suggesting superior antioxidant capacity of UW-CSS owing to the combination of GSH and allopurinol.[133]

PRESERVATION BY HYPOTHERMIC MACHINE PERFUSION

In the late 1960s, HMP, developed by Belzer, was used by many centers in the United States to preserve kidneys because it was considered the best and only way to transport organs from the donor to the recipient center.[81] Belzer and coworkers were able to preserve canine kidneys for 72 hours using the HMP technique[18] and introduced the HMP technique clinically 1 year later.[19] With the introduction of an "effective" cold storage preservation solution such as EuroCollins, the number of kidneys preserved by machine decreased. In the United States today, about 10% of kidneys are preserved by machine preservation (Fig. 9-5). In more recent years, a small increase can be observed, presumably as it has become generally accepted that kidneys from DCD donors are better preserved using machine perfusion preservation.

Although modern HMP systems are smaller, lighter, and more sophisticated than the original machine used by Belzer (Fig. 9-6), the principles of HMP have not changed. Machine perfusion generates a controlled continuous or pulsatile recirculating flow of the preservation solution at 0°C to 4°C. This continuous flow allows complete perfusion of the organ promoting a complete washout of blood and subsequent tissue equilibration with preservation solution. Until now, technologies used in preservation machines had remained almost identical for decades, using roller pumps simulating a pulse wave at a low pressure setting. A new machine perfusion system was developed employing centrifugal

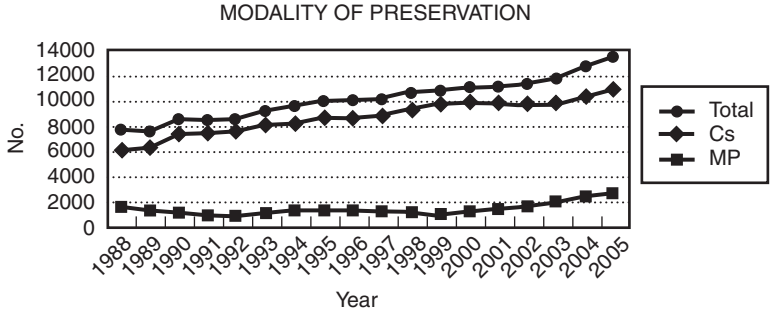

Figure 9–5 Number of kidneys preserved by cold storage (Cs) or machine perfusion (MP). (Data from Organ Procurement and Transplantation Network.)

Figure 9–6 Professor F. O. Belzer with the first "transportable" machine perfusion system.

pumps that allow higher pressures under hypothermic and normothermic conditions using acellular solutions and blood.[172] This machine was shown to result in better porcine kidney preservation compared with cold storage.[105]

Beneficial effects claimed on behalf of machine perfusion are a low incidence of DGF, the possibility of online viability assessment, the ability to provide metabolic support during perfusion, and the potential to add pharmacological agents to the perfusate. In kidney preservation, in animal experiments and in historical controlled clinical studies, HMP has been shown to provide better early graft function compared with static cold storage.[48,51] In addition, when kidneys derived from extended, marginal, or non–heart-beating (NHB) donors were analyzed, HMP was found to be beneficial.[16,19,119,129,145] In most studies, no randomization was used, and patient numbers were not large enough to allow extrapolation of the results. Wight and colleagues[180] reported an excellent meta-analysis based on aggregated results of current literature concerning HMP versus static cold storage, showing a 20% reduction in DGF. DGF is the compilation of accumulated risk factors and depends on the presence or absence of independent donor, preservation, and recipient characteristics.[127] Possibly, some of the detrimental effects caused by these risk factors, which make a kidney susceptible to injury and result in DGF, can be reduced with HMP. The occurrence of DGF after transplantation requires continuation of dialysis and is associated with an increased incidence of acute rejection and inferior long-term outcome.[123,125]

Although individual studies suggest potential benefits of HMP, such as less DGF, less acute rejection, and better short-term and long-term function at reduced cost, no comparative study of these modalities has been performed under strict conditions.[153] For this reason, a European multicenter prospective randomized clinical trial was started in The Netherlands, Belgium, and Germany[126] comparing for the first time a U.S. Food and Drug Administration–approved transportable perfusion machine with cold storage. The results of the trial suggest that there is a beneficial effect of HMP over cold storage both in terms of fewer cases of DGF and graft failure in the first 6 months after transplantation.[184,185]

Overall, experimental and clinical data imply that HMP improves renal preservation. Because modern, portable, and stand-alone HMP systems for kidney preservation are now available, allowing user-friendly transportation within international organ sharing systems, a broader clinical application of HMP should be considered to reduce the impressively high DGF rate of 60% to 85% in NHB donor kidneys.[41,134,182]

RENAL PRESERVATION STARTS IN THE DONOR

In the early days of organ transplantation, all cadaver donor grafts were retrieved from victims of cardiac death.[84,111,174] When legal definitions for brain death (Harvard Criteria) became available in the late 1960s,[8] most centers established transplant programs based on organ retrieval from heart-beating, brain-dead donors, avoiding the warm ischemic damage that NHB donor organs by definition have sustained.[98]

In recent decades, indications for transplantation have been extended, although not accompanied by a substantial increase in donors. In an effort to enlarge the donor pool, living donation has made a valuable contribution to kidney transplantation programs.[57,142] Such programs could never yield sufficient new donor organs to bridge the global gap between supply and demand. Many centers are now actively re-establishing the practice of DCD or NHB donation,[93] although actual numbers are still small in many countries.

In the United States, a gradual increase in living donors has been observed such that almost 50% of all kidney transplants in 2005 were from living donors (Fig. 9-7). In Europe, the growth of living donor programs has been more modest than in the United States, but is continuously increasing.

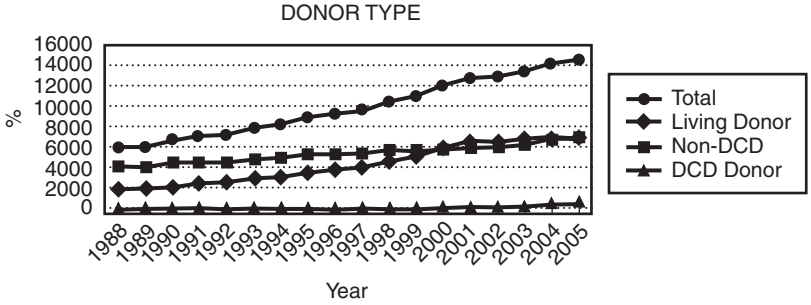

Figure 9–7 Gradual increase in (living) donor number in the United States. DCD, deceased cardiac death donor. (Data from Organ Procurement and Transplantation Network.)

A striking phenomenon is that now, more than 3 decades after the definition of brain death, DCD or NHB donation is being revisited. To date, in The Netherlands, DCD donation contributes 40% of all transplanted cadaver donor kidneys. Transplant outcome achieved with kidneys from living donors is far superior compared with grafts obtained from cadaver donors.[160] The persistent donor organ shortage has led, however, to a gradual shift toward accepting suboptimal donors. The use of older and more marginal donors is now routine, and the number of NHB donors has increased significantly. As mentioned earlier, in the 1980s, the typical donor was younger than age 30, was fairly healthy, and had died of traumatic cerebral injury. Today, the average donor is older than 50, and the main cause of death is intracranial hemorrhage. The improvements that have occurred in immunosuppressive treatment of the recipient, organ preservation, reduction of cold ischemia time, and better allocation of donor organs based on matching have been masked to some extent by the use of lower quality donors. In the past, much effort was directed toward post-transplant immunosuppression and better treatment of complications. Now, risk factors and conditions before organ retrieval in the donor also need to be recognized for their impact on donor organ viability.

Brain-Dead Donors and Preservation

To date, most cadaver donor organs are still retrieved from cerebrally injured brain-dead or cardiac death donors. The condition of the patient before retrieval and preservation, together with the efficacy of preservation, determines the viability of the organ at the time of transplantation. Brain death induces pathophysiological changes in the donor kidney that have a negative impact on the outcome after transplantation. Ischemia of the brain results in nonfunction of the central nervous system and is associated with pertinent hemodynamic instability, hormonal changes, and diminished perfusion. This abnormal physiological state induces proinflammatory changes in the potential donor organs that negatively affect function and cause an increased chance of acute rejection.

Before 1997, the concept of donation after brain death did not exist in Japan. Patients who would be considered brain dead and eligible for organ donation in the United States or Europe were kept in a coma in Japan until cardiac arrest occurred. This presented Nagareda and coworkers[116] with the unique opportunity to investigate the time course of the effects of brain death on the kidney for 48 days. Their study revealed that the mean urinary sodium output increased during the first 14 days, mean urine osmolarity was above normal on the first day but decreased gradually, and urine volume during the first 14 days was high as a consequence of the cerebral injury–related diabetes insipidus. On histological examination, degenerative changes of renal structures were found, including vacuolization, atrophy, and necrosis of renal proximal and distal tubules. Advancing glomerulitis and progressing periglomerulitis expressed inflammatory changes. Fibrosis and proliferation of the arterial intima and glomerular endothelium reflected the structural changes in the kidney.

In experimental conditions in rats, renal function is already adversely affected during 4 hours of brain death followed by inferior results after reperfusion in an isolated perfused kidney model. In the isolated perfused kidney, urine volume and glomerular filtration rate were significantly higher than in controls.[166] Potassium excretion was increased in these kidneys, possibly explained by the depletion of ATP in these kidneys, which can trigger the opening of ATP-sensitive potassium channels. An impaired sodium/potassium homeostasis also was observed after brain death in a renal slice model.[178] Organs also can become more prone to ischemia-reperfusion injury; livers derived from brain-dead rats are more susceptible to hypothermic preservation–induced injury. This susceptibility was shown by a decreased survival after 20 hours of cold storage when compared with living donor livers stored for the same time.[167]

Renal tubular damage as a consequence of brain death can be observed in urine as well. Brush-border enzymes, such as alkaline phosphatase and alanine amino peptidase, and the lysosomal enzyme *N*-acetyl-beta-D-glucosaminidase[166] are released into the urine. Kidney injury molecule 1 is a more recently discovered brush-border enzyme that is considered a marker of tubular damage (e.g., in ischemia-reperfusion injury).[59,85,173] As a result of brain death, kidney injury molecule 1 was massively upregulated. It can be detected on the luminal side of the renal cortical tubule and is shed into the urine,[148] which may simplify viability assessment of potential donor organs.

Immunological Activation

In ischemia-reperfusion injury, a clear-cut correlation was found between endothelial injury and acute rejection. This association between the innate immune response and subsequent alloreactivity could be explained by Matzinger's danger hypothesis.[108] An increased immunogenicity also is observed in the brain-dead donor organ. Endothelial activation is present with the upregulation of adhesion molecules (E-selectin, P-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1) that promote the rolling, adhesion, diapedesis, and subsequent leukocyte migration into the interstitium of the kidney.[95,103,158,168,169] Multiple cytokines and chemokines play a role in the immunological response to cerebral injury. Upregulation of interleukin-1, interleukin-2, interleukin-6, tumor necrosis factor-α, transforming growth factor-β, interferon-γ, vascular endothelial growth factor, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, monocyte chemotactic protein-1, and osteopontin has been reported.[103,104,146,147,158] The expression of the major histocompatibility complex class II is increased as well.[158] Amplification of cytokines, chemokines, and adhesion molecules causes a chemotactic gradient that promotes the influx of leukocytes to the kidney. T cells, macrophages, and polymorphonuclear neutrophil leukocytes all are found in higher quantities in donor kidneys during brain death.[121,158,168,169]

After reperfusion, a large difference in neutrophil infiltration and P-selectin expression can be observed between living and cadaver grafts. Koo and associates[95] showed that 53% of cadaver renal allografts had increased neutrophil infiltration versus 0% of living related grafts. P-selectin expression was increased in 44% of cadaver grafts and 9% of living related grafts.

In a syngeneic animal model of renal transplantation, Kusaka and colleagues[103] investigated short-term inflammatory changes to the kidneys. Leukocyte infiltration reaches its

peak at 24 hours after transplantation in this syngeneic transplant model and corresponds with the levels of E-selectin and P-selectin. After this period, the extent of immunological activation gradually decreases, but histological changes to the kidney still can be observed. Allotransplant experiments have shown that after experimental brain death, recipients of brain-dead donor kidneys had a greatly increased acute rejection rate.[130] When kidney allografts are treated with cyclosporine to prevent acute rejection, long-term renal function is adversely affected by brain death compared with syngeneic transplants. The state of brain death also can enhance the development of chronic renal transplant dysfunction.[131]

Protection and Repair

Not only detrimental or degenerative changes occur during cerebral injury and preservation; protective or recuperative mechanisms are induced as well. There is increased expression of the cytoprotective genes heme oxygenase-1 (HO-1), heat-shock protein 70, and manganese superoxide dismutase.[121,146,147] Kunzendorf and coworkers[101] showed that a prolonged duration of brain death positively influences long-term graft survival. The mechanism behind this observation could be the delayed induction of protection or initiation of repair. In another study, increased HO-1 expression at organ retrieval was correlated with outcome after renal transplantation in the living donor setting.[121] Expression of HO-1 was not related to graft survival in deceased donor kidneys. Donor HO-1 gene polymorphisms have been associated with transplantation outcome.[12] In a liver transplant study, livers with an initial low HO-1 expression before transplantation, but a high HO-1 expression after reperfusion, had superior outcome compared with livers with high HO-1 expression at organ harvest.[70] These observations indicate that the ability to induce HO-1 is important, and not the high expression of HO-1 per se. Two different mechanisms should be considered here: Although the increase in expression of HO-1 in living donors may initiate protection against the potential injuries to the kidney during transplantation and thereafter, in deceased donors, HO-1 may be a reflection of the level of stress to the kidney owing to brain death. These compromising changes in the donor suggest that there is a need to develop treatment regimens for application during the state of brain death and before retrieval and initiation of preservation.

The use of pharmacological interventions to provide optimal conditions for the donor organ and prevent the decline of renal function is expected to become an important part of the entire donation and transplantation process. Reducing hemodynamic instability is crucial to maintain normal perfusion of organs. The use of catecholamines for this purpose would benefit renal transplant outcome. Caution is needed, however, because interventions that can be beneficial to one organ may be detrimental to another; this was shown by Schnuelle and associates[144] in their analysis of catecholamine use in the donor. Although renal transplant survival was increased, liver transplant outcome was not improved, and cardiac results seemed to be adversely influenced by catecholamine administration in the donor.[143] A randomized prospective clinical trial is currently under way to assess the effects of donor pretreatment with dopamine.

The use of immunomodulators such as corticosteroids or recombinant soluble P-selectin glycoprotein ligand immunoglobulin has shown some promising results in experimental models.[68] Counteracting inflammatory changes in the deceased donor kidney improved function and survival after transplantation.[69] Steroid treatment is effective in modulating the immune response in human organ donors.[100] The use of carbamoylated erythropoietin was shown to decrease breath death–induced inflammation effectively[52] and protect against ischemia-reperfusion injury.[86] Because all organs exhibit inflammatory changes as a result of brain death, immunomodulating treatment has a high probability of being beneficial for all transplanted organs.

The induction of protective mechanisms, such as HO-1 upregulation, is an important development in donor pretreatment. Initiation of protective pathways can diminish brain death–related damage and ischemia-reperfusion injury. The products created during heme degradation by HO-1 are involved in cytoprotective processes. In addition, immunomodulating effects of HO-1 could be useful in the improvement of deceased donor transplantation. Another option is the addition of gaseous substances to the breathing air of brain-dead donors. Carbon monoxide has shown a beneficial effect in modulating ischemia-reperfusion injury,[2] and inhalation of low-dose carbon monoxide after experimental renal transplantation prevents the development of chronic allograft nephropathy.[117]

To date, many challenging opportunities do exist to counteract the deleterious effects of brain death in combination with preservation of the donor kidney. A better characterization and understanding of the mechanisms of injury and repair that play a role during massive cerebral injury, followed by ischemia-reperfusion and its effect on potential donor organs, would lead to novel treatment options. As a result, the outcome after cadaver donor organ transplantation may improve and approach that of living donors.

Deceased Cardiac Death Donation

The use of DCD donors (in Europe often referred as NHB donors) to enlarge the donor pool is a logical step because the potential pool of these donors is many times larger than the amount of available DBD donors.[97,98] In the late 1980s and early 1990s, a few hospitals had already reintroduced DCD protocols. The group from Maastricht, led by Kootstra, was one of the pioneering centers.[80] In 1995, at the First International Workshop on NHB Donors in Maastricht, consensus was reached about donor management protocols, and four different categories of NHB donors were defined (Table 9-2).[96]

The practice of DCD donation has increasingly become part of transplant programs throughout the world. Within Eurotransplant, 6% of all kidney donors in 2005 were DCD donors. Of these donors, 91% came from The Netherlands.

Table 9–2 **Maastricht Classification of Non–Heart-Beating Donors**

Category	Description	Procurement
I	Dead on arrival	Uncontrolled
II	Unsuccessful resuscitation	Uncontrolled
III	Awaiting cardiac arrest	Controlled
IV	Cardiac arrest while brain dead	Uncontrolled

In The Netherlands, 47% of all donors were DCD donors in 2005, mostly Maastricht category III.[10] In Spain, although nationwide only 4% of the donor pool consists of DCD donors, the Hospital Clinico in Madrid developed a well-established NHB program, with approximately 25% of all cadaver donors being DCD (percentages adopted from the website for the Spanish National Transplant Organization, www.ont.msc.es). In the United Kingdom, 11% of all cadaver kidney grafts came from NHB donors in 2005.[11] Worldwide, several centers in the United States and Japan have started extensive DCD programs.[49,77,122,159]

At present, more than 10 years after the Maastricht workshop, many centers have published results of their DCD programs. NHB grafts have a significantly inferior function in the short term, with reported DGF rates of 48% to 94% compared with 19% to 46% for organs retrieved from DBD donors. For primary graft nonfunction, these rates are 4% to 14% and 1% to 8%.* Medium-term and long-term graft survival and acute rejection rates do not differ between these two types of donors.[40,41,47]

Retrieval and preservation of DCD kidneys may involve different approaches compared with living and DBD kidneys. As in DCD donation, blood circulation is no longer present owing to the cardiac arrest, and an important cornerstone of DCD donor management is the reduction of warm ischemia. The major difference between warm and cold ischemia is the rate at which injury develops in the donor kidney. Detrimental effects of ischemia are much more pronounced as long as organ cooling has not yet been initiated. Because metabolism is decreased by approximately 50% for every 10°C of organ cooling, only when hypothermia has slowed down tissue metabolism does accumulation of ischemic injury decrease.[29] Rapid institution of cooling is essential. This cooling can be accomplished in several ways, ranging from an emergency laparotomy with direct aortic cannulation to total body cooling by an extracorporeal pumping device. The Maastricht group and others have advocated the use of a double-balloon, triple-lumen catheter for rapid onset of cooling.[80] Although especially useful for uncontrolled (categories I, II, and IV) NHB kidney-only donors, NHB multiorgan donation is impossible with this technique because only the kidneys are cooled.

Reliable and objective data on the technical effectiveness of cooling by this approach are lacking, as are data for all other cooling techniques. Few groups have measured whether the desired temperature of 0°C to 4°C is ever reached in the time that elapses between the beginning of cooling and organ procurement.[140] Also, the time span needed to reach adequate cooling via various techniques is largely unknown. Future research directed at characterizing and improving cooling dynamics during donor management may be relevant for marginal organs. For this reason, a team from Groningen is currently developing an extracorporeal perfusion system that can be used for in situ cooling of abdominal organs, including the kidneys.

Before cooling is instituted, other actions also can be taken to minimize the amount of injury that donor organs sustain. Management of uncontrolled NHB category I kidney donors by rapid (<15 minutes) emergency service response and continuation of resuscitation after declaration of cardiac death may be useful.[4,5,139] Short-term graft function is similar to kidneys derived from controlled NHB donors (DGF 68%, primary graft nonfunction 6%). Promising results also have been obtained by artificial normothermic recirculation after cardiac arrest of NHB categories II and IV kidney donors, before consent is obtained and cooling is begun.[164,165] A completely different improvement in NHB donor management may emerge someday from donor pretreatment.

In the clinical transplantation setting, cold ischemia time is considerably longer than warm ischemia time, and for every additional 6 hours of cold ischemia time, the likelihood of DGF increases by approximately 25%.[56,145] In NHB donation, warm ischemia and cold ischemia have additive detrimental effects. This is shown by animal studies, in which prolonged cold ischemia after a warm ischemia insult rendered donor kidneys less suitable for transplantation.[37,61] These studies also illustrate that HMP cannot prevent the cold ischemic deterioration of a graft that has sustained a prolonged period of warm ischemia.

To resolve this dilemma, several groups have suggested switching to normothermic (or near-normothermic) machine perfusion as the preferred method for NHB kidney preservation. Normothermic machine perfusion does support metabolism at an almost-normal rate, and by adding oxygen to the perfusate, it prevents further ischemic damage to the graft. In contrast to HMP or cold storage, it can address essential physiological needs of the organ. Several studies have shown that normothermic machine perfusion is superior to HMP or cold storage preservation of severely warm ischemia–damaged NHB donor kidneys.[34-38,154] Apart from this, normothermic machine perfusion may offer a more reliable method for ex vivo pretransplant functional assessment of a kidney graft, based on urine production, perfusion dynamics, and biochemical injury markers in the perfusate.[155]

OUTLOOK

An increasing awareness that ischemia-reperfusion injury does determine a significant part of the outcome after transplantation has stimulated the research of preservation damage and the development of new preservation solutions and methods. A relatively new machine preservation solution developed at the University of Amsterdam is Polysol. Its composition is based on the fact that metabolism is still present at 4°C. Polysol is a classic preservation solution enriched with amino acids, vitamins, and antioxidants.[25,28] Many components in Polysol have not been evaluated separately yet, however. In experimental liver preservation studies, superiority over University of Wisconsin machine perfusion solution (UW-MPS) was seen in isolated perfused models of NHB and steatotic livers. Compared with UW-MPS, Polysol improved functional parameters (e.g., oxygen consumption, ammonia clearance, urea production, and damage markers).[25,27] Transplant data in experimental and clinical preservation are required to determine the efficacy of Polysol. Based on its "metabolic support" design, beneficial effects of Polysol can be expected, especially in damaged organs.

Another new and now clinically available preservation solution is IGL-1, developed by the Lyon group. IGL-1 builds on the heritage of UW-CSS and Celsior.[22,65] It combines the extracellular composition of Celsior with the colloidal support of UW-CSS using PEG instead of HES. In a porcine kidney autotransplantation model with IGL-1, PEG was found

*References 3, 4, 6, 17, 40, 49, 55, 77, 83, 92, 93, 112, 118, 120, 122, 136, 139, 159, 181, 182.

to limit influx of macrophages by approximately 50%.[78] Polymers, such as PEG, spontaneously bind to cell and tissue surfaces and sterically stabilize the underlying surface from interactions with other components. The main advantage of this "immunocamouflage" is that it directly modifies inherent immunogenicity of donor tissue.[64,87] PEG does not exert any aggregating effects on red blood cells, and in combination with the extracellular composition of IGL-1, this should improve the washout of blood.[15,113,170]

Rat and porcine transplantation studies of liver and kidney have shown encouraging results in terms of organ function after transplantation following preservation with IGL-1.[21,23,132] The first preliminary clinical results in renal transplantation with IGL-1 showed a reduction in DGF compared with kidneys preserved with UW-CSS (5.7% versus 13.8%). Also, less apoptosis was seen using terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) techniques when kidneys were preserved in IGL-1 compared with UW-CSS.[14] Until now, patient numbers have been too small to draw clinical conclusions, however, and a randomized controlled multicenter study is needed to confirm the encouraging initial results. Given its extracellular composition and the beneficial effects of PEG, IGL-1 should be considered as a potential promising successor to UW-CSS.

Despite the fact that static cold storage preservation methods have facilitated many transplant programs throughout the world, it seems that the increasing challenge to maintain viability in marginal or extended criteria donor organs has now touched the limits of cold storage preservation. Even with beneficial additives and enriched compositions, static cold storage, at best, slows down ischemic damage. To improve organ viability further, a more dynamic preservation method is needed to better fulfill metabolic demands of damaged organs. Many groups are switching gears and are revisiting the possibilities of HMP or investigating the possibilities of normothermic (or near-normothermic) perfusion of donor organs.[73,75,171]

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Chapter 10

Histocompatibility in Renal Transplantation

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HISTORICAL BACKGROUND

In the 1960s, the study of histocompatibility was stimulated as the early pioneers of clinical kidney transplantation realized that immunological mechanisms were responsible for allograft destruction. In 1961, the introduction of chemical immunosuppression using first 6-mercaptopurine followed soon after by azathioprine and corticosteroids enabled short-term and medium-term success. Forty percent to 50% of cadaver grafts were lost, however, as a result of immediate or early graft failure owing to irreversible rejection in the first year, and thereafter there was an insidious decline in graft function. These early experiences severely limited the success of human allotransplantation and led to the study of compatibility of transplanted tissue, which over the following 40 years gave rise to the specialty of histocompatibility and immunogenetics.

The antigens of the ABO blood group system were the first human histocompatibility antigens identified. The vascular endothelium of the donor organ forms an interface with the recipient blood, and expression of ABO blood group antigens on capillary endothelium serves as a target for circulating natural antibodies to blood group A and B glycoproteins. ABO incompatibility leads to complement activation, thrombosis, and hemorrhage (collectively termed hyperacute rejection [HAR]). With only rare exception, ABO blood group–mismatched transplants fail as a result of immediate humoral hyperacute or acute vascular rejection; the requirement for donor and recipient ABO blood group compatibility was quickly established (see also Chapter 22).[95]

The first human leukocyte antigens (HLAs) were discovered in 1958 and subsequent years by Jean Dausset, Rose Payne, and Jon van Rood.[112] During the next few years, many more HLAs were characterized using antibodies in sera obtained from multiparous women and from patients after multiple blood transfusions. Such antibodies also were shown in patients after allograft rejection,[67] and antibodies present in recipient sera before renal transplantation that reacted against donor lymphocytes, either by leukoagglutination or by cytotoxicity, were associated with HAR.[48,124] HLAs were quickly recognized as the human equivalent of the major histocompatibility complex (MHC), previously identified in inbred rodents, the products of which control the recognition of self and foreign antigens.[38] The dual requirements for blood group compatibility and a negative pretransplant donor lymphocyte crossmatch have virtually eliminated HAR; most modern-day transplant surgeons have not encountered (or should not encounter) a case.

Evidence for the major role of HLAs as the dominant transplantation antigens of the human MHC arose from transplants performed using genetically related donors. Despite limited knowledge of HLA polymorphism and definition of only a few HLA class I and class II specificities, it was possible to assign familial HLA haplotypes whereby the genes encoding an individual's HLA type are inherited en bloc. Graft survival was shown to correlate with the number of HLA haplotypes shared between donor and recipient, with 90% graft survival between HLA-identical siblings compared with 70% or 60% when sharing one or no HLA haplotypes.[74] The impact of HLA compatibility on cadaver donor kidney transplantation is more controversial, however, because many factors may confound any clinical benefit in terms of graft outcome. These factors include the limited number of HLA specificities identified, the logistics of matching for such a diverse polymorphic antigenic system, the increased ischemia time that may be associated with the matching process, and a diminishing effect of HLA matching in the presence of more potent immunosuppressive regimens that can override rejection. After the definition of the class II HLAs, however, there was a much more clear-cut association between matching and graft outcome.[116,117]

Despite a complete allelic HLA match, 10% of kidney transplants performed using an HLA-identical sibling donor failed, and 40% of HLA-identical bone marrow transplant recipients still have acute graft-versus-host disease. The occurrence of immunological rejection on the background of HLA identity is likely to result from differences caused by polymorphic proteins at minor histocompatibility complex loci encoded outside the HLA region.[39] Several minor histocompatibility complex antigens have been identified as immunogenic targets in graft-versus-host disease after bone marrow transplantation, including the male antigen H-Y (encoded on the short arm of the Y chromosome), although there is no convincing evidence for a role in solid organ transplantation.

HLA SYSTEM

The HLA system encoded on the short arm of chromosome 6 is the most intensively studied region of the human genome. The region spans more than 4 Mb and contains greater than 250 expressed genes, making it the most gene-dense region characterized to date.[45] Of relevance to transplant clinicians and immunologists is that about 28% of these genes encode proteins that have immune-related functions. Originally discovered in the late 1950s as the equivalent of the human MHC, HLA incompatibility was identified as the principal stimulator of graft rejection. At that time, nothing was known, however, about the natural evolution and role of HLA.

HLA is now recognized to have a central role in immune recognition for the defense against foreign pathogens and neoplasia, mediating T cell signaling through the presentation of self and foreign antigens in the form of short protein fragments (peptides) recognized by self-HLA restricted T lymphocytes (see Chapter 2). Recognition of nonself peptides in the context of self-HLA (i.e., altered self) is the function of the T cell antigen receptor and elicits a powerful immune response. The extensive polymorphism of HLA has evolved to enable efficient binding of peptides from the vast array of potentially pathogenic organisms that invade and colonize human bodies. The evolutionary pressures to develop and maintain diversity vary with time and geographical area. As a consequence, HLA has adapted differently according to geographical region and ethnic group, and HLA phenotypes differ across populations throughout the world.

HLA Genes and Their Products

The HLA system is a complex multigene family consisting of more than ten loci. HLA types are codominantly inherited on a maternal and paternal haplotype and transmitted as a single mendelian trait (Fig. 10-1); an individual can express two alleles at each locus. The genes encoding HLA and their corresponding glycoprotein products are divided into two classes according to their biochemical and functional properties: HLA class I and HLA class II. Between these genes are the so-called class III genes that encode some immune-related proteins, such as complement factors (C2, C4A, C4B, properdin factor B), tumor necrosis factor, lymphotoxin α and β, and heat-shock proteins. The steroid 21-hydroxylase is encoded by the gene *CYP21B*, which is in close proximity to HLA-DR.[9]

HLA Class I

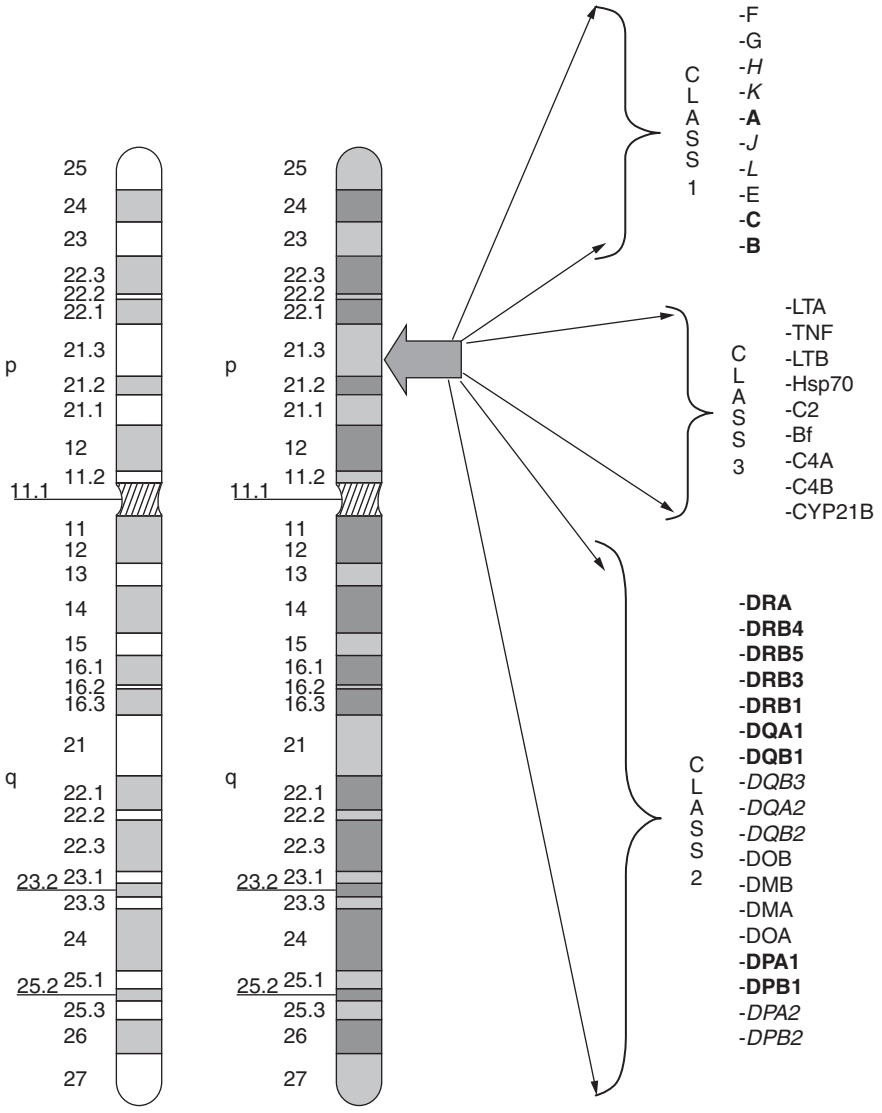
HLA class I genes span 2 Mb at the telomeric end of the 6p21.3 region of chromosome 6. This region encodes the classic transplantation antigens (HLA-A, HLA-B, and HLA-C) that are expressed on virtually all nucleated cells.[17] Genes of the HLA class I loci encode the 44-kD heavy chains that associate with intracellular peptides present within the cytoplasm (Fig. 10-2). The tertiary structure is stabilized on the cell surface by noncovalent association with β_2-microglobulin, a nonpolymorphic 12-kD protein encoded on chromosome 15. The heavy chain consists of three extracellular immunoglobulin-like domains (α_1, α_2, and α_3), a hydrophobic transmembrane region, and a cytoplasmic tail. The two extracellular domains distal to the cell membrane (α_1 and α_2) are highly polymorphic and fold to form a peptide-binding cleft consisting of eight strands forming an antiparallel beta-pleated sheet, overlaid by two alpha helices (Fig. 10-3). The cleft accommodates peptides 8 to 10 amino acids long that are mostly derived from "endogenous" proteins present within the cell cytoplasm. The major areas of amino acid polymorphism line the sides and base of the cleft and govern the peptide-binding repertoire of the HLA molecule. In contrast, the α_3 domain (proximal to the cell membrane) is highly conserved and acts as a ligand for CD8 expressed on T lymphocytes.[88] This interaction confers HLA class I restriction on $CD8^+$ T lymphocytes, which have a predominantly cytotoxic function and form the basis for cellular immunity to intracellular pathogens such as viruses.

There are other class I loci, and knowledge about their expression and function is only beginning to emerge (Table 10-1). HLA-H, HLA-J, HLA-K, and HLA-L are pseudogenes and HLA-N, HLA-P, HLA-S, HLA-T, HLA-U, HLA-V, HLA-W, HLA-X, HLA-Y, and HLA-Z are gene fragments that are not transcribed or translated. HLA-G is expressed on placental trophoblast cells, implicating a possible involvement in fetal-maternal development. HLA-E, HLA-F, and HLA-G have limited polymorphism (9, 20, and 23 alleles) and are known to act as ligands for natural killer cell inhibitory receptors (e.g., CD94). These loci may prove to be important in certain experimental xenograft models and in bone marrow transplantation (where natural killer cells are involved in the rejection process), but they have not been shown to be relevant in clinical solid organ transplantation. There is, however, an emerging role for these molecules in innate immunity to persistent viruses such as cytomegalovirus, and they may prove to have an important role in post-transplant viral defense.

HLA Class II

The HLA class II region consists of three main loci, HLA-DR, HLA-DQ, and HLA-DP. The glycoprotein products are heterodimers with noncovalently associated alpha and beta chains of molecular weight 33 kD and 28 kD. Both chains have two extracellular immunoglobulin-like domains—a transmembrane region and a cytoplasmic tail (see Fig. 10-2). The distal membrane domains α_1 and β_1 form a peptide-binding cleft similar to, but less rigid than, that of HLA class I, accommodating peptides 10 to 20 amino acids long that are derived predominantly from ingested (endocytosed or phagocytosed) extracellular (exogenous) proteins. The β1 domains of HLA-DR, HLA-DQ, and HLA-DP are highly polymorphic and govern the peptide-binding repertoire.

Figure 10–1 Genomic organization of the HLA region on chromosome 6. HLA antigens are codominantly inherited en bloc as a haplotype from maternal and paternal chromosomes. Italic type indicates pseudogenes. Normal type indicates "nonclassic" HLA genes with no known role in clinical solid organ transplantation. Bold type indicates genes encoding HLA products with clinical relevance to solid organ transplantation.



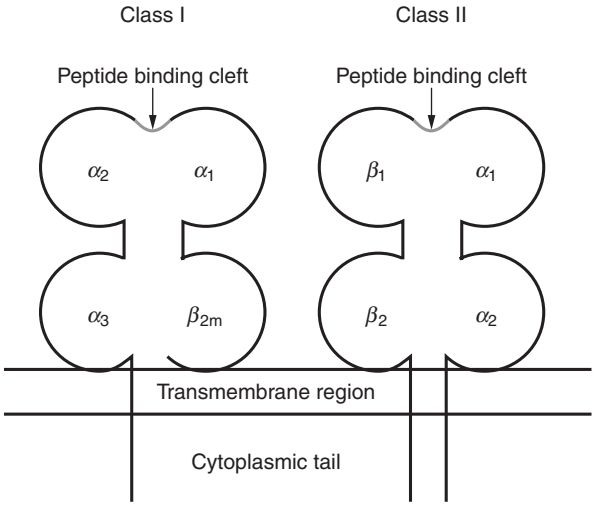


Figure 10–2 Schematic representation of the domain structure of HLA class I and class II.

They are constitutively expressed on cells with immune function, such as B lymphocytes, activated T lymphocytes, and antigen-presenting cells (monocytes, macrophages, and cells of dendritic lineage). HLA class II expression can be induced on most cell types during inflammatory responses (including allograft rejection) by cytokines such as interferon-γ and tumor necrosis factor-α.[18,28,29] The β_2 domain associates with CD4 on T lymphocytes with predominately helper/inducer function and confers HLA class II restriction (Fig. 10-4) and forms the basis of cellular and humoral immunity to circulating pathogens such as bacteria.

HLA Polymorphism and Nomenclature

Early investigations into HLA polymorphism used relatively crude alloantisera that were able to distinguish only a few antigens. There was international collaboration between HLA serologists to identify specificities defined by common sets of reagents, and a nomenclature system to denote these polymorphisms was devised in a series of International Histocompatibility Workshops. Nearly half a century later, these simple techniques have been complemented by molecular methods capable of resolving HLA variants at the DNA

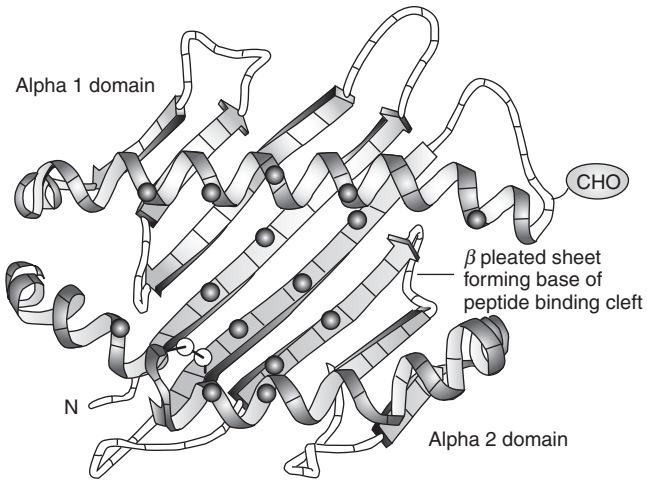


Figure 10–3 Ribbon diagram of the peptide-binding cleft of HLA class I. The peptide-binding cleft is formed by two alpha helix protein chains (alpha 1 and alpha 2) that overlay a beta-pleated sheet. The *boxes* indicate amino acid positions that form the protein structure, and as an example of the HLA polymorphism, the *dots* denote the amino acid residues that differ between two HLA-A specificities (HLA-A1 and HLA-A2) that line the base and sides of the peptide-binding cleft (and govern the peptide-binding repertoire for antigen presentation) and those that face the outer surface toward the T cell receptor (governing the self-restricted or alloreactive T cell specificity).

sequence level and identifying single amino acid polymorphisms that are indistinguishable by serology. For example, there are currently 19 HLA-DR specificities defined by serological methods compared with more than 450 sequence variants (alleles) detected using DNA-based typing methods. The number of newly defined alleles identified is still increasing rapidly and has now surpassed even the highest expectations of the early pioneers.

Concomitant with the ever-increasing complexity of the HLA region, a nomenclature system has been developed to assign accurately HLA loci and their alleles.[89] This nomenclature system encompasses the methodology (serology, biochemistry, and DNA sequencing) and level to which the HLA genes and their products have been resolved. The nomenclature is complex and, to individuals outside the field, can be confusing.

Resolution of HLA Typing Methods

Serologically based HLA typing uses alloantisera and monoclonal antibodies that bind to tertiary epitopes of the cell surface HLA glycoproteins. There is a high degree of sequence homology between HLA specificities, and identical amino acid sequence motifs are often shared between groups of antigens.[2,57] These related structures give rise to cross-reactivity because many HLA-specific antibodies bind epitopes that are shared between HLA specificities. Serologically detectable HLA epitopes that are common between different specificities are called public or supertypic determinants and form cross-reactive groups (CREGs), whereas epitopes that are unique to an antigen are termed private determinants. In some cases, antigens that were originally defined as single specificities could be subdivided into two or more specificities called splits. For example, some alloantisera were able to discriminate the broad HLA-A9 specificity into two subgroups, A23 and A24. These specificities are annotated as HLA-A23(9) and HLA-A24(9), where the A23 and A24 denote the split specificity and the number in parentheses (9) denotes the broad specificity.

The degree of HLA compatibility between transplant donors and recipients can be considered at many different levels of resolution, depending on the HLA typing method (Table 10-2); this can range from single amino acid differences detected by high-resolution, DNA sequence–based

Table 10–1 HLA Genes and Their Products

Name	Molecular Characteristics
HLA-A	Class I alpha chain
HLA-B	Class I alpha chain
HLA-C	Class I alpha chain
HLA-E	Associated with class I 6.2-kb Hind III fragment
HLA-F	Associated with class I 5.4-kb Hind III fragment
HLA-G	Associated with class I 6-kb Hind III fragment
HLA-H	Class I pseudogene
HLA-J	Class I pseudogene
HLA-K	Class I pseudogene
HLA-L	Class I pseudogene
HLA-N	Class I gene fragment
HLA-P	Class I gene fragment
HLA-S	Class I gene fragment
HLA-T	Class I gene fragment
HLA-U	Class I gene fragment
HLA-V	Class I gene fragment
HLA-W	Class I gene fragment
HLA-X	Class I gene fragment
HLA-Y	Class I gene fragment
HLA-Z	Class I gene fragment (located in HLA class II region)
HLA-DRA	DR alpha chain
HLA-DRB1	DR beta 1-chain determining specificities DR1 to DR18
HLA-DRB2	Pseudogene with DR beta-like sequences
HLA-DRB3	DR beta 3-chain determining DR52 found on DR17, DR18, DR11, DR12, DR13, and DR14 haplotypes
HLA-DRB4	DR beta 4-chain determining DR53 found on DR4, DR7, and DR9 haplotypes
HLA-DRB5	DR beta 5-chain determining DR51 found on DR15 and DR16 haplotypes
HLA-DRB6	DRB pseudogene found on DR1, DR2, and DR10 haplotypes
HLA-DRB7	DRB pseudogene found on DR4, DR7, and DR9 haplotypes
HLA-DRB8	DRB pseudogene found on DR4, DR7, and DR9 haplotypes
HLA-DRB9	DRB pseudogene, probably found on all haplotypes
HLA-DQA1	DQ alpha chain
HLA-DQB1	DQ beta chain determining specificities DQ1 to DQ9
HLA-DQA2	DQ alpha chain–related sequence, not known to be expressed
HLA-DQB2	DQ beta chain–related sequence, not known to be expressed
HLA-DQB3	DQ beta chain–related sequence, not known to be expressed
HLA-DOA	DO alpha chain
HLA-DOB	DO beta chain
HLA-DMA	DM alpha chain
HLA-DMB	DM beta chain
HLA-DPA1	DP alpha chain
HLA-DPB1	DP beta chain
HLA-DPA2	DP alpha chain–related pseudogene
HLA-DPB2	DP beta chain–related pseudogene
HLA-DPA3	DP alpha chain–related pseudogene

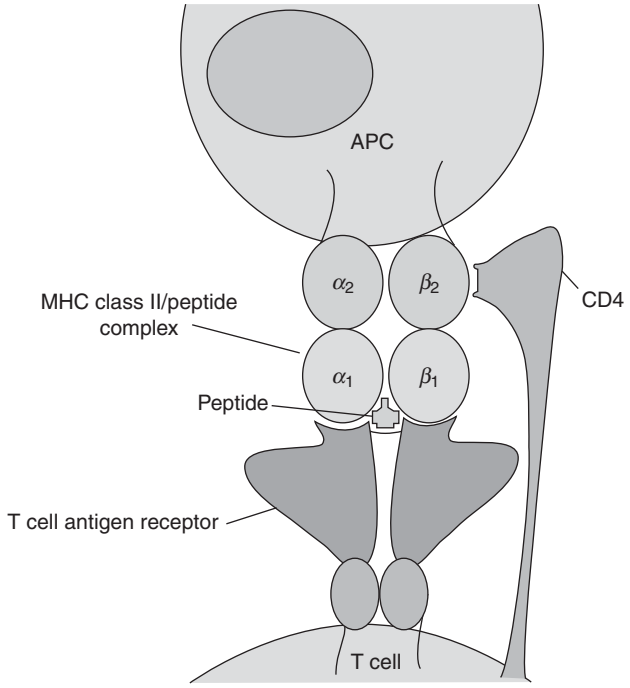


Figure 10–4 Cartoon diagram depicting the presentation of peptide by an HLA class II molecule on an antigen-presenting cell (APC) to the antigen receptor on $CD4^+$ T helper cells. (Redrawn from Taylor CJ, Metcalfe S: The Immunobiology of Organ Transplantation. In Klinck JR, Lindop MJ [eds]: Anesthesia and Intensive Care for Organ Transplantation. London, Chapman & Hall, 1998.) (See color plate.)

methods (allele matching) to broad and split specificity matching or matching for the small number of common CREGs. The influence of all levels of donor and recipient HLA compatibility has been considered in cadaver donor renal transplantation.[106] Although strongly implicated for negating graft-versus-host disease after unrelated bone marrow transplantation, a role for high-resolution allele matching in renal transplantation has not been firmly established. However, matching for serologically defined amino acids, specificities, and CREGs all have been reported to benefit transplant outcome.[49,101,120,126] Generally, the more discerning the matching criteria, the greater the correlation with graft outcome.

World Health Organization Nomenclature for HLA

Many HLA genes have been characterized and cloned and have been given official designations using the following principles. The genes are prefixed by the letters HLA followed by the loci or region (e.g., HLA-A, HLA-B, or HLA-D). The HLA-D region has several subregions denoted HLA-DR, HLA-DQ, HLA-DP, HLA-DO, and HLA-DM (see Fig. 10-1). These are followed by the letters A or B to define the gene encoding the alpha and beta chain gene product of that subregion, respectively (e.g., HLA-DRB genes code for the DR beta-chain protein product). Where there is more than one A or B gene within a subregion, a corresponding number is given (e.g., HLA-DRB1) (see Fig. 10-1 and Table 10-1).

Each allele has a unique 4-digit number prefixed by an asterisk (*) where DNA sequence–based information is available. The first 2 digits identify the broad specificity based on homology between allele "families." These digits usually correlate with the serological specificity, for instance HLA-B*27 correlates with the serological specificity HLA-B27. For most serologically defined antigens, there is further polymorphism, however, detectable at the DNA and amino acid sequence level. Where sequence information is available, the third and fourth digits denote the precise allele. For example, there are 35 subtypes (allele variants) of HLA-B27 involving amino acid substitutions at 23 positions. These are represented as HLA-B*2701, HLA-B*2702, and so forth. In cases where a DNA base change does not alter the amino acid sequence (termed silent substitution or synonymous variants), fifth and sixth digits are applied to differentiate the noncoding base change (e.g., HLA-DQB1*050301 and HLA-DQB1*050302).

Some alleles or genes contain a sequence defect that prevents normal antigen expression at the cell surface. Nonexpressed alleles (null alleles) are indicated using the suffix "N" (e.g., HLA-DRB4*010301N), whereas alleles with low expression or soluble (secreted) alleles carry the suffix "L" or "S." Further mutations have been detected outside the coding region, and additional digits have been added to indicate these intronic polymorphisms (e.g., HLA-DRB4*01030102N, HLA-A*24020102L, HLA-B*44020102S).

The HLA-DR and HLA-DP alpha chains are less polymorphic (DRA is diallelic), and the HLA-DRB1 or HLA-DPB1 allele (which code for the main polymorphic amino

Table 10–2 **Resolution of HLA Typing Methods and Their Application to Renal Transplantation**

HLA Typing Resolution	Method
HLA allele matching	High-resolution DNA sequence-based typing*
Split HLA specificity matching	Serology and low-resolution (generic) DNA typing†
Broad HLA specificity matching	Serology and low-resolution (generic) DNA typing
HLA-B, HLA-DR matching	Serology and low-resolution (generic) DNA typing
Epitope matching	Serologically defined cross-reactive groups
	Serologically defined motifs/determinants
	Single amino acid residues
	Linear peptides and conformational epitopes
	Supertypic antigen matching
	Triplet amino acid mismatches (HLA Matchmaker)

*High-resolution DNA typing can be translated into low-resolution serological equivalents (allele families).

†Low-resolution HLA typing by polymerase chain reaction uses DNA primers designed to identify polymorphisms at a level comparable to serology.

Adapted from Taylor CJ, Dyer PA: Maximising the benefits of HLA matching for renal transplantation: alleles, specificities, CREGs, epitopes or residues? Transplantation 68:1093-1094, 1999.

acid determinants present on the beta chain) is usually annotated alone. In contrast, both the HLA-DQ alpha and beta chains are polymorphic. To describe one of these alleles precisely, definition of both the A and the B alleles may be required (e.g., HLA-DQA1*0101 and HLA-DQB1*0501). Although the alpha and beta chain protein products of the A and B gene pairs associate preferentially, there is also the possibility of the formation of novel hybrid molecules (e.g., HLA-DRA and HLA-DQB1*0402). A complete list of recognized HLA genes and their expressed products can be found at www.bmdw.org (Bone Marrow Donors Worldwide; HLA information).

Extended HLA Haplotypes

The HLA region displays strong linkage disequilibrium whereby certain HLA alleles are inherited together as a conserved HLA haplotype. Extended HLA haplotypes involving HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ commonly exist within and between ethnic groups. The probability of locating an HLA-matched unrelated donor is greatly improved because common HLA haplotypes are frequently found within a population (e.g., HLA-A*0101, HLA-B*0801, HLA-C*0701, HLA-DRB1*0301, HLA-DRB3*0101, HLA-DQA1*0501, HLA-DQB1*0201).[108] These extended haplotypes also involve the class III region with the previous example being linked to the tumor necrosis factor-α promoter polymorphism (TNFA2) associated with increased production of this proinflammatory cytokine. Linkage within the HLA class I region has been extended a further 4 Mb telomeric to HLA-A, to the class I–like gene *Hfe*. A point mutation that substitutes cystine or tyrosine at position 282 of the Hfe protein is the major cause of hereditary hemochromatosis and explains the weaker association of this disease observed with HLA-A3 more than 20 years ago. There is only relatively weak linkage centromeric to HLA-DQ because of a recombination "hot spot" between HLA-DQ and HLA-DP.

HLA on the Web

Information concerning the HLA system is rapidly expanding, and articles are always out of date by the time they go to print. Numerous Internet websites with useful links are regularly updated, however. The following websites provide contemporary articles and information concerning HLA genes, nomenclature, polymorphism, DNA, and amino acid sequences for lay and professional readers:

www.bmdw.org
www.ashi-hla.org/index.htm
www.bshi.org.uk
www.efiweb.org
www.anthonynolan.org.uk
www.sanger.ac.uk

HLA MATCHING

It was first noted 40 years ago that HLA matching between donor and recipient was associated with better transplant and patient survival.[62,79,110] Matching for the class I HLA-A and HLA-B antigens influenced survival, but matching for the class II HLA-DR antigens had the most powerful effect.[116,117] Over the years, there has been an overall improvement in transplant survival and a decrease in the survival advantage conferred by HLA matching.[11,62] The improvement can be attributed to numerous factors, but one of the most powerful is advancement in the potency of immunosuppression. This was clearly demonstrated in a local comparison of transplant survival in patients receiving azathioprine and prednisolone; cyclosporine and prednisolone; and cyclosporine, azathioprine, and prednisolone triple therapy: 1-year transplant survival rates were 65%, 69%, and 81% respectively.[104] In this analysis, HLA-DR compatibility still had a marked effect on the post-transplant clinical course, with an increased incidence of rejection in HLA-DR–mismatched grafts, the socioeconomic effects of which were increased use of immunosuppressive drugs, longer hospital stays, and higher 3-month creatinine levels.[105]

A beneficial effect of HLA matching still can be shown in analyses of large datasets and national and international databases,[11,52,75] but as already discussed, the genes of the HLA region are highly polymorphic, and HLA typing can be performed at many different levels of resolution. In solid organ transplantation, the effects of HLA matching reported are generally based on matching at the HLA-A, HLA-B, and HLA-DR loci, but the definition of a match may vary according to whether matching is considered only at the level of "broad" specificities, or whether the associated "splits" also are considered. For example, a donor and recipient who type as HLA-A2, HLA-A24(9) and HLA-A2, HLA-A23(9), where HLA-A23 and HLA-A24 are both splits of HLA-A9, may be considered to have no HLA-A mismatches at the broad antigen level, but as having one mismatch at the split antigen level. Reports have suggested an additional benefit of matching at the split antigen level.[75] The effects of matching other HLA loci have been analyzed. Matching for HLA-DQ has been variously reported as having either a beneficial effect[121] or no effect on transplant outcome.[7,27] Registry analysis has shown that HLA-DPB matching has an effect on the transplant survival of regrafts, but not of first transplants[70]; a report has shown this effect to result from matching for certain immunogenic HLA-DPB epitopes.[51]

Analyses of the effects of matching at the level of the CREGs have been performed, but the conclusions regarding the benefit on outcome are conflicting.[60,93,96,98,126] This conflict may be partly because of the complexity in the serological cross-reactions, whereby the antigens can be grouped in slightly different ways, and the grouping used differs between analyses. Furthermore in these analyses, a proportion of the CREG-matched transplants also are matched for HLA-A, HLA-B, and HLA-DR in a conventional sense, and this may explain an observed benefit of matching CREGs.[52,106]

In analyzing the effect of HLA on transplant outcome, it is important that other factors known to have a strong influence on outcome are taken into account. In a rigorous multivariate analysis of factors influencing the outcome of primary deceased donor transplants in a cohort of transplants performed in the United Kingdom from 1986 to 1993, the year of transplant, donor and recipient age, waiting time to transplant, diabetes in the recipient, donor cause of death, exchange of kidneys, cold ischemia time, and HLA mismatching were found to influence transplant survival (death with function treated as failure). The best transplant survival was achieved in transplants that had no mismatches at HLA-A, HLA-B, and HLA-DR (000 mismatch grade). Other well-matched transplants, termed *favorably matched* transplants,

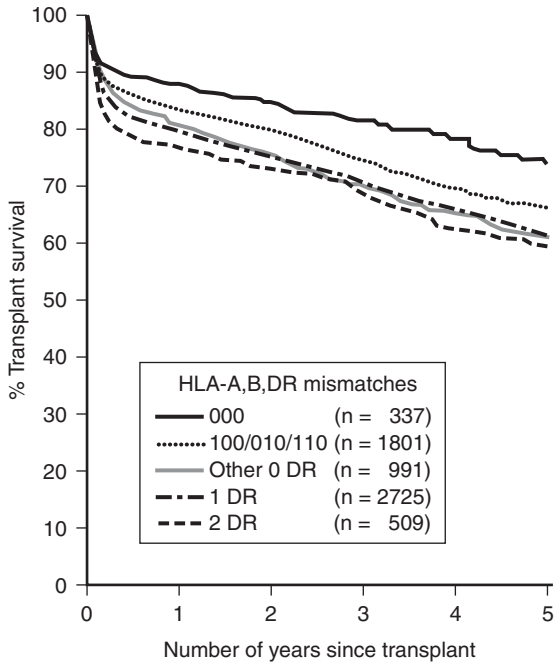


Figure 10–5 Survival of first cadaver renal allografts by HLA match grade. The curves differ significantly ($P = .0001$). (From Morris PJ, Johnson RJ, Fuggle SV, et al: Analysis of factors that affect outcome of primary cadaveric renal transplantation in the UK. Lancet 354:1147, 1999.)

with a maximum of one HLA-A and one HLA-B antigen mismatched in the absence of mismatches at HLA-DR (110, 100, and 010 mismatch grades) had a significantly improved survival over transplants of all other match grades (Fig. 10-5).[68] An analysis of factors influencing the long-term outcome of these transplants revealed that for patients with transplants functioning after 6 years, only older donor age and diabetes had a significant detrimental influence on survival.

The influence of HLA mismatch on outcome of first deceased donor transplant has been investigated in a more recent cohort of patients in the United Kingdom, transplanted between 1995 and 2001. As a result of the allocation policy, the more recent transplants were significantly better matched than the previously analyzed cohort (1986 to 1993), where 46% transplants were 0 DR mismatched, and 10% had 2 DR mismatches compared with 60% 0 DR mismatched and only 3% 2 DR mismatches in the 1995 to 2001 cohort. In a multivariate analysis, there was no longer an effect of HLA-A mismatching, but the levels of HLA-B and HLA-DR mismatching were statistically significant: level 1, 000 mismatches; level 2, 0 DR and 0/1 B mismatches (relative risk 1.06, 95% confidence interval 0.87-1.30); level 3, 0 DR and 2 B or 1 DR and 0/1 B mismatches (relative risk 1.22, 95% confidence interval 0.99-1.49); level 4, 1 DR and 2 B or 2 DR mismatches (relative risk 1.41, 95% confidence interval 1.11-1.80).

In many countries, HLA matching is one of the factors considered in the allocation of kidneys. There has been considerable discussion about HLA matching and the allocation of organs, and this has been the subject of numerous reviews and articles.[23,31,44,56,69,100,119] One disadvantage of allocation based on HLA matching is that patients with rare HLA types are difficult to transplant. The allocation scheme in the United Kingdom has been revised recently, and although HLA matching is still an important feature, the scheme contains factors to increase access to transplantation for patients with rare HLA types. One feature is to decrease the number of specificities used for matching by defaulting HLA-A, HLA-B, and HLA-DR specificities with a frequency of less than 2% in the donor population to a more frequent counterpart, based on serological and sequence similarity. For example, on the United Kingdom transplant waiting list, 0.2% of patients are HLA-A36, a specificity found in 0.05% of United Kingdom donors. By mapping HLA-A36 to its closest common counterpart HLA-A1, HLA-36 typed patients have access to HLA-A1 donors, which constitute 18% of the donor pool. The simulations of transplant activity suggest that this strategy will increase the transplant rate for the most difficult-to-match patients by 11% and for ethnic minority patients by 14%. The strategy was incorporated into the allocation scheme implemented in April 2006, and the early results suggest the strategy is effective in achieving transplants for these patients.

SENSITIZATION

Routes of Sensitization

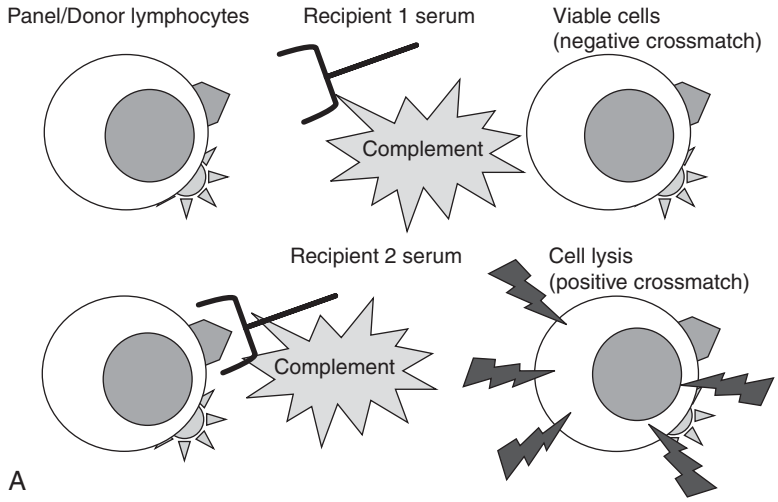
An individual can become sensitized to HLA alloantigens as a result of blood transfusion, pregnancy, or previous organ transplantation. Transplantation of poorly HLA-matched kidneys can result in allosensitization to the mismatched HLA antigens. In the United Kingdom, an audit of the national transplant waiting list showed that 20% of patients waiting for a first transplant were sensitized, but 77% of patients waiting for a second transplant were sensitized.[32] Approximately 20% of pregnant women produce HLA-specific antibodies to paternally derived fetal HLA antigens. The use of erythropoietin for the treatment of patients with anemia has decreased the use of blood transfusion in renal patients with a consequent decrease in the number of patients becoming sensitized by this route. It would be expected that the use of leukocyte-depleted blood would prevent allosensitization, but there is evidence to suggest that this is not the case.[123] Furthermore, HLA-specific antibodies may be detected in patients who have not been exposed to these classic routes of sensitization. These idiopathic HLA antibodies may result from cross-reactivity with infectious agents and are usually IgM.

Antibody Detection and Specificity Definition

In recent years, there have been significant advances in the technology for the detection and definition of HLA antibodies, and these can be used to define precisely the specificity of HLA antibodies in serum samples and to elucidate a patient's sensitization profile. The available technologies are briefly described next.

Complement-Dependent Cytotoxicity

Complement-dependent cytotoxicity (CDC) was the first technique routinely used for HLA antibody detection and for the crossmatch test (Fig. 10-6). In this assay, lymphocyte target cells are used to detect complement-fixing IgG and IgM antibodies present in patient's serum samples after the addition of rabbit complement. IgM antibodies can be



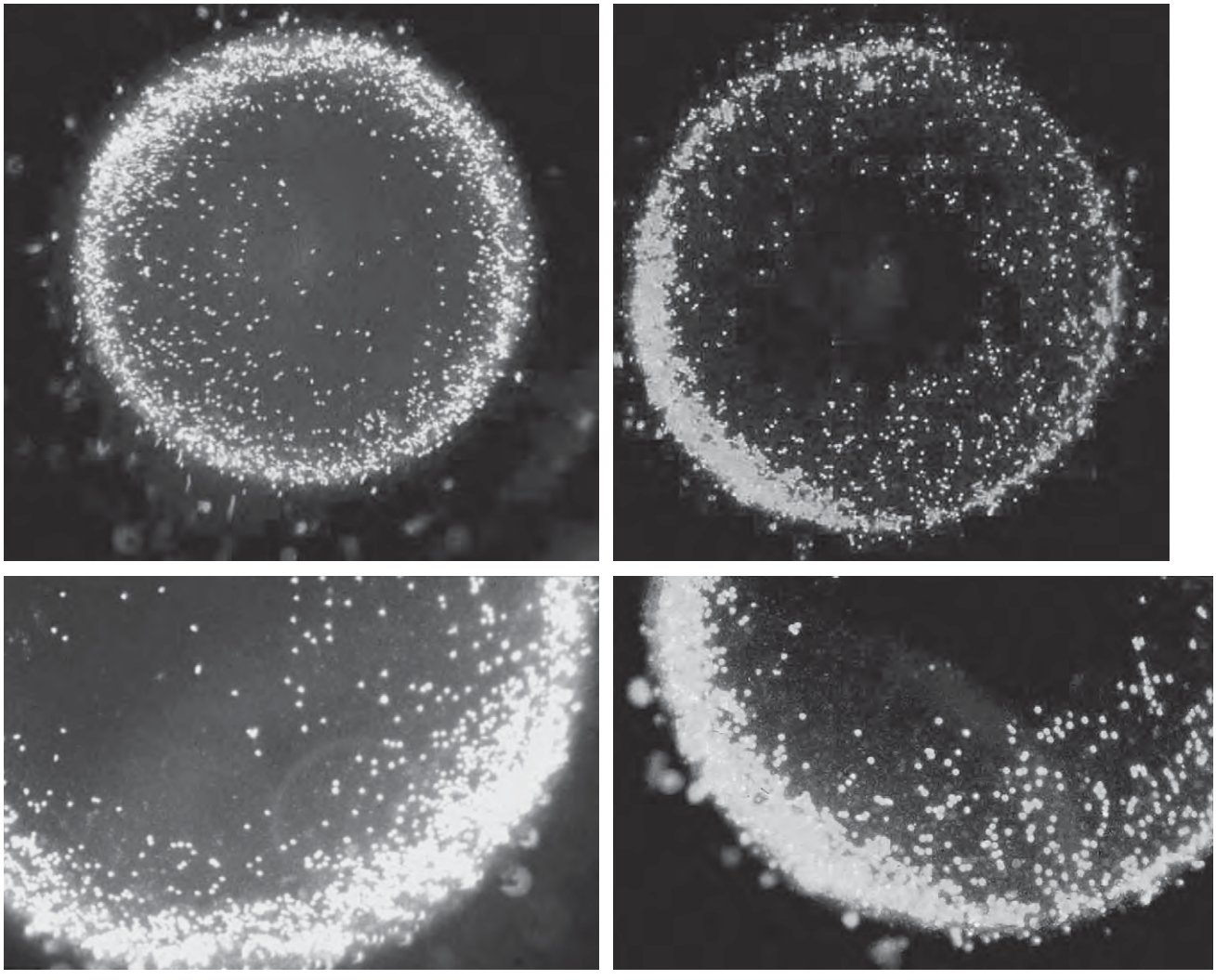


Figure 10–6 **A,** Lymphocytotoxic crossmatch test. Panel or donor lymphocytes are incubated with recipient serum in the wells of a microtiter (Terasaki) tray, followed by the addition of rabbit complement. After a second incubation period, vital stains (e.g., acridine orange and ethidium bromide) are added, and the wells are viewed using fluorescent (ultraviolet) microscopy to determine cell viability. **B**, Lymphocytotoxic (complement-dependent cytotoxicity) crossmatch results. *Left*, Viable lymphocytes take up acridine orange and appear a yellowish orange color (negative crossmatch). *Right*, Lysed cells (have pores in the lymphocyte cell membrane caused by antibody binding and complement activation) take up ethidium bromide and appear a brown color (positive crossmatch). (See color plate.)

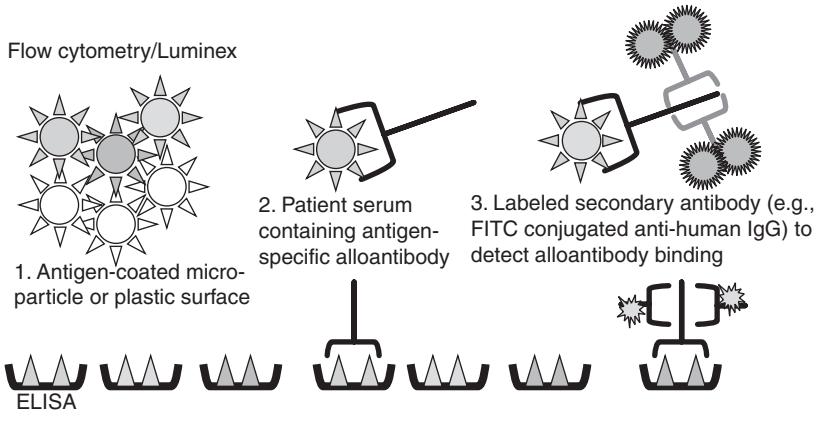
differentiated from IgG antibodies by the use of dithiothreitol (DTT). DTT reduces the disulfide bonds in the IgM pentamer and consequently renders negative a reaction due to IgM. Serum samples are tested against panels of cells that can be "random" or alternatively can be "selected" to represent the spectrum of HLA types in the population. The technique can be used for specificity definition, but the results are often expressed as the percentage of the panel to which the sample has reacted (% panel reactive antibody [%PRA]). This term has limited value, and its use is now strongly discouraged because the figure entirely depends on the composition of the panel used for testing. If a patient has a monospecific antibody to a specificity that is common in a population, and a random panel is used, the %PRA is high, but if the panel has been carefully selected to cover rare and common specificities, the %PRA value for the same antibody may be low. Values for %PRA cannot be compared between panels or laboratories.

There are other limitations of the CDC technique. Only complement-fixing antibodies are detected, and the sensitivity of the technique depends on the viability of the target cells and the particular batch of rabbit complement used. Both HLA and non-HLA antibodies are detected. Although the use of DTT can differentiate IgM from IgG antibodies, this does not indicate the specificity of the antibody. Reactivity resulting from an IgM HLA-specific antibody would be indistinguishable from reactivity of an IgM autoreactive antibody. Autoantibodies are frequently weak or nonreactive with lymphocytes from patients with chronic lymphocytic leukemia, however, and including these cells in a panel can be useful in elucidating a patient's antibody profile.[118] Alternatively, serum samples can be preabsorbed with autologous cells to remove autoreactive antibodies, before screening for alloreactivity.

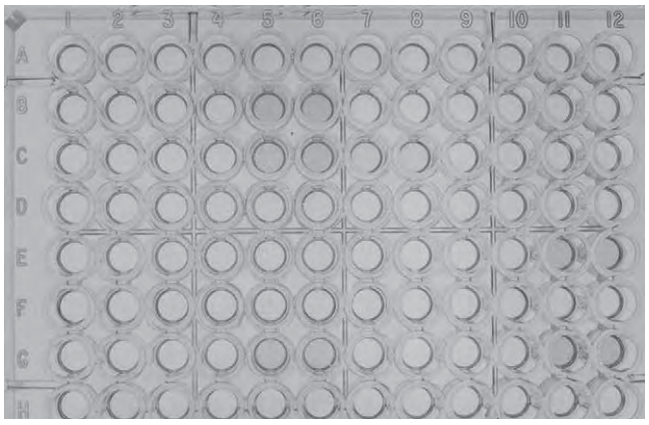
There have been a number of approaches used to increase the sensitivity of the CDC test, including increasing the incubation times, the wash (Amos) technique, and augmentation with antihuman globulin. In the Amos technique, unbound serum is washed from the cell suspension before the addition of rabbit complement, removing the anticomplement factors in the serum. In the antihuman globulin augmentation CDC test, anti–kappa light chain is added to the washed cells before the addition of complement. The techniques that include wash steps preferentially detect IgG antibodies because the lower affinity IgM antibodies become detached during the washing process.

Enzyme-Linked Immunosorbent Assays

The targets in an enzyme-linked immunosorbent assay (ELISA) are soluble HLA antigens coated onto plastic, and this is termed a *solid-phase assay* (Fig. 10-7). These commercially available kits have immediate advantages over CDC in that the test does not rely on viable cells, and only HLA antibodies are detected. The overall sensitivity of ELISA is greater than CDC. Two different types of ELISA are routinely used—assays to detect the presence or absence of HLA antibodies that can be used as a prescreen of a patient's



A



B

Figure 10–7 **A,** Schematic representation of antibody screening using solid-phase binding assays (enzyme-linked immunosorbent assay [ELISA] and flow cytometry/Luminex). *1*, Purified HLA proteins (either pooled HLA specificities or single antigen specificities) coated onto a solid phase (microtiter tray [ELISA] or microparticles [flow cytometry/Luminex]) are incubated with patient serum. *2*, HLA-specific antibodies bind to the antigen-coated solid phase, and nonspecific antibodies are washed off. *3*, IgG HLA-specific antibodies bound to the antigen-coated solid phase are detected using a conjugated (e.g., alkaline phosphatase [ELISA] or fluorescein isothiocyanate [FITC] [flow cytometry/Luminex]) anti–human IgG secondary antibody and detected by colorimetric analyses (ELISA, e.g., using p-nitrophenyl phosphate) or fluorescent signal (flow cytometry/Luminex after excitation by a laser). **B**, Example of HLA-specific antibody screening by ELISA. The plastic surface of each well in the microtiter tray is coated with pooled HLA specificities. Patient serum containing IgG HLA-specific antibodies is determined and quantified by colorimetric analyses (brown denotes a positive result). (**B**, See color plate.)

serum sample and assays that are designed for antibody specification. The assays have been shown to be reliable for the detection of IgG antibodies, but less so for the detection of IgM, probably because of the washing steps required and the lower affinity of IgM antibodies.

Flow Cytometry

The original use of flow cytometry in antibody screening was as a test to determine the presence or absence of antibody. Pools of HLA-typed target cells from chronic lymphocytic leukemia patients,[43] lymphoblastoid cell lines,[42,54] or peripheral blood lymphocytes,[92] constructed to cover the most frequent HLA specificities and cross-reactive groups, have been used. Flow cytometry is more sensitive than CDC and primarily detects IgG antibodies; this offers the advantage that IgM autoreactive antibodies are not detected. Although less frequent, however, IgG autoreactive antibodies will be detected by this method.

There have been advances in commercial products for antibody detection and specification with the development of antigen-coated microparticles (see Fig. 10-7). Kits of microparticles allow the detection of HLA-A, HLA-B, HLA-Cw, HLA-DR, HLA-DQ, and HLA-DP antibodies, and exquisitely specific microspheres coated with a single antigen can be purchased to elucidate highly complex antibody profiles.[78] These assays are more sensitive than CDC; they primarily detect IgG, but also can be modified to detect IgM.[47] A further development is an assay that uses multiplexed microparticles and allows the simultaneous detection and specification of multiple antibodies in a serum sample.[33] Many laboratories are rapidly gaining experience with this technology, although the precise relationship between antibodies identified using such sensitive techniques compared with conventional methods and their clinical relevance has not been fully evaluated.

Antibody Screening Strategies

The aim of an antibody screening strategy is to determine whether the patient has developed HLA alloantibodies and, if so, the antibody class and specificity of the antibodies. All laboratories supporting renal transplantation have an antibody screening strategy, but may use different approaches and technologies to achieve the goal. One common strategy, because many patients are unsensitized, is to screen samples first with a sensitive method to detect the presence of HLA antibody and then to perform further testing and analysis to determine the specificity of the antibodies in samples that are positive. To perform effective antibody screening, samples should be obtained regularly from patients on the waiting list, on at least a 3-monthly basis. Information about the nature and timing of potential sensitizing events is important in a patient's sensitization profile. If sensitization occurs, additional samples are required (e.g., 14 and 28 days after a transfusion with blood products).[6]

Patient Sensitization Profile and Definition of Unacceptable Specificities

The cumulative information obtained from an antibody screening program, together with knowledge of the potential sensitizing events, enables the laboratory to develop a sensitization profile for patients on the transplant waiting list. The sensitization profile is based on the complete sensitization history for the patient and includes the timing of appearance or disappearance of antibody reactivity, the specificity and antibody class of HLA antibodies, and the presence or absence of autoreactive antibodies.

The characterization of HLA antibody specificities for a patient enables the definition of unacceptable HLA mismatches in a donor. HLA mismatches from a previous transplant and mismatched paternal specificities in multiparous women also may be considered unacceptable specificities. In countries and regions where there is exchange of kidneys, these unacceptable specificities are registered with the Organ Exchange Organisation and facilitate efficient allocation of organs and prevent unnecessary shipping of organs to patients where the crossmatch would be positive. All of the information obtained through regular antibody screening of patients awaiting transplantation is crucial in interpreting the results of a crossmatch test and in assessing the immunological risk of transplantation for a patient.

DONOR CROSSMATCH

Kidney transplantation in patients with donor-specific sensitization has a significant detrimental impact on graft survival, with most transplants undergoing hyperacute or acute humoral rejection. Recipient antibodies against donor histocompatibility antigens bind to the vascular endothelium of the transplanted organ, which disrupts the intercellular junctions and causes release of cell surface heparin sulfate and loss of the antithrombotic state, leading to rapid uncontrollable activation of the thrombotic and complement cascades. The resultant intravascular coagulation and interstitial hemorrhage can lead to graft destruction within minutes or hours after revascularization.

Hyperacute allograft rejection was first reported in the 1960s and was associated with recipient antibodies that agglutinate donor leukocytes, whereas recipients with no detectable antibodies had a good prognosis. The donor leukocyte agglutination assay was soon replaced by the more robust CDC assay, in which recipient serum is incubated with donor lymphocytes in the presence of complement followed by the addition of vital dyes to visualize cell lysis (see Fig. 10-6B). Using these techniques, in 1965, Terasaki and colleagues[110] reported a case of immediate failure of a kidney transplanted from a brother to a sister who had lymphocytotoxic donor-reactive antibodies. A year later, Kissmeyer-Nielsen and colleagues[48] reported a series of 21 consecutive kidney allografts, with 2 cases of immediate failure (which they termed HAR) in multiply-transfused and multigravid recipients who had high-titer (1:512) donor leukocyte agglutinating antibodies. This report was soon followed by seminal papers from Terasaki and colleagues[111] and Williams and colleagues[124] with larger series of transplants (218 and 132 transplants, respectively), each with seven cases of HAR, all having circulating pretransplant donor reactive cytotoxic antibodies. Patel and Terasaki[77] concluded that "the ethics of transplanting kidneys without prior knowledge of the crossmatch test, or across a known positive crossmatch result can reasonably be expected to be questioned in the face of this evidence." This statement established a mandate to perform a prospective pretransplant crossmatch, and the dogma arose that a positive crossmatch was an absolute contraindication to transplantation.

Crossmatch Techniques and Their Clinical Relevance

Complement-Dependent Lymphocytotoxic Crossmatch

The donor lymphocytotoxic crossmatch using the CDC techniques was established in the 1960s and has remained a cornerstone for determining donor and recipient compatibility. The standard National Institutes of Health crossmatch technique involves the incubation of donor lymphocytes isolated from peripheral blood, lymph node, or spleen with recipient sera in the wells of a microtiter (Terasaki) tray, followed by the addition of rabbit serum as an exogenous source of complement (see Fig. 10-6A). Recipient cytotoxic antibodies (predominantly IgM, IgG3, and IgG1) that bind donor cells cause activation of the classic complement pathway resulting in cell lysis, the extent of which can be quantified by the addition of vital stains and determination of viability by microscopy (see Fig. 10-6B). A high percentage of cell death above background levels is interpreted as a "positive crossmatch" with the potential to damage a transplanted kidney. Ensuring a negative pretransplant lymphocytotoxic crossmatch using this basic technique has virtually eliminated HAR, but in its simplest form the CDC crossmatch carries several major drawbacks and has been subject to many modifications.

During the 1970s, it emerged that not all lymphocytotoxic antibodies that cause a positive crossmatch are specific for donor histocompatibility antigens, and that some antibodies display autoreactivity, causing in vitro lysis of the patient's own cells in the CDC assay. In 1976, Stastny and Austin[97] reported a successful transplant using an HLA-identical sibling donor with a positive autologous and donor lymphocyte crossmatch. Larger studies confirmed that a positive crossmatch caused by non-HLA (autoreactive) lymphocytotoxic antibodies could be safely ignored,[16,83,115] with transplant survival rates being equivalent to, or even higher than, transplants with a negative crossmatch.[19] Taylor and colleagues[103] characterized these autoantibodies as polyreactive IgM, capable of low-affinity binding to multiple antigens owing to weak electrostatic interactions.[103] Depending on antibody titer or affinity or both, the antigens may display in vitro cytotoxicity to autologous and third-party (panel) B lymphocytes alone, or T and B lymphocytes, and are often negative or only weakly reactive with B lymphocytes from patients with chronic lymphocytic leukemia.

The good sensitivity but poor specificity of the CDC assay in preventing HAR prompted numerous technical modifications. These included the Amos wash technique, which removed nondamaging low-affinity IgM antibodies and anticomplement immune complexes and was effective at improving the assay specificity. It was noted, however, that sensitized patients and patients receiving a regraft had a higher incidence of primary graft nonfunction or delayed graft function and poorer graft survival, despite a negative donor lymphocytotoxic crossmatch. This poor outcome was assumed to be caused by low antibody levels below the threshold of detection of the standard National Institutes of Health CDC assay, or by noncomplement fixing antibodies (e.g., IgG2 and IgG4) that are not detected by CDC. Two further modifications were introduced to address this: extended postcomplement incubation times (increased from 1 hour to 2 hours), and the addition of antihuman globulin to enhance the detection of low-level IgG bound to donor cells. Although inconclusive, these modifications were perceived as beneficial, particularly in sensitized patients and second grafts, and were widely adopted in Europe and North America.

B Cell Crossmatch

Further advances came with the discovery by Ting and Morris in 1978[116] of the strong effect of HLA-DR matching on graft outcome that prompted investigators to consider the clinical relevance of HLA-DR–specific antibodies in rejection. Numerous studies were undertaken using separated donor B lymphocytes (that express HLA class I and class II) as targets in the crossmatch test. The results of the analyses were contradictory and ranged from showing no effect, enhanced graft survival, and poor graft survival. These findings now can be explained by the heterogeneous antibodies that cause a positive B cell crossmatch. Most studies did not differentiate between nondamaging (autoreactive) and potentially harmful (HLA specific) B lymphocyte–reactive antibodies. The clinical interpretation of a B cell crossmatch result is impossible without definition of the specificity of the antibody; d'Apice and Tait[20] showed that most positive donor B cell crossmatches are not caused by HLA-DR–specific antibodies. In the studies where antibody specificity was defined, it was clear that most positive B cell crossmatches are caused by non–HLA-specific, usually B cell–autoreactive, antibodies that are not harmful to a transplant. A minority of positive B cell crossmatches are caused by HLA class II–specific antibodies that can be deleterious to transplant outcome, but are unlikely to cause HAR. The presence of unusually high-titer HLA-DR–specific antibodies can cause HAR, however, and such antibodies are more common in patients with previous graft rejection.[1,4,65,91]

Further indirect evidence of the importance of HLA class II–specific sensitization and the B cell crossmatch is indicated by the poor survival of HLA-DR–mismatched regrafts, most of which were performed with no knowledge of the patient's HLA class II sensitization status or the pretransplant donor B cell crossmatch result. In contrast, in single-center and larger multicenter reports in which detection of HLA class II–specific sensitization and performing a pretransplant donor B cell crossmatch is routine, regraft survival is equivalent to that of primary grafts.[104]

Crossmatch Serum Sample Selection (Timing)

An essential feature of the immune system is immunological memory and its ability to produce a rapid and vigorous secondary response on re-exposure to antigens to which an individual is already primed. To avert the risk of rejection caused by an anamnestic memory response, crossmatch regimens include serum samples obtained throughout a recipient's time on the transplant waiting list, selected to represent peak periods of sensitization. The introduction of calcineurin-based immunosuppression in 1982 prompted Cardella and colleagues[10] to question the relevance of "historical" allosensitization in patients in whom antibody levels had declined over time. They reported a series of 15 transplants in which the donor crossmatch was positive using noncurrent (historical) serum samples, but negative using serum obtained at the time of transplantation (so-called peak positive current negative), with graft survival rates (60% at 1 year) equivalent to their negative

crossmatch transplants. Similar outcomes were confirmed by other groups, and many concluded that memory B cell responses were short-lived and could be adequately controlled by immunosuppression. A significant proportion of transplants still underwent irreversible acute rejection, however, and past donor reactive sensitization was particularly associated with poor regraft survival.

Immunoglobulin Class and Specificity

The aforementioned findings of acceptable primary graft survival, but poor regraft survival associated with an historical positive crossmatch prompted further modification of the CDC crossmatch assay to identify the immunoglobulin class and specificity of antibodies causing a positive crossmatch. Patient crossmatch serum was preincubated with a reducing agent (2-mercaptoethanol and DTT) to distinguish IgM and IgG antibodies in the donor CDC crossmatch assay.[3,12] In addition, Taylor and colleagues[102] defined the antibody specificity (HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ) using a cytotoxicity inhibition assay to distinguish accurately between HLA class I–specific, HLA class II–specific, and non-HLA–specific antibodies causing a positive donor T cell or B cell (or both) crossmatch. The studies found acceptable primary and second graft survival associated with historical IgM HLA-specific sensitization, but poor graft survival with historical IgG HLA-specific antibodies. These results indicated that past allosensitization events that resulted only in a transient primary response and IgM alloantibody production could be readily controlled by conventional cyclosporine-based immunosuppression, whereas secondary responses (denoted as IgG positive) that commonly occur after pregnancy and previous transplant rejection indicate immunological priming accompanied by T cell and B cell memory that is poorly controlled by immunosuppression. A number of studies provided corroborative evidence that historical DTT-resistant (IgG), CDC-positive crossmatches were immunologically high risk, whereas IgM alloantibodies could be safely ignored and the use of DTT has been widely adopted in the donor crossmatch assay.[109]

Flow Cytometry Crossmatch Test

Although the CDC crossmatch was effective at averting HAR, a number of transplants still had primary nonfunction or delayed graft function, and this was particularly prevalent in sensitized patients and regrafts. This indicates that early graft dysfunction in sensitized recipients may be caused by low levels of antibody, below the sensitivity threshold of the conventional CDC crossmatch. Garovoy and colleagues[34] addressed this question using a flow cytometry crossmatch test (Fig. 10-8) capable of detecting weak IgG antibodies that were undetectable by CDC. In this retrospective study, there was a higher incidence of delayed graft function and graft failure

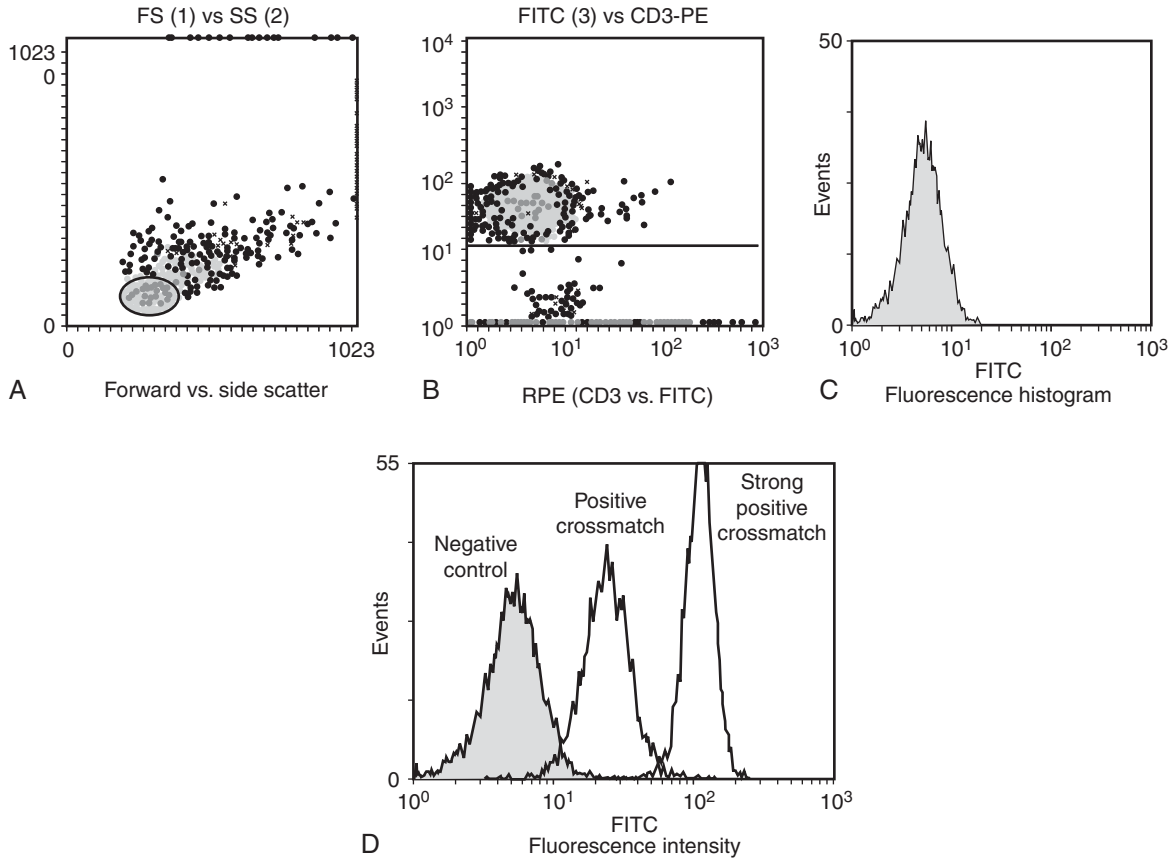


Figure 10–8 Flow cytometry crossmatch test. **A**, Cells pass through a laser beam, and "forward and side light scatter" (FS and SS) is detected by photomultiplier tubes. An "electronic gate" is used to select cells of morphological interest (in this case, lymphocytes). **B**, T lymphocytes are identified using a recombinant phycoerythrin (RPE)–labeled CD3-specific antibody, and HLA-specific IgG bound to cells is identified with fluorescein isothiocyanate (FITC)–labeled antihuman IgG. **C**, Light emission is detected and displayed as a "fluorescence histogram." **D**, Increased FITC fluorescence (a shift to the right on the fluorescence histogram) is a measure of HLA-specific IgG bound to T lymphocytes above that of the negative control indicating a positive crossmatch. (See color plate.)

in the presence of a pretransplant flow cytometry–positive (but CDC-negative) donor crossmatch, indicating a pathogenic role for weak, sublytic, HLA-specific antibodies. Other studies quickly corroborated this finding, but a significant proportion of patients had an uneventful clinical course, despite a positive flow cytometry crossmatch. These data showed a high sensitivity, but lower specificity of a positive flow cytometry crossmatch in predicting early graft dysfunction caused by antibody-mediated rejection. Many centers were concerned that false-positive crossmatches would unnecessarily deny patients the opportunity of a transplant and were deterred from adopting the technique in routine clinical practice.[53] Nevertheless, the predictive value of a positive result was high in sensitized patients and second grafts, which carry an increased immunological risk of rejection, and the increased assay sensitivity is widely used in such scenarios.[14,46]

Crossmatch Policies and Clinical Interpretation

The purpose of a pretransplant donor crossmatch is to detect donor-specific sensitization that is predictive of hyperacute, acute, and chronic rejection (cellular and humoral) and to ensure appropriate therapeutic strategies are in place that are effective at controlling the ensuing rejection response. The crossmatch strategy must define the immunological risk by distinguishing antibodies that would be harmful, and the type of rejection response that is likely to occur. Because of the intricate relationship between this strategy and the clinical program, crossmatch strategies vary between centers, depending on laboratory and clinical facilities and expertise.

Crossmatch Veto: Which Antibodies Are Harmful?

It is important to distinguish damaging from nondamaging antibodies, and in this context the crossmatch can be viewed as a risk assessment for antibody-mediated rejection (Table 10-3). Donor-specific antibodies that are predictive of HAR (e.g., CDC-positive or strong positive flow cytometry crossmatch detecting IgG HLA class I–specific and HLA class II–specific antibodies present at the time of transplantation) in most cases constitute an absolute veto to transplantation, unless preemptive antibody removal (desensitization) and post-transplant immunological monitoring programs are instituted (see Chapter 22). Weaker IgG HLA-specific antibodies that are detectable only using flow cytometry crossmatch assays (i.e., CDC-negative) are associated with delayed graft function and acute humoral rejection and should be considered a contraindication to transplantation.[32] There is accumulating evidence that hitherto undefined HLA-DP–specific antibodies are commonly found in patients with graft rejection, and this suggests that it is necessary to avoid retransplantation in patients with donor-reactive HLA-DP antibodies.[80]

The prognostic relevance of historical IgG HLA class I–specific and class II–specific positive crossmatches that are negative at the time of transplantation has not been rigorously addressed using the diverse armory of modern immunosuppressive options. In this scenario, although HAR would not occur, early acute humoral or accelerated cellular rejection (or both) that is refractory to treatment with conventional calcineurin-based immunosuppressive agents is likely.[12,102,109] It has been suggested that historical IgG alloantibodies act as a marker for T cell priming and the presence of antigen-specific memory helper and cytotoxic T lymphocytes.[73,84] Such cells display cyclosporine resistance, and their rapid reactivation on repeat exposure to alloantigen elicits a powerful rejection response. Transplantation in patients with past IgG donor HLA–specific sensitization should be approached with caution and requires an augmented immunosuppressive therapy designed to control secondary (memory) T cell or B cell (or both) responses.

There is no doubt that IgM non–HLA-specific lymphocytotoxic antibodies that cause a positive donor B cell or T and B cell crossmatch are benign and have no harmful effect on transplant survival. In addition, good graft survival is reported with historical IgM donor HLA–specific positive crossmatches, which also can be safely ignored.[87,102] Many centers believe this also is true for current IgM donor HLA–specific antibodies despite their potential to bind vascular alloantigens and activate complement. High-titer IgM antibodies are thought to cause HAR in ABO blood group–incompatible transplants and in discordant xenotransplantation, and IgM alloantibodies may exhibit potential to

Table 10–3 **Risk Assessment for Antibody-Mediated Rejection**

Crossmatch Result: IgG HLA-Specific Antibodies	Contraindicated*	High Risk†	Intermediate Risk‡	Low Risk
Current Positive				
Cytotoxicity	•			
Flow cytometry			•	
Historical Positive				
Cytotoxicity		•		
Flow cytometry			•	
Current and Historical Negative				
Cytotoxicity			•§	•
Flow cytometry				•

*Contraindicated unless the donor-specific antibody can be removed with desensitization protocols.

†High risk of antibody-mediated rejection in the first month post-transplant requiring additional treatment or antibody removal; post-transplant antibody monitoring essential; augmented immunosuppression may be indicated.

‡Augmented immunosuppression may be indicated; post-transplant antibody monitoring advisable.

§Because of the variable sensitivity of the cytotoxic crossmatch, a negative result does not indicate a low risk, particularly for sensitized patients or in the absence of comprehensive antibody specificity data for the patient.

From Fuggle SV, Martin S: Toward performing transplantation in highly sensitised patients. Transplantation 78:186, 2004.

cause HAR. Most IgM HLA-specific antibodies have low affinity, however, and appear only transiently after blood transfusion, whereas persistent high-titer IgM HLA-specific antibodies with potential to cause HAR are rare.

Pretransplant Donor Crossmatch Testing

Prolonged cold ischemia time is a significant and controllable factor that has a detrimental effect on cadaver donor kidney transplant outcome. There is a progressive detrimental effect of cold ischemia time on transplant outcome with 90% survival at 1 year for organs transplanted within 20 hours compared with 83% for organs transplanted at more than 30 hours (relative risk 1.9) (data from United Kingdom Transplant). It is essential that cadaver donor organ allocation and crossmatch policies are designed to ensure a safe decision making process and minimize delays in transplantation associated with the allocation process. Recent technical advances have facilitated HLA typing and donor crossmatch strategies that can identify suitable recipients before completion of the organ retrieval operation and remove delays caused by histocompatibility testing.

Many histocompatibility laboratories receive donor peripheral blood obtained early in the donation process, before beginning the retrieval operation. This early receipt enables prospective donor HLA typing using a combination of molecular and serological techniques and completion of local and national allocation algorithms to identify potential recipients before organ donation. In addition, modern cell separation techniques using immunomagnetic particles enable the recipient crossmatch to be performed using donor peripheral blood. In selected cases (e.g., nonsensitized patients with low immunological risk), archived sera collected within the last 3 months can be used in the crossmatch test, which can be completed before patient admission. In cases in which a patient's antibody profile has been completely characterized, and comprehensive data concerning allosensitization events are available, a negative donor crossmatch can be predicted with absolute certainty. It such cases, it is possible to omit a pretransplant crossmatch.[107] With the adoption of these and similar crossmatch policies, there is no histocompatibility-associated cold ischemia time.

STRATEGIES FOR TRANSPLANTING SENSITIZED AND HIGHLY SENSITIZED PATIENTS

Patients with HLA antibodies reactive with a high proportion of a donor pool are difficult to transplant, and special strategies are required to find suitable kidneys for these patients. Highly sensitized patients have been defined as patients with a %PRA value of 85% or more, but a more meaningful definition may be patients who would have a positive crossmatch with greater than 85% of available donors.

To find a crossmatch-negative kidney, highly sensitized patients need access to a large donor pool. Numerous approaches have been adopted for transplanting highly sensitized patients. Eurotransplant introduced an Acceptable Mismatch Program for highly sensitized patients. In this program, extensive antibody screening was performed to identify "windows" in the patient's immune repertoire. The HLA antigens of cells unreactive with the patient's serum were the "windows" or specificities to which the patient had not made antibodies. The Acceptable Mismatch Program includes minimal mismatching criteria of full HLA-DR compatibility or matching for one HLA-B specificity and one HLA-DR specificity. Of patients entered in the program, 43% are transplanted within 6 months, and 58% are transplanted within 21 months.[13] When this system was first introduced, the antibody screening was performed on carefully selected cells that had only one mismatched antigen with the highly sensitized patient. This approach was extremely labor intensive and would be possible only in a laboratory that had access to very large panels of HLA-typed cells. The advent of solid-phase assays with single antigen preparations, or cell lines expressing single HLA antigens,[128] greatly expedites this type of approach.

A computer algorithm HLA Matchmaker developed by Duquesnoy may assist in defining acceptable mismatches.[24,25] In the algorithm, an HLA specificity is represented as a string of amino acid triplets, and it is possible to compare HLA specificities and identify mismatched triplets. The theory is that if there are no triplets mismatched, the specificity would not be recognized, and an immune response would not be generated. Clearly, HLA antigens are not linear sequences; nor are the amino acids in a protein in triplets; nevertheless, the algorithm has been shown to assist in the process of specifying a patient's sensitization profile.[37]

In the United Kingdom, the approach has been different. In the national kidney allocation scheme implemented in 1998, highly sensitized patients were prioritized for well-matched transplants with no HLA-A, HLA-B, or HLA-DR mismatches between the donor and recipient (000 HLA-A, HLA-B, or HLA-DR mismatch grade).[30] Sensitization data also were collected nationally and used for allocation purposes. The data collected were designed to capture an expert view of the patient's sensitization status, based on the patient's history, antibody screening data, and the policy of the local transplant unit. Rather than reporting sensitization as a %PRA value, the key data registered were unacceptable specificities and HLA reactivity in a patient's serum that could not be accounted for from the unacceptable specificities defined. This was termed *residual reaction frequency*, and in highly sensitized patients if this figure was zero (i.e., the antibody profile has been completely specified), the highly sensitized patients also were eligible for favorably matched transplants. These were transplants where there was a maximum of one HLA-A and one HLA-B mismatch in the absence of mismatches at HLA-DR (denoted 100, 010, 110 HLA-A, HLA-B, HLA-DR mismatches). This policy resulted in a threefold increase in the number of highly sensitized patients transplanted, 62% of these transplants having a 000 mismatch grade.[32]

The basis for national kidney allocation has changed more recently in the United Kingdom, but the 2006 allocation scheme has retained the priority given to highly sensitized patients for 000 mismatched transplants and access to other, less well-matched kidneys for highly sensitized patients where the antibody profile is completely specified. One of the benefits of defining antibody profile is to make it possible to estimate a patient's chance of receiving a transplant and make informed decisions about the best therapeutic option for a patient. In the United Kingdom, a "matchability score" is calculated for all patients on the transplant waiting list, by comparing a patient's HLA type, unacceptable specificities, and blood group against a file of

10,000 United Kingdom donors.[31] Patients with a high matchability score are likely to be easy to transplant with a well-matched transplant. This is particularly informative in planning transplantation for children, who are given high priority for national kidneys. Patients with a low matchability score are less likely to receive an offer of a deceased donor kidney through the national scheme, and alternative approaches to transplantation may be explored.

Antibody Removal

There has been a resurgence of interest in using antibody removal techniques to reduce donor-specific HLA antibody before transplantation (see Chapter 22).[35] Two main approaches are being used with successful outcomes: high-dose intravenous immunoglobulin[36,122] used for transplantation with living or deceased donors, and plasmapheresis combined with low-dose cytomegalovirus hyperimmune globulin[66,90,94,127] used for transplantation with living donors.

In considering patients for antibody removal, it is important for the HLA specificity and titer of the antibodies to be determined before beginning antibody removal; this may help determine whether this approach is appropriate for a particular patient. During antibody removal, it is important to monitor antibody levels to determine the effectiveness of the treatment regimen. The plasmapheresis and hyperimmune globulin regimen can be monitored using a solid-phase assay, but the high-dose intravenous immunoglobulin regimen requires the use of a sensitive cytotoxicity assay because the intravenous immunoglobulin interferes with the solid-phase assays. Most centers advocate that a final crossmatch against the potential donor is performed, regardless of the methods used for monitoring. After transplantation, antibody rebound usually occurs, and monitoring antibody levels provides valuable information to indicate whether additional antibody removal is required. Experience in performing transplants after desensitization is mounting, but because of the complexity in the management before and after transplantation, it may be that in the longer term, patients are referred to specialist centers for transplantation after antibody removal.

Paired Exchange

Paired exchange, or living donor exchange, is another possible option for patients who have a potential living donor, but for reasons of HLA or ABO antibody incompatibility the transplant cannot proceed.[85,86] In such schemes, donors and recipients are paired, and crossover transplants are undertaken. Simple systems pair recipients and their respective donors, but it is possible that multiple exchanges can be undertaken. There is a well-established living donor exchange scheme in South Korea,[76] and other regional and national schemes have been reported from the United States.[22,59] In Europe, the encouraging results of the first year of the Dutch national living donor kidney exchange program have been reported,[21] and in the United Kingdom paired exchange became possible in September 2006 because of a change in legislation, and the first exchanges are planned for early in 2007.

Combined Transplants

One further approach for transplanting highly sensitized patients is to perform a combined liver-kidney transplant in the presence of a positive crossmatch. The rationale for this procedure is that the liver, or soluble antigen derived from the liver, is capable of absorbing the donor-specific antibody and protects the kidney from HAR. This in vivo absorption has been successfully performed with no reported HAR or accelerated acute rejection episodes, but as yet only a few patients have been treated.[40,72]

POST-TRANSPLANT MONITORING

There is an expanding literature on the development of HLA antibodies after renal transplantation and consideration of the role these antibodies may play in transplant failure.[8,113] The proportion of recipients developing antibodies has been reported to range between 12% and 60%.[61] This figure not only is influenced by the sensitivity of the assay system, but also by clinical factors, such as the nature and degree of mismatching between the donor and the recipient and the immunosuppressive regimens.

The appearance of donor-specific antibodies has been shown to be associated with a poorer outcome and with the occurrence of acute and chronic rejection. In recent reports in which serial post-transplant serum samples were analyzed, donor-specific HLA antibodies were strongly predictive of allograft failure being detected before chronic rejection or transplant failure.[55,125] A large international prospective trial that included more than 4500 patients from 36 units also concluded that HLA antibody production predicts transplant failure.[114]

Mismatched HLA antigens are important stimuli for an alloimmune response, but antibodies to nonclassic polymorphic MHC antigens also may contribute. The MHC-related chain A and B antigens (MICA and MICB) are expressed on epithelia in response to cellular stress and on endothelium in vitro. In the kidney, MICA and MICB expression has been reported on tubular epithelia.[41,81] Antibodies to MICA were first reported in the sera of transplant recipients,[99,131] but because the antigens are not expressed on lymphocytes,[129,130] MICA and MICB antibodies would remain undetected in standard antibody screening and crossmatch tests. Reports in which MICA has been included as one of the targets studied have shown the presence of MICA antibodies in transplanted patients, and a higher incidence of antibodies was found in patients whose transplants failed.[63,64]

The histological detection of immunoglobulins bound to the transplant endothelium is difficult because antibody is rapidly removed from the endothelium. After activation of the classic complement pathway, however, one of the components, C4d, remains covalently bound to the endothelial surface, acting as an imprint of antibody binding. Following the original observation by Feucht and colleagues[26] that C4d deposition on the endothelium of renal peritubular capillaries was a marker of acute humoral rejection, this has become an accepted diagnostic tool. There is strong evidence that circulating donor-reactive antibodies are associated with C4d deposition in the transplant,[5,15,58,71] and that this is highly specific for antibody-mediated rejection.[50] The Banff 97 classification of renal allograft rejection has been modified such that the definition of acute humoral rejection includes C4d deposition, histological evidence of graft injury, and donor-reactive antibodies.[82]

Currently, HLA matching, definition of sensitization, and donor crossmatch are making an important contribution to

successful renal transplant programs. It is an exciting time as many of the traditional boundaries are being challenged to enable transplantation of patients who previously would have been unlikely to be transplanted.

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Chapter 11

Surgical Techniques of Renal Transplantation

John M. Barry • Peter J. Morris

Renal transplantation is a major surgical procedure that includes a vascular component and a urological component. Although in the past it was common for a general or vascular surgeon to do the vascular component of the implantation and a urologist to do the urological component of the operation, today the entire procedure generally is performed by a transplant surgeon, regardless of his or her background training as a general surgeon, vascular surgeon, or urologist. The recipient, who is uremic and usually being maintained on hemodialysis or peritoneal dialysis, often is a poor-risk patient with comorbid disease (e.g., diabetes, cardiovascular disease, obesity). If poorly dialyzed, the recipient has a significant degree of platelet dysfunction with a resultant tendency to bleed. The need for meticulous techniques cannot be stressed enough, bearing in mind that the operation could be the only opportunity that the patient may have to obtain a successful kidney transplant, which can change the quality of life dramatically.

PREPARATION OF RECIPIENT

The general preparation and selection of recipients for transplantation is discussed in Chapter 4. On admission for transplantation, a careful history and physical examination is required to ensure that there is no immediate contraindication to major surgery, and particular attention should be paid to the patient's fluid and electrolyte status. The patient may require dialysis before going to surgery because of fluid overload or a high potassium level. Although dialysis may delay surgery by several hours, this should not influence the decision to dialyze the patient before surgery, bearing in mind that if the patient is to receive a cadaver donor kidney, there is a significant chance of delayed graft function.

Immunosuppression, whatever the protocol, is often begun before the patient goes to surgery. Although there is no hard evidence that preoperative immunosuppression is necessary, the rationale is that a loading dose of a calcineurin inhibitor ensures a better blood level in the first 12 hours because most patients are unable to take oral medication in the first 24 hours after surgery.

The use of preventive antibiotics is advised because although the operation is a clean one, the patient is uremic and will be immunosuppressed, which puts the patient at high risk for wound infection. There is always a possibility of contamination of a cadaver donor kidney, and the combination of a vascular procedure with a urological procedure increases the risk of infection in the vicinity of the vascular anastomosis. An infection of the vascular anastomosis with subsequent secondary hemorrhage is a catastrophic complication, resulting in loss of the kidney, compromise of distal circulation, and a threat to life. The case for preventive antibiotics is a strong one. In the Oxford Unit, cefuroxime, 1.5 g intravenously, is given with the induction of anesthesia.

After the induction of anesthesia, a central catheter is inserted into the internal jugular vein or into the subclavian vein. Insertion of the catheter is facilitated by the use of duplex ultrasound. Although a central line is not essential intraoperatively, it facilitates management because many patients who have been on long-term hemodialysis are dehydrated and require significant amounts of fluid to maintain a central venous pressure of 7 to 10 cm H_2O. Other aspects of the induction of anesthesia and monitoring during the operative procedure are discussed in Chapter 13.

A balloon catheter is inserted into the bladder with full aseptic technique (see later) on the operating table. The skin

should be prepared carefully in the operating room, first with thorough removal of hair with clips followed by preparation of the skin of the abdominal wall with an antimicrobial agent, such as povidone-iodine or chlorhexidine gluconate. It is wise to prepare the entire abdomen from nipples to midthighs, especially in a recipient with vascular disease, because the original incision may need to be extended or abandoned and the opposite iliac fossa opened.

SITE

Although traditionally the right iliac fossa was used for implantation of the kidney since the early descriptions,[30,38,49,50,66] today it is more usual to place the left kidney in the right iliac fossa and the right kidney in the left iliac fossa, other things being equal. This approach places the pelvis and ureter anteriorly, which to some extent facilitates the urological tract reconstruction, particularly if a subsequent urological complication requires surgical intervention. If a continuous ambulatory peritoneal dialysis catheter or stoma were emerging from one side of the abdomen, the contralateral side would usually be chosen. In the presence of polycystic kidneys, one would choose the side of the smaller polycystic kidney, assuming that there was room for the transplanted kidney below it. Often, one polycystic kidney has to be removed to make room for a transplanted kidney, and this preferably is done as a separate operative procedure before transplantation because a large polycystic kidney is removed more easily as a transperitoneal procedure, and one or more of the cysts may harbor bacteria. It can be done at the time of the transplant procedure, however, through the same extended retroperitoneal approach or through a midline incision with extraperitoneal placement of the kidney transplant. In the case of nephrectomy for polycystic kidney disease, a fluoroquinolone antibiotic is often used instead of a cephalosporin because of better antibiotic penetration into renal cysts with the former.[19] In children, in whom the vascular anastomoses of the renal vessels may be to the aorta and vena cava because of the size of the kidney, the right side is preferred because the kidney is placed behind the cecum and ascending colon. Simultaneous pancreas and kidney transplantations usually are accomplished by a vertical midline transperitoneal approach, and torsion of the renal pedicle with kidney transplant thrombosis has been reported.[70] This torsion can be prevented by nephropexy or retroperitoneal placement of the kidney graft. The iliac fossa can be developed by inserting an index finger into the prevesical space just lateral to the midline.

INCISION

An oblique Rutherford Morison[40] or curvilinear incision is made in the right or left lower quadrant of the abdomen beginning almost in the midline and curving upward parallel to the inguinal ligament and approximately 2 cm above it and ending just above the anterior superior iliac spine of the iliac crest. In a child or small adult, this incision can be carried up to the costal margin to increase exposure (Fig. 11-1).[42] The external oblique muscle and fascia are divided in the line of the incision and split to the lateral extent of the wound.

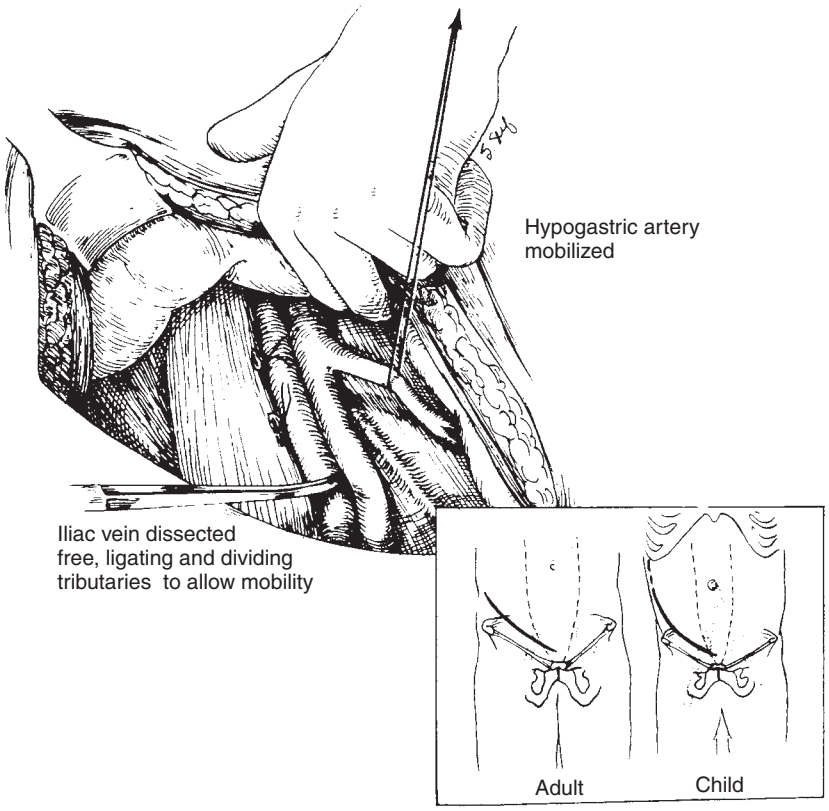

Figure 11–1 Iliac vessels dissected free. *Inset*, Incision for adult; incision for child. (From Lee HM: Surgical techniques in renal transplantation. In Morris PJ [ed]: Kidney Transplantation. London, Academic Press/Grune & Stratton, 1979, p 146.)

This incision is carried medially onto the rectus sheath to permit retraction or division of part of the rectus muscle for later exposure of the bladder. To expose the peritoneum, either the internal oblique and transverse muscles are divided with cautery in the line of the incision, or the confluence of the oblique muscles and the rectus sheath is divided medially lateral to the rectus muscle as a pararectus incision, which avoids division of the internal oblique and transversus muscles. The inferior epigastric vessels are ligated and divided, but if there are multiple renal arteries, the inferior epigastric vessels should be preserved in the first instance in case the inferior epigastric artery is required for anastomosis to a lower polar renal artery or if a chevron incision with division of the superior epigastric vessels had been used to remove an ipsilateral kidney, gallbladder, or spleen. Although division of the spermatic cord was advocated in early descriptions of the procedure and was common practice for many years, it should not be done and rarely is required for adequate exposure. The spermatic cord is not cut, but freed laterally, which allows it to be retracted medially.[29] The round ligament can be divided.

PREPARATION OF OPERATIVE BED

After exposure of the transversalis fascia and peritoneum, the transversalis fascia is divided, and the peritoneum is reflected upward and medially to expose the psoas muscle and the iliac vessels. At this stage, a self-retaining retractor is inserted. We find the Bookwalter retractor system satisfactory because it provides excellent exposure and allows the assistant to have both hands free to assist with the anastomoses. Depending on whether the internal iliac artery is to be anastomosed to the renal artery of the transplant kidney or whether the renal artery with a cuff of aorta is to be anastomosed to the external iliac artery, dissection proceeds in the first instance to expose the external, common, and internal iliac arteries. The lymphatics that course along and over the vessels must be ligated with a nonabsorbable suture such as silk and divided, rather than cauterized, to prevent the later occurrence of a lymphocele (see Chapter 26). The surgeon must be careful not to mistake the genitofemoral nerve for a lymph vessel. It lies on the medial edge of the psoas muscle, and a branch may cross the distal external iliac artery. If the internal iliac artery is to be used, it is important to mobilize a length of the common and external iliac arteries so that the internal iliac artery can be rotated laterally without kinking at its origin and so that the vascular clamps can be applied to the common and external iliac arteries when the internal iliac artery is short. Care is taken to inspect the origin of the internal iliac artery, if this is to be used, for any evidence of atheroma and, similarly, any atheromatous disease in the common or external iliac artery should be noted. If there are two or more renal arteries not on a cuff of aorta, the dissection of the internal iliac artery is extended distally to expose the initial branches of the internal iliac artery, some of which may be suitable for anastomosis to individual renal arteries.

Having completed the exposure of the appropriate iliac arteries, dissection of the external iliac vein is begun. If a left kidney with a long renal vein is available, dissection of the external iliac vein alone generally allows a satisfactory anastomosis without tension. If a right kidney, which has a short renal vein, is to be used, or a left kidney, in which a short renal vein has been provided, is to be used, or if the recipient is obese, the internal iliac vein and usually one or two gluteal veins can be ligated with silk and divided.[29,64] This technique allows the common and external iliac veins to be brought well up into the wound, particularly if the internal iliac artery is divided, and this facilitates the performance of a tension-free anastomosis. The peritoneum is reflected further up laterally to prepare the final pocket for the kidney in the parapsoas gutter. Temporary placement of the cold kidney graft into the wound assists in the selection of the recipient artery and vein for revascularization.

When the kidney has been prepared and is ready for implantation, the vessels are now ready for clamping. Heparin is commonly administered in a modest dose of 30 IU/kg.

Vascular clamps are applied to the external iliac artery proximally and distally if an end-to-side anastomosis is to be performed, and if the internal iliac artery is to be used, a vascular clamp is applied to the internal iliac artery at its origin or to the common and external iliac arteries. The vein is clamped proximally and distally with vascular clamps or isolated between tourniquets, or a Satinsky side clamp is used. After division of the internal iliac artery distally, the lumen is flushed out with heparinized saline. Similarly, if the external iliac artery or common iliac artery is to be used, an appropriate-sized arteriotomy is made, usually enlarged with a vascular punch, and the lumen is flushed out again with heparinized saline. The venotomy similarly is flushed out with heparinized saline, and if a valve is present at the site of the venotomy, it should be removed carefully. Before making the arteriotomy or venotomy, the surgeon should mentally visualize the kidney in situ in its parapsoas gutter and the course that the renal artery and vein would take to ensure the optimal site for the anastomoses.

PREPARATION OF KIDNEY

A varying degree of dissection of the kidney is required when it is received in ice. In the case of a cadaver kidney, in which the kidneys usually have been removed as part of an en bloc procedure, considerable dissection needs to be performed, and this should be done carefully and with a good light on a back table with the kidney in a bowl of ice slush. The dissection of the cadaver kidney usually is done in advance of the transplant procedure in case some anomaly is present that would preclude going ahead with the transplant. In the dissection, great care must be taken in protecting the blood supply to the ureter, and the so-called golden triangle should not be broached (see Chapter 27).

A kidney from a living donor generally has a single renal artery, but there may be additional arteries. In this case, reconstruction usually is done on the back table, and either the arteries are joined together at their orifices to form a common trunk (Fig. 11-2),[42] or a smaller artery is anastomosed end-to-side to a larger renal artery. It is imperative that a lower polar artery be revascularized because this almost certainly gives rise to the ureteric blood supply. It also is possible to use the epigastric artery to revascularize a lower polar artery, but in general it is preferable to anastomose a lower polar artery to the major renal artery, either end-to-side or as a common trunk. It also is possible to use a portion of saphenous vein as a graft bridge. A small upper polar artery, if thought to be too small to anastomose safely to the major

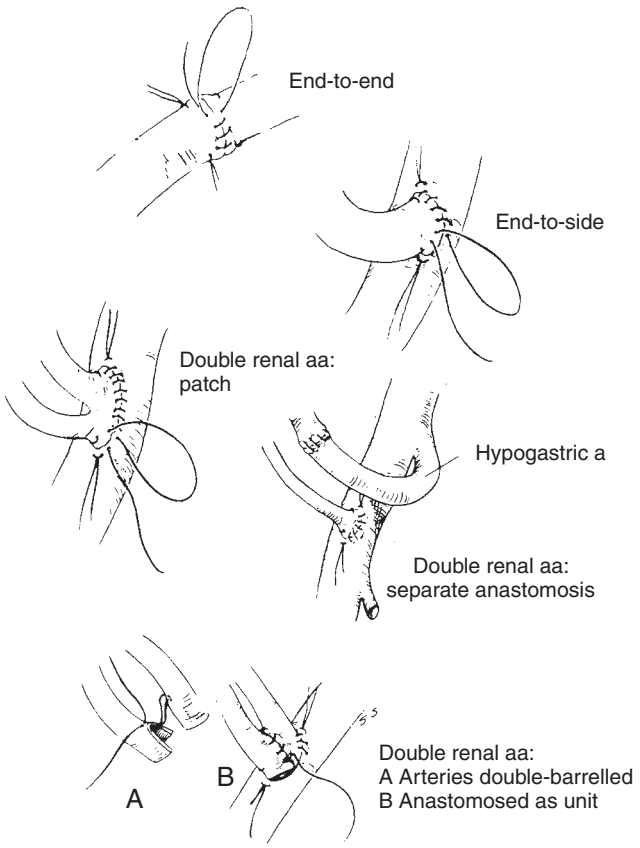

Figure 11–2 Variations of renal artery anastomoses. (From Lee HM: Surgical techniques in renal transplantation. In Morris PJ [ed]: Kidney Transplantation. London, Academic Press/Grune & Stratton, 1979, p 150.)

renal artery, may be ligated, provided that it supplies less than one eighth of the kidney (which should be evident on perfusion of the kidney after removal).

A cadaver kidney usually has a renal artery or arteries arising from an aortic patch, and this patch should be trimmed to an appropriate size and used for anastomosis to the external iliac artery. If two renal arteries are widely separated on the aortic patch, the patch may be divided to allow separate implantation into the external iliac artery, or one may be implanted end-to-side and the other to a branch of the internal iliac artery.

If there is more than one renal vein, smaller veins can be ligated, assuming that there is one large renal vein. If two renal veins are of equal size and are not arising from a Carrel patch, there is some risk of subsequent venous infarction if one vein is ligated, and it is preferable to join the veins to form a common trunk for the subsequent anastomosis. A short right renal vein can be extended with donor inferior vena cava or external iliac vein, or with a recipient renal vein if a native nephrectomy had been done as a preliminary procedure under the same anesthetic.

When the kidney finally is prepared and ready for implantation, a technique that we use in Oxford ensures that the kidney remains cool during the time of the anastomoses. A surgical glove (size 8) is used, with the fingers knotted and the ends cut off. The glove is packed partially with crushed ice, and the kidney is inserted, with care being taken always to have the upper pole of the kidney at the finger end of the glove ("fingers up"). More crushed ice is inserted into the glove, and the glove is tied at its wrist end. A 1.5-cm hole is made over the vessels, which can be brought through this opening in turn. This technique not only keeps the kidney cool during the anastomosis,[57] but also facilitates handling the kidney because an artery clamp can be placed on the glove itself to allow the kidney to be held in position during the procedure. When the anastomoses are completed, the glove is removed, and the kidney is reperfused.

REVASCULARIZATION

The question of whether the arterial anastomosis or the venous anastomosis should be done first depends on the final position of the kidney and the ease with which the second anastomosis may or may not be done. In general, if the renal artery is to be anastomosed to the internal iliac artery, the arterial anastomosis should be done first because this enables the renal vein to be positioned appropriately. If the renal artery is to be anastomosed end-to-side—usually with a cuff of aorta—to the external iliac artery, it is preferable to do the venous anastomosis first, then the end-to-side arterial anastomosis can be positioned correctly.

Arterial Anastomosis

The internal iliac artery is anastomosed end-to-end to the renal artery with 5-0 or 6-0 monofilament vascular suture using a three-point anastomosis technique, as described by Carrel in 1902,[16] or a two-point anastomosis (Fig. 11-3).[42] If there is a disparity between the renal artery and the internal iliac artery, the renal artery being considerably smaller in diameter, the renal artery should be spatulated along one side to broaden the anastomosis. If one side of the renal artery is spatulated, care should be taken to place the spatulation of the renal artery appropriately, taking into consideration the final curve of the internal iliac artery and the renal artery so that one or the other would not be kinked when the kidney is placed in its final position (Fig. 11-4).[42] If both arteries are small, at least one third of the anastomosis should be performed with interrupted sutures to allow for expansion. In a child or a small adult with small arteries, the whole anastomosis should be performed with interrupted sutures unless the recipient arteriotomy is greater than 5 mm in diameter.

An end-to-side anastomosis of the renal artery to the external iliac artery usually is performed using an appropriately trimmed cuff of aorta attached to the renal artery. An arteriotomy appropriately placed is performed in the external iliac artery, then the anastomosis is done with a continuous 5-0 or 6-0 monofilament vascular suture (see Fig. 11-2).[42]

Venous Anastomosis

The renal vein is anastomosed end-to-side, usually to the external iliac vein using a continuous 5-0 monofilament vascular suture, with the initial sutures placed at either end of the venotomy (Fig. 11-5).[42] An important aspect of this technique is the placement of an anchor suture at the midpoint of the lateral wall, which allows the external iliac vein and the renal vein on the lateral side of the anastomosis to be drawn clear of the medial wall of the anastomosis. This technique avoids any possibility of the back wall being caught up

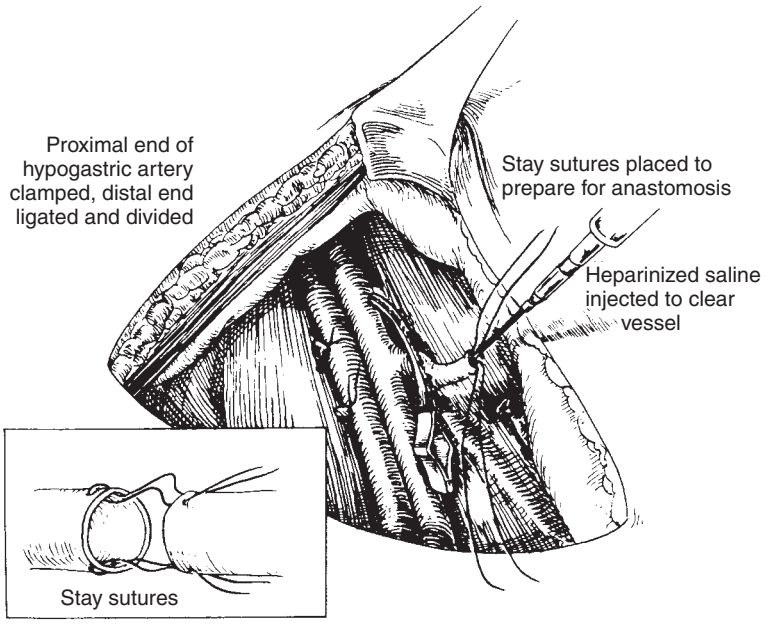

Figure 11–3 Hypogastric artery ligated and divided, the lumen flushed with heparinized saline. (From Lee HM: Surgical techniques of renal transplantation. In Morris PJ [ed]: Kidney Transplantation. London, Academic Press/Grune & Stratton, 1979, p 148.)

in the suture while the medial wall is being sutured. The renal vein may be anastomosed to the external iliac vein lateral or medial to the external iliac artery. This anastomosis depends on the length of the renal vein lateral to the artery, but if the external and common iliac vein has been mobilized as described earlier, usually even with a short vein the venous anastomosis can be performed medial to the artery. Wherever the anastomosis is positioned, it is important to ensure that the renal vein is under no tension, and care should be taken that the vein is not twisted before starting the anastomosis. When a small child receives an adult kidney, it is sometimes necessary to shorten the renal vein to prevent kinking, especially when the vein is anastomosed to the inferior vena cava.

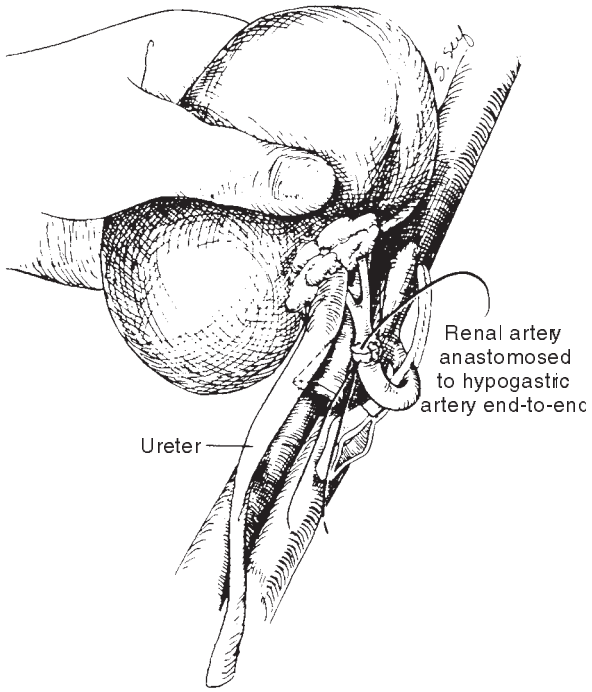

Figure 11–4 Anastomosis of the renal artery to hypogastric artery. (From Lee HM: Surgical techniques of renal transplantation. In Morris PJ [ed]: Kidney Transplantation. London, Academic Press/Grune & Stratton, 1979, p 149.)

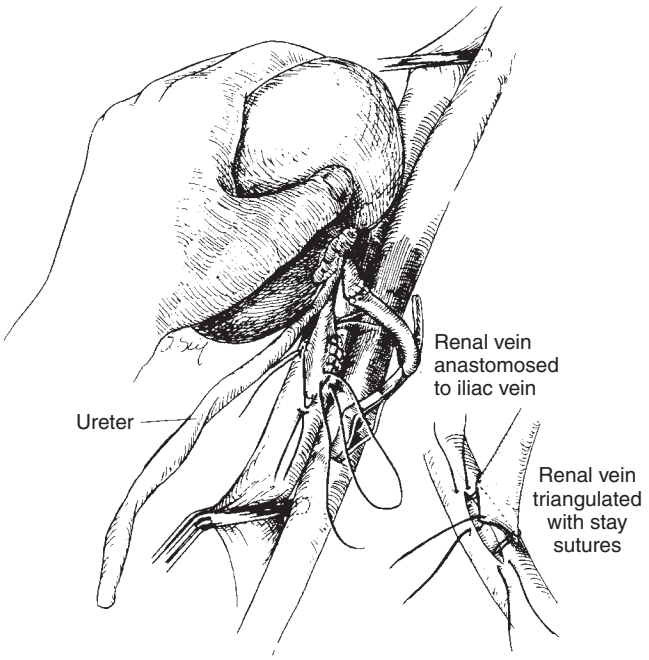

Figure 11–5 Vein anastomosis with triangular stay sutures in place. (From Lee HM: Surgical techniques of renal transplantation. In Morris PJ [ed]: Kidney Transplantation. London, Academic Press/Grune & Stratton, 1979, p 151.)

RECONSTRUCTION OF THE URINARY TRACT

After renal revascularization, the kidney is placed in what is to be its final position, and re-establishment of urinary tract continuity begins. Transplantation of the left kidney into the right iliac fossa and the right kidney into the left iliac fossa reverses the normal anterior-to-posterior relationship of the vein, artery, and collecting system and positions the renal pelvis and ureter of the kidney transplant so that they are the most medial and superficial of the hilar structures.[49] This positioning simplifies primary and secondary urinary tract reconstruction, especially if pyeloureterostomy, ureteroureterostomy, or pyelovesicostomy is to be done. The factors that determine the type of urinary tract reconstruction are the length and condition of the donor ureter, the condition of the recipient's bladder or bladder substitute, the condition of the recipient's ureter, and the familiarity of the surgeon with the technique.

Suture material is an individual choice. Although urinary tract reconstruction with nonabsorbable sutures has been described,[31,48] it leaves the recipient with the risk of stone formation if the suture material is chronically exposed to urine. Currently available synthetic absorbable sutures have characteristics suitable for the immunocompromised kidney transplant recipient who has the potential for delayed wound healing.[58] In vivo strength retention is poorest with natural fibers (plain gut and chromic gut), better with synthetic braided materials (polyglycolic acid and polyglactin), and best with synthetic monofilament materials (polyglyconate and polydioxanone). Monofilament suture has less tissue drag than braided suture, but knot security is better with braided suture. We have found polydioxanone to be satisfactory and use 3-0 for bladder closure and 4-0 or 5-0 for ureteric or renal pelvic anastomoses.

Ureteroneocystostomy

Ureteroneocystostomy is the usual form of urinary tract reconstruction. Its advantages are (1) it can be performed regardless of the quality or presence of the recipient ureter, (2) it is several centimeters away from the vascular anastomoses, (3) the native ureter remains available for the treatment of ureteric complications, and (4) native nephrectomy is unnecessary. The goal is to create a 2- to 3-cm submucosal tunnel with muscle backing of the ureter so that when the bladder contracts, there is a valve mechanism to prevent reflux of urine up the ureter.[43,53,55,68]

The genitals are prepared with an antiseptic solution, and a lubricated balloon retention catheter is passed into the urinary bladder or bladder substitute. The catheter is connected to a sterile Y-tube system (Fig. 11-6).[37] This system has a bag filled with an antibiotic solution on one line and a collection bag on the other. With this system, the bladder can be filled, irrigated, drained, and refilled during the procedure. It is especially helpful when the bladder is difficult to identify because of pelvic scar tissue, recipient obesity, or reduced capacity. After initially accommodating a small volume, the defunctionalized bladder often accepts more fluid 1 or 2 hours into the transplantation procedure.[4]

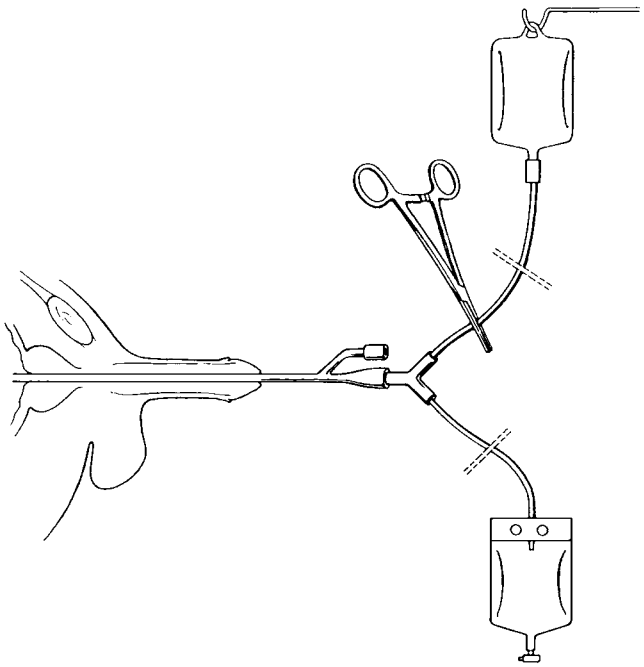

Figure 11–6 Y-tube system for rinsing, filling, and draining bladder or bladder substitute. (From Kostra JW: Kidney transplantation. In Kremer B, Broelsch CE, Henne-Bruns D [eds]: Atlas of Liver, Pancreas, and Kidney Transplantation. Stuttgart, Georg Thieme Verlag, 1994, p 128.)

Transvesical Ureteroneocystostomy

The technique for transvesical ureteroneocystostomy is similar to that described by Merrill and colleagues[49] in the first successful kidney transplant from a twin (Fig. 11-7).[42] The dome of the bladder is cleared off, and stay sutures or Allis clamps are placed on either side of a proposed vertical midline incision. The urinary bladder is drained, and an incision is made through all layers of the anterior bladder wall. A padded retractor is placed into the dome of the bladder to expose the trigone. A point clear of the native ureter is selected, and a transverse incision is made in the mucosa. A submucosal tunnel is created with a right-angle clamp or Thorek scissors for about 2 cm. The clamp or scissors is punched through the bladder, and the muscular opening is enlarged to accept the kidney transplant ureter. The ureter is drawn under the spermatic cord or round ligament and into the bladder, where it is transected at a length that prevents tension or redundancy. The cut end of the ureter is incised for 3 to 5 mm and approximated to the bladder mucosa with fine absorbable sutures. The inferior suture includes the bladder muscularis to fix the ureter distally and to prevent its movement in the submucosal tunnel. The padded retractor is removed, and the cystotomy is closed with a single layer of 3-0 absorbable suture, although some surgeons use a two-layer or three-layer closure. The bladder can be refilled to check for leakage, and points of leakage can be repaired with one or two interrupted sutures. Some surgeons use two bladder mucosal incisions about 2 cm apart[65]; when this technique is used, the proximal bladder mucosal incision is closed with a fine absorbable suture.

Extravesical Ureteroneocystostomy

Compared with the transvesical procedures, the extravesical techniques are faster, a separate cystotomy is not required,

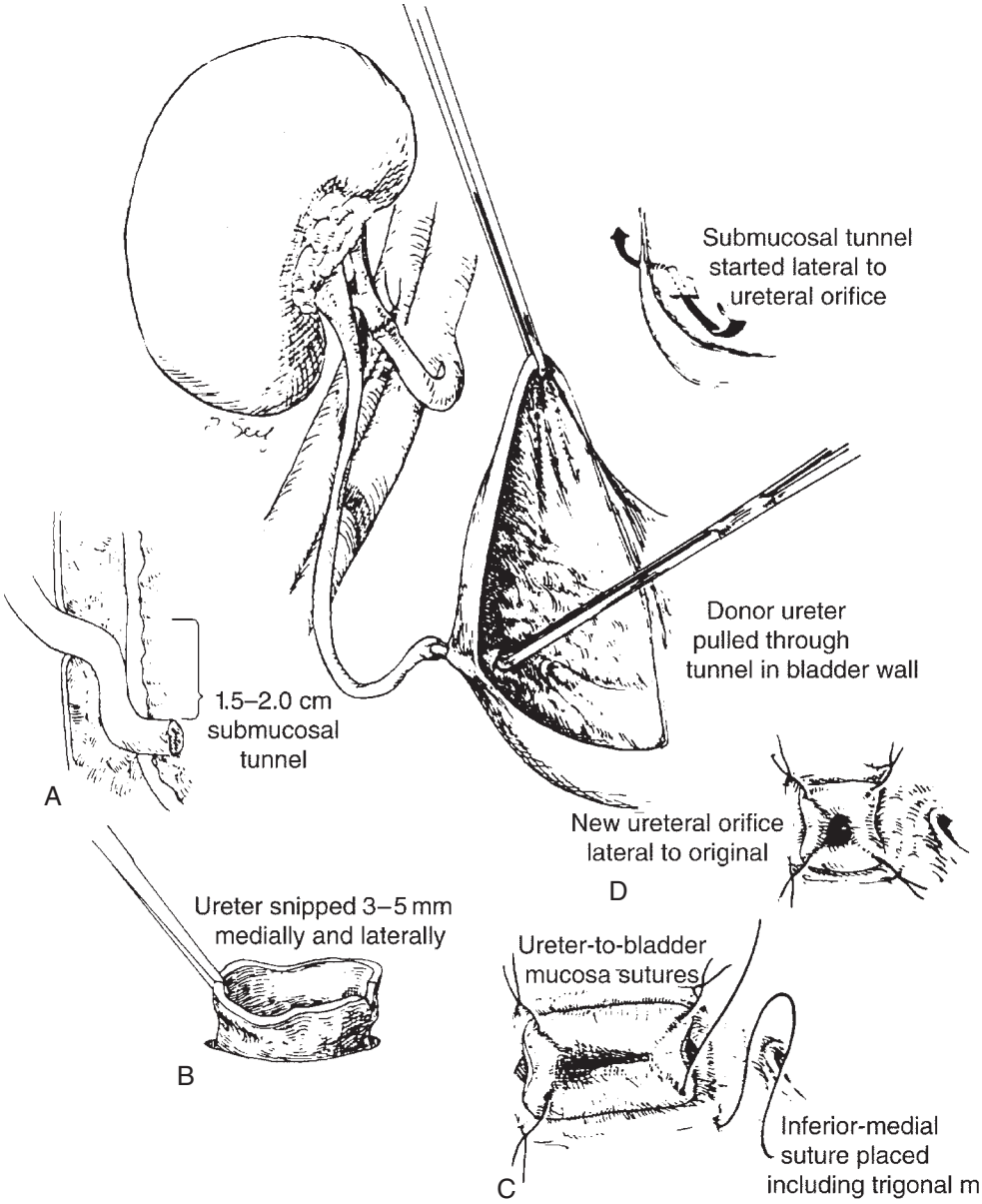

Figure 11–7 **A-D**, Transvesical ureteroneocystostomy. (From Lee DM: Surgical techniques of renal transplantation. In Morris PJ [ed]: Kidney Transplantation. London, Academic Press/Grune & Stratton, 1979, p 153.)

and less ureteric length is necessary (Fig. 11-8). These factors should reduce operating time, bladder spasms, and hematuria, and improve the probability of adequate distal ureteric blood supply. Extravesical techniques are based on the procedure described by Lich and associates.[43] Extravesical ureteroneocystostomy was adapted for renal transplantation by Woodruff in 1962,[72] and it is well illustrated by Konnak and colleagues (see Fig. 11-8).[36] A subsequent modification was the addition of a stitch to anchor the toe of the spatulated ureter to the bladder to prevent proximal slippage of the ureter in the submucosal tunnel with loss of the antireflux valve and disruption of the ureteric anastomosis.[9,14]

The bladder is distended with an antibiotic solution through the urethral catheter. The lateral surface of the bladder is cleared of fat and the peritoneal reflection, a padded retractor is placed medially, another is placed inferolaterally, and a third retractor is placed cephalomedially to hold the peritoneum and its contents out of the way. It is important to place the ureter under the spermatic cord or round ligament to prevent post-transplant ureteric obstruction. A T-shaped or longitudinal oblique incision is made for approximately 3 cm until the bladder mucosa bulges into the incision. The bladder is partially drained, and the mucosa is dissected away from the muscularis to make a submucosal tunnel for the ureter. The bladder mucosa is grasped with atraumatic forceps, the urinary bladder is drained, and an incision is made in the mucosa. The ureter is laid in the trough, spatulated, and anastomosed to the bladder mucosa with running or interrupted fine absorbable sutures. A horizontal or vertical mattress suture is placed in the toe of the ureter and passed submucosally through the seromuscular layer of the bladder

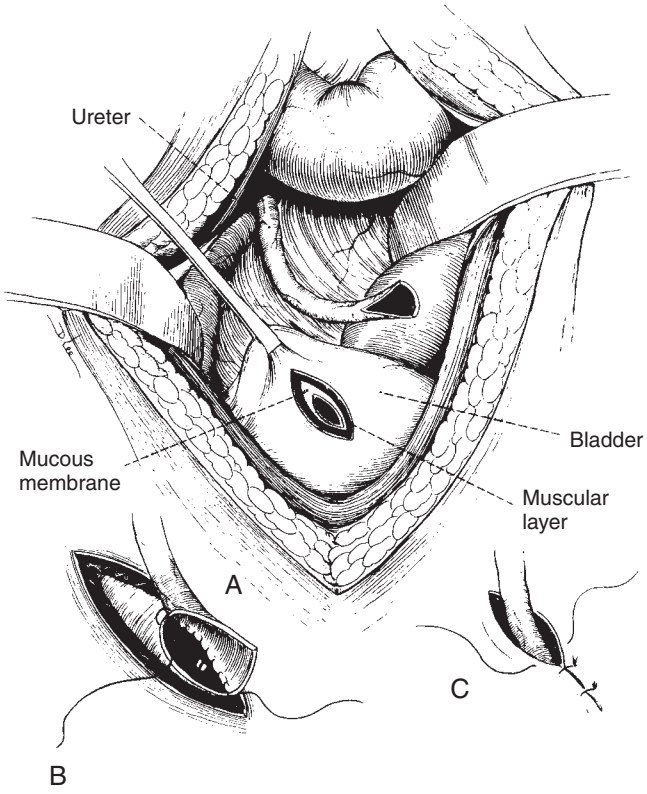

Figure 11–8 **A-C**, Extravesical ureteroneocystostomy. (From Konnak JW, Herwig KR, Turcotte JG: External ureteroneocystostomy in renal transplantation. J Urol 108:380, 1972.)

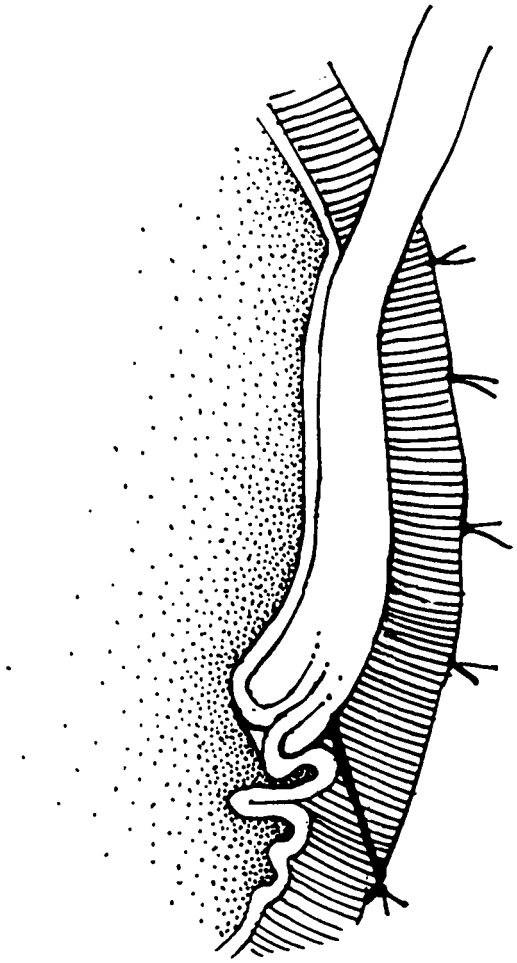

Figure 11–9 One or two mattress sutures to anchor toe of transplant ureter to full-thickness bladder. This prevents ureteric slippage in the submucosal tunnel. (From Hinman F Jr: Ureteral reconstruction and excision. In Hinman F Jr [ed]: Atlas of Urologic Surgery, 2nd ed. Philadelphia, WB Saunders, 1998, p 799.)

and tied about 5 mm distal to the cystotomy (Fig. 11-9). The seromuscular layer is closed over the ureter with interrupted sutures so that the proximal one or two sutures can be removed if the ureteric lumen has been compromised by the closure.

The one-stitch[63] and two-stitch[46] extravesical ureteroneocystostomies are modifications of the Lich procedure in which one or two mattress sutures are placed full thickness through the spatulated ureter and the bladder without an attempt at mucosa-to-mucosa approximation (Fig. 11-10).[46] If the ureter lies too loosely in the partial cystotomy, the seromuscular layer is closed over the ureter with interrupted stitches.

The parallel-incision extravesical ureteroneocystostomy commonly is used in the Oregon program (Fig. 11-11).[3,23] The setup is the same as for a modified Lich procedure. Parallel incisions are made in the lateral bladder about 2 cm apart until the bladder mucosa bulges into both incisions. The bladder is drained partially, and a submucosal tunnel is created between the two incisions. The ureter is drawn through the tunnel, transected, spatulated, and anastomosed to the bladder mucosa with interrupted fine absorbable sutures. Sometimes extra stitches are placed between the quadrant sutures to prevent urinary leakage. A vertical or horizontal mattress suture is used to anchor the toe of the ureter to the urinary bladder. This suture is tied about 5 mm distal to the cystotomy. Finally, the distal cystotomy is closed with a running fine absorbable suture. The parallel-incision extravesical ureteroneocystostomy has been slightly modified by Caparros and associates[15] by application of the one-stitch principle with no suture approximation of the ureteric and bladder mucosa, and by Knechtle,[34] who described a longitudinal distal bladder incision.

Double Ureters

Double ureters can be managed simply by leaving them in their common sheath, trimming them to appropriate length, spatulating them, and either anastomosing the medial edges together with a running fine absorbable suture (Fig. 11-12)[17,56] or joining them, one on top of the other, with a single stitch from the toe of the upper one to the heel of the lower one (Fig. 11-13).[5] The conjoined ureters can be treated as a single ureter by any of the previously described ureteroneocystostomy techniques. The submucosal tunnel needs to be made a bit wider. Others have used a separate ureteroneocystostomy for each of the ureters.[69] These same techniques can be used for the en bloc transplantation of pediatric kidneys or the transplantation of two adult kidneys, stacked one on top of the other,[47] into one recipient. Fjeldborg and Kim[22] described a pyeloureteric anastomosis in which both renal pelves are joined after dividing the ureters at their ureteropelvic junctions and suturing the posterior walls together,

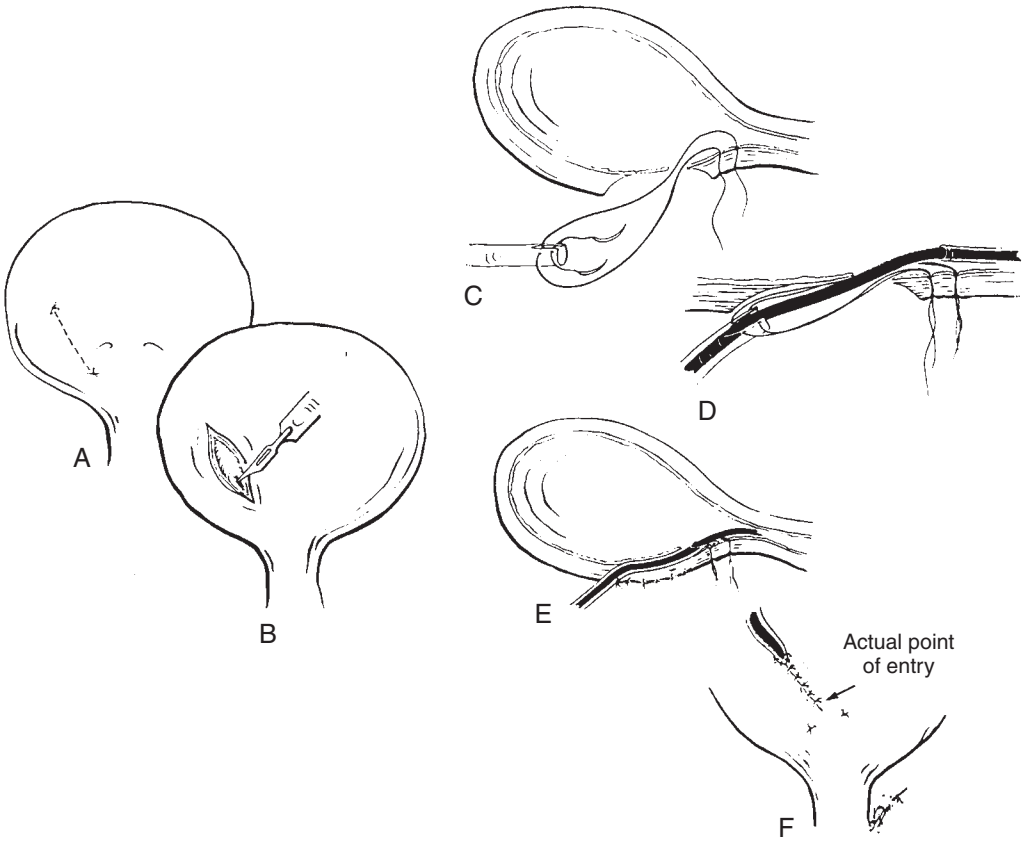

Figure 11–10 **A-F**, Extravesical ureteroneocystostomy without mucosa-to-mucosa anastomosis. This also is done without a stent. (From MacKinnon KJ, Oliver JA, Morehouse DB, et al: Cadaver kidney transplantation: emphasis on urologic aspects. J Urol 99:46, 1968.)

leaving the anterior halves for anastomosis with the recipient ureter (Fig. 11-14).

Augmented Bladder

It is important to know the blood supply of an augmentation patch so as not to interfere with it during the renal transplant procedure. With the possible exception of stomach, development of a submucosal tunnel for ureteroneocystostomy is usually easier in the bladder itself. Ureteric stents are usually used.

Pyelopyelostomy

Pyelopyelostomy has been used for orthotopic renal transplantation, usually in the left flank.[24] The native kidney is removed, and the kidney transplant is revascularized with the native renal artery or the splenic artery and the native renal vein. The proximal ureter and renal pelvis of the kidney transplant are opened medially, and the native renal pelvis is anastomosed to the kidney transplant renal pelvis with a running fine absorbable suture. After completion of one wall, a double-pigtail ureteric stent is passed with or over a wire through the native ureter into the bladder, and the wire is withdrawn to allow the bladder curl to form. Its position in the bladder is confirmed by reflux of bladder irrigant up the stent. The proximal coil is placed in the renal pelvis of the kidney transplant, and the remaining half of the suture line is completed. Compared with ureteroneocystostomy, an advantage of urinary tract reconstruction with the native renal pelvis or ureter is the ease with which subsequent retrograde pyelography, stent placement, or ureteroscopy can be accomplished through the normally positioned ureteric orifice.

Pyeloureterostomy and Ureteroureterostomy

Pyeloureterostomy and ureteroureterostomy usually are done when the transplant ureter's blood supply seems to be compromised, when the urinary bladder is difficult to identify because of pelvic scar, when the bladder does not distend enough for a ureteroneocystostomy, or when the surgeon prefers one of them to ureteroneocystostomy.[25,39,41] The techniques for ureteropyelostomy and ureteroureterostomy are similar (Fig. 11-15). The posterior, or back wall, anastomosis of the kidney transplant pelvis or ureter to the side or to the spatulated end of the native ureter is completed; a double-pigtail ureteral stent is placed, and the anterior suture line is completed. The proximal native ureter is managed by (1) leaving the native kidney in situ and using the side of the native ureter for the anastomosis, (2) ipsilateral nephrectomy and proximal ureterectomy, or (3) ligation of the proximal ureter with the obstructed native kidney left in situ. Although Schiff and Lytton[59] and Lord and colleagues[45] have described the safety of native ureteral ligation with kidney transplant urinary tract reconstruction, we prefer to leave the native ureter in continuity with its kidney and to anastomose the pelvis or ureter of the renal transplant to the

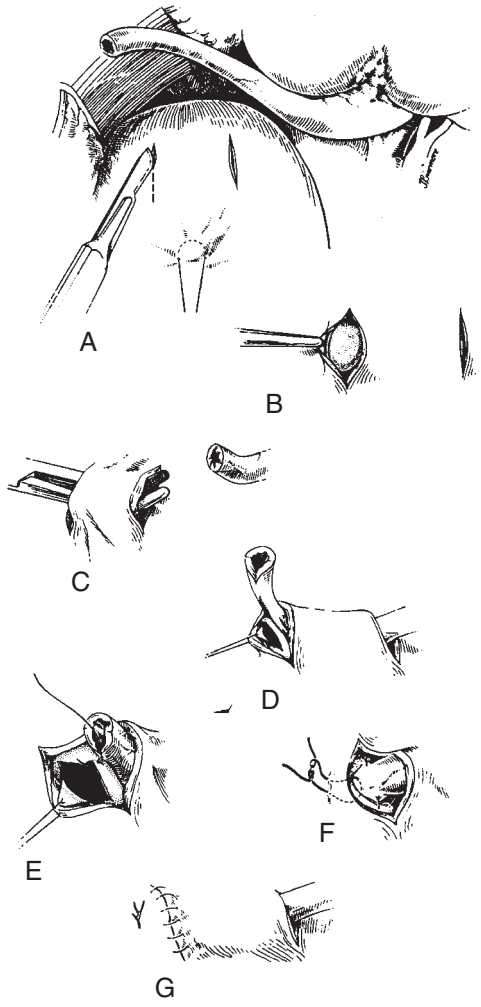

Figure 11–11 **A-G**, Parallel-incision extravesical ureteroneocystostomy. The adequacy of the submucosal tunnel is judged by pulling the ureter through it. (From Barry JM: Unstented extravesical ureteroneocystostomy in kidney transplantation. J Urol 129:918, 1983.)

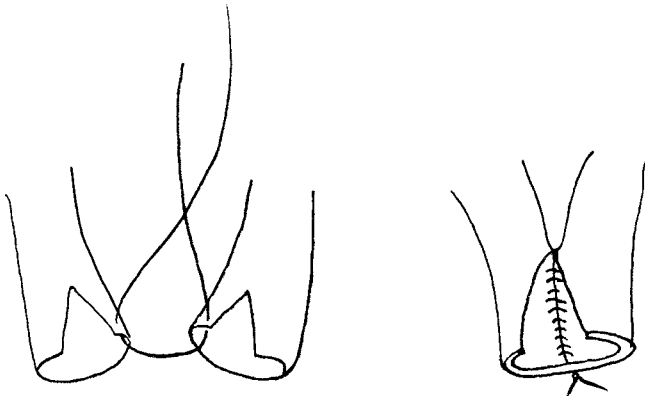

Figure 11–12 Management of double ureters to make them into a single ureteric orifice.

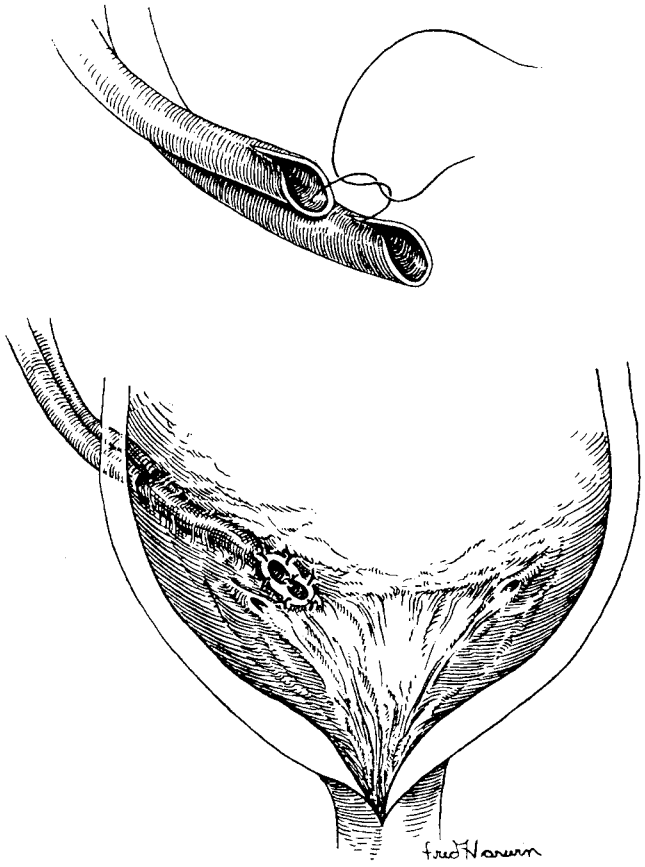

Figure 11–13 Alternative method of managing double ureters. (From Barry JM, Pearse HD, Lawson RK, et al: Ureteroneocystostomy in kidney transplants with ureteral duplication. Arch Surg 106:345, 1973.)

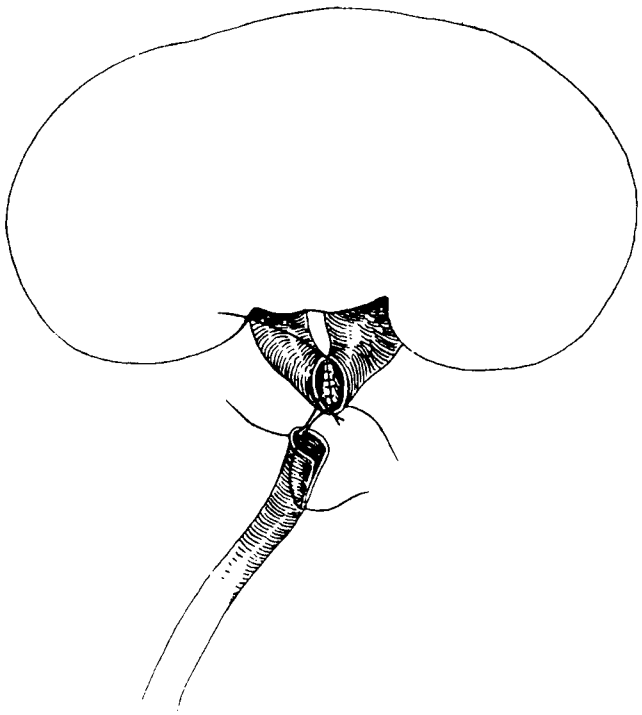

Figure 11–14 Management of double ureters by pyelopyelostomy followed by conjoined pyeloureterostomy. (From Fjeldborg O, Kim CH: Double ureters in renal transplantation. J Urol 108:377, 1972.)

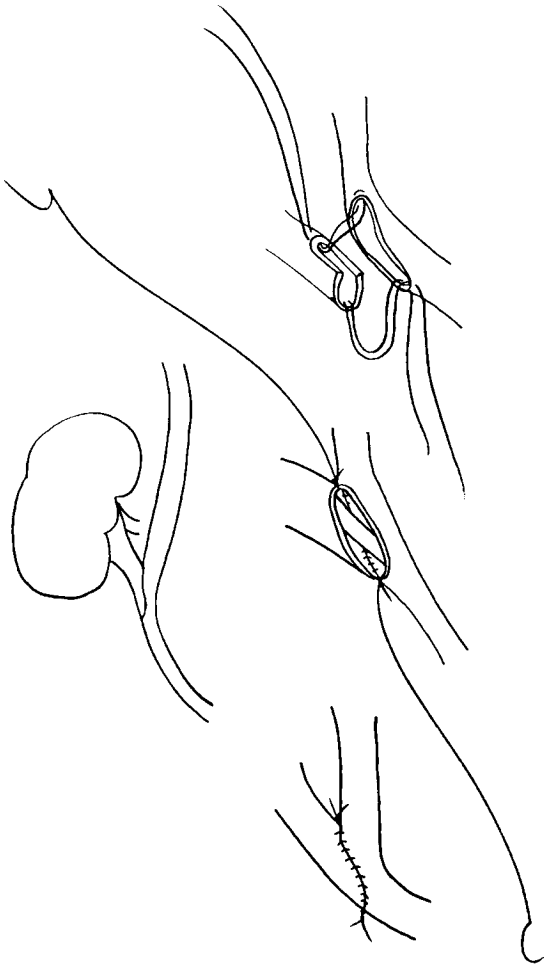

Figure 11–15 Ureteropyelostomy and ureteroureterostomy. A double-pigtail stent is placed after the backwall suture line has been completed.

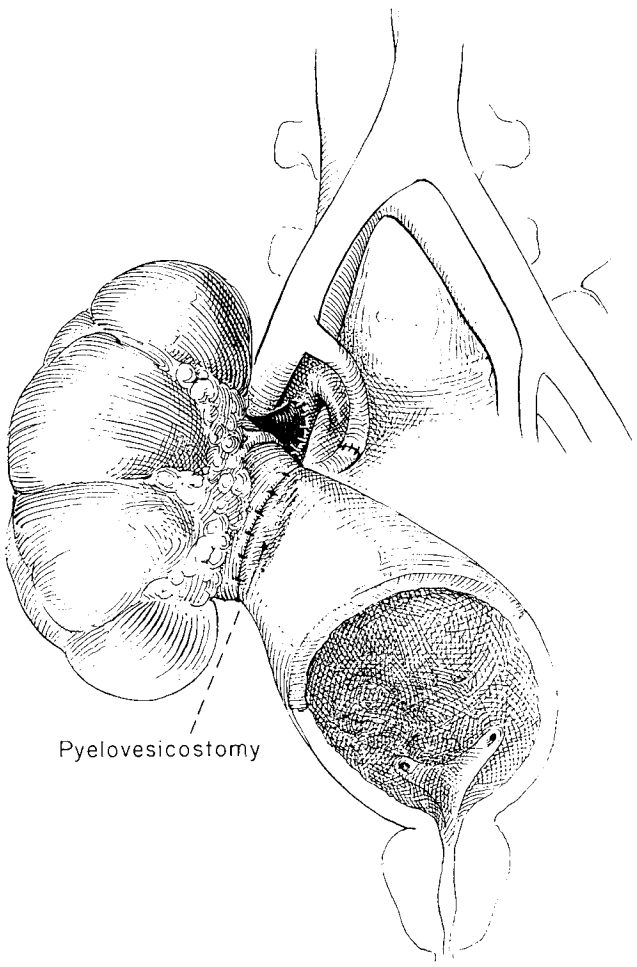

Figure 11–16 Pyelovesicostomy. (From Firlit CF: Unique urinary diversions in transplantation. J Urol 118:1043, 1977.)

side of the native ureter. This technique ensures a good blood supply to the native ureter and removes an obstructed, hydronephrotic kidney from the differential diagnosis of a post-transplant problem.

Pyelovesicostomy

Pyelovesicostomy has been described by Bennett,[8] Herwig and Konnak,[28] and Firlit[21] for urinary tract reconstruction when the native ureter and the renal transplant ureter are unsuitable or become so (Fig. 11-16).[21] The bladder must reach the renal pelvis without tension; a bladder extension with a psoas hitch or Boari flap may be necessary.

Ureteroenterostomy

Ureteroenterostomy into an intestinal conduit or an intestinal pouch has been successful.[26,32] It is performed by slight distention of the conduit or pouch with antibiotic-containing irrigant and then using one of the extravesical ureteroneocystostomy techniques. Successful anastomosis of the transplant ureter to the afferent limb of a Koch pouch has been described.[27] If it is difficult to identify the intestinal conduit or pouch because of surrounding intestines, the addition of methylene blue to the irrigant stains the conduit or pouch and makes it easy to find.[71] This topic is discussed more completely in Chapter 12.

Ureteric Stents

We use ureteric stents when there is concern about urinary leakage or temporary obstruction because of edema, periureteral bleeding, or a thickened bladder; when pyelopyelostomy, pyeloureterostomy, or ureteroureterostomy has been performed; or when the ureter has been anastomosed to an intestinal conduit or pouch. The length of the stent is determined by the estimated distance between the renal pelvis of the kidney graft and the bladder or its substitute. A double-pigtail 5F × 12 cm stent is the most common type and size used when the transplanted kidney is located in the iliac fossa. A prophylactic ureteric stent for all kidney transplant ureteroneocystostomies was shown by Pleass and colleagues[54] in a randomized prospective trial to reduce the incidence of urological complications.

Management of Catheter and Stent

The urinary bladder or reservoir catheter usually is removed on postoperative day 5 after a urine specimen is tested at the bedside for nitrites and sent for bacterial culture and after

a single dose of a broad-spectrum antibiotic has been administered. If the urine is shown to be infected, an antibiotic is chosen based on sensitivity results and is prescribed for 10 to 14 days. If a stent had been placed and attached to the indwelling bladder or reservoir catheter, the stent comes out as the catheter is withdrawn. If not, the stent is removed in the outpatient clinic 6 to 12 weeks later.

CLOSURE

Many units obtain a biopsy specimen of the kidney routinely before closure of the wound. This biopsy can be used to provide baseline histology and can provide evidence of ischemic reperfusion injury or early antibody-mediated damage (see Chapters 24 and 25). Methods of closing the wound vary, but, in general, closure with loop nylon in two layers—internal oblique and transverse muscles followed by external oblique—is common practice, with subcuticular nylon or polyglactin closure of the skin.

The question of drainage is controversial because of the risk of providing a portal for entry of microorganisms. If drainage is required, it should be a closed system of suction drainage, and drains should be removed at the earliest opportunity. The exit site of the drain is cleaned daily with an antimicrobial solution and dressed until the drain is removed.

In the past, a capsulotomy of the transplanted kidney before closure was advocated[29,62] by carefully splitting the renal capsule at its convex surface from pole to pole, but not stripping it. This technique was proposed to prevent ischemic injury when the kidney swells as a result of edema; there is no evidence that this is the case, and this practice has now been abandoned.

PEDIATRIC RECIPIENT

For older children, the transplant procedure is the same as for adults if their weight is more than 20 kg.[7,20,52] The renal vessels are anastomosed end-to-side to the iliac vessels or aorta and vena cava.[10,13]

In smaller children (weight <20 kg), the right extraperitoneal space can be developed by extending the incision to the right costal margin,[51] or a transperitoneal approach can be used.[67] In the case of the latter, the abdomen is opened through the midline incision from the xyphoid to the pubis, and the posterior peritoneum is incised lateral to the ascending colon, which is reflected medially. The terminal portion of the vena cava is dissected over 3 to 4 cm, ligating and dividing two to three lumbar veins posteriorly. The terminal aorta also is dissected free at its junction with the right common iliac artery. A partial occluding clamp is used to isolate the vena cava and aorta, and the renal vein is anastomosed to the vena cava first in an end-to-side technique with sutures of 5-0 monofilament vascular suture (Fig. 11-17).[42] The renal artery is anastomosed to the common iliac or terminal aorta in an end-to-side fashion using 5-0 or 6-0 monofilament vascular suture. The renal artery is usually brought in front of the vena cava, but sometimes behind the vena cava. Another approach is to dissect the inferior vena cava, proximal common iliac veins, common iliac arteries, inferior mesenteric artery, and aorta and to control the venous system with tourniquets and the arterial system with a combination of vessel loops and vascular clamps. Use of a 5- or 6-mm aortic punch prevents coaptation of the recipient aorta and renal artery occlusion if significant hypotension occurs. Careful observation of the recipient hemodynamic response on clamping and declamping the vena cava and aorta is required.

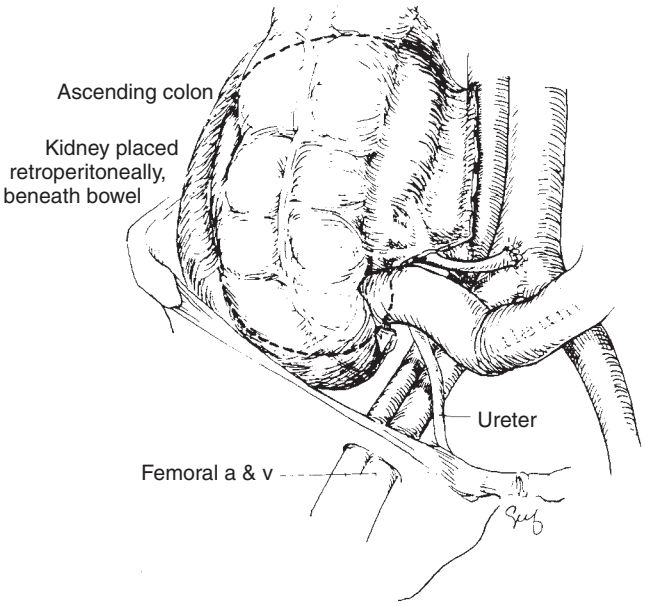

Figure 11–17 Renal transplant in small children (<20 kg). (From Lee HM: Surgical techniques of renal transplantation. In Morris PJ [ed]: Kidney Transplantation. London, Academic Press/Grune & Stratton, 1979, p 159.)

The ascending colon is placed back over the anterior surface of the kidney. No fixation is necessary. The ureter is brought down retroperitoneally crossing the common iliac artery at its midpoint and is implanted into the bladder as a ureteroneocystostomy.

Calne[11] expressed concern about the development of stenosis of the vascular anastomosis in growing children as a result of the use of continuous sutures. He advised performing at least half of the anastomosis with interrupted sutures in children. This advice may apply when one performs end-to-side anastomosis of the renal artery or the renal vein, but Starzl and colleagues[67] stated that after end-to-side anastomosis, there is little likelihood of the development of relative stenosis as a result of growth of the child.

PEDIATRIC DONOR

When a child's kidney is used as a donor kidney for an adult or child recipient, the surgical technique is essentially the same as has been described. Because of the small size of the renal vessels, however, use of aortic and vena caval patches generally is necessary. Interrupted sutures are used by some surgeons for at least half the circumference of the anastomosis. When pediatric kidneys are very small, double kidneys are transplanted en bloc into adults and bigger children.[1,18,33,44,60]

For en bloc transplantation, both kidneys are removed with a segment of aorta and vena cava. The cranial ends of the aorta and vena cava are oversewn. The caudal ends of the aorta and vena cava are anastomosed end-to-side to the iliac vessels. The superior poles of the kidneys are sutured to the sides of the aorta to prevent the torsion or kinking of renal vascular pedicles. Ureters are implanted to the bladder

separately using the extravesical approach or are joined together to form a common funnel, as described earlier. Another technique is to remove segments of the recipient's external iliac artery and vein and anastomose the tubular aorta and inferior vena cava into the defects. A third technique is to incise longitudinally the posterior aorta and inferior vena cava and anastomose these vascular patches to the iliac vessels. A fourth useful technique is to remove a segment of aorta and vena cava below the renal vessels and to reanastomose these segments to the aorta and vena cava above the renal vessels. This technique allows the kidneys to be placed quite low over the iliac vessels and provides a short distance for the ureters to traverse to the bladder.

TRANSPLANT NEPHRECTOMY

Removal of a graft that has undergone chronic rejection and has been in place for many months or years can be extremely difficult and should be performed by an experienced transplant surgeon. The usual approach for the transplant nephrectomy is through the original transplant incision. An abdominal incision may be preferred in small children, particularly if the transplantation was performed intra-abdominally. One also may use the abdominal approach to control the iliac artery system in case of a mycotic aneurysm or a perinephric abscess, in which a potential exists for blowout of vessels.

In the early postoperative period, removal of the transplant in toto is simple with easy identification of the renal pedicle structures. The long-standing transplanted kidney should be removed subcapsularly to lessen the technical difficulty and the bleeding. After deepening the incision sharply to the false capsule, which is incised, the kidney is freed subcapsularly with blunt dissection all around the kidney. The capsule around the hilum has to be incised to get outside it again so as to isolate the pedicle. The pedicle is mass clamped with a Satinsky clamp and divided to remove the kidney. Many surgeons use monofilament vascular suture to oversew the vessels as well as for the ligature. One also may dissect the artery and the vein at this time and ligate them separately, but this is difficult, especially if end-to-side anastomoses have been used. Sometimes the segmental renal arteries and venous branches are ligated and divided as they appear during dissection within the renal hilar scar. Meticulous hemostasis should be obtained with the use of electrocautery. The wound is irrigated with a liberal amount of topical antibiotic solution. It is wise to use prophylactic antibiotics. The technique of deep wound closure depends on the quality of the tissues and the experience of the surgeon, and it can range from interrupted wound closure with buried, absorbable retention sutures to a single-layer closure with synthetic monofilament sutures.

If the wound is grossly contaminated or infected, it should be left open with packing, with secondary closure in mind. Insertion of drains should be avoided because it may increase the incidence of infection, and if drains are considered necessary, a closed system of drainage should be used for a short time.[2,6,35,61]

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Chapter 12

Transplantation and the Abnormal Bladder

Julie Franc-Guimond • Ricardo González

The ability of the bladder to store urine at low pressure and to empty completely at intervals is essential to preserve the integrity of the kidneys and to achieve continence. Although an abnormal lower urinary tract is not a contraindication to renal transplantation, bladder dysfunction needs to be addressed before renal transplantation.

Children with end-stage renal disease at risk for bladder dysfunction include patients with known congenital urological anomalies, such as posterior urethral valves, prune-belly syndrome, neurogenic bladder dysfunction, bladder exstrophy, Hinman syndrome, and Ochoa syndrome, and patients with vesicoureteral reflux who have recurrent urinary tract infections.[59,91,106] Familiarity with the evaluation and the management of patients with an abnormal lower urinary tract is important because more recent series report that such patients represent 20% to 30% of renal transplant recipients depending on the given transplant population.[2,35] A large proportion of children with the diagnosis of neurogenic bladder required renal transplantation in some series. In some of the above-mentioned conditions, particularly neurogenic bladder, renal failure is preventable with good management and patient education.

The management of the child with an abnormal lower urinary tract who is awaiting renal transplantation presents a unique series of challenges. When renal failure results from underlying urological anomalies (e.g., posterior urethral valves, prune-belly syndrome, neurogenic bladder), it can be assumed that the abnormal bladder that contributed to the destruction of the native kidneys might adversely influence the outcome of the transplant. Many reports have shown that bladder dysfunction can negatively affect graft function if left untreated. Reinberg and colleagues[93] first pointed this out in 1988. Correction of structural anomalies and optimization of storage and emptying functions of the bladder are often recommended before transplantation. We also support the concept of doing all anticipated reconstructive procedures on the lower urinary tract, including procedures needed to achieve continence, before transplantation.

Urinary diversion has been shown to be safe in renal transplantation.[57,70] With the development of innovative reconstructive techniques, and with the acceptance of clean intermittent catheterization, a permanent incontinent diversion is rarely required today. Instead, patients who require bladder reconstruction can benefit from an augmentation cystoplasty, which is a more attractive alternative. Refunctionalization of the urinary reservoir can be accomplished in patients with a previously defunctionalized bladder in anticipation of renal transplantation.

Although favorable long-term results have been reported, bladder augmentation with gastrointestinal segments carries a significant lifelong complication risk.[109] Despite possible complications, bladder reconstruction has major relevance in the pediatric renal transplant population with small noncompliant bladders. It remains unproven, however, that the benefits of bladder augmentation in cases of posterior urethral valves and renal failure outweigh the risks of reconstruction.

Kelly and coworkers[57] first reported renal transplantation into an ileal conduit in 1966. Marshall and colleagues[70] introduced the concept of combining augmentation cystoplasty with renal transplantation in 1982. Since then, the compatibility of bladder reconstruction and renal transplantation in all age groups has been documented often, but most authors report small series, and controlled studies are lacking. Nonetheless, most publications confirm that patients with dysfunctional bladders treated with an augmentation cystoplasty or a continent diversion may be successfully transplanted, despite the increased morbidity.[47,48,84]

CAUSES OF ABNORMAL BLADDERS

Pediatric end-stage renal disease and its management are unique owing to the high incidence of underlying urological disease—hence the high incidence of patients with possible dysfunctional bladders. Bladders that seem to have normal function initially may become abnormal over time, such as seen in cases of valve bladders. Classically, pediatric patients identified with abnormal bladders carry the following diagnoses: posterior urethral valves, prune-belly syndrome, urethral hypoplasia/atresia and neurogenic bladder, vesicoureteral reflux with renal dysplasia, bladder exstrophy, and cloacal anomalies. Most renal transplant recipients identified as having a dysfunctional bladder are diagnosed and treated before adulthood.

ASSESSMENT OF BLADDER FUNCTION

All patients with known or suspected genitourinary abnormalities require evaluation. Patients without lower urinary tract abnormalities need specific therapy only rarely.[43,107] Other authors have reported, however, that some patients with end-stage renal disease not related to urological problems have abnormal lower urinary tracts when evaluated before renal transplantation. The abnormality is often secondary to prolonged anuria or polyuria, depending on the initial disease.[37] Also, certain urological diseases occasionally may not be obvious or may not have contributed to the progression to end-stage renal disease, such as occult urinary tract neoplasms or urolithiasis, but still need attention. A complete evaluation of the urinary tract before renal transplantation is necessary to limit unforeseen problems occurring after transplantation. If this assessment is consistently adhered to, only in very rare situations does a potential recipient have to be denied the opportunity of receiving an allograft based on preexisting urological diseases. A Spanish group evaluated patients based on the following indications: (1) lower urinary tract symptoms, (2) defunctionalized bladder, and (3) complex urological history (e.g., reflux, neurogenic bladder, urethral valves). The investigators found that 45% of the patients showed abnormal urodynamic studies.[33]

The evaluation starts with a complete history, including voided volumes and frequency, incontinent episodes, and presence of nocturia or nocturnal enuresis. In anuric patients, the history before the onset of anuria is very valuable. In most cases of lower urinary tract anomalies, a voiding cystourethrogram is valuable to outline the bladder contour, evaluate urethral anatomy, and determine the presence of reflux to the native ureters. Noninvasive urodynamics including the pattern of the uroflow examination, the maximal and average flow rate, and the residual urine measured by bladder scanning are invaluable. In most patients without symptoms, a normal uroflow examination and the absence of residual urine are sufficient to rule out significant bladder dysfunction.

Invasive urodynamic studies, including cystomanometry with or without simultaneous intrarectal pressure measurements and electromyography of the pelvic floor, are needed when the bladder capacity and compliance are questionable. The simultaneous performance of a voiding cystourethrogram and cystomanometry (videourodynamics) is most useful in these cases.

The pretransplant urological evaluation aims to diagnose, treat, and optimize any preexisting urological disease.[19,56,91] Cystoscopy is indicated in cases in which the urinary flow is abnormal, residual urine volumes are elevated, or the urethra is difficult to catheterize.

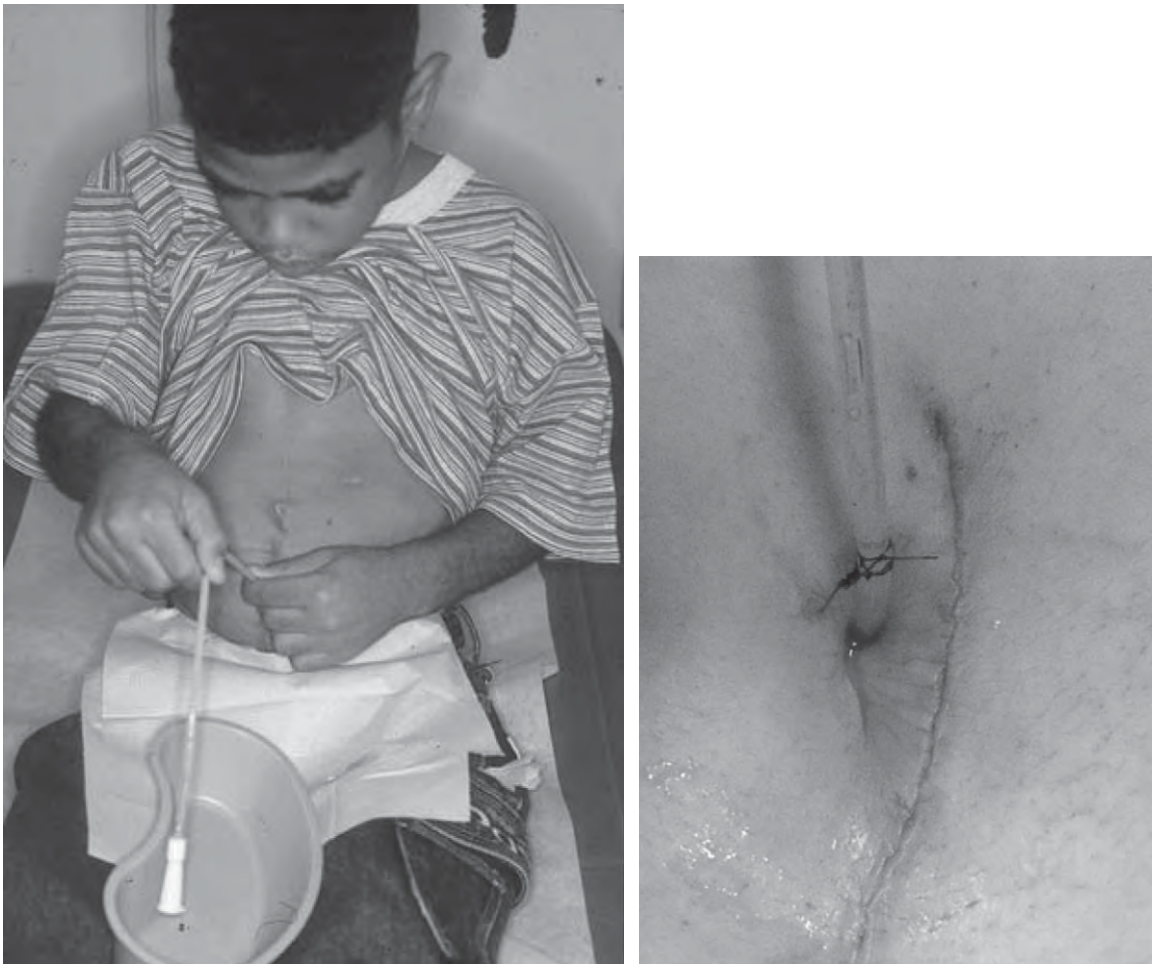
After the evaluation is completed, decisions need to be made regarding the adequacy of the lower urinary tract. Criteria for a usable bladder relate to bladder capacity, bladder compliance, the bladder's ability to empty completely, and urinary continence. The presence of vesicoureteral reflux also should be taken into consideration.

Bladder capacity varies with age. Known formulas exist to determine if the bladder capacity for age is satisfactory for a given patient. With the capacity of the newborn bladder at about 30 to 60 mL, and bladder capacity increasing by about 30 mL/yr each year almost until puberty, bladder capacity in childhood may be reasonably well estimated by a simple formula (capacity in ounces = age in years + 2),[63] although numerous other formulas have been proposed.[13,52,53,55] Although most calculations use the patient's age assuming that the body habitus is within normal limits, this is often not the case in patients with spina bifida and end-stage renal disease. A formula based on weight, such as 7 mL/kg, should be used for that population.

Bladder compliance is defined as the change in bladder pressure for a given change in volume. It is calculated by dividing the volume change (ΔV) by the change in detrusor pressure (ΔP_{det})—compliance = $\Delta V/\Delta P_{det}$—and is expressed in mL/cm H_2O. Decreased bladder compliance implies a poorly distensible bladder in which the pressure/volume curve is steep, and the pressure rise is rapid for low-volume increases. The lowest full resting pressure is preferable regardless of the maximal bladder capacity. In the presence of reduced compliance, medical management can be attempted first, but if the problem remains despite of the use of anticholinergics, bladder augmentation needs to be performed.

The question of what pressure is dangerous for the upper tracts has no straightforward answer. McGuire and associates[75] stated that sustained detrusor pressures greater than 40 cm H_2O put the upper tracts at risk. Also, the bladder capacity and compliance should always be looked at together, and the overall medical and surgical approaches should address both to obtain an optimal storage phase. The optimal bladder capacity is difficult to evaluate or predict in polyuric patients. A polyuric child with end-stage renal disease may have a bladder capacity that, although normal for age or weight, may be inadequate to handle an extremely large diuresis, yet it is often difficult to predict what capacity would be adequate after transplantation. This is a common dilemma in children with posterior urethral valves.[68]

Normal bladder emptying implies complete emptying without dyssynergia or the use of Credé's maneuvers. When the emptying phase is inadequate, the bladder is emptied periodically by means of catheterization either urethrally or through a continent catheterizable channel using the surgical principles described by Mitrofanoff[79] and Monti and colleagues[81] positioned preferably in the umbilicus or at the level of the lower quadrants (Figs. 12-1 to 12-3). Finally, incontinence can sometimes be treated medically with anticholinergics if the problem is related to uninhibited bladder contractions. Incontinence caused by decreased bladder outlet pressure always requires surgical attention,



A B

Figure 12–1 **A**, Catheterization through an umbilical stoma in a patient who had a continent catheterizable channel accomplished using the Mitrofanoff principle. **B**, Larger view of an umbilical stoma in another patient. (See color plate.)

however, using various types of bladder neck procedures (urethral sling, Young-Dees-Leadbetter bladder neck procedure, artificial urinary sphincter implantation, or injection of bulking agents in the bladder outlet) (Figs. 12-4 and 12-5). Artificial urinary sphincter implantation is compatible with renal transplantation.[84]

A functional bladder may need to be re-evaluated over time if the waiting time for renal transplantation is prolonged or if new lower urinary tract symptoms occur. It also is known that bladder dysfunction in children and adolescents occurs after transplantation, even when the bladder was normal before renal transplantation, warranting careful follow-up.[50,115]

MEDICAL MANAGEMENT OF AN ABNORMAL BLADDER

Small and poorly compliant bladders can be managed initially with anticholinergics often combined with clean intermittent self-catheterization. The response to anticholinergic agents should be evaluated not only by the clinical symptoms but also urodynamically. Clean intermittent self-catheterization also is essential to treat hypocontractile bladders with incomplete emptying. The compatibility of clean intermittent self-catheterization with immunosuppression and renal transplantation is well established.[40] Some patients ultimately may require bladder augmentation or urinary diversion before transplantation if urodynamic parameters do not improve or worsen over time.[105]

SURGICAL MANAGEMENT OF AN ABNORMAL BLADDER

Urinary Diversion

Decreased bladder capacity and compliance not responsive to anticholinergic agents should be treated surgically by means of bladder augmentation. The compatibility of intestinal diversions with renal transplantation has been reported often, but the numbers of patients included in controlled studies are often small. Since the 1980s, bladder augmentation and continent reservoir have gained popularity over urinary diversion because they are more socially suitable options for most patients. Nonetheless, patients can be considered for transplantation with an incontinent urinary diversion, such as an ileal conduit. These patients should be appropriately assessed before transplantation occurs. Investigation of such patients particularly should include

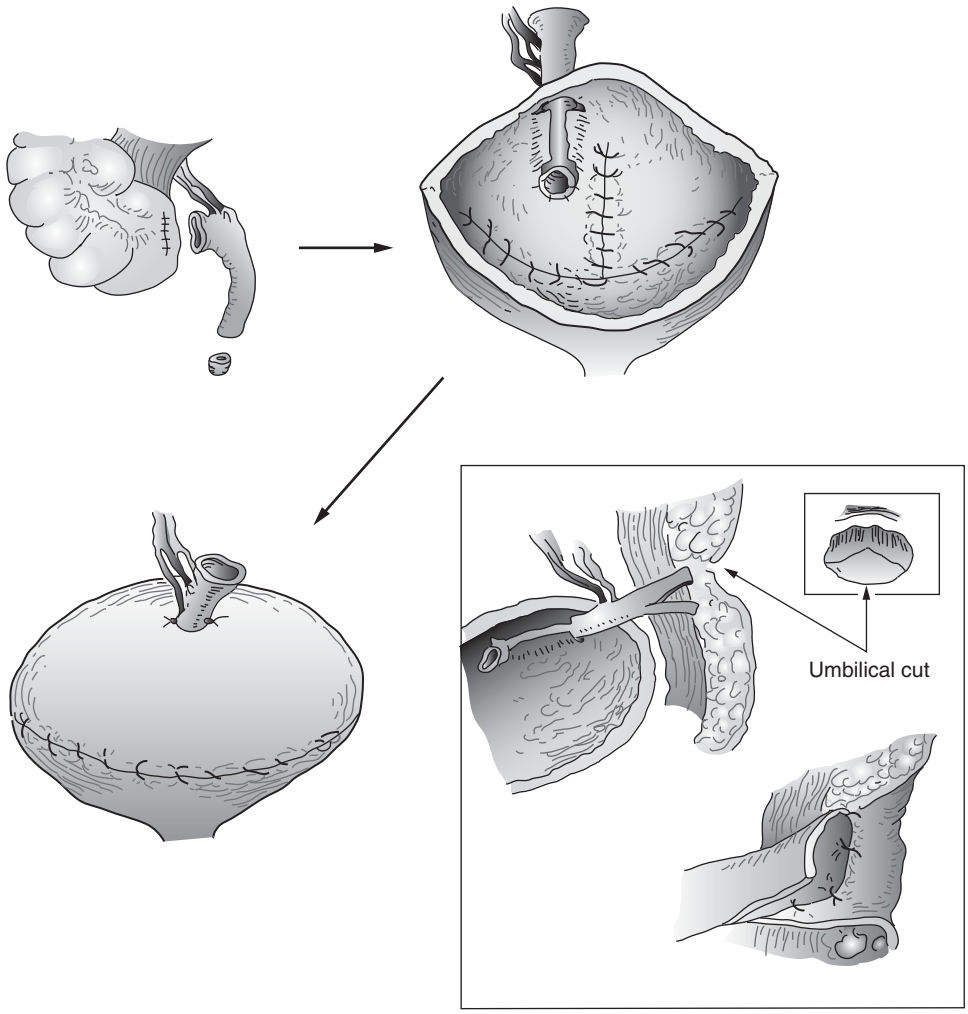


Figure 12–2 Illustration showing the creation of an appendicovesicostomy using the Mitrofanoff principle.

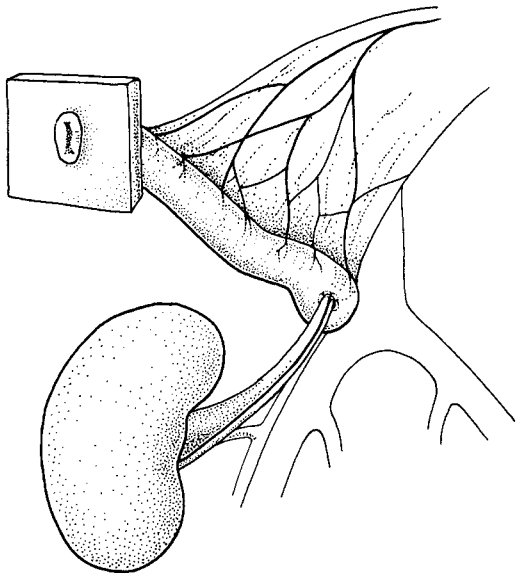


Figure 12–3 Illustration showing the creation of a continent catheterizable channel with a bowel segment using Monti's principle.

a contrast study of the conduit to evaluate its course and length before transplantation. Also, the possibility of urinary undiversion before transplantation should be considered in selected cases. Careful assessment of the native defunctionalized bladder before kidney transplantation may reveal a usable lower urinary tract in many patients. Most of these bladders need some kind of rehabilitation, however. An assessment of the continence mechanism also is mandatory.

Bladder Reconstruction

Augmentation cystoplasty performed using various bowel segments is now used routinely for treatment of reduced bladder compliance and capacity, but the use of intestinal tissue to increase the size of the bladder is not a modern idea. In 1888, Tizzoni and Foggi[114] reported an animal model of bladder augmentation by connecting a loop of ileum to the bladder neck. During the late 19th and early 20th centuries, there were various attempts at lower urinary tract substitution, usually involving some form of rectal pouch.[65,73,96,102] In the preantibiotic era, results in humans were poor, tempering enthusiasm for such surgical techniques. During the 1950s, interest in cystoplasties was renewed, with Couvelaire[25] and Gil-Vernet[41] reporting good results using

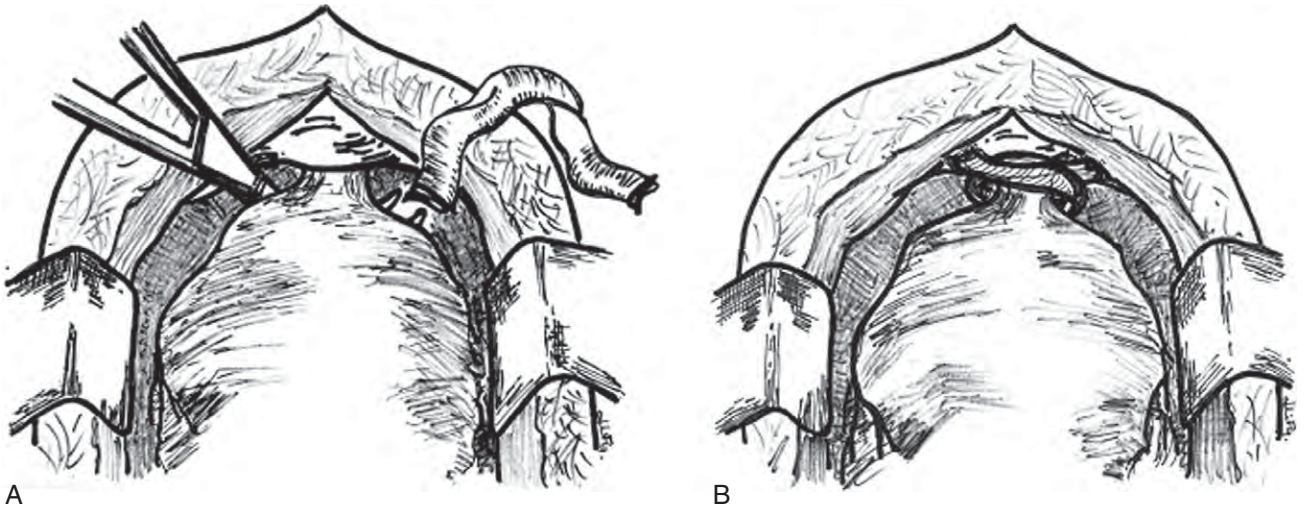


Figure 12–4 Illustration showing how the installation of a sling can be done in pediatric patients. The sling (allograft fascial sling or autologous) is transferred around the bladder neck and crossed anteriorly. The ends are secured with permanent sutures and anchored suprapubically to Cooper's ligaments.

large and small bowel segments. During subsequent years, use of these procedures increased rapidly as their technical aspects were better defined. The use of clean intermittent catheterization also broadened considerably the applicability of these surgical techniques because appropriate urinary drainage is needed for patients with neurogenic bladder and others unable to empty spontaneously.[66]

The principal indication for bladder augmentation is a small-capacity, poorly compliant bladder that precludes storage of urine for a reasonable amount of time at a "safe" pressure, allowing continence and avoiding damage to the upper tracts (Fig. 12-6).[89] Incontinence also may be a problem, and it should be addressed simultaneously.

Augmentation cystoplasty has been used in a diverse group of patients, including patients with neurogenic bladder (especially due to myelomeningocele), exstrophy, posterior urethral valves, bilateral ectopic ureters, inflammatory disorders (including tuberculosis and interstitial cystitis), and

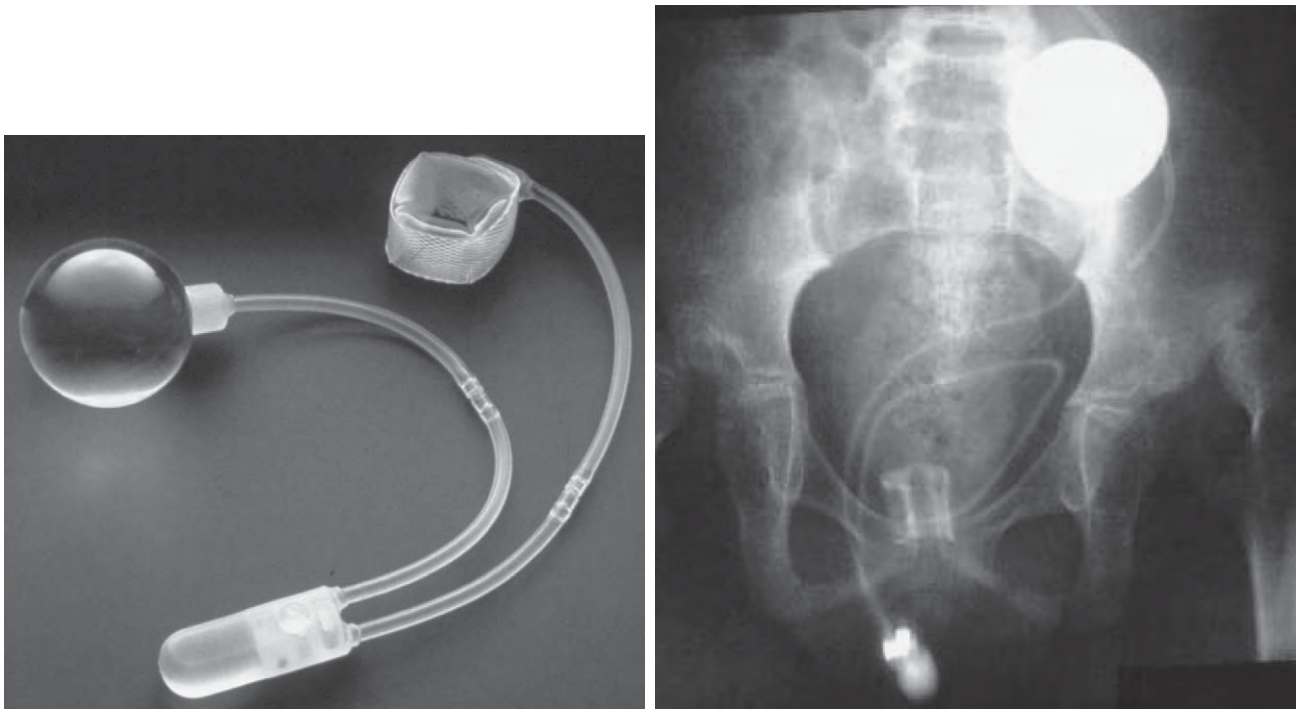


Figure 12–5 **A**, AMS-800 artificial urinary sphincter. **B**, Plain radiograph of the abdomen showing the presence of an artificial urinary sphincter that contains contrast media within the system, allowing good visualization of the device.

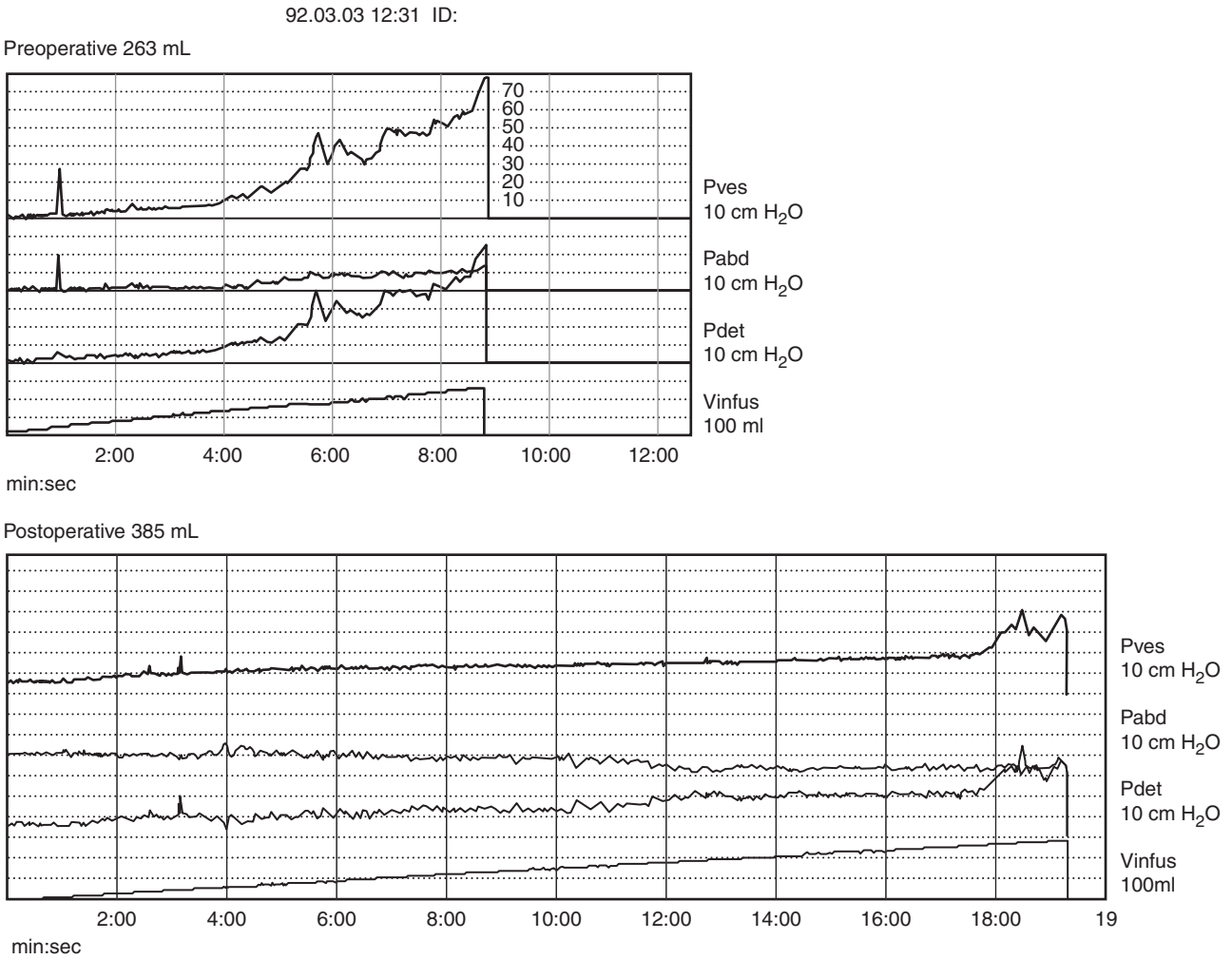


Figure 12–6 Preoperative and postoperative urodynamic studies performed in a patient who underwent a bladder augmentation. The bladder compliance, defined as the measure of the bladder's storage capability or Δ volume/Δ pressure calculated for any volume increment, was decreased before surgery. Pves, bladder pressure; Pabd, abdominal pressure; Pdet, detrusor pressure; Vinfus, Infused volume.

miscellaneous causes. Although the ideal material for bladder augmentation has not been developed, we do know its ideal properties. It should be easily available as a viable graft, easily shaped, compliant, easily accessible for periodic endoscopic examination, impermeable, and exempt of mucus production. Although bowel does not meet all of these criteria, most notably the latter two, in many ways it has served well for many years. Sigmoidocystoplasty and ileocystoplasty have become standard techniques (Fig. 12-7).

Because of the relatively high morbidity of intestinal cystoplasty, there is renewed interest in alternative techniques, such as seromuscular augmentation, various alloplastic or biodegradable scaffolds, and in vitro culture with subsequent grafting of autologous urothelium. These alternative procedures all have been reported to avoid inclusion of intestinal mucosa in the urinary tract while creating a compliant bladder of adequate capacity. Although encouraging results have been reported in animals and humans, each technique is associated with its own limitations and disadvantages. Nonetheless, we prefer to use the seromuscular colocystoplasty lined with urothelium rather than conventional surgical techniques when feasible and appropriate for a given patient (Fig. 12-8).[54]

CONSIDERATIONS IN MANAGEMENT OF AN ABNORMAL BLADDER

Reflux

High-grade vesicoureteral reflux that is left untreated after transplantation is accompanied by a higher risk of urinary tract infections even if it was not a problem before transplantation.[15] Surgical options for treatment—ureteral reimplantation or nephrectomy—have been associated with a reduced risk of infection after transplantation.[15,34] Endoscopic injections also have been used to treat children with vesicoureteral reflux awaiting renal transplantation.[5,44,58]

Timing

More challenging for pediatric urologists is the question of when to augment the bladder in children with posterior urethral valves. Bladder dysfunction and urinary incontinence in children with a history of posterior urethral valves is more common in the presence of renal insufficiency. One of the factors that contribute to a relative decreased storage capacity and incontinence in such cases is polyuria from renal tubular dysfunction. The bladder that seems inadequate before

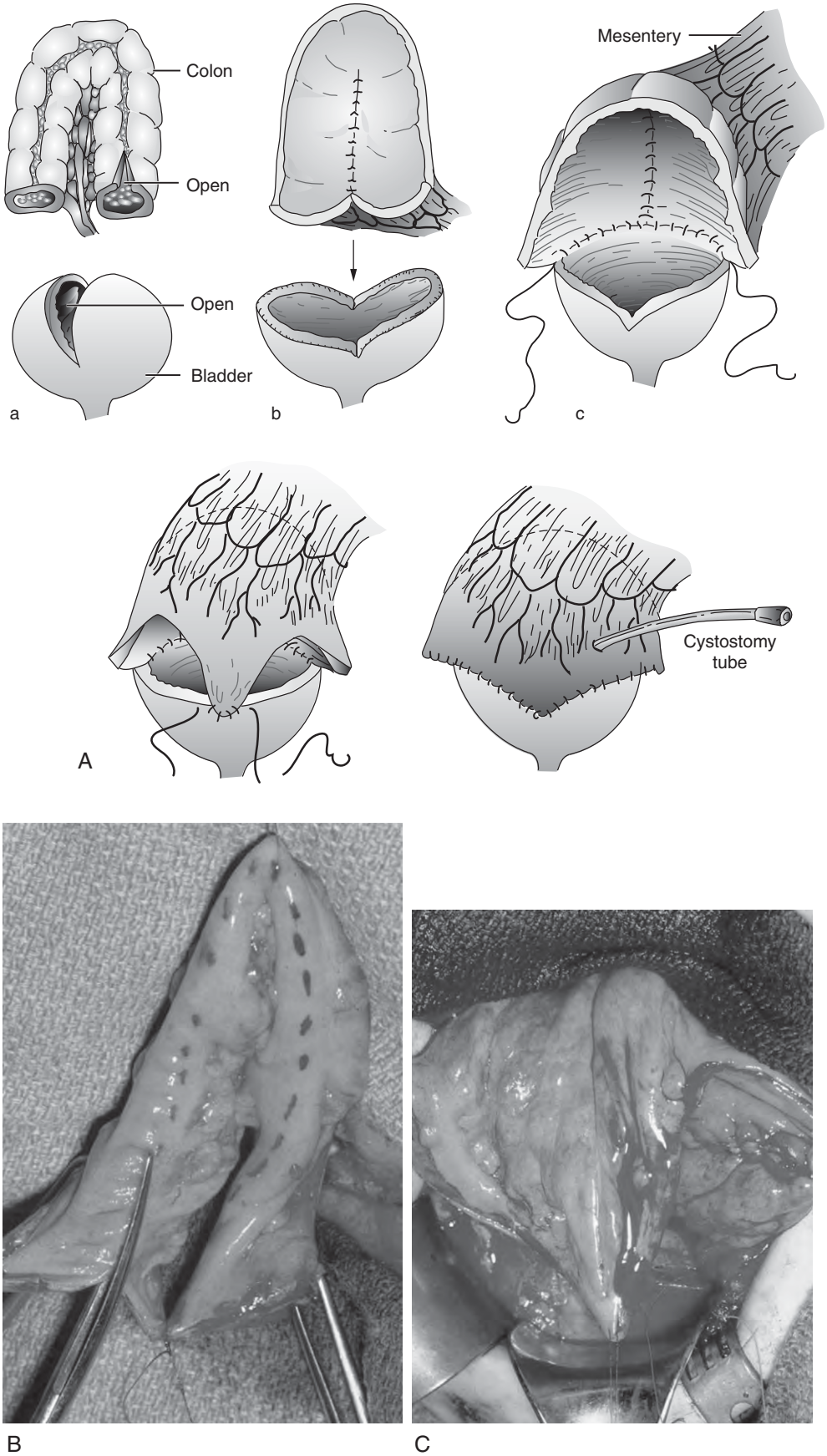
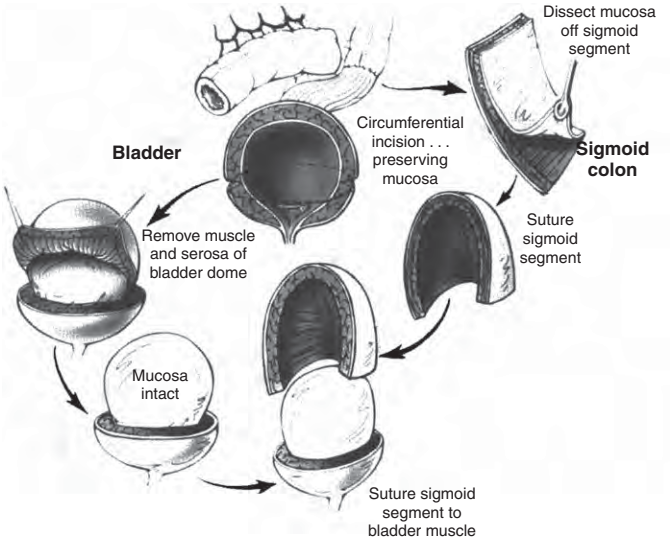


Figure 12–7 **A**, Bladder augmentation using a detubularized segment of bowel. **B**, Bowel segment used for bladder augmentation is detubularized on its antimesenteric border and folded in half to form a U shape. **C**, The U-shaped flap is anastomosed to the opened bladder beginning in the midline posteriorly. (See color plate.)



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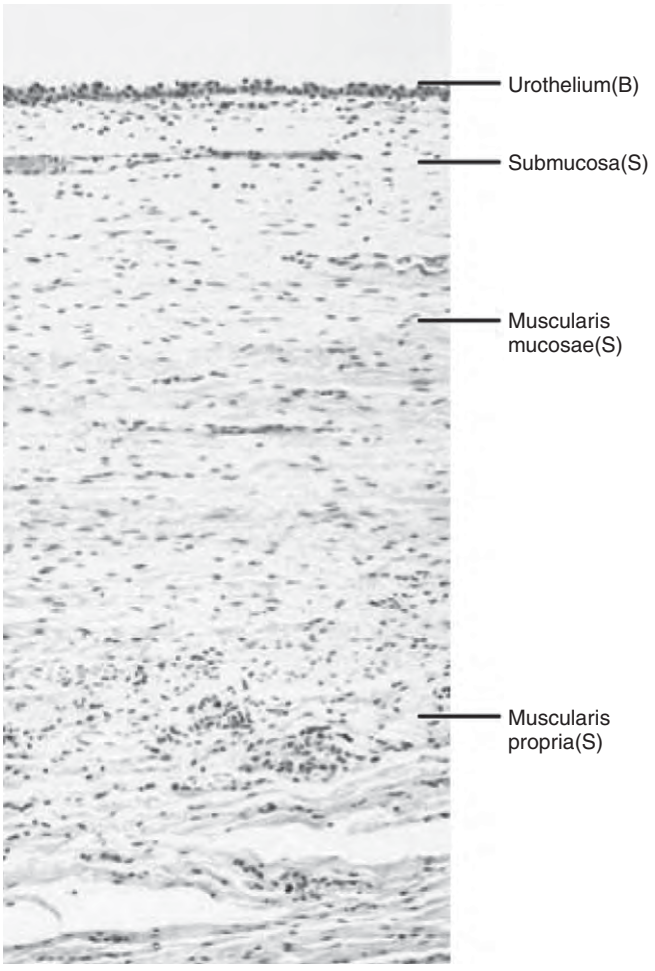

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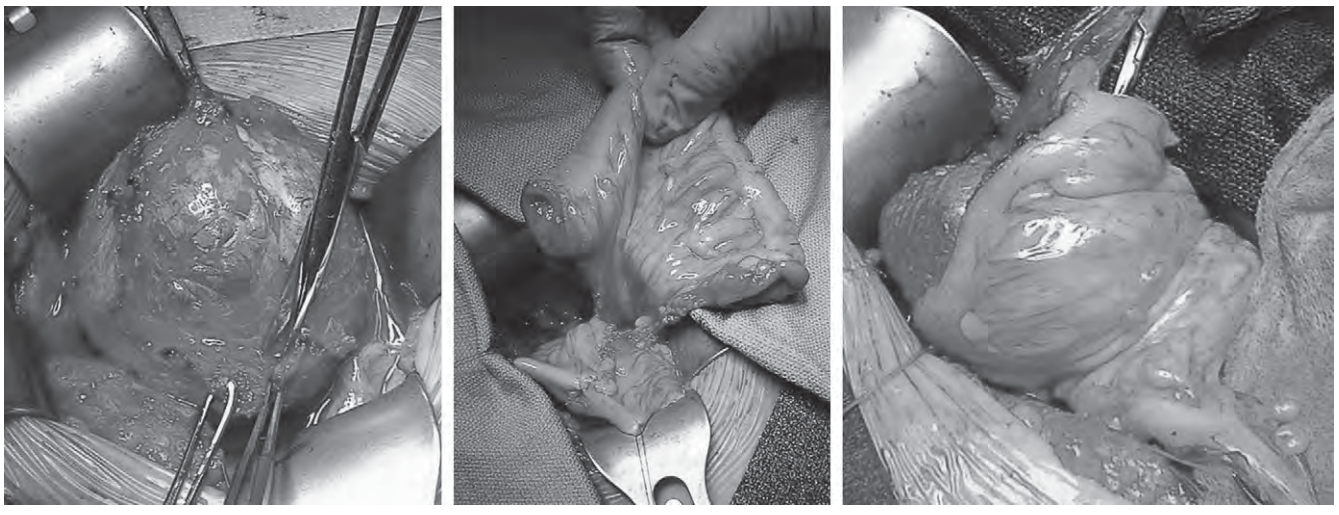

C D E

Figure 12–8 **A**, Seromuscular colocystoplasty lined with urothelium. **B**, Pathological aspect of the bladder wall after a seromuscular colocystoplasty lined with urothelium showing the juxtaposition of the urothelium next to submucosal and muscular layers of the colonic segment. B, bladder; S, sigmoid. **C**, Removal of the detrusor over the dome of the bladder with the urothelium kept intact. **D**, Isolation of a colonic segment, which will be detubularized. **E**, Intestinal segment, from which the mucosa has been dissected off, is used to cover the exposed dome of the bladder. (**B**-**E**, See color plate.)

the renal transplant may behave normally when the polyuria resolves. A bladder that has inadequate capacity and compliance for a given urine output may contribute to or accelerate the progression of renal failure.[68]

The timing and type of bladder augmentation relative to the transplantation warrants comment. Most authors have performed the augmentation before the transplantation. This seems to be a safe approach but presents a management problem when the patient is anuric and expecting a cadaver donor organ. Cycling the augmented bladder by clean intermittent self-catheterization is necessary while waiting for a kidney to become available. The small number of cases in which the bladder was augmented after transplantation attests to the feasibility of such an approach when needed. Nevertheless, it is generally recommended that if a conduit or a bladder augmentation is needed, it should be done several weeks before transplantation, although ureterocystoplasty may be performed simultaneously.[86]

Segment to Use

As mentioned earlier, sigmoidocystoplasty, ileocystoplasty, and variants such as the seromuscular colocystoplasty lined with urothelium[54] have become standard techniques for reconstructive procedures, and this is also true for the transplant population. Nonetheless, alternatives exist. Bellinger[12] described, in 1993, the technique of ureterocystoplasty using a detubularized segment of dilated ureter to augment the bladder. Soon after this initial publication, other reports of ureterocystoplasty were published.[22,51,92,117] These series show that in many ways ureterocystoplasty may be a good technique for bladder augmentation. It produces a compliant reservoir lined with urothelium, avoiding metabolic complications, mucus production, and the cancer risk of a heterotopic epithelium. Ureteral tissue should be used to augment the bladder when possible.[49,64,110] This approach is not frequently practical, however, because it is applicable only in a highly select group of patients with unilateral megaureter and poorly functioning kidney, and it may not provide sufficient functional capacity. The most common candidate is a boy with posterior urethral valves with a noncompliant bladder. These children are often polyuric, however, and, in our experience, the increase in capacity obtained with the ureter is often insufficient for the high urine output.

Gastrocystoplasty, another option, was initially thought to be a great idea, but as the popularity of gastrocystoplasty increased, so did awareness of the potential complications, such as severe metabolic derangements and the so-called hematuria-dysuria syndrome (bladder/urethral pain, hematuria in the absence of infection, skin excoriation), which has been seen in 36% of patients after gastric augmentation.[85] Another concern is having gastric tissue in the bladder of an anuric patient because of the risk of peptic perforation. We were the first to describe this complication,[94] and others made similar observations.[38,68,110] The widespread use of gastrocystoplasty seems to be fading given the serious potential complications; patients with preexisting gastrocystoplasty must be followed carefully for possible hypochloremic alkalosis and treated effectively with suppression of acid production, especially in the oliguric phase of disease.

Management of Anuric Patients

The management of a patient with a bladder augmentation or reservoir before transplantation is problematic, particularly if the patient is anuric or oliguric. This issue is magnified in the patient on a cadaver donor waiting list because the bladder or neobladder must be kept sterile so as not to miss possible opportunities to use a well-matched organ. We usually recommend daily bladder irrigations and instillation of an antibiotic solution. Instillation of aminoglycosides, which is usually safe, may lead to complications in patients with end-stage renal disease.[29]

Prophylactic Antibiotics

Other vexing problems are maintaining bladder sterility until the transplant is performed and preventing urinary tract infections after renal transplantation. Suboptimal bladder function in transplant recipients is linked to an increased risk of urinary tract infections, which could affect graft outcome.[26] This is particularly true for patients with small, noncompliant bladders.[56] Most authors recommend identification and normalization of bladder dysfunction before renal transplantation.[19,38,64,67]

Clean Intermittent Catheterization

Most patients with an augmented bladder and all patients with a continent diversion empty by clean intermittent catheterization. Although clean intermittent catheterization results in virtually universal bacteriuria, the safety of clean intermittent self-catheterization in renal transplantation has withstood the test of time,[6,40] and renal recipients performing clean intermittent self-catheterization can expect outcomes comparable to outcomes of children with normal bladder function.[21,28,38,49,67]

Complications of Reconstructive Lower Urinary Tract Procedures

The many metabolic and surgical complications observed with intestinal cystoplasties have been well published in the medical literature. One of the first reports of metabolic disturbances associated with intestinal urinary diversion appeared in 1931.[17] Since this initial publication, a wide variety of metabolic disturbances associated with use of intestine in the urinary tract have been reported. The main problem is a functional mismatch. Bowel mucosa and urothelium have different absorptive properties, the latter being impermeable to most electrolytes and ammonia under normal conditions. The severity of the disturbance is affected by the segment of bowel used, the amount of time spent in contact with urine, and the concentration, composition, and pH of the urine.[60-62,87] Other metabolic anomalies have been observed in addition to the hyperchloremic acidosis typical of ileal and colonic augmentation, including growth retardation,[82] malignancy,[8,36,74] interrupted bile acid recirculation,[7] and impaired clearance of drugs.[16] Urolithiasis formation in the augmented bladder or urinary reservoir is another complication of intestinal bladder substitution and augmentation that is seen in 30% of patients after augmentation cystoplasty, but it also can be seen in patients on clean intermittent self-catheterization

without augmentation.[9] The predominant stone composition is triple phosphate, usually identified in the reconstructed lower urinary tract.[14] The predisposition for stone formation in these patients may be due to infection, mucus retention, or the presence of foreign bodies.[88] Surgical complications of intestinal cystoplasty also are well known—perhaps the most devastating is spontaneous perforation of the bowel segment.[4,11,24,32,42,100,103,108]

Surgical Complications of Renal Transplantation into Reconstructed Abnormal Bladders

Our experience and the reviewed literature suggest that transplantation can be performed safely in patients with reconstructed bladders and urinary diversions with acceptable graft survival and function. Some authors reported an increased incidence of urological complications, such as urinary leak, ureteral stenosis, symptomatic urinary tract infections, metabolic acidosis, and calculi. There are few controlled studies that permit meaningful comparisons between results of transplantation in native versus reconstructed bladders. Comparison among reported series is difficult because some fail to define the source of the graft, which is one of the best-known determinants of graft survival. Some series combine patients with bladder augmentation with patients with diversions; this is problematic because it is well recognized that nonrefluxing ureteroenterostomies, in contrast to ureteroneocystostomies, carry a risk of stenosis of greater than 10%.[112] Nevertheless, one retrospective controlled study that included mostly adult patients with urinary diversion failed to show any differences with control patients with normal bladders.[116]

There is little question that in patients who must have bladder augmentation to attain continence or prolong life of the native kidneys, such as patients with neurogenic bladder or after cystectomy, renal transplantation can be accomplished with satisfactory results. The "catastrophic results" reported by one author[3] in a few cases are not the rule in the published literature or our experience.

Results of Pediatric Series

Most authors agree that although more complicated, it is feasible to proceed with renal transplantation in patients who are known to have an abnormal bladder with good results. Nahas and colleagues[83] reported on 24 patients (mean age 27.6 years), 21 of whom had the enterocystoplasty performed before transplantation. Seventeen transplants were from living donors. This is the largest series from a single center. In their series, the graft survival at a mean of 5 years was 78%, and the mean serum creatinine level was 141 μmol/L. Four patients died with functioning grafts. One patient died of bladder cancer 25 years after the augmentation, which was done because of tuberculosis of the bladder. The surgical complications mentioned included ureteral stenosis in two patients and a lymphocele in another. Urinary tract infections occurred at least once in 56% of patients, and 32% required hospitalization.

The largest pediatric series reported is by Hatch and coworkers,[49] which consists of a retrospective review of children operated on in 16 North American centers over 28 years. The series includes patients with bladder augmentation ($n = 17$) and patients with urinary diversion ($n = 13$). Of the transplants, 45% were from living related donors. A surgical complication rate of 19% was reported. Surgical complications consisted of renal artery stenosis ($n = 1$), urinary leak and fistula ($n = 2$), bladder calculus ($n = 1$), and wound dehiscence ($n = 1$), or were related to the cutaneous stoma ($n = 2$). Five patients developed metabolic acidosis (four augmented). The incidence of postoperative urinary tract infections was not reported. Graft survival by donor type was not reported. The mean serum creatinine level for all patients was 133 μmol/L at 5 years and 221 μmol/L at 9 years. The graft survival was not significantly different for augmentation and diversion groups (78% versus 46%), but the trend suggests better results in the augmented group. More recently, Martin and associates[71] and DeFoor and coworkers[27] published great results using enterocystoplasties.

Another multi-institutional review from 15 centers in France[99] included 20 patients with bladder augmentation, 8 with continent diversion, and 23 with incontinent diversion who received deceased donor renal transplants. The graft survival was 76% at 5 years, and there were no statistical differences between patients with augmentation or diversion. Data on renal function were not reported. Thirteen of 51 patients required repeat operations, including three for ureteral complications, three for lithiasis, and one for adenocarcinoma of the pouch. The incidence of urinary tract infections was 18%.

Another report from France[38] included 14 children (10 posterior urethral valves), all with bladder augmentation (10 performed before transplantation). The graft survival was 84% and 73% at 5 years and 10 years, respectively. The serum creatinine level was less than 124 μmol/L in 9 of 14 patients after a mean follow-up of 80 months. Complications included symptomatic urinary tract infections in four patients, metabolic acidosis in two, lithiasis in two, and hematuria-dysuria syndrome in the only patient who underwent augmentation with stomach.

Koo and associates[64] reported on 18 children (mean age 8.4 years); 4 had an enterocystoplasty, 2 had a ureterocystoplasty, and 7 had a diversion (5 continent, 2 incontinent). The remaining five patients were transplanted into their native bladders. Eight had a history of posterior urethral valves. Fifteen patients received kidneys from living related donors. Graft survival at a median follow-up of 4.4 years was 81%, and the mean serum creatinine level was 124 μmol/L. Complications included ureteral stenosis in two patients, incontinence in one, lithiasis in two, and stomal stenosis in one. Allograft thrombosis occurred in two patients. Metabolic acidosis was observed in 12 patients, and urinary tract infections were seen in 10.

Power and colleagues[90] published results of 17 cadaver donor renal transplantations in 16 patients with spina bifida (mean age 20 years). Eight patients had enterocystoplasty, five had ileal conduits, and three had native bladders that emptied by clean intermittent self-catheterization. Graft survival was 65% at 53 months, and the mean creatinine level was 113 μmol/L. There were two deaths after failed transplantation.

A report of nine children (seven augmentations, two continent diversions) from three centers included patients with posterior urethral valves ($n = 3$), urogenital sinus anomalies ($n = 2$), and miscellaneous conditions ($n = 4$).[110] Five augmentations were accomplished with stomach.

Two patients had artificial urinary sphincters. Graft survival (initial transplantation) was 56% at 29 months. At last follow-up, eight of nine patients were dialysis-free, and the mean creatinine level was 106 μmol/L. Complications occurred in five patients, including small bowel obstruction ($n = 1$), hematuria-dysuria syndrome ($n = 1$), stomal stenosis ($n = 1$), and ureteral obstruction ($n = 2$).

Nguyen and colleagues[84] reported 17 patients with a mean age of 20 years who underwent 20 transplantations (14 living related donors). This was a retrospective controlled study, which included 7 patients with previously defunctionalized bladders, and 10 with either augmentation or diversion. There were no statistical differences in graft survival (70%) and patient survival (88%) among augmented/diverted bladders, previously defunctionalized bladders, and control patients. Mean serum creatinine level was 80 μmol/L for the previously defunctionalized bladders at 5 years and 106 μmol/L in the diversion/augmentation group at more than 5 years. There were no surgical complications in the previously defunctionalized bladders. In contrast, in patients with bowel incorporated into the urinary tract, there were four ureteral complications, one wound dehiscence, and one lithiasis. One patient developed metabolic acidosis, and four had urinary tract infections. Three other series looked at graft survival among augmented/diverted cases; although they reported better results in the diverted groups, the differences are not significant.[76,77,98]

A report on 13 patients transplanted into small bladders that had been defunctionalized for 3 to 20 years but not augmented (3 posterior urethral valves) indicated a graft survival of 62% at 4 years.[69] There were no surgical complications. Another seven patients considered to have unusable bladders underwent transplantation into an existing urinary conduit. Their graft survival was 57% at 4 years. Patient survival was comparable.

In contrast, Alfrey and coworkers[3] reported kidney transplantation in eight patients with an enterocystoplasty as bladder augmentation. In five patients, the augmentation was taken down before the transplant, and in three the kidney was transplanted into the augmentation. Of those three patients, all had severe urinary tract infections. One died, one lost the graft, and another was being considered for an incontinent diversion. In contrast, the patients whose augmentation was taken down fared well, and the authors concluded that augmented bladders represent a significant risk in kidney transplantation.

Rigamonti and colleagues[98] published a distinctive study that looked at long-term results. From September 1987 to January 2005, 255 patients (161 males and 94 females) with a median age of 14 years (range 7 months to 39 years old) received 271 kidney transplants. The cause of end-stage renal disease was lower urinary tract disease in 83 cases. Among them, 23 had undergone bladder augmentation ($n = 16$) or incontinent urinary diversion ($n = 7$). Cumulative graft survival rates of all cases transplanted was 69.4% after 15 years; in the two investigated groups, augmented group and diverted group, graft survival was 80.7% (augmented group) and 55.5% (diverted group) (P value not significant). The Italian authors concluded that bladder augmentation or urinary diversion is an appropriate management strategy when the native bladder is unsuitable and yields similar results to those obtained in the general population with normal lower urinary tracts.

Additional publications warrant comment. In a retrospective controlled study from Sweden[116] involving four institutions during a 15-year period, the outcomes of transplantation in patients with continent and incontinent diversion were compared with patients with normal bladders. The only difference among the groups was the surgical time, which was longer in the diverted group. Graft survival (70% versus 74%) and patient survival at greater than 5 years were similar. Likewise, there was no statistical difference in the 5-year serum creatinine level, but the data presented suggest a tendency toward a higher serum creatinine in the continent diversion group. Another controlled study published in 1994 by Griffin and coworkers[45] stated that graft survival and patient survival were comparable, with graft survival being 70% at 5 years for both groups and patient survival being 82% and 90%.

Riedmiller and associates[97] reported 12 patients (7 children) with renal transplantation (all cadaver donors) into continent diversion (4 with posterior urethral valves). Technical difficulties led to the need for reoperations in 6 of 12 patients, including 1 child requiring a second transplantation. At 32 months of follow-up, the mean creatinine level was 115 μmol/L, and 11 of 12 initial grafts were functioning. Bacteriuria was present in all cases, but no episodes of pyelonephritis were recorded. All of the aforementioned studies are summarized in Table 12-1.

Posterior Urethral Valves

Renal transplantation in patients with posterior urethral valves is unique. It is well known that many of these children have bladder dysfunction with poor compliance,[20] and the proportion may be higher in children who have renal failure. Although many uncontrolled studies suggest that renal transplantation into the valve bladder is associated with good results,[23,101] close examination of every controlled study reported to date indicates that patients with renal transplantation into nonreconstructed valve bladders exhibit higher creatinine levels at the end of 5 years compared with controls. This higher creatinine level has been observed in virtually all studies reported and has been attributed to bladder dysfunction.[2,18,46,93] In 1997, Salomon and colleagues[104] reported worse results of transplantation in children with posterior urethral valves and symptomatic bladder dysfunction than with children without such symptoms. The graft survival may be normal or marginally decreased in these cases.[30,101] It has been tempting to pursue an aggressive approach to the valve bladder in hopes of improving the life span of the native kidneys and improving the results of renal transplantation. Others[10] have shown, however, that patients with posterior urethral valves managed by a limited intervention approach had better outcomes than patients who underwent extensive urological procedures. Nonetheless, transplantation into a nonreconstructed valve bladder and into an augmented bladder can yield acceptable graft survival rates.[28] Lacking controlled studies of patients with posterior urethral valves to define the possible advantages and risks of lower urinary tract reconstruction, no recommendations can be made based on the available evidence as to the indications of bladder augmentation in this condition.

In addition, one study indicates that the rate of post-transplantation urinary tract infections is greater in patients with a history of posterior urethral valves, regardless of the

Table 12–1 **Significant Series Reporting on Graft and Patient Survival in Transplant Recipients with Reconstructed Bladders or Urinary Diversions**

Reference	No. Patients	No. Transplants			Mean Age (yr)	Cystoplasties							Graft Survival Rate	Mean Serum Creatinine	Patient Survival Rate
		Total	LRD	DD		EC	GC	UDB	UC	CP	CD	ID			
Nahas et al[83]	24	25	17	8	27.6	24							78% at 5 yr	141 μmol/L at 5 yr	86% at 5 yr
Hatch et al[49]	30	31	14	17	12.1	11	1		5		1	12	78% at 5 yr (cystoplasties); 46% at 5 yr (urinary diversions); 60% at 10 yr (overall survival)	133 μmol/L at 9 yr; 221 μmol/L at 9 yr	100% at 5 yr
Rischmann et al[99]	51	51		51	NA	19	1				8	23	76% at 5 yr	NA	100% at 47 mo
Fontaine et al[38]	14	14		14	12.1	13	1						84% at 5 yr; 73% at 10 yr	<124 μmol/L at 80 mo (in 9/14 patients)	86% at 10 yr
Koo et al[64]	18	21	15	6	8.4	4			2		5	2	81% at 4.4 yr	124 μmol/L at 4.4 yr	100% at 4.4 yr
Power et al[90]	16	17		17	20.2	8						5	65% at 53 mo	113 μmol/L at 53 mo	88% at 53 mo
Sheldon et al[110]	9	12	8	4	9.8	1	5		1		2		56% at 29 mo	106 μmol/L at 29 mo	100% at 29 mo
Nguyen et al[84]	17	20	14	6	20	2		7				8	70% at >5 yr	106 μmol/L at 5 yr (EC and ID); 80 μmol/L at 5 yr (UDB)	88% at >5 yr
MacGregor et al[69]	20	24	14	10	23			13				7	62% at 4 yr (UDB); 57% at 4 yr (ID)	NA	85% at 4 yr
Alfrey et al[3]	10	8	NA	NA	12.8	3		7					NA	NA	88%
Warholm et al[116]	22	22	NA	NA	32						5	17	93% (cases and controls) at 1 yr* 70% (cases) versus 74% (controls) at 5 yr*	119 μmol/L (cases) versus 137 μmol/L (controls) at 1 yr* 147 μmol/L (cases) vs 132 (controls) at 5 yr*	3 (cases) versus 4 (controls) deaths*
Riedmiller et al[97]	12	13		13	21.8						12		92% at 32 mo	115 μmol/L at 32 mo	100% at 32 mo
Martin et al[71]	7	7		7	38.4	7							100% at 48 mo	150 μmol/L	100% at 48 mo
McInerney et al[76]	21	21			NA	8						13	100% at 4.6 yr and 3.2 yr for conduits (8) and cutaneous ureterostomies (5); 75% of EC		
Rigamonti et al[98]	23	23			19 (EC) and 17 (ID)	16						7	55.5% (EC) and 80.7% (ID) at 15 yr		
Thomalla et al[113]	8	8				8							50% (follow-up 6 mo-7 yr)		
DeFoor et al[27]	20	20	15	5	4.5 (when reconstructed)	14				6			82% (7.3 yr follow-up)	106 μmol/L	
Griffin et al[45]	23	28				20					3		70% at 5 yr (similar to controls)		82% versus 90% (controls) at 5 yr
Mendizabal et al[77]	15	18	1	17	13	4			3		1	6	77% and 62% at 1 yr and 5 yr		93%

*Not statistically significant.

CD, continent diversion; CP, continent procedure; DD, deceased donor; EC, enterocystoplasty; GC, gastrocystoplasty; ID, incontinent diversion; LRD, living related donor; NA, not available; UC, ureterocystoplasty; UDB, undiverted bladder.

presence of reflux.[80] This information is important, not to discourage renal transplantation in young patients with a history of posterior urethral valves but rather to pay particular attention to bladder care in these cases. It would seem rational to do everything feasible to optimize bladder function before transplantation by improving emptying, decreasing storage pressures, and providing adequate capacity. When evaluating these bladders, it must be remembered that what is considered adequate bladder capacity and compliance varies with the obligatory diuresis of a given patient. Inadequate capacity in a polyuric child with end-stage renal disease may become acceptable after the transplant when the urine output normalizes. Measures such as clean intermittent self-catheterization and anticholinergics should be used if indicated.[3]

Prune-Belly Syndrome

The first renal transplant in a patient with prune-belly syndrome was reported in 1976 by Shenasky and Whelchel[111] followed by other single case reports.[31,78] In 1998, Fontaine and colleagues[38] reported on a controlled study done retrospectively, indicating that the results of renal transplantation with regard to graft survival and function in cases of prune-belly syndrome were comparable to those of controls. These results confirm previously reported results by Reinberg and coworkers in 1989,[95] which is not surprising because bladder storage pressures are low in most cases of this syndrome. Later, an Italian group published their experience with a series of five boys and reported good results as well, but they stressed the need to address the lack of abdominal wall musculature by performing abdominal wall reconstruction in selected patients.[39] A unique complication specific to renal transplantation performed in patients with prune-belly syndrome is torsion of the graft, attributed to the laxity of the abdominal wall or improper fixation of the kidney.[1,72]

FOLLOW-UP

Abnormal bladders must be assessed urodynamically before and after transplantation. Adequacy of urinary storage and drainage must be reassessed after renal transplantation even in patients known to have a normal lower urinary tract before the transplantation because they also may exhibit abnormal bladder function. A Swedish study published in 2005 showed that abnormal bladder capacity was found in 26%, abnormal urinary flow in 50%, and residual urine in 32% of the patients, and there was no significant difference in bladder or renal function in children with urinary tract malformations and those with normal urinary tracts.[50] Prophylactic antibiotics are now given for the first 6 months, and urinary tract infections must be treated promptly. With these measures, good results, similar to those in patients without urological problems, can be obtained.

CONCLUSION

End-stage renal disease caused by congenital genitourinary anomalies is common, especially in pediatric patients. Integrity of the lower urinary tract is mandatory, and proper investigation should be done in a given population. Graft implantation into the native bladder is always preferred. Surgical correction may be required, however, if the bladder is unsuitable. Planning ahead is crucial, and a multidisciplinary approach is advocated if possible. Bladder reconstruction and procedures to correct incontinence should be done before transplantation when clinically indicated.

Bladder reconstruction, although not exempt from complications, is an acceptable method for patients with abnormal lower urinary tracts who are candidates for renal transplantation. The use of bladder reconstruction before transplantation in children with posterior urethral valves is different, however. The appropriate management of the valve bladder in patients requiring renal transplantation is still in question because results tend to vary between studies. In such cases, the complications associated with the reconstructed bladder should always be balanced against the possible risks of performing renal transplantation into an abnormal bladder. Finally, even if the reported series of renal transplantation into abnormal bladders are small, and there are few controlled studies, the graft and patient survival rates in most series seem to be comparable to the rates for transplants into nonreconstructed bladders.

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Chapter 13

Anesthesia for Patients Undergoing Renal Transplantation

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Patients with end-stage renal failure may receive replacement therapy by dialysis or by renal transplantation from a living related donor or a cadaver donor. Many factors that contribute to end-stage renal disease—generalized atherosclerosis, uncontrolled hypertension, and diabetes mellitus, among others—also increase the perioperative cardiovascular risk associated with anesthesia and surgery. Chronic renal failure increases the risks of ischemic heart disease and poor anesthetic outcome.

In 1996, in one of the first reports of the anesthetic problems associated with renal transplantation, Strunin[187] reported a 56% mortality rate within 3 months of surgery. More recent data show a considerable improvement. Solomonson and coworkers[183] observed a 30-day mortality rate of 2.8% in patients undergoing formation of an arteriovenous fistula, whereas Humar and coworkers[96] reported a 6.1% overall perioperative cardiac complication rate among 2694 renal transplant recipients, and Gill and Pereira[74] reported a 4.6% first-year all-cause mortality rate in 23,546 adult first-transplant patients, with greater than 25% of these being secondary to cardiac causes. The main predictors of adverse outcome are a past history of pretransplant cardiac disease or myocardial infarction within the previous 6 months and age older than 40 years.

A patient with end-stage renal disease scheduled for renal transplantation presents the anesthesiologist with many clinical problems. Successful outcome depends on a clear understanding of the clinical issues of renal failure; the influence of renal failure on the pharmacokinetics, metabolism, and pharmacodynamics of anesthetic drugs; the correct management of the intercurrent problems that caused the renal failure; and the choice of an appropriate anesthetic technique for renal transplantation.

CLINICAL PROBLEMS RELEVANT TO ANESTHESIA FOR RENAL TRANSPLANTATION

Cardiovascular Disease

The two main cardiovascular effects of chronic renal failure are arterial hypertension and atherosclerosis and hyperlipidemia leading to ischemic heart disease. Hypertension and ischemic heart disease are common complications in patients presenting for renal transplantation. The incidence of preoperative hypertension is about 80% in patients undergoing renal transplantation.[192] Hypertension of chronic renal

failure often is a consequence of volume expansion secondary to salt and water retention.[19] It usually can be controlled with dialysis and appropriate antihypertensive therapy. In patients in whom the hypertension cannot be controlled by dialysis alone, it has been suggested that an abnormal relationship exists among plasma renin activity, intravascular fluid volume, and blood pressure. There also may be an inappropriate level of sympathetic activity.[36] Patients needing treatment in addition to dialysis are often refractory to single agents and require large doses of combinations of antihypertensive drugs (e.g., β-adrenoceptor blocking drugs, calcium channel blockers, vasodilators, and angiotensin-converting enzyme inhibitors), which all may combine to produce significant drug interactions with volatile and intravenous anesthetic agents.[172,176]

In a post-transplant patient in whom there is correction of the uremia and fluid imbalance, persistence of hypertension may be due to acute or chronic rejection of the allograft, the presence of native diseased kidneys, or transplant artery stenosis. Cyclosporine therapy also may produce hypertension; this often is accompanied by renal dysfunction. It seems to be a direct vasoconstrictor response and an action of cyclosporine on intracellular calcium homeostasis. Cyclosporine reduces renal tubular sensitivity to aldosterone. Other contributory factors include the presence of cyclosporine-induced hypomagnesemia and the renal production of thromboxane A_2.

Despite seemingly adequate therapy, 50% to 70% of patients receiving a renal transplant experience marked swings in blood pressure during surgery (± 30% shifts from the awake preinduction value) and show exaggerated vascular responses to induction of anesthesia, laryngoscopy, and intubation and extubation. Induction of anesthesia is best achieved by combining a hypnotic agent supplemented with an opioid (remifentanil, fentanyl, alfentanil, or sufentanil). Patients receiving antihypertensive or antianginal treatment (especially patients receiving β-adrenoceptor blocking drugs, angiotensin-converting enzyme inhibitors, and calcium channel blockers) should receive their "regular" therapy as part of their premedication. In patients not receiving therapy and presenting for surgery with elevated blood pressure, Stone and colleagues[185] showed the efficacy of preoperative oral β-adrenoceptor blockade as an adjunct to premedication in reducing the hemodynamic lability in response to surgical stress and its associated incidence of myocardial ischemia (Fig. 13-1).

Patients with renal failure, especially patients on dialysis, are prone to develop accelerated atherosclerosis. Left ventricular function may be compromised further by uremic cardiomyopathy and pericarditis.

Anemia

Anemia has been a major problem in the anesthetic management of patients with renal failure. Hemoglobin concentrations in patients receiving hemodialysis before transplantation often were 6 to 8 g/100 mL with hematocrit values of 20% to 25%, although this is now uncommon with the more liberal use of erythropoietin in anemic patients with renal failure, at least in the Western world. The normal picture is that of a normochromic, normocytic anemia of complex origin that usually is due to impaired erythropoiesis secondary to decreased erythropoietin synthesis and release.

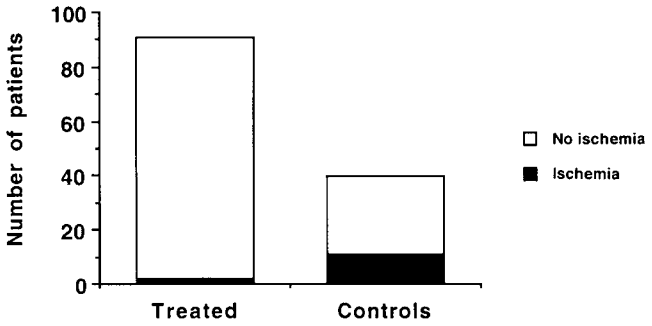

Figure 13–1 Reduction in the incidence of myocardial ischemia during surgery after pretreatment of untreated or poorly treated hypertensive patients with oral β-adrenoceptor antagonists. (From Stone JG, Foex P, Sear JW, et al: Myocardial ischemia in untreated hypertensive patients: effect of a single small oral dose of a beta-adrenergic blocking agent. Anesthesiology 68:495, 1988.)

Other factors include a decreased red blood cell life span, increased hemolysis and bleeding, repeated blood loss during hemodialysis, aluminum toxicity, uremia-induced bone marrow suppression, and iron, folate, and vitamins B_6 and B_{12} deficiencies.[64]

In the absence of a correction of the anemia, there are compensatory mechanisms for the reduction in oxygen-carrying capacity. At a hemoglobin concentration of 6 to 8 g/100 mL, the oxygen-carrying capacity of the blood is about 50% normal (i.e., about 10 mL oxygen per 100 mL blood). The normal tissue arteriovenous oxygen difference is 5 mL oxygen per 100 mL blood, although the heart extracts two to three times this amount. Various compensatory mechanisms exist to overcome the decrease in oxygen-carrying capacity, including an increase in cardiac output and an increase in the red blood cell 2,3-diphosphoglycerate. The latter causes a shift of the oxygen dissociation curve to the right, improving tissue oxygenation. The shift seems to be greater in uremic patients who are well managed by hemodialysis compared with patients in renal failure who are poorly managed or are not on dialysis. This difference may reflect the influence of acidosis on the oxyhemoglobin dissociation curve. Severe anemia also affects the blood-gas partition coefficient for the volatile anesthetic agents, with an increase in the rate of onset and recovery from anesthesia.

Respiratory System

Between dialysis sessions, pulmonary congestion and edema often are seen with a resultant hypoxemia and hypocapnia. The use of peritoneal dialysis can aggravate the problem because the intraperitoneal fluid causes diaphragmatic splinting with basal pulmonary atelectasis and shunting. Uremic lung is a radiological entity characterized by perihilar pulmonary venous congestion secondary to fluid retention. Uremia can cause pleuritis. Immunosuppressed transplant patients are more susceptible to pulmonary infections, with preexisting disease often exacerbated by airway instrumentation and general anesthesia.

Acid-Base Status and Electrolyte Imbalance

Patients with renal failure have an impaired ability to excrete water, electrolytes, and free acids. The presence of a metabolic

acidosis with its associated electrolyte disturbances (hyponatremia, hyperchloremia, and hyperkalemia) may cause problems with respect to the adequacy of reversal of residual neuromuscular blockade at the end of anesthesia.

With the introduction of routine dialysis in most patients before transplantation, preoperative electrolyte disturbances have largely disappeared. Of more importance is the blood potassium concentration. At serum concentrations greater than 7 mmol/L, abnormal electrocardiogram (ECG) changes are common, with the possibility of developing ventricular tachycardia and ventricular fibrillation. A high potassium level before anesthesia is potentially dangerous and must be avoided. Evidence exists, however, that uremic patients can tolerate mild-to-moderate degrees of hyperkalemia (see later). It is probably safe to administer anesthesia in the presence of higher than normal potassium concentrations, unless there are ECG changes associated with hyperkalemia (high-peaked T waves, decreased amplitude of the R wave, widened QRS complexes, and progressive diminution of P wave amplitude).

Methods available for the preoperative correction of hyperkalemia include glucose-insulin therapy and administration of bicarbonate (these are acute temporary methods rather than being corrective), or continuous hemofiltration or hemodialysis leading to increased potassium elimination. Situations that may increase further the plasma potassium concentration (including infusions of stored blood and hypoventilation causing respiratory acidosis) are best avoided.

Coagulation

Some patients show persistent heparinization after hemodialysis before transplantation.[82] A few uremic patients, normally patients who are inadequately dialyzed or undergo transplantation before requiring dialysis, exhibit a separate hemorrhagic diathesis.

Several abnormalities of the coagulation factors have been described (platelet dysfunction, decreased levels of platelet factor III resulting in poor adhesiveness, and thrombocytopenia). Laboratory investigations show no alteration in prothrombin or partial thromboplastin time, but the bleeding time is prolonged. The decrease in platelet factor III occurs because of accumulation of toxic endogenous waste products, including guanininosuccinate, phenol, and phenolic acid. These products are removed by adequate dialysis with a return to normal platelet function.

Other methods of treatment of uremic coagulopathy include platelet transfusion, cryoprecipitate, and infusions (0.3 μg/kg) over 15 minutes of desmopressin acetate. Desmopressin acetate acts to increase the activity of coagulation factors VIII and XII, von Willebrand's factor, and high-molecular-weight kininogen.[130,131]

Despite these theoretical problems, the blood loss during transplantation normally is less than 500 mL. If blood loss occurs, it may be rapid, and all replacement fluid should be administered through large venous catheters.

Central Nervous System

The central nervous system features of uremia are initially malaise and reduced mental ability. Other manifestations include myoclonus, seizures, coma, and death. Patients complain of pruritus, which tends to be severe at night and at rest and is relieved by movement. Peripheral neuropathies also may occur, especially in the lower limbs, and may involve the autonomic nervous system, leading to postural hypotension. Dialysis is associated with neurological sequelae, such as the dysequilibrium syndrome. This sequela arises from sudden changes in extracellular volume and electrolyte composition and cerebral edema. The dysequilibrium syndrome is characterized by dehydration, weakness, nausea and vomiting, hypotension, and occasionally convulsions and coma. Treatment should be symptomatic and aggressive.

Endocrine System

Diabetic nephropathy is a common cause of end-stage renal disease and may be accompanied by accelerated atherosclerosis. Severe coronary artery disease may be a significant presenting feature of the triad of diabetes, hypertension, and hyperlipidemia (the so-called syndrome X).[204] Diabetes may lead to an autonomic neuropathy that can cause gastroparesis and hemodynamic instability. Long-term problems in diabetic patients include stiffening of the temporomandibular joints and difficulty with laryngoscopy and intubation (see later).

Uremic osteodystrophy encompasses many separate skeletal problems, including osteomalacia, osteosclerosis, and osteitis fibrosa cystica—the last-mentioned developing as a result of secondary hyperparathyroidism. As renal function decreases, phosphate excretion declines, resulting in hyperphosphatemia. Failure to convert 1-hydroxyvitamin D to 1,25-dihydroxyvitamin D leads to a reduced absorption of calcium, which results in hyperparathyroidism. The sequela is bone demineralization, making patients susceptible to spontaneous fracturing of long bones and vertebrae.

Gastrointestinal Tract

Common gastrointestinal symptoms in uremic patients are anorexia, nausea and vomiting, gastrointestinal hemorrhage, and diarrhea. Most of these problems are attenuated by the introduction of dialysis before transplantation. Renal failure patients have delayed gastric emptying in addition to an increase in acidity and gastric volume.[186] Patients benefit from administration of a histamine H_2-receptor antagonist as part of premedication. A rare, but important, complication of end-stage renal disease is ascites (accompanied by hypoalbuminemia), which may lead to splinting of the diaphragm and basal pulmonary atelectasis with resultant hypoxemia.[75]

Immune System

Uremia impairs normal immune mechanisms, and these mechanisms may be obtunded further by administration of glucocorticoids and immunosuppressant drugs for treatment of the underlying renal pathology (e.g., systemic lupus, scleroderma, nephrotic syndrome). As a result, sepsis remains a major cause of morbidity and mortality. Particular attention should be paid to strict aseptic technique when inserting a urinary catheter, inserting invasive monitoring devices, or administering peripheral infusions.

Preoperative Assessment

Preoperative assessment should lead to optimization of any persistent serious cardiorespiratory complications, such as congestive heart failure, ECG abnormalities resulting from

myocardial ischemia, and autonomic dysfunction in patients with diabetes mellitus. With increasing numbers of elderly and diabetic patients being accepted for renal transplantation, careful assessment of cardiorespiratory function is needed before placement on the transplant list. This assessment should include referral, when appropriate, for an anesthetic consultant opinion and clear indication in the case notes of special problems relating to the individual patient at the time of surgery. Patients maintained on hemodialysis usually undergo a dialysis session at some point during the 24- to 36-hour period before transplantation. Predialysis and postdialysis weight and electrolyte status should be recorded.

A common complication of dialysis in the preoperative transplant recipient is hypotension, which is predominantly due to ultrafiltration-induced hypovolemia. However, it also may be due to the reduction in plasma osmolality, to reflex sympathetic inhibition, or to the autonomic neuropathy associated with diabetes mellitus or systemic sepsis, and to electrolyte abnormalities (especially hypokalemia, hyperkalemia, and hypocalcemia). Treatment is administered by infusing normal saline and reducing the rate of ultrafiltration if it occurs during preoperative dialysis.

Protection of Veins, Shunts, and Fistulas

Functional shunts or fistulas should be protected carefully during surgery, with the sphygmomanometer cuff placed on the other arm. Venous lines should be restricted when possible to peripheral veins, preferably on the dorsum of the dominant hand, with the preservation of all forearm and antecubital fossa veins.

Nonanesthetic Drugs Given during Renal Transplantation

The policy relating to immunosuppressive therapy varies among units, but the anesthesiologist may be required to institute the appropriate therapy (e.g., glucocorticoids, azathioprine, cyclosporine, antilymphocytic globulin, OKT3, tacrolimus, sirolimus, and alemtuzumab [Campath]) during the perioperative period.

INFLUENCE OF RENAL DISEASE ON PHARMACOKINETICS AND PHARMACODYNAMICS OF DRUGS USED DURING ANESTHESIA

Many important changes occur in the uptake, disposition, metabolism, and excretion of drugs given to patients with chronic renal failure, as follows:

1. Altered absorption: Because of gastric stasis, there are delays in the uptake of orally administered drugs.
2. Altered apparent volumes of distribution: Because of increased extracellular and intracellular fluid volumes, apparent volumes of drug distribution of water-soluble compounds are increased.
3. Altered plasma protein binding and free drug fraction: Plasma concentration of albumin (binding site for acidic drugs) is usually decreased in uremia, whereas concentration of α_1-acid glycoprotein (binding of basic drugs) is increased.
4. Altered drug and xenobiotic metabolism: This is a variable effect that can include reduced renal breakdown of insulin and glucagon, increased hepatic clearance (owing to increased free fraction) of drugs such as phenytoin and nifedipine, and decreased hepatic clearance of erythromycin, propranolol, and verapamil.
5. Altered drug elimination: This effect occurs as a result of the decreased glomerular clearance of filtered drugs (e.g., aminoglycosides, β-lactams, vancomycin, digoxin) and of active metabolites (e.g., morphine-6- glucuronide and normeperidine), and competition for the carriers involved in the excretion of acidic drugs.

Premedicant Drugs

Anticholinergic Drugs

Atropine and glycopyrronium are eliminated by the kidney (20% to 50% of the total dose).[107] Because these agents are usually administered only as single doses, however, accumulation with toxic side effects is unlikely.

Antacids and Prokinetic Drugs

The handling of H_2-histamine receptor antagonists, such as cimetidine and ranitidine, is largely unaltered by end-stage renal disease. Similarly, the disposition of proton-pump inhibitors (omeprazole, lansoprazole, and rabeprazole) is not changed in renal failure.

Metoclopramide is eliminated via the kidney unchanged (<20%) and as the *N*-4-sulfate (≤50%) and *N*-glucuronide. The kinetics of metoclopramide are complex because the elimination half-life is dose dependent after intravenous and oral administration. When given to patients with end-stage renal disease, there is a significant reduction in clearance (16.7 L/hr compared with 52.5 L/hr) and prolongation of the terminal half-life (13.9 hours compared with 2.8 hours).[10] This is not the result of reduced renal clearance, but rather impaired metabolism and alteration in the amount of drug-glucuronide conjugates undergoing enterohepatic recirculation. In a study in patients maintained on hemodialysis, Lehmann and colleagues[120] found altered kinetics of metoclopramide and reported significant side effects (especially drowsiness, restlessness, and diarrhea) after single doses of 10 mg. Hemodialysis does not affect metoclopramide elimination from the body, and clearance of the drug after dialysis is unaltered.[206]

Benzodiazepines

The kinetics and dynamics of the benzodiazepines are altered in patients with acute or chronic renal failure.

DIAZEPAM

Although Andreasen[3] found no correlation between the serum albumin concentration and protein binding of diazepam in patients in acute renal failure, Kangas and associates[103] showed a decrease in the plasma protein binding of diazepam in patients with chronic renal failure. In a further investigation of the disposition of diazepam in patients with chronic renal failure, Ochs and coworkers[147] found an increase in the apparent volume of distribution and increased systemic clearance, both secondary to an increase in the free unbound drug fraction (from 1.4% to 7.9%). There was no difference, however, in free drug clearance in

the uremic and healthy patients, although there was a smaller volume of distribution in the renal failure group.

MIDAZOLAM

The kinetics of the short-acting, water-soluble benzodiazepine, midazolam, are of greater interest to the anesthesiologist. Total drug clearance and apparent volumes of distribution were significantly greater in patients with chronic renal failure than in healthy controls.[196] These changes are secondary to an increased free drug fraction (6.5% compared with 3.9%). There were no differences in unbound drug kinetics, and the elimination half-life was similar in the two groups (4.6 to 4.9 hours). In patients with impaired renal function, there was no correlation between onset time of midazolam sedation and the free drug fraction; this may have been due to inherent alterations in drug sensitivity in the uremic patient. Because the increased free fraction of unbound drug is rapidly distributed to the richly vascularized tissues, it is probably advisable to give intravenous midazolam slowly, titrating dose to effect. In this way, the anesthesiologist can minimize any effects of relative overdosage of free drug to the heart and brain.

OTHER BENZODIAZEPINES

Odar-Cederlof and colleagues[148] investigated the disposition of oxazepam in patients with renal failure. After an oral dose of 0.2 mg/kg, there was significant prolongation of the terminal half-life (range 5.9 to 25 hours in healthy subjects, and 24 to 91 hours in uremic patients), decreased plasma protein binding in renal failure, and an increased fecal excretion of the drug. Initial data analysis suggests unaltered systemic drug clearance; if correction is made for the decreased absorption of the oral drug in uremia, there is reduced clearance of oxazepam in renal failure. Altered clearance of oxazepam was not confirmed in a subsequent study by Murray and associates.[144]

Single-dose studies with lorazepam indicated no alterations in the terminal half-life in renal failure[194]; however, the same authors described impaired drug elimination after long-term administration to two patients with uremia.[195] Although temazepam is widely used as a premedicant, there are few data on its kinetics or dynamics in patients with end-stage renal disease. A single oral dose study in dialysis patients by Kroboth and colleagues[119] indicates that lower maximal plasma drug concentrations are achieved together with an increased free drug fraction compared with healthy subjects.

Induction Agents

The most widely used hypnotic agent for induction of anesthesia in patients undergoing renal transplant surgery is probably propofol, although some anesthesiologists may favor etomidate or a barbiturate. There also are reports of ketamine's use as part of a total intravenous technique.

Propofol

Kinetic studies after induction and maintenance with propofol (diisopropyl phenol) show no major alteration in terminal half-life or clearance in patients with renal failure,[56,99,109] although Ickx and colleagues[99] reported a greater apparent volume of distribution of propofol in patients with end-stage renal disease. There is no significant effect of end-stage renal disease on the plasma protein binding of propofol (Table 13-1).[43]

In a comparison of propofol induction doses in healthy patients and patients with end-stage renal disease, Goyal and associates[79] found a greater dose requirement for attaining hypnosis and a bispectral index monitor level of 50 in patients with renal failure. They attributed these effects to the increased cardiac output accompanying the anemia seen in renal failure.

Morcos and Payne[142] and Kirvela and coworkers[109] reported the cardiovascular effects of an induction dose of propofol (2 to 2.5 mg/kg) preceded by fentanyl (3 to 5 μg/kg) in adequately volume-loaded end-stage renal failure patients and compared the data with data from healthy subjects. This induction sequence caused significant vasodilation in all patients, with 24% to 30% decreases in systolic blood pressure and 22% to 32% decreases in diastolic pressure in the healthy subjects, and similar changes of 19% to 39% and 14% to 39% in the renal disease patients. In the study by Kirvela and coworkers,[109] the maintenance of adequate antihypertensive therapy in the uremic patients up to the time of surgery may have contributed to the cardiovascular stability. Infusions of propofol also have been used for maintenance of anesthesia for renal transplantation.[50,112]

Table 13–1 **Influence of End-Stage Renal Disease on Disposition Kinetics of Commonly Used Intravenous Induction Agents**

	Patients with Normal Renal Function				Patients with Impaired Renal Function			
	$T_{1/2}el$	Cl_p	V_{ss}	FF	$T_{1/2}el$	Cl_p	V_{ss}	FF
Propofol: Kirvela et al[109]	1714	11.8	19.8	—	1638	12.9*	22.6	—
Ickx et al[99]	420†	33.5†	5.8†	—	513†	32†	11.3†	—
Midazolam: Vinik et al[196]	296	6.7	2.2	3.9	275	11.4*	3.8*	5.5*
Etomidate: Carlos et al[25]	—	—	—	24.9	—	—	—	43.4*
Thiopental: Burch et al[20]	611	3.2	1.9	15.7	583	4.5*	3*	28*
Christensen et al[33]	588	2.7	1.4	11	1069	3.9*	3.2*	17.8*

*$P < .05$ versus healthy subjects.

†Median values.

Cl_p, systemic clearance (mL/kg/min); FF, free or unbound fraction of drug (%); $T_{1/2}el$, elimination half-life (min); V_{ss}, apparent volume of distribution at steady state (L/kg).

Note: Mean values are given except where indicated.

Etomidate

The dynamic properties of the carboxylated imidazole in patients with impaired cardiovascular function may be useful. The well-documented side effects of etomidate on the adrenal gland (to suppress steroidogenesis) are short-lived and would be of little relevance in transplant patients concurrently receiving a glucocorticoid for immunosuppression. There are no formal studies of the disposition of etomidate in patients with renal failure; several authors have reported a significant decrease in the plasma protein binding of etomidate in patients with uremia.[25,138]

Barbiturates

Although there is evidence for an increased sensitivity of patients in chronic renal failure to barbiturate drugs, thiopental is still used by some anesthesiologists for induction of anesthesia in patients undergoing transplantation. When given as an induction dose comparable to that used in healthy patients, thiopental induces prolonged unconsciousness. Dundee and Richards[60] showed that the duration of effect was related to the blood urea concentration. Various causes have been proposed, including increased blood-brain barrier permeability, increased free plasma barbiturate concentrations in uremic patients, qualitative plasma albumin abnormalities leading to decreased drug binding, and abnormal cerebral uptake and metabolism of the barbiturate.

Burch and Stanski[20] and Christensen and associates[33] formally investigated the disposition of thiopental in patients with chronic renal failure. Burch and Stanski[20] found an unaltered total drug elimination half-life, but an increased free drug fraction. There were no differences compared with healthy patients in unbound drug apparent volumes of distribution and systemic clearance. The increased free drug fraction results in higher brain concentrations of thiopental. If there is assumed to be no alteration in brain or cardiovascular sensitivity to thiopental in the patient with chronic renal failure, the rate of administration rather than the drug dose should be decreased during induction of anesthesia.[20]

This hypothesis is supported by the studies of Christensen and colleagues,[32,33] who have found no differences in the dose of thiopental (milligrams per kilogram) required to induce anesthesia successfully in healthy patients and in patients with renal failure; there also were no differences in arterial and venous drug concentrations at the point of hypnosis. This finding also suggests that there is no alteration in brain sensitivity to the thiobarbiturate. The kinetics of pentobarbital (an active metabolite of thiopental) are unaltered in patients with end-stage renal disease.[158]

Ketamine

Ketamine is probably best avoided for induction of anesthesia for transplantation because it causes increases in heart rate and blood pressure, which may be deleterious in a patient with preexisting hypertension or coronary arterial disease. In end-stage renal disease, elimination of the metabolites of ketamine (especially the active norketamine and the glucuronide conjugates) is reduced.[118]

There are reports of ketamine being used successfully as part of a total intravenous anesthetic technique for renal transplantation, in combination with fentanyl-droperidol, fentanyl-propofol, or remifentanil-propofol. There are, however, no dynamic or outcome data available against which to judge these techniques in renal transplantation.

Opioid Drugs

Renal failure has significant effects on the disposition, metabolism, and excretion of many opioid drugs. Most are biotransformed into inactive or less active compounds, which are excreted in the urine or bile (e.g., pethidine [meperidine], alfentanil, fentanyl, sufentanil, and morphine). Of particular interest to the anesthesiologist has been the introduction of the esterase-metabolized drug remifentanil, where the disposition and dynamics are not significantly altered by chronic renal impairment.

Morphine

Morphine is still the most widely used drug for the provision of perioperative and postoperative analgesia, but inappropriate dosing can result in important effects. There are many reports in the literature of prolonged or exaggerated clinical effects when morphine was given to patients with chronic renal failure. What is the basis of these observations? Olsen and colleagues[149] compared the plasma protein binding of morphine in healthy patients and in patients with renal failure and found an increased free drug fraction (from 65% to 70% to 75%) in the presence of uremia.

Morphine is metabolized primarily in the liver, where it undergoes glucuronidation to the 3-glucuronide (M3G) (the main metabolite, ≤50%) and the 6-glucuronide (M6G) (about 10%). Other metabolites in humans include *N*-demethylation to normorphine. Although M6G is a more potent analgesic than the parent drug, M3G has been shown in animal models to antagonize the dynamic properties of morphine and M6G.[182] Any change in the concentrations of these metabolites (or the parent drug) could have considerable dynamic sequelae. When the influence of end-stage renal disease on morphine disposition and metabolism has been examined in awake[1,169,205] and anesthetized patients,[31,175] most studies show renal failure to have little effect on morphine clearance, but to result in the accumulation of the various metabolites—M3G, M6G, and normorphine (Fig. 13-2 and Table 13-2). In patients undergoing transplantation who received 10 mg of morphine intravenously as a supplement to nitrous oxide–oxygen anesthesia, we found the elimination half-lives of the derived glucuronides M3G and M6G ranged from 300 to 920 minutes (M3G) and 220 to 900 minutes (M6G).[175] These estimates are prolonged compared with values of 100 to 200 minutes in healthy anesthetized patients. Loetsch and colleagues[127] determined the half-lives of M3G and M6G in healthy volunteers to be of the order of 2.8 to 3.2 hours (M3G) and 1.7 to 2.7 hours (M6G), whereas Hanna and coworkers[84] reported the half-lives of M6G when given by intravenous administration to patients with renal failure to be similar to the values reported in our study.

The longer half-lives of M3G and M6G (41 to 141 hours and 89 to 136 hours) reported by Osborne and colleagues[152,153] and Sawe and Odar-Cederlof[169] in patients with impaired renal function could be clinically important in the prolonged effect of the parent drug. These higher concentrations of M6G may account for the profound analgesia and sedation seen in the uremic patient who has received large doses of morphine or papaveretum.[153]

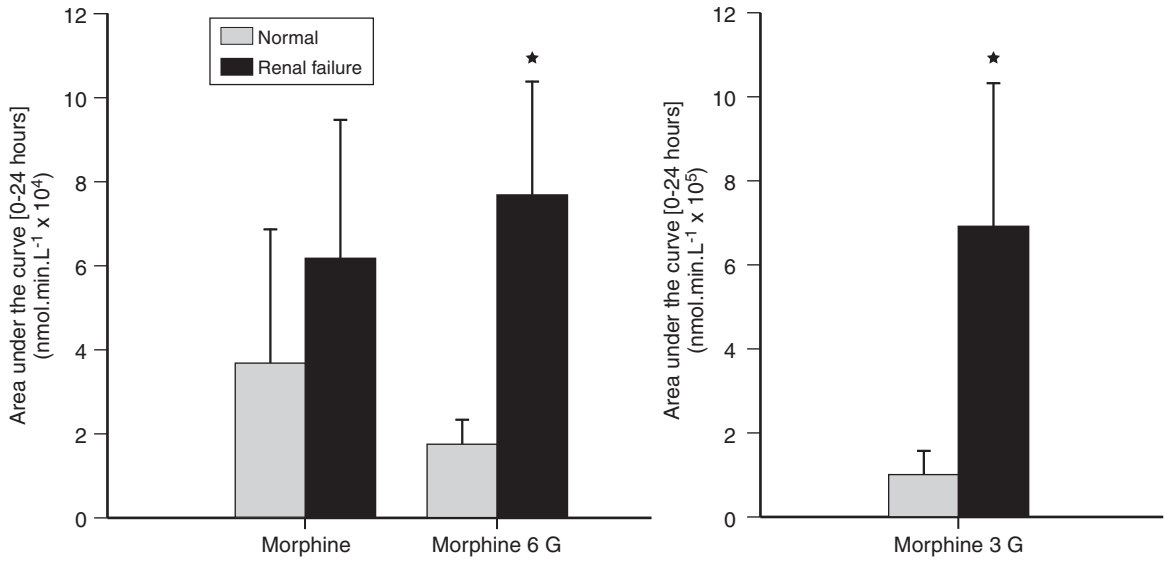

Figure 13–2 Areas under the concentration versus time curve for morphine and its two metabolites, morphine-6-glucuronide and morphine-3-glucuronide in 5 anesthetized healthy subjects with normal renal function (*gray columns*) and in 11 patients with renal failure receiving kidney grafts (*solid columns*) (*$P < .05$). (Adapted from Sear JW, Hand CW, Moore RA, et al: Studies on morphine disposition: influence of renal failure on the kinetics of morphine and its metabolites. Br J Anaesth 62:28, 1989.)

Although we would expect chronic renal impairment to result in larger areas under the concentration-time curve (AUCs) for M3G and M6G, our data[175] and those of Mazoit and coworkers[133] and Sloan and colleagues[181] also show greater AUCs for the parent drug morphine. These data suggest that the kidney itself may play a role in morphine disposition and metabolite elimination. The studies of Mazoit and Sloan suggest that approximately 30% to 35% morphine elimination may be by nonurinary excretion, nonhepatic degradation (i.e., potentially by renal parenchymal metabolism). Our data[175] and the data of Osborne and associates[152] offer another explanation: the increased plasma morphine concentrations (and larger AUCs) could have occurred by hydrolysis of one or another of the accumulating glucuronides (probably M3G) back to the parent compound. This would tend to reduce the AUC M3G-to-M6G ratio. In Osborne's study,[152] there was a mean value of about 5 in the healthy patients, and 3.9 and 4.5 in the groups of patients with end-stage renal disease. In our patients, similar ratios were found in the healthy anesthetized patients and in the patients undergoing renal transplantation (8 and 9).[175] Although we found no difference in the AUC M3G-to-M6G ratio between the healthy patients and patients with end-stage renal disease undergoing transplantation, this model for

Table 13–2 **Influence of Chronic Renal Failure on Disposition of Opioids in Anesthetized Patients**

	Patients with Normal Renal Function				Patients with Impaired Renal Function			
	$T_{1/2}el$	Cl_p	V_{ss}	FF	$T_{1/2}el$	Cl_p	V_{ss}	FF
Morphine: Chauvin et al[31]	186†	21.3	3.7	—	185†	17.1	2.8*	—
Sear et al[175]	307†	11.4	3.8	—	302†	9.6	2.4*	—
Osborne et al[152]	102†	27.3	3.2	—	120†	25.1	2.8*	—
Fentanyl: Duthie[61]	405†	14.8	7.7	—	594*	11.8	9.5	—
Sear and Hand[174]	175†	17.1	2.7	—	229†	18.5	3.6	—
Bower[14]	—	—	—	20.8	—	—	—	22.4
Koehntop and Rodman[116]	—	—	—	—	382†	7.5	3.1	—
Alfentanil: Chauvin et al[30]	90†	3.1	0.3	11	107†	3.1	0.4*	19*
Bower and Sear[15]	120†	3.2	0.4	10.3	142†	5.3*	0.6	12.4*
Sufentanil: Davis et al[51]	76†	12.8	1.3	—	90†	16.4	1.7	—
Sear[171]	195†	18.2	3.6	7.8	188†	19.2	3.8	8.6
Remifentanil: Hoke et al[95]	4.0	33.2	0.19	—	4.9	35.4	0.25	—
Dahaba et al[49]	16.4	46.3	0.57	—	18.9	28.0	0.36	—
Oxycodone: Kirvela et al[108]	138†	16.7	2.39	—	234†	12.7	3.99	—

*$P < .05$ versus healthy subjects.

†Mean residence time (rather than elimination half-life).

Cl_p, systemic clearance (mL/kg/min); FF, free or unbound fraction of drug (%); $T_{1/2}el$, elimination half-life (min); V_{ss}, apparent volume of distribution at steady state (L/kg).

Note: Mean values are given throughout except for oxycodone, for which medians are given.

studying drug disposition has limitations because the pharmacokinetics of morphine may be influenced by the onset of graft function.

Hasselstrom and Sawe[87] showed that renal clearance of morphine and M6G exceeds creatinine clearance, suggesting there may be an active secretion process in the kidney. The relationship between creatinine clearance and the renal clearances of morphine, M3G, and M6G also has been studied by Milne and colleagues[139] in intensive care unit patients with variable degrees of renal impairment. For all three compounds, there was a linear relationship between free drug clearance and creatinine clearance. The unbound clearance of morphine exceeded that of creatinine, whereas the clearances of M3G and M6G were similar. The ratios of the plasma concentrations of M3G to morphine and M6G to morphine ranged from 4 to 170, and 0.79 to 51. Similar values have been reported by Petersen and colleagues[154] in terminal cancer patients with impaired renal function receiving subcutaneous morphine. The mean plasma concentration ratio of M3G to M6G was 5 (similar to the ratios of AUCs seen in the study of Osborne and associates[152]). The unbound fractions for morphine, M3G, and M6G were 74%, 85%, and 89%, respectively; the first figure was significantly greater than that determined by Olsen and colleagues.[149]

Can we relate these kinetic changes to the preoperative renal status of the transplant recipient? Sawe and Odar-Cederlof[169] showed a significant correlation between the M3G half-life and the plasma urea concentration. Although we did not find a significant correlation between the AUCs of M3G and M6G and the immediate postoperative 24-hour creatinine clearance in the patients undergoing transplantation, there was an association between the creatinine clearance and the elimination half-life of the two glucuronide metabolites ($r = .87$ and $r = .63$; $P < .01$ and $P < .05$). There was no relationship between creatinine clearance and morphine clearance.

MORPHINE BY INFUSION OR AS PATIENT-CONTROLLED ANALGESIA

When infusions of morphine are administered to patients with impaired renal function, there is accumulation of M6G to give the clinical picture of a persistently narcotized patient.[88,153,179] The importance of M6G also can be seen in the case reported by Covington and colleagues,[44] in which severe respiratory depression was observed in a patient with end-stage renal disease receiving morphine patient-controlled analgesia (PCA) for postcholecystectomy pain; the blood morphine concentration was within the therapeutic range, but the M6G level was significantly elevated. Similar data have been described by Carr and associates,[26] in which the PCA dose requirements after cadaver renal transplantation ranged from 3 to 4.7 mg/hr compared with 4.6 to 23.6 mg/hr in patients with normal renal function undergoing lower abdominal surgery. As might be expected, the former group showed considerably greater AUCs for M6G.

D'Honneur and colleagues[58] have studied the transfer of morphine and its metabolites across the blood-brain barrier. Fourteen patients (six with end-stage renal disease) received a single oral dose of morphine before the onset of continuous spinal anesthesia for peripheral vascular or orthopaedic surgery. Plasma concentrations of morphine, M3G, and M6G were greater in the renal failure patients, but only the glucuronide concentrations (not the parent drug) were greater in the cerebrospinal fluid of the renal patients. This study did not address the key issue, however, of whether the higher cerebrospinal fluid concentrations of M6G were associated with greater respiratory depression, or sedation, or prolonged analgesia.

There is no question regarding the analgesic and central nervous system depressive effects of M6G, but its effect on respiration is more uncertain. A reduction in M6G binding at the μ_2 receptor may be one reason why the effect of this metabolite on respiration varies. Although M6G crosses the blood-brain barrier slowly, once in the central nervous system, its effects can be dramatic and prolonged.[4] M6G may exist in two forms—an extended hydrophilic molecule and a folded, more lipophilic compound, which remains in the fatty tissues of the brain. The latter configuration may explain why after discontinuation of morphine dosing or dialysis, the central nervous system effects of the drug persist for long periods, as the M6G only slowly re-equilibrates back across the blood-brain barrier into the systemic circulation.

INFLUENCE OF RENAL FAILURE ON OTHER MORPHINOIDS

Similar alterations in the disposition and dynamics of codeine, dihydrocodeine, and propoxyphene (with active metabolite accumulation) have been observed in patients with renal failure.[7,72,80] These drugs are best avoided for postoperative pain relief. Whether these altered kinetics of morphine and its congeners are the sole explanation for their prolonged dynamic effects is uncertain. Uremia is itself associated with central nervous system depression; the increased sensitivity to central nervous system depressant drugs also may be due to increased receptor responsiveness or increased meningeal or cerebral permeability.

Fentanyl, Alfentanil, and Sufentanil

Because of the exaggerated dynamic effects of morphine and its metabolite M6G, many anesthesiologists prefer to provide intraoperative analgesia with drugs of the phenylpiperidine type. Only a small fraction of each of the three main drugs (fentanyl, alfentanil, and sufentanil) is excreted by the kidney unchanged, and their metabolites are inactive.

The disposition of fentanyl was first studied in awake patients with end-stage renal failure by Corall and coworkers,[42] who showed an increased clearance. Other studies confirm these findings, with all showing wide interindividual variability in kinetic parameters and no differences in the disposition of fentanyl in renal transplant patients compared with comparable patients undergoing lower abdominal surgery.[61,116,174] Although Koehntop and Rodman[116] found an inverse relationship between the degree of azotemia and fentanyl clearance, Bower[14] and Sear and Hand[174] showed no alteration in fentanyl binding in patients with uremia, and no relationship between preoperative creatinine or urea and the disposition parameters of fentanyl.

Similarly, chronic renal failure has no effect on drug binding to plasma proteins or disposition of sufentanil.[51,70,171] There are case reports of prolonged narcosis after administration of sufentanil to patients with chronic renal failure. These cases are probably due to alterations in the dynamics of the opioid in the uremic patient.[70,203] Studies examining the disposition of alfentanil in anesthetized patients with chronic renal failure showed an increased free drug fraction, together with greater total drug clearance

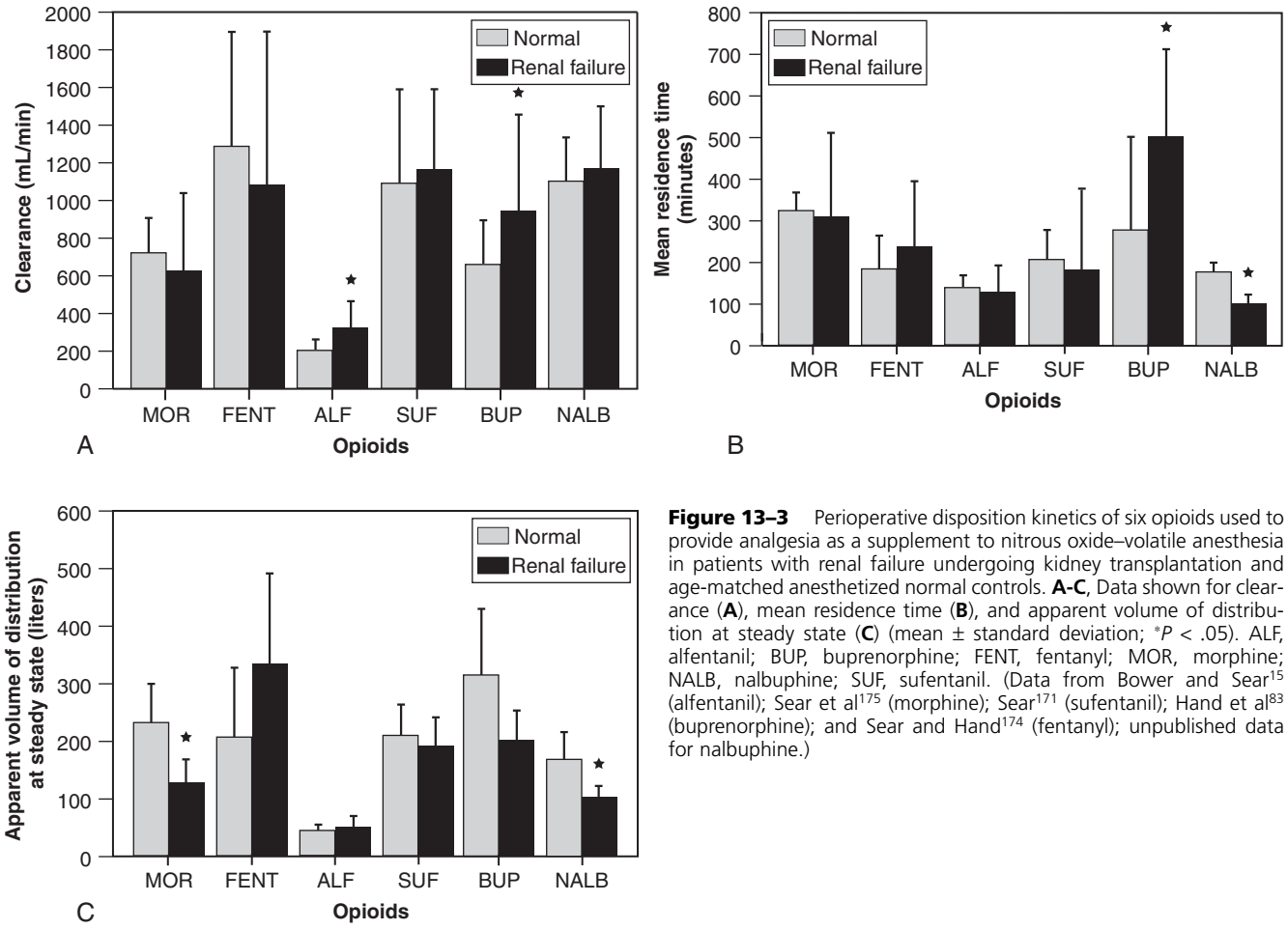

Figure 13–3 Perioperative disposition kinetics of six opioids used to provide analgesia as a supplement to nitrous oxide–volatile anesthesia in patients with renal failure undergoing kidney transplantation and age-matched anesthetized normal controls. **A-C**, Data shown for clearance (**A**), mean residence time (**B**), and apparent volume of distribution at steady state (**C**) (mean ± standard deviation; $^{*}P < .05$). ALF, alfentanil; BUP, buprenorphine; FENT, fentanyl; MOR, morphine; NALB, nalbuphine; SUF, sufentanil. (Data from Bower and Sear[15] (alfentanil); Sear et al[175] (morphine); Sear[171] (sufentanil); Hand et al[83] (buprenorphine); and Sear and Hand[174] (fentanyl); unpublished data for nalbuphine.)

rates and volumes of distribution. There were no differences, however, in the free drug apparent volume of distribution or clearance.[15,30]

Figure 13-3 summarizes data from our own studies examining the perioperative disposition of morphine, the three phenylpiperidine drugs, and buprenorphine and nalbuphine when given to provide analgesia during surgery for renal transplantation in patients receiving a balanced anesthetic technique with controlled ventilation to normocapnia. The main kinetic changes are the increased total drug clearances of alfentanil and buprenorphine and the longer mean residence time for buprenorphine.

Remifentanil

Although remifentanil also is a piperidine derivative, its elimination does not depend on either hepatic metabolism or renal elimination, but rather plasma and tissue nonspecific esterase hydrolysis. In healthy individuals, remifentanil clearance is high (25 to 45 mL/kg/min), and its major metabolite (GI 90291) has only minimal analgesic activity.

The kinetics of remifentanil are unaltered in awake patients with end-stage renal disease.[95] Because of its short half-life (4 to 9 minutes), the drug is best given by continuous infusion; it also has a short context-sensitive half-time (3 minutes) after prolonged periods of infusion—implying that there would be a rapid offset of its analgesic and respiratory depressant effects at the end of a surgical procedure. The dynamics of remifentanil were unaffected in renal failure patients.

In anesthetized patients with renal failure undergoing fistula surgery, Dahaba and associates[49] found no alteration of the distribution half-life of remifentanil, but significantly smaller estimates for clearance and a longer elimination half-life. There were higher blood concentrations in the renal patients. Hoke and colleagues[95] also showed that the main metabolite had a longer terminal half-life and reduced clearance in patients with renal failure. The main differences between Dahaba's study and the earlier publication of Hoke and colleagues[95] relate to the influence of anesthesia in the study of Dahaba and associates[49] and the preoperative hemodialysis of Dahaba's patients compared with awake subjects and predialysis hypervolemic patients in the Hoke study. In a separate study in which remifentanil was infused to intensive care unit patients with renal impairment, Breen

and colleagues[17] found no prolongation of the dynamic effects of the opioid, even after a 72-hour continuous infusion, when the patients with renal failure were compared with other intensive care unit patients who had normal renal function.

Pethidine (Meperidine)

There are few kinetic data on the disposition of pethidine in patients with renal failure. The drug is mainly metabolized in the liver, with only 1% to 5% excreted unchanged in the urine. Chan and coworkers[28] showed that the systemic clearance of pethidine depends on renal function, with accompanying reduced excretion of the metabolite norpethidine. Burgess and colleagues[21] assessed the dynamics of pethidine in patients with end-stage renal disease. They found that the renal failure group had a reduced ventilatory response to carbon dioxide, but subcutaneous administration of 1 mg/kg of pethidine did not exaggerate the effect. Whether this observation is transferable from the laboratory to the ward scenario remains untested.

As with morphine, Szeto and colleagues[189] found that when repeated doses of pethidine are given to patients in chronic renal failure, the *N*-demethylated metabolite, norpethidine, accumulates. This compound is about half as potent as an analgesic, but has greater convulsant activity than the parent drug. Szeto and colleagues,[189] Armstrong and Bersten,[5] and Hassan and associates[86] all have reported patients in renal failure in whom increased plasma ratios of norpethidine to pethidine were associated with excitatory signs. Hemodialysis provides a suitable method of treatment, with a plasma meperidine clearance of 50 mL/min and an average reduction in the normeperidine concentrations of 26% over 3 hours of dialysis.[86]

Other Intraoperative Opioids

The kinetics and dynamics of buprenorphine and oxycodone have been studied in patients undergoing renal transplantation. Hand and coworkers[83] found that renal impairment had little effect on parent drug kinetics of buprenorphine, but there were significant increases in the plasma concentrations of two metabolites (buprenorphine-3-glucuronide and norbuprenorphine). There was no evidence, however, that the latter resulted in any prolonged drug action.

Oxycodone disposition has been studied in healthy anesthetized patients and patients undergoing cadaver transplantation. Kirvela and coworkers[108] found a prolonged elimination half-life owing to a reduction in clearance and an increase in the volume of drug distribution. There also were higher plasma concentrations of the metabolite noroxycodone. The authors did not comment on any dynamic consequences of their findings, however.

Neuromuscular Relaxant Drugs

The neuromuscular blocking drugs are a group of ionized, water-soluble compounds that are freely filtered at the glomerulus. Most relaxants have low (<50%) plasma protein binding, and changes in plasma albumin concentrations (which may occur in patients with end-stage renal disease) are unlikely to affect the drugs' disposition. If the drug is normally excreted unchanged via the kidney, however, the kinetics and dynamics would be altered in patients with renal failure. Table 13-3 lists the extent of urinary excretion in the elimination of the various muscle relaxants.

Table 13–3 **Renal Excretion of Neuromuscular Blocking Drugs**

Quaternary Amines	
Suxamethonium	<10%
Gallamine	>95%
Benzylisoquinolinium Compounds	
Tubocurarine	31-45%
Methyltubocurarine	42-52%
Atracurium	10%
Doxacurium	25-30%
Mivacurium	<10%
Cisatracurium	?
Aminosteroid Compounds	
Pancuronium	35-50%
Vecuronium	15-20%
Pipercuronium	38%
Rocuronium	9%

Note: Expressed as a mean percentage (or range) of total drug elimination.

Depolarizing Neuromuscular Relaxants

Because potassium homeostasis is altered in patients with renal failure, concerns have been raised over the use of suxamethonium and the possibility of exaggerated hyperkalemic responses leading to adverse cardiac effects. Way and colleagues[200] showed the increase in potassium in patients on hemodialysis to be comparable with that seen in normal healthy subjects, however. Koide and Waud[117] observed no difficulties with the use of the drug as long as the plasma potassium was less than 5.5 mmol/L. Numerous case reports, case series, and controlled studies[191] suggest that suxamethonium can be used safely for rapid-sequence intubation as long as there is no associated uremic neuropathy or preoperative hyperkalemia. Repeated doses of suxamethonium are best avoided, however.

To minimize the increase in plasma potassium levels seen after suxamethonium, numerous pretreatments have been evaluated, including predosing with a nondepolarizing neuromuscular blocking agent, benzodiazepines (e.g., flunitrazepam, diazepam), or magnesium sulfate. Only the studies of Koide and Waud[117] and Radnay and coworkers[157] assessed the efficacy of these pretreatments in patients with renal failure. Koide and Waud[117] found that tubocurarine did not prevent the increase in potassium. Radnay and coworkers[157] showed that hexafluorenium prevented the increase in potassium; however, this latter compound is no longer available for clinical use.

There is a further aspect to the hyperkalemic response. In chronic renal failure (but *not* in acute renal failure), there are adaptive changes in the kidneys and the gut to prevent hyperkalemia. Despite the patient with chronic renal failure having a chronically increased extracellular potassium level (E_{K+}), the intracellular concentration (I_{K+}) also is increased. As a result, the ratio (I_{K+}) to (E_{K+}) is unaltered. Depolarization of the cardiac cell membrane with resulting cardiac arrhythmias would occur only with a change in the intracellular-to-extracellular potassium ratio. It is probably safe to use suxamethonium for intubation in the presence of a clinically increased potassium. In a patient with chronic renal failure with preoperative hyperkalemia (>6 mmol/L), there is less supportive evidence, however, because a small increase in the

potassium level may trigger an arrhythmia. Although nondepolarizing neuromuscular blocking drugs may prevent the onset of muscle fasciculations, they do not block the increase in plasma potassium concentrations.

For a rapid-sequence intubation in renal transplant recipients, *in the absence of hyperkalemia,* suxamethonium remains the drug of choice, although large doses of some nondepolarizing drugs (especially atracurium, cisatracurium, mivacurium, and rocuronium) offer an alternative in patients with hyperkalemia. A further issue with the use of suxamethonium relates to decreased activity of the enzyme pseudocholinesterase in patients being treated for renal failure by hemodialysis; however, this does not seem to be a significant problem with current hemodialysis techniques.

Nondepolarizing or Competitive Neuromuscular Relaxants

Nondepolarizing relaxants can be broadly divided into agents showing significant alteration in the kinetics and duration of effect in end-stage renal disease patients (and not useful in the anesthetic management of transplant recipients), and agents for which renal failure has little effect on the drug's dynamics.

DRUGS SHOWING SIGNIFICANT ALTERATIONS IN PHARMACODYNAMICS IN RENAL DISEASE

Many authors have reported kinetic and dynamic interactions between chronic renal failure and tubocurarine, d-methyl tubocurarine, gallamine, and pancuronium. The altered dynamics of these agents relates to significant renal excretion for their elimination, and these drugs should no longer be used for neuromuscular blockade in renal transplant recipients. Pipecuronium and doxacurium are newer neuromuscular blockers that are excreted mainly unchanged by the kidney, and renal failure causes a prolonged elimination half-life and reduced clearance.[23,24,27,39,68] With other relaxants now widely available, there is no place for any of these drugs as part of the anesthetic technique for renal transplantation.

DRUGS MOST SUITED FOR USE IN PATIENTS UNDERGOING RENAL TRANSPLANTATION

Atracurium. Initial clinical studies by Hunter and colleagues[97] found no difference in the duration of neuromuscular blockade from an initial dose of atracurium or from repeated doses when the drug was administered to patients with normal function compared with patients who were anephric. This finding was confirmed by the dynamic-kinetic studies of Fahey and associates[66] and De Bros and coworkers,[54] who showed that onset time, duration of action, recovery time (from 25% to 75% initial twitch height), and disposition kinetics were unaltered in patients with renal failure (Table 13-4). Another study by Hunter and colleagues[98] compared the properties of atracurium, vecuronium, and tubocurarine in healthy patients and in patients with renal failure. After bolus dosing, atracurium and vecuronium were little affected by renal failure, but tubocurarine was longer acting and less predictable, and inappropriate for use in these patients.

Atracurium may be administered by bolus dosing or continuous infusion to maintain neuromuscular blockade. Even after prolonged infusion, rapid recovery has been reported in patients with renal failure,[166] although Nguyen and colleagues[145] showed a prolonged recovery rate and longer time to 90% recovery of twitch height when administering atracurium by infusion to anephric patients anesthetized with nitrous oxide and increments of fentanyl. The breakdown of atracurium is by Hoffmann degradation and ester hydrolysis, although Fisher and colleagues[67] showed that 50% of total systemic clearance cannot be accounted for by either of these mechanisms.

An important metabolite of atracurium is laudanosine. Laudanosine is normally eliminated in the urine. When administered intravenously in high doses to animals, laudanosine has been reported to cause excitatory electroencephalogram activity. When given to nephrectomized cats, these electroencephalogram changes of nonepileptiform spike activity were seen only when the plasma concentrations were 8 to 10 times those observed in patients during continuous infusions of atracurium.[100] Ward and coworkers[197] investigated the relationship between renal function and plasma concentrations of laudanosine. After single doses of atracurium (0.3 mg/kg), there were no effects of renal failure on the disposition of atracurium or its two metabolites, laudanosine and the associated monoquaternary alcohol. Peak laudanosine concentrations were not significantly different in healthy patients and patients with renal failure.

Table 13–4 **Disposition of Neuromuscular Blocking Drugs in Patients with Chronic Renal Failure**

	Patients with Normal Renal Function			Patients with Impaired Renal Function		
	$T_{1/2}el$	Cl_p	V_{ss}	$T_{1/2}el$	Cl_p	V_{ss}
Tubocurarine: Sheiner et al[178]	84	2.4	0.25	132*	1.5*	0.25
Pancuronium: McLeod et al[136]	104	1.8	0.34	489*	0.3*	0.24
Atracurium: Fahey et al[66]	21	6.1	0.19	24	6.7	0.26
De Bros et al[54]	17	5.9	0.14	21	6.9	0.21
Vecuronium: Lynam et al[129]	53	5.3	0.20	83*	3.2*	0.24
Cisatracurium: Eastwood et al[62]	30	4.2	—	34	3.8	—
Mivacurium: Head-Rapson et al[89] Cis-cis	68	3.8	0.23	80	2.4*	0.24
Cis-trans	2	106	0.28	4.3	80	0.48
Trans-trans	2.3	57	0.21	4.2	47	0.27
Rocuronium: Szenohradszky et al[188]	71	2.9	0.26	97	2.9	0.21
Cooper et al[41]	104	3.7	0.21	97	2.5*	0.21

*$P < .05$.
Cl_p, systemic clearance (mL/kg/min); $T_{1/2}el$, elimination half-life (min); V_{ss}, apparent volume of distribution at steady state (L/kg).
Note: Mean values are given.

Other studies by Fahey and colleagues[65,66] have observed higher plasma laudanosine concentrations in cadaver transplant recipients, however, compared with healthy anesthetized subjects after larger doses of atracurium. In both of these studies and in the study by LePage and associates,[121] peak plasma laudanosine concentrations were considerably lower than the concentrations associated with electroencephalogram excitation in the anesthetized dog.[29] Although elimination of laudanosine is principally via the kidney, there is some evidence in humans that other organs (e.g., the liver) may be involved in its elimination.

Vecuronium. Lynam and coworkers[129] studied the kinetics of 0.1 mg/kg of vecuronium in patients receiving cadaver renal allografts and a control group of healthy patients, where anesthesia was maintained with nitrous oxide in oxygen and 1% end-tidal isoflurane. There were no significant effects on the elimination half-life or systemic clearance of vecuronium, but the duration of neuromuscular blockade and recovery from blockade were significantly prolonged in the renal failure group. Several other studies also have suggested the accumulation of vecuronium in patients with renal failure.[12,34,122,184] Because of the clinical importance of any dynamic interaction with renal failure, Beauvoir and colleagues[11] conducted a meta-analysis of the available data. Based on six studies, they found renal failure to cause a significant increase in the duration of effect (measured as the time from injection to 25% recovery of twitch height), but no effect on the onset time, or the 25% to 75% recovery time. Part of the explanation for these dynamic effects may lie in the biotransformation of vecuronium. It is metabolized by hepatic hydrolysis to yield three desacetyl metabolites—3-desacetyl vecuronium, 17-desacetyl vecuronium, and 3,17-desacetyl vecuronium. The first of these is estimated to have the potency of about 80% of the parent drug, and there is good evidence that 3-desacetyl vecuronium accumulates in patients with renal failure.[177] A more recent study by Sakamoto and associates[167] has confirmed that the duration of action of vecuronium is prolonged in patients with end-stage renal disease mainly as a result of a higher sensitivity to the drug (rather than the result of kinetics alterations).

OTHER FACTORS AFFECTING DURATION OF NEUROMUSCULAR BLOCKADE

Potentiation of neuromuscular blockade may occur in patients with metabolic acidosis; the acidosis also opposes the reversal by neostigmine. In the uremic patient undergoing transplant surgery, potentiation of blockade also may occur secondary to hypokalemia, hypocalcemia, hypermagnesemia, parenteral or topical use of some aminoglycoside antibiotics, furosemide, mannitol, and methylprednisolone. Caution should be exercised to ensure complete return of neuromuscular function if multiple increments or infusions of the drug are used during prolonged surgery in anephric patients.

Newer Neuromuscular Blocking Drugs

There are three new neuromuscular blocking agents that show differing disposition profiles in patients with end-stage renal failure (see Table 13-4).

MIVACURIUM

Mivacurium is a short-acting benzylisoquinolinium that is metabolized by plasma esterases and presumably also in the liver. In healthy subjects, De Bros and colleagues[53] showed an elimination half-life of 17 minutes and a high clearance (54 mL/kg/min). Early studies suggested that the kinetics and duration of effect of mivacurium were not prolonged in renal failure.[40]

Similar to atracurium, mivacurium is formulated as a number of stereoisomers (cis-trans, 37%; trans-trans, 57%; and the less active cis-cis, 6%). Phillips and Hunter[155] showed a prolonged duration of action of mivacurium in renal failure patients compared with patients with normal renal function. In another study, Head-Rapson and colleagues[89] examined the kinetics and dynamics of the isomers in anesthetized patients with renal failure. Although clearance of the cis-cis isomer was significantly reduced in renal failure, the disposition of the other two isomers was not. The clearance of each isomer correlated significantly with plasma cholinesterase activity. The median infusion rate required to achieve a common level of neuromuscular blockade (T1/T0: 10%) was similar in patients with renal failure compared with healthy subjects.

CISATRACURIUM

Cisatracurium is one of the ten stereoisomers of atracurium and has the advantage of being three times more potent and releasing less histamine in animals. In contrast to the parent compound, its metabolism is mainly by Hoffmann degradation with no ester hydrolysis. Studies by Kisor and associates[113] confirm that the Hoffmann pathway accounts for about 77% of total body clearance, 23% of organ clearance, and 16% of renal clearance. The drug has an elimination half-life of 23 minutes and clearance in healthy subjects of 5.2 mL/kg/ min. The main metabolites are laudanosine and a monoquaternary acrylate.

Two more recent studies have examined the dynamics and disposition of cisatracurium in renal failure. Boyd and coworkers[16] found that at a dose of $2 \times ED_{95}$ (0.1 mg/kg), onset times were longer in the renal failure group, but recovery was not affected. The clearance of cisatracurium was decreased by 13%, and the half-life was longer (34.2 minutes versus 30 minutes). Although plasma concentrations of laudanosine were elevated in patients with renal failure, the peak values were about one tenth of the values seen after atracurium.[62] In the only study directly comparing these relaxants, Jirasiritham and colleagues[101] found no differences in the hemodynamic responses to anesthesia and surgery in renal failure patients receiving atracurium or cisatracurium as the neuromuscular blocking agent.

ROCURONIUM

Rocuronium has a rapid onset of effect and intermediate duration of action and may be an alternative to suxamethonium for rapid-sequence intubation. Being a steroid molecule, it is primarily metabolized in the liver, with only 9% of the injected dose being recovered unchanged in the urine. In a comparison of its kinetics and dynamics in healthy anesthetized subjects and patients undergoing cadaver renal transplantation, Szenohradszky and colleagues[188] found that renal failure altered drug distribution, but not systemic clearance. Cooper and colleagues[41] found a decreased clearance of the relaxant in patients with renal failure during isoflurane anesthesia.

Several studies have examined the dynamics of rocuronium in patients with chronic renal failure, with differing outcomes.

In an initial study with the drug, Khuenl-Brady and colleagues[106] found no differences in onset, duration of effect, or recovery after doses of 0.6 mg/kg and three maintenance doses of 0.15 mg/kg during isoflurane anesthesia. There also were no significant differences in drug dynamics in the study of Cooper and colleagues.[41] More recently, Robertson and colleagues[164,165] investigated the dynamics and kinetics of the relaxant during propofol infusion anesthesia. After a single dose of 0.6 mg/kg, renal failure had no effect on the onset of neuromuscular block, but was associated with a prolonged duration of effect. This finding can be explained by a decrease of 39% in drug clearance in renal failure patients, coupled with an 84% prolongation of the mean residence time. When administered in a smaller dose of 0.3 mg/kg, however, there were no kinetic or dynamic differences.

In a single-dose comparison of vecuronium, atracurium, cisatracurium, and rocuronium in healthy controls and in patients undergoing renal transplantation, there were no significant differences within patient groups of onset time or duration of action; however, the recovery index was slower in the renal patients for all four neuromuscular blocking drugs. There also was a prolonged duration of effect after repeat doses of rocuronium and vecuronium in the renal patients.[57] Despite the chemical similarity of rocuronium to vecuronium, and the observations of Della Rocca and associates,[57] rocuronium has a possible role in providing neuromuscular blockade for patients with renal impairment, although most anesthesiologists would currently favor atracurium or cisatracurium as the drug of choice.

Anticholinesterases

All anticholinesterases are excreted through the kidney by glomerular filtration and tubular secretion. The pharmacokinetics of neostigmine, pyridostigmine, and edrophonium have been studied in patients with chronic renal failure by Cronnelly and Morris.[47] Significant decreases in the clearance of anticholinesterases are seen in anephric patients, although pharmacokinetics parameters similar to those in patients with normal renal function can be shown in patients to whom the drugs are given approximately 1 hour after receiving a living related renal transplant (Table 13-5). Because a greater percentage (75%) of an intravenous dose is excreted by the kidney, the terminal half-life of pyridostigmine is more prolonged than that of neostigmine in patients with renal failure.

A more recent development to the reversal of neuromuscular blockade has been the introduction of ORG 25969 (Sugammadex).[73] This is a cyclodextrin molecule specially designed to bind rocuronium and bring about the reversal of neuromuscular blockade by a physicochemical interaction. To our knowledge, there are no available published data on its use in renal transplant recipients, but it could offer an important pharmacological advance in the conduct of renal transplant anesthesia.

Inhalational Anesthetic Agents

All volatile anesthetic agents are, to some extent, myocardial depressants and may reduce the cardiac output and blood flow to the transplanted kidney. Some agents (particularly enflurane and sevoflurane) are biotransformed in the liver, resulting in increased serum levels of inorganic fluoride. This ion can lead to the development of high output renal failure.

When used to provide anesthesia for patients undergoing living related donor renal transplantation, Wickstrom[202] observed that administration of 2.4 MAC-hour enflurane (mean duration 189 minutes) caused a peak fluoride concentration of 21 μmol/L (MAC-hour is the product of minimum alveolar concentration of a volatile anesthetic agent and time). In 1 of 10 patients, the serum fluoride concentration increased significantly, however, to 40 μmol/L. There also has been a case report of deterioration in renal transplant function when enflurane was given to provide anesthesia for vascular access surgery.[126] Enflurane should not be used to supplement anesthesia for renal transplantation. In contrast, its isomer isoflurane undergoes only limited biotransformation to inorganic fluoride and is one of the agents of choice for a balanced anesthetic technique.[76,135]

The two newer volatile agents (desflurane and sevoflurane) differ in their molecular stability and extent of biotransformation. Desflurane does not undergo breakdown either by the liver or by contact with soda lime, and after 1 MAC-hours anesthesia, the increase in inorganic fluoride is less than 1 μmol/L. Desflurane also has no deleterious effect on routine laboratory tests of renal function when given to patients with chronic renal disease.[63,125,207]

Sevoflurane is less stable, with about 3% of the absorbed dose undergoing hepatic biotransformation. After prolonged anesthesia of an average 13.4 hours to patients with normal renal function, peak serum fluoride concentrations of 42.5 μmol/L have been reported—with 5 of 10 patients exceeding the assumed nephrotoxic threshold of 50 μmol/L.[114]

Table 13–5 **Influence of Renal Disease on Disposition of Anticholinesterases**

	Subjects with Normal Renal Function			Anephric Patients			Living Related Donor Renal Transplants			Renal Fraction of Total Clearance (%)
	$T_{1/2}$el	Cl_p	V_{ss}	$T_{1/2}$el	Cl_p	V_{ss}	$T_{1/2}$el	Cl_p	V_{ss}	
Neostigmine	80	9	0.7	183*	3.4*	0.8	104	9.4	1	54
Pyridostigmine	112	8.6	1.1	379*	2.1*	1	83	10.8	1	76
Edrophonium	110	9.6	1.1	206*	2.7*	0.7	87	9.9	0.9	66

*$P < .05$ versus transplant patients and subjects with normal renal function.
Cl_p, systemic clearance (mL/kg/min); $T_{1/2}$el, elimination half-life (min); V_{ss}, apparent volume of distribution at steady state (L/kg).
Adapted from Cronnelly R, Morris RB: Antagonism of neuromuscular blockade. Br J Anaesth 54:183, 1982.

There were no cases of gross renal dysfunction, however. Similarly, Higuchi and colleagues[92] found no evidence of any impairment in urinary concentrating ability to antidiuretic hormone administration after 10.6 MAC-hours sevoflurane anesthesia despite a mean plasma fluoride concentration of 41.9 μmol/L, and 1 of 11 patients having a plasma fluoride concentration greater than 50 μmol/L.

Subsequent studies by Kharasch and colleagues[105] have shown that it is the increased systemic fluoride concentration that causes nephrotoxicity. Neither the peak concentration nor the duration of the fluoride concentration increase alone is sufficient, however, to predict the occurrence of renal damage. Rather the site of the fluoride production is important. For sevoflurane, this site is extrarenal. When administered to patients with end-stage renal disease, sevoflurane (1% to 2.5%) supplementing nitrous oxide–oxygen anesthesia caused higher postoperative levels of blood and urea creatinine and serum and urinary β_2-microglobulins.[146] There were no differences, however, between these patients and controls in serum fluoride levels, the rate of elimination, or AUC fluoride-time. The patients with renal failure, however, had lower urinary fluoride concentrations compared with the healthy controls. Similar data have been found in studies in which low-flow sevoflurane and isoflurane have been compared in patients with impaired renal function.[37,91]

In another study in patients with chronically impaired renal function, peak serum fluoride concentrations were significantly higher after sevoflurane administration compared with enflurane (25 μmol/L versus 13.3 μmol/L), but no permanent deterioration of preexisting renal insufficiency was observed.[38] Goldberg and associates[76] examined inorganic fluoride concentrations in patients receiving isoflurane or sevoflurane anesthesia. The latter group contained three patients in whom the fluoride concentration exceeded 50 μmol/L, and two who had increased postoperative plasma urea and creatinine concentrations at 24 hours postoperatively. There was no evidence that sevoflurane caused further deterioration of renal function.

Sevoflurane also is degraded to a vinyl metabolite (compound A) when administered by low flow with a carbon dioxide absorber containing either Baralyme or to a lesser extent soda lime. Reductions in anesthetic fresh gas flow and an increase in temperature would result in increased compound A concentrations. Although this metabolite is nephrotoxic in rats, there is no evidence to support nephrotoxicity in humans.[71,104,134] The safety of sevoflurane in patients with renal impairment is unclear. Finally, no renal toxicity has been reported after inhalation of desflurane.[63]

CHOICE OF ANESTHETIC TECHNIQUE AND OUTCOME FOR RENAL TRANSPLANTATION

Although use of a balanced anesthetic technique (opioid with volatile supplementation) is the method of choice for renal transplantation, some authors have described other approaches.

Regional Techniques for Transplantation

When Vandam and colleagues[193] described the use of regional anesthetic techniques for renal transplantation, it was to avoid the complications of general anesthesia in uremic patients; however, this is no longer a major problem with modern dialysis techniques. In a subsequent review of regional anesthesia, Linke and Merin[124] cited its advantages as the avoidance of neuromuscular blocking drugs and endotracheal intubation, the reduced likelihood of regurgitation and pulmonary inhalation by a patient with a full stomach, and the provision of a pain-free awake postoperative patient.

In chronic renal failure, the onset of sensory analgesia occurs faster after subarachnoid blockade because of the combined effects of the metabolic acidosis causing a greater degree of ionization and a reduction in the volume of the epidural space secondary to distention of the epidural and spinal veins by a hyperdynamic circulation. The duration of sensory and motor blockades was shorter (20%) in the patients with renal failure because the increased cardiac output in these patients resulted in a faster washout of the local anesthetic from its site of action.[151,156]

There is concern over the possibility of extradural hematoma formation in patients with a disordered coagulation system. Basta and Sloan[8] reported the first case of an epidural hematoma in a patient with chronic renal failure about 60 hours after catheter placement. Other possible complications include difficulty in handling major blood loss in a vasodilated patient, an unpredictable response of a hypertensive renal patient on drug therapies to vasopressors, the maintenance of an awake patient's well-being during a long procedure, and the medicolegal complexity of a postoperative peripheral neuropathy.

Comparison of Different General Anesthetic Techniques

Other approaches used for renal transplant anesthesia include neuroleptanesthesia[123] and total intravenous techniques (e.g., the combination of propofol and alfentanil,[112] and ketamine supplemented by infusions of fentanyl-droperidol, fentanyl-propofol, or remifentanil-propofol). Studies have compared the different volatile agents as supplementation and have compared total intravenous and regional anesthesia. All volatile agents cause a dose-related decrease in mean arterial pressure. When halothane, enflurane, and isoflurane have been used as volatile supplementation in patients undergoing living related donor renal transplantation, they have been shown to have no influence on postoperative renal function.[48] Administration of a fluid challenge of 1000 mL 0.154M sodium chloride resulted in similar increases of arterial and central venous pressures, regardless of the choice of anesthetic agent.[45,46]

Patients with impaired renal function may develop cardiac dysrhythmias secondary to alterations in plasma electrolyte concentrations. There is an additional risk of acute hemodynamic changes occurring during transplantation owing to the release of catecholamines and renin from the revascularized kidney.[69] The effects of endogenous catecholamines may result in the development of ventricular dysrhythmias. Neither isoflurane nor desflurane significantly sensitizes the myocardium to these amines, and isoflurane and desflurane are the volatile agents of choice as the supplement to a nitrous oxide in oxygen-opioid anesthetic for renal transplantation.

Outcome studies have compared epidural and nitrous oxide–isoflurane anesthesia,[2] isoflurane versus desflurane anesthesia,[125] fentanyl-isoflurane versus propofol-alfentanil[6] or propofol-remifentanil total intravenous techniques,[140]

and combined spinal-epidural versus general anesthesia.[81] None of these anesthetic techniques seems to affect the outcome of transplantation.

Other Anesthetic-Related Complications after Renal Transplantation

The major postoperative anesthetic complications are vomiting and pulmonary aspiration; cardiac arrhythmias, which can lead to cardiac arrest; pulmonary edema; hypotension and hypertension; and delayed respiratory depression. Cardiovascular complications in the transplant recipient are responsible for about 33% of all mortality[59]; about 50% of all patients have arterial hypertension. Although hypertension is usually a reflection of chronic rejection or excess renin release from the patient's native kidneys, rarer causes include the effects of the immunosuppressive drugs (particularly cyclosporine), recurrent glomerulonephritis, and transplant renal artery stenosis. Transplant patients also seem to be at greater risk of developing left ventricular hypertrophy if the treatment of hypertension requires two or more antihypertensive therapies.[85]

The post-transplant patient also may manifest diabetes mellitus; this occurs in 3% to 16% of all recipients, with 4% of these patients requiring insulin. Usually the onset of hyperglycemia occurs within the first 3 months of transplantation or following the first bolus dose of steroid for the treatment of kidney graft rejection. Predisposing factors include preoperative glucose intolerance and the presence of HLA B28.

With increasing awareness of the surgical risk factors present in renal transplant patients, careful perioperative monitoring has led to low rates of perioperative mortality (0.03% to 0.06%). Factors leading to increased perioperative risk in renal transplantation include recipient age greater than 60 years, coronary artery disease, and diabetes mellitus.[190]

STIMULUS TO EARLY ALLOGRAFT FUNCTION

Loop diuretics or mannitol, or both, may be used to promote a diuresis from the grafted kidney. Use of mannitol (the reduced form of the 6-carbon sugar mannose) has been criticized, but there is evidence to suggest that it may have a protective role as a free radical scavenger preventing free radical–induced reperfusion injury. Mannitol reduces the incidence of impaired renal function immediately after transplantation from 55% to 14%.[201] It also has been shown to improve renal blood flow by a greater percentage than can be accounted for by plasma volume expansion alone.[102] It is a small molecule that equilibrates slowly with the interstitial fluid compartment and so causes an increased circulating blood volume. Mannitol is freely filtered by the renal glomerulus and is not reabsorbed in the distal tubules. Because of its osmotic effect, sodium and water also are excreted; this may lead to increases in the serum potassium by 0.7 mmol/L.

Moote and Manninen[141] examined the influence of mannitol on serum electrolytes in patients undergoing renal transplantation. A dose of 50 g of mannitol (four times the dose used in Oxford) increased the central venous pressure and decreased the serum concentrations of sodium, chloride, and bicarbonate. The increase in potassium was small, but this may assume clinical importance in patients also receiving a blood transfusion. The thiazide diuretics and furosemide are not open to the same criticism, although their use should be coupled with preloading of the patients with isotonic (0.154M) saline.

Besides use of mannitol and diuretics to establish a diuresis, it is important to maintain an adequate circulating volume. Dawidson and colleagues[52] found that urine output is delayed after reperfusion in patients in whom the blood volume was less than 70 mL/kg. Rehydration requirements can be estimated from the central venous pressure, using normal saline as the initial volume expander. If more than 40 to 90 mL/kg is required, colloid solutions should be added. The administration of this fluid load also acts as a physiological stimulus to urine production. This stimulus is important because most analgesic and inhalation anesthetic agents increase circulating antidiuretic hormone levels.[9]

ANESTHESIA FOR LIVING RENAL TRANSPLANTATION

Renal transplantation increasingly involves living related or unrelated donation. This section considers physiological and anesthetic principles underlying laparoscopic nephrectomy in the donor and outlines our practices in Oxford for the recipient. (See also Chapter 8.)

Physiological Consequences of Laparoscopic Surgery

The general effects of carbon dioxide on the cardiovascular system have been described fully elsewhere[35]; they include the mechanical consequences of pneumoperitoneum, neurohumoral responses, systemic absorption of the carbon dioxide, and physiological effects of patient posture. Insufflation of carbon dioxide to create a pneomoperitoneum decreases renal blood flow,[170] leading to transient intraoperative kidney dysfunction; there is interest as to whether this leads to a greater incidence of delayed graft function compared with open surgery. Although a greater incidence of delayed graft function was found in a series of cases reported by London and colleagues,[128] this was not subsequently confirmed by Biancofiore and associates.[13]

The need for the Trendelenburg position, coupled with the increase in arterial carbon dioxide tensions, can result in increased cerebral blood flow, whereas the increased intra-abdominal pressure and central hemodynamic effects of the pneumoperitoneum tend to reduce cerebral blood flow through the reduction in cardiac output. Studies of the head-down posture in an animal model of laparoscopy caused increases in intracranial pressure, however, of 150%. An analysis of possible renal protective strategies has shown loop diuretics, mannitol, atrial natriuretic peptide analogues, and dopamine by infusion to have no positive effect. Fenoldopam may be useful, however. The only useful protective approach against renal damage is to ensure adequate circulatory volume and optimal renal blood flow.[173] The aim should be to keep intra-abdominal pressure low (<12 mm Hg), to ensure a positive fluid balance, and to maintain an adequate urinary output with mannitol and furosemide as needed.

Monitoring during Laparoscopic Nephrectomy

Monitoring should include electrocardiography, blood pressure by a noninvasive method, pulse oximetry, end-tidal anesthetic and carbon dioxide tensions, temperature, and urine output. Some authorities also advocate measurement of the central venous pressure in the donor patient to ensure normovolemia and avoid the risk of underperfusion of the donated kidney. The aim in the donor is to promote a diuresis, aiming for a urine flow of 300 to 500 mL/hr. Although this goal can be partially achieved with fluid loading, mannitol (in a dose of 1 to 2 g/kg) also should be used. In addition to promoting urine flow, mannitol aids the preservation of the donor renal tissue with conservation of renal function and protects the donor against cerebral swelling secondary to any increased cerebral blood flow. Additional doses of furosemide may be needed.

Postoperative Pain

Despite the minimally invasive approach (which usually involves a separate incision for the retrieval of the donor kidney), there is a requirement for initial use of strong analgesics. The provision of PCA may be a useful adjunct to local analgesia infiltration. Use of morphine or similar opioids may increase the incidence of postoperative nausea and vomiting, delay the return of normal function, and prolong hospital stay. Little evidence is available regarding the provision of epidural analgesia in the kidney donor. A more recent advance in the use of laparoscopic techniques has been the introduction of a "gasless laparoscopic assisted donor nephrectomy," which avoids the effects of high circulating carbon dioxide tensions.[199]

Anesthesia for the Transplant Recipient

The following practices have been used in Oxford since the 1980s and are based on the physiological and pharmacological principles outlined previously. We use the same strategy for patients receiving either a cadaver or a living related graft.

Premedication is important because many patients are anxious at the time of transplantation; suitable attenuation of this anxiety may be achieved with an orally administered benzodiazepine (usually temazepam, 10 to 20 mg). Intramuscular premedication is avoided because of the tendency of the uremic patient to bleeding disorders. Vagolytic drugs (e.g., atropine) are given intravenously at the time of induction of anesthesia if clinically indicated, such as when suxamethonium is used to facilitate intubation, or when a combination of an opioid plus one of the hemodynamically neutral muscle relaxants is administered. The avoidance of pronounced bradycardias is particularly important in patients receiving long-term β-adrenoceptor blockade for the treatment of ischemic heart disease and hypertension. β-Adrenoceptor blocking drugs, calcium channel blockers, and other antihypertensive and antianginal therapies are continued up to the morning of surgery.

The routine prophylactic administration of antacids may be advocated for patients with symptoms of esophageal reflux; a single dose of sodium citrate (30 mL) in the anesthetic room is appropriate. Histamine H_2-receptor antagonists (e.g., ranitidine 150 mg orally) or proton-pump inhibitors (e.g., omeprazole) are given with the premedication to reduce gastric hyperacidity. Phenothiazine antiemetics and metoclopramide should be administered with care because they may cause prolonged sedation and extrapyramidal side effects in patients with renal failure (see earlier).

Anesthesia is best induced with a sleep dose of propofol coupled with a loading dose of fentanyl, 3 to 6 μg/kg, or an infusion of remifentanil, 0.05 to 0.1 μg/kg/min. For patients with poor cardiac reserve, etomidate, 0.3 mg/kg, may be preferred. Using the combination of a hypnotic and an opioid, the anesthesiologist can minimize the hemodynamic response to induction of anesthesia, laryngoscopy, intubation, and surgical incision. Neuromuscular blockade is provided by atracurium or cisatracurium in doses of 0.6 mg/kg or 0.15 to 0.4 mg/kg. To maintain neuromuscular blockade, increments of either drug are given when indicated clinically with neuromuscular transmission monitored using a peripheral nerve stimulator. An alternative technique involves continuous infusion of either relaxant (atracurium, 6 to 8 μg/kg/min, or cisatracurium, 1 to 2 μg/kg/min). For the patient in whom there is the added problem of an inadequate period of fasting before surgery, suxamethonium, 1 to 1.5 mg/kg, should be used to aid intubation.

Maintenance of anesthesia is achieved with isoflurane to supplement nitrous oxide; this has the advantages of nonrenal elimination and may be given with high inspired oxygen concentrations in severely anemic patients. Alternatively, with a remifentanil infusion, an air-oxygen-isoflurane mixture may be used. The arterial blood carbon dioxide tension should be kept at normocapnia or mild hypocapnia, and monitored by end-tidal carbon dioxide sampling. Short periods of hypoventilation can lead to hemoglobin desaturation, whereas excess hyperventilation with low arterial carbon dioxide tensions causes a shift of the oxyhemoglobin dissociation curve to the left. Intraoperative analgesia can be provided by intravenous morphine (10 to 15 mg).

At the end of surgery, anesthesia is discontinued, and residual muscular paralysis is reversed with neostigmine. The muscarinic effects may be blocked by atropine or glycopyrrolate. Glycopyrrolate is preferred in patients with associated hypertensive or ischemic heart disease to avoid excessive tachycardia. An important interaction for the anesthesiologist to be aware of is that between cyclosporine and muscle relaxants. Sidi and colleagues[180] found a greater incidence of postoperative respiratory failure in transplant patients receiving cyclosporine as the immunosuppressant drug. After extubation, all transplant patients should receive oxygen for 12 to 24 hours postoperatively.

Monitoring during Anesthesia

The high incidence of ischemic and hypertensive heart disease in these patients makes it essential to monitor the ECG and blood pressure continuously during induction of anesthesia, the perioperative period, and the immediate postoperative period. Blood pressure should be measured noninvasively, with the cuff placed on the nonfistula arm. Because of improvements in the preoperative preparation of kidney transplant recipients, and because excessive blood loss is the exception rather than the rule, arterial cannulation is only rarely needed for the perioperative monitoring of blood pressure. The aim should be to maintain the systolic blood pressure close to the patient's normal blood pressure.

Measurement of the central venous pressure is as important as measurement of the blood pressure in patients undergoing renal transplantation. We use a triple-lumen catheter inserted under ultrasound guidance into the internal jugular or subclavian vein. Intraoperative fluids are given generally as Hartmann's solution rather than normal saline (0.154M sodium chloride) because the large volumes of the latter have been found to cause episodes of hyperkalemia and a hyperchloremic metabolic acidosis.[150] Unless the recipient becomes ketoacidotic, the increased bicarbonate concentration (from metabolism of the lactate) is not significant. Hydroxyethyl starch solutions are more useful than gelatins to achieve plasma expansion and result in a greater increase in the central venous pressure. Blood is given to maintain a hemoglobin concentration of approximately 10 g/dL. With this fluid strategy, we aim to increase the central venous pressure by 4 to 8 mm Hg by the time of revascularization. In practice, we aim for a central venous pressure of 10 to 15 mm Hg in patients with good left ventricular function and 12 mm Hg in patients in whom function is impaired and older patients (>55 years old).

Postoperative fluid requirements depend on early renal function, but should be aimed at keeping the central venous pressure at its intraoperative level. In our practice, this equates to a regimen of urine output plus 50 to 100 mL/hr. Replacement fluids are given as crystalloid (equal volumes of 5% dextrose and Hartmann's solution), supplemented by colloid in cases of a decrease in central venous pressure accompanied by arterial hypotension. Persistent hypotension in the presence of an adequate central venous pressure (6 to 10 mm Hg) normally responds to a vasoconstrictor agent, such as norepinephrine. The accurate assessment of fluid balance postoperatively may be difficult in a predialysis patient who still has native urine production; after living related transplantation, there may be a major response by the kidney to the high osmotic load of creatinine, urea, and other solutes with urine outputs of 40 L over the first 24 hours. Urine output tends to return to normal volumes by 24 to 48 hours. Because of this high fluid flux, the patient's temperature should be carefully monitored intraoperatively, and heat balance should be maintained by warming all infused fluids and the use of convection heaters (e.g., the Bair Hugger, Arizant Healthcare Inc; Eden Prairie, MN). Other causes of a massive diuresis include the onset of the diuretic phase of acute tubular necrosis, characterized by large volumes of dilute urine.

With increasing availability of blood gas analysis, and near-patient testing systems, measurement of electrolytes and hemoglobin during the operative procedure has become more routine. There have been reports of sudden increases of plasma potassium levels leading to arrhythmias and cardiac arrest.[93] Several factors may be responsible, such as the administration of mannitol[143] or stored blood, severe metabolic acidosis, and hyperkalemia or hyperglycemia.[77] The cause of hyperkalemia or hyperglycemia is unknown. Prevention of this complication assumes greater significance in diabetic patients undergoing renal transplantation (see later). If urine output is more than 300 mL/hr, the serum sodium and potassium levels should be checked frequently. If output is greater than 1000 mL/hr, potassium supplements (10 mmol/L) may be needed. The excretion of large fluid volumes also may lead to intravascular and intracellular volume depletion manifesting as tachycardia (either ventricular tachycardia or atrial fibrillation) or seizures.

In patients with poor renal output in the absence of dehydration, electrolytes should be checked every 6 hours, and accurate weight should be obtained every 24 hours. Dialysis should be avoided during the first 24 hours postoperatively, but it is indicated when there is massive weight gain, severe hypertension, fluid overload with pulmonary edema, or a severe metabolic acidosis or hyperkalemia.

There are no indications for the use of "renal doses" of dopamine in the transplant recipient. Studies fail to show any efficacy of dopamine by infusion for improving renal function. Inotropic doses may be useful, however, in patients with poor myocardial function.

The diabetic patient undergoing renal transplantation also should receive an infusion of glucose (5 g/hr). The blood glucose level is titrated to normoglycemia (4 to 8 mmol/L) with a separate infusion of soluble human insulin.

Postoperative Care

Because of the multiple pathologies exhibited by transplant patients, they should receive postoperative care in the intensive care unit, where controlled oxygen therapy and full monitoring can be provided. The correct positioning of the triple-lumen catheter must be checked by radiography in the recovery area. If controlled ventilation is needed, admission to the intensive care unit is required. Strict monitoring of fluid input and output is essential; there should be regular monitoring of the ECG, blood pressure, heart rate, central venous pressure, and oxygen saturation by pulse oximetry.

Analgesia in the Postoperative Period

Analgesia should be titrated according to patient demand. The choice of drugs (opioids and oral non-narcotic analgesics) must be considered carefully because accumulation of active metabolites of pethidine and morphine may occur in a patient with a nonfunctioning allograft. Excessive use of opioids may lead to delayed respiratory depression, sedation, and convulsions (all related to parent drug and active metabolite accumulation). PCA may aid the more efficient and safe titration of dosage to desired effect in the uremic patient, although there have been reports of excessive sedation and respiratory depression after use of PCA in end-stage renal disease patients.[44] In Oxford, as in many other units, a morphine or fentanyl PCA is used with a bolus dose of 1 to 2 mg of morphine administered with a lockout of 5 to 10 minutes, or a bolus dose of 20 μg of fentanyl and a 3- to 6-minute lockout. We do not administer a background infusion of opioid as part of the PCA.

Although only a few nonsteroidal anti-inflammatory drugs (NSAIDs) are eliminated unchanged via the kidney, there is evidence of reduced clearance of ketoprofen, fenoprofen, naproxen, and carprofen in renal failure as a result of probable deconjugation of acyl glucuronide metabolites. More importantly, NSAIDs also can cause reversible kidney damage with reduction of renal blood flow and glomerular filtration rate. They also can cause edema, interstitial nephritis, and papillary necrosis in the kidney. These effects are probably caused by the action of the NSAIDs on prostaglandin synthesis—the latter being integral for renal blood flow and glomerular filtration rate autoregulation. These drugs should be avoided in the post-transplant patient and in *all* patients with renal impairment.

Vascular and Peritoneal Access for Dialysis

Surgery for shunt insertion or fistula creation may be done under general or regional anesthesia. The anesthetic agent may be infiltrated locally, but for vascular access in the upper limb, anesthesia is best achieved by brachial plexus blockade. The associated sympathetic nerve block abolishes vasospasm and ensures vasodilation. The duration of brachial plexus anesthesia in end-stage renal disease patients is decreased, however, by 39%.[18] This decrease was thought to be the result of metabolic changes present in uremia (e.g., hyperkalemia) and the increase in cardiac output secondary to anemia. Two later publications failed to support these earlier data, however.[132,137] Bupivacaine disposition is unaltered after supraclavicular plexus blockade in uremic patients,[163] and there is no direct correlation between the shortening of anesthetic action and the severity of anemia or uremia. Although bupivacaine is the agent of choice for local anesthetic procedures, a report by Gould and Aldrete[78] described cardiotoxic effects of bupivacaine after its use in normal doses in a patient with end-stage renal disease.

Other drugs suitable for this group of patients include lidocaine, prilocaine, mepivacaine, ropivacaine, and levobupivacaine. Compared with bupivacaine, lidocaine, mepivacaine, and prilocaine have a faster onset of anesthesia but a shorter duration. Generally, the normal maximal doses for bupivacaine and other local anesthetic agents (Table 13-6) should be decreased by 25% in end-stage renal disease patients because any accompanying acidosis would have the effect of decreasing the central nervous system threshold to the toxic effects of local anesthesia.

The use of regional anesthesia in a patient with uremic neuropathy generally is contraindicated. Similarly, use of vasoconstrictors such as epinephrine to prolong local anesthetic action is best avoided because of the risk of cardiac arrhythmias after systemic absorption in the acidotic, hyperkalemic patient. Other limitations to the use of regional anesthesia are the presence of a bleeding tendency, patient acceptance, and the possible inadequate duration of analgesia. For more complex procedures (e.g., the insertion of arteriovenous grafts, thigh shunts, or continuous ambulatory peritoneal dialysis cannulation), general anesthesia usually is preferable.

ANESTHETIC MANAGEMENT OF DIABETIC PATIENTS UNDERGOING RENAL OR COMBINED KIDNEY-PANCREAS TRANSPLANTATION

The combined problems of diabetes and uremia are common because patients with juvenile-onset diabetes that developed before age 30 years have a 1:5 chance of renal complications. At present in the United Kingdom, about 10% of all renal transplants are in diabetic patients, but in the United States, the figure is more than 40%. Hence, an increasing number of units are being faced with uremic diabetic patients for transplantation. There also is a move toward the combined transplantation of kidney and pancreas in these patients. The problems presented for the anesthesiologist by the diabetic patient may be considered under the following headings:

1. Influence of uremia on carbohydrate metabolism
2. Preoperative assessment of the patient
3. Anesthetic and postoperative management
4. Management of kidney-pancreas transplantation

Influence of Uremia on Carbohydrate Metabolism

The influence of uremia on carbohydrate metabolism has been extensively reviewed by de Fronzo and coworkers.[55] The main defect seems to be a systemic insulin antagonism. Coupled with this hyperglycemic response, there is a glucose-induced hyperkalemia. Its exact cause is uncertain, although hypoaldosteronism and hyporeninism have been suggested.

Preoperative Assessment of the Patient

The preoperative assessment of the patient does not differ significantly from that of the nondiabetic patient undergoing renal transplantation apart from the additional factor of achieving optimal glycemic control before surgery is started. All patients with diabetes present an increased risk to the anesthesiologist, especially related to the complicating factors of hypertension and coronary artery disease.[22] This increased

Table 13–6 **Maximal Safe Doses of Local Anesthetic Agents***

	Plain Solution (mg)	With Added Epinephrine (1:200,000) (mg)	Relative Duration of Sensory Block (hr)
Lidocaine	300	500	1.5
Bupivacaine	175	250	8
Mepivacaine	300	500	1.5
Etidocaine	300	400	8
Prilocaine	400	600	1.5
Chloroprocaine	600	650	0.75
Procaine	500	600	1
Ropivacaine	250	—	1
L-Bupivacaine	175	—	1

*These doses are based on a 70-kg body weight. Doses should be decreased by 25% in acidotic patients to avoid signs of central nervous system toxicity (e.g., lightheadedness, dizziness, disorientation, euphoria, dysarthria, slurring of speech, progressing to twitching and generalized convulsions).

risk includes a greater need for intraoperative blood pressure support and aggressive treatment of any hypotension with vasoconstrictor drugs. In the diabetic patient with renal failure, there is an even higher risk of perioperative myocardial ischemia and infarction, both of which may be silent because of the accompanying autonomic neuropathy. Other potential complications are the increased risk of wound infection and a prethrombotic state compared with nondiabetic renal transplantation.[160]

Heino[90] observed an increased perioperative morbidity and mortality in diabetic patients undergoing renal transplantation compared with nondiabetic transplant recipients. In a follow-up of 413 patients, Heino[90] showed that diabetic patients with end-stage renal disease had a higher incidence of preoperative ischemic ST-T wave changes (62.2% versus 39.8% in nondiabetic uremic patients) and higher incidences of pulmonary congestion (14.5% versus 5.2%) and pleural effusions on chest radiography (10.1% versus 4.5%). There were, however, no differences in the frequency of perioperative complications, although the diabetic patients had a greater mortality during the first postoperative month.

Many diabetics presenting for transplantation are poorly controlled and show lability of the blood glucose concentration. Concurrent administration of thiazide diuretics, diazoxide, and β-adrenoceptor blocking drugs may complicate glucose homeostasis further. If the patient is normally maintained on continuous ambulatory peritoneal dialysis, it should be continued up to 1 hour before surgery. In general, the anesthesiologist should aim for a blood glucose concentration of 4 to 8 mmol/L.

Anesthetic Technique

After induction of anesthesia, uremic patients with diabetic neuropathy may show a greater systolic pressor response to intubation and other noxious stimuli.[110] This response is due to an increased sensitivity to circulating catecholamines and a loss of baroreceptor control. These same authors also have shown that diabetic patients exhibit greater Q-Tc dispersion,[111] with an associated increased risk of sudden cardiorespiratory arrest.[162] The measurement of Q-T dispersion by 24-hour Holter ECG monitoring does not seem, however, to be a sensitive method per se to detect the cardiac autonomic neuropathy in these patients.[111]

Because of the possible association of diabetes with gastroparesis and an increased gastric residual volume, all diabetic uremic patients should undergo a rapid-sequence intubation using suxamethonium, unless there is hyperkalemia.[161] Other suitable drugs include large doses of atracurium or cisatracurium, coupled with the application of cricoid pressure. The handling of opiates during anesthesia in a diabetic patient with end-stage renal disease has not been researched in depth; Koehntop and colleagues[115] found an increased clearance of alfentanil (6.4 mL/kg/min versus 4.1 mL/kg/min) in the diabetic patient compared with the nondiabetic uremic patient.

Other potential anesthetic problems in these patients include temporomandibular joint rigidity and difficulties in intubation caused by tissue glycosylation.[94,159,168] Although Hogan and associates[94] reported that 32% of diabetic patients undergoing renal or simultaneous kidney-pancreas transplantation had a difficult grade laryngoscopy, more recent data from the Mayo Clinic found an overall incidence of difficult laryngoscopy of 2.1% in a series of 725 patients.[198] This latter figure compared with a 1% incidence of difficulty in renal failure patients without diabetes mellitus. If there is any concern, the anesthesiologist should use suxamethonium as the relaxant of choice or use fiberoptic techniques to aid intubation.

Anesthesia for Kidney-Pancreas Transplantation

Simultaneous kidney-pancreas transplantation is increasingly being used for the management of diabetic end-stage renal disease. Patients have creatinine clearance less than 40 mL/min and are on dialysis or are very close to needing it. The present results indicate a 1-year graft survival of 85% and a patient survival at 1 year greater than 94%. Simultaneous kidney-pancreas surgery offers numerous challenges, however, including a prolonged operation (5 to 7 hours), careful metabolic control, and provision of effective analgesia without postoperative respiratory depression. Patients undergoing simultaneous kidney-pancreas transplantation are type 1 diabetics; there is no evidence to support simultaneous kidney-pancreas transplantation in patients with type 2 diabetes mellitus and insulin resistance. All patients have evidence of secondary diabetic complications (e.g., retinopathy, vasculopathy, neuropathy). Contraindications to simultaneous kidney-pancreas transplantation include untreatable coronary artery disease, irreversible pulmonary or hepatic dysfunction, and recent myocardial infarction or significant left ventricular dysfunction. Relative contraindications include age older than 55 years, symptomatic cerebrovascular or peripheral vascular disease, severe aortoiliac disease, and body mass index greater than 30.

Preoperative assessment should include a thorough investigation of the cardiovascular system including ECG, echocardiogram, and thallium stress scan. In the presence of coronary disease, the patient should undergo angiography with percutaneous coronary intervention or coronary artery bypass graft if appropriate. Other important factors include examination for autonomic failure, with evidence of cardiac denervation, gastroparesis, and orthostatic hypotension. Preoperative electrolytes usually show the serum potassium level to be 5 to 6 mmol/L; cancellation is not warranted unless the serum potassium level is significantly outside these limits. All cardiac medications should be continued preoperatively.

For the conduct of anesthesia, our own practice is to premedicate with the combination temazepam, metoclopramide, and ranitidine. We use general anesthesia based on a fentanyl- or remifentanil-isoflurane-air-oxygen technique. There is some evidence to advocate insertion of a thoracic epidural because this provides a reduced incidence of venous thromboembolism, a reduction of respiratory complications, and first-rate postoperative analgesia. We avoid high-dose epidural local anesthetic regimens until the patient is stable at the end of surgery because they can make assessment of the patient's volume status difficult. There are large fluid shifts in these patients, and any period of hypotension secondary to hypovolemia is worsened by peripheral vasodilation. The use of epidural opiates (diamorphine or fentanyl) may be a useful adjunct. There is no place for NSAIDs because of the risk of renal impairment in the new graft and

the risk of gastrointestinal and surgical bleeding. The choice of immunomodulation therapies varies among different centers.

Careful monitoring is crucial in these patients. Our choice includes two large peripheral lines, invasive blood pressure measurement (this allows us to follow the swings in blood pressure and facilitates regular blood gas sampling), central venous pressure (with maintenance of central venous pressure at 10 to 12 mm Hg; this may require large fluid volumes at declamping), peripheral and core temperature monitoring, perioperative clotting studies (and if concerned about the development of a coagulopathy, thromboelastogram studies should be undertaken), and monitoring of neuromuscular blockade. Fluid requirements are given as Hartmann's solution via a fluid warmer. The hemoglobin should be maintained at greater than 8 g/dL, with monitoring of the serum K^+ and blood glucose, especially at unclamping of kidney and pancreas. Glucose control is provided by an infusion of 50% dextrose (10 to 15 mL/hr) and a separate infusion of a short-acting insulin. Diabetic control also is influenced by intraoperatively administered steroids given for immunosuppression and the use of intraoperative mannitol. Reperfusion of the pancreatic graft also can lead to an *increase* in blood glucose levels, however, owing to the release into the circulation of graft preservation fluid that contains high glucose concentrations.

CONCLUSION

Although Strunin[187] reported an immediate perioperative mortality of 16% after renal transplantation, more recent series have recorded immediate mortality rates of 0.03% to 0.6%. With present-day anesthetic techniques, the incidence of delayed extubation (owing to inadequate ventilatory performance) also is low (<3% of patients).

There is no single correct technique for the anesthetic management of patients in end-stage renal failure. Effective and safe anesthesia for the renal transplantation patient depends on an understanding of the pathophysiology and biochemistry of uremia, and its effect on the pharmacokinetics and metabolism of the drugs used. As the criteria for accepting patients into renal transplantation programs broaden, the anesthesiologist is likely to be faced with increasing problems of the interaction of other intercurrent diseases and multiple drug therapies.

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Chapter 14

Early Course of the Patient with a Kidney Transplant

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A successful long-term outcome for a new kidney transplant recipient depends on the early perioperative management and course after surgery. Important factors affecting long-term outcome include the occurrence of delayed graft function (DGF)[7,55]; episodes of acute rejection[7]; early surgical complications,[5] such as obstruction, urine leak, or vascular complications; and sepsis.[1] Toxicity from calcineurin inhibitors can lead to chronic transplant nephropathy later in the post-transplantation course.[36] Donor and recipient factors affect long-term outcome, particularly the use of expanded criteria donors[33] or highly sensitized recipients. The early management and amelioration of risk factors in the immediate postoperative period may lessen their long-term negative impact and improve outcome.

OVERVIEW

Perioperative Management

Management of the transplant recipient begins in the immediate preoperative period. An initial assessment of the recipient includes a careful assessment of pretransplant fluid status to determine the need for dialysis and a careful physical examination to exclude potential contraindications to transplantation, such as significant cardiac disease or vascular insufficiency, which could preclude successful surgery. Knowledge of the donor status also is helpful in the early postoperative management of the transplant recipient. With an ideal donor or a living related donor, the expected outcome is an immediately functioning transplant that may preclude post-transplant dialysis. Expanded criteria donors (donor age >60 years or age 50 to 59 years with death due to cerebrovascular accident, history of hypertension, or creatinine level >1.5 mg/dL) have a higher likelihood of DGF, which can lead to volume overload and the need for urgent dialysis.[12] Technical considerations include the need for vascular reconstruction, which may prolong surgery and contribute to postoperative DGF. Recipient factors also affect the early postoperative course. Significant risk factors for early post-transplant dysfunction include pretransplant sensitization, obesity, younger or older age, and anatomical considerations that complicate the surgery.

In the early perioperative period, attention to fluid and electrolyte balance is crucial. Careful monitoring of urine output is essential, and any decrease in urine flow must be evaluated carefully. A decrease in urine volume may be due to acute tubular necrosis, hypovolemia, urinary leak, ureteric obstruction or, most significantly, vascular thrombosis. Assessment of the patient's volume status with the measurement of central venous pressure may help eliminate hypovolemia as a cause of decreasing urine output. DGF can be ascertained further with a nuclear scan or duplex ultrasonography to assess perfusion of the graft and to exclude renal artery or vein thrombosis. Duplex ultrasonography also allows the diagnosis of a urinary complication.

Measures to decrease the likelihood of DGF often are used during the operative procedure and in the perioperative period. Maintenance of adequate blood pressure and fluid status may be accomplished with intravenous albumin[17] or crystalloid, the latter being preferable. Shorter cold ischemia or pulsatile perfusion of the donor organ also may decrease the likelihood of postoperative DGF. Some centers have used intra-arterial calcium channel blockers, such as verapamil, to improve renal blood flow.[16] It is common practice to administer mannitol (12.5 g) about 10 minutes before the kidney is reperfused, which helps to trigger an osmotic diuresis and might be protective. Oral calcium channel blockers have been used to decrease the incidence of DGF.[15] There is controversy about the early initiation of calcineurin inhibitors because of the potential for nephrotoxicity. Some centers delay the use of calcineurin inhibitors until there is

Table 14–1 Early Surgical and Medical Complications after Transplantation

Surgical/Mechanical	Medical
Obstruction	Acute rejection
Hematuria	Delayed graft function
Urinoma	Acute cyclosporine/ tacrolimus nephrotoxicity
Arterial stenosis	Prerenal/volume contraction
Arterial thrombosis	Drug toxicity
Renal vein thrombosis	Infection
Postoperative hemorrhage	Recurrent disease
Lymphocele	

established diuresis. If additional immunosuppression is desired, polyclonal or monoclonal anti–T cell antibodies may be used.

Graft Dysfunction

Early complications of renal transplantation may be mechanical/surgical or medical. Early medical problems are more common than post-transplant surgical problems (Table 14-1). The most common early post-transplant medical problem is DGF, which occurs in 20% of patients who received kidneys from ideal deceased donors and in nearly 40% of patients in whom the donors were older than age 55 years.[8] After or concomitant with DGF, acute rejection may become a significant clinical problem.[52,55,58] Other reasons for early medical complications include acute cyclosporine or tacrolimus nephrotoxicity, prerenal azotemia, other drug toxicity, infection, and early recurrent disease. An uncommon but serious post-transplantation medical problem is thrombotic microangiopathy, formerly called hemolytic-uremic syndrome. Thrombotic microangiopathy may be induced by rejection or as a secondary event from cyclosporine, tacrolimus, or sirolimus therapy.[39]

Mechanical problems usually are the result of complications of surgery or specific donor factors, such as multiple arteries, that lead to post-transplantation dysfunction. Mechanical/surgical factors include obstruction of the transplant, hematuria, urine leak or urinoma, and vascular problems such as renal artery or vein stenosis or thrombosis. Postoperative bleeding is another potential complication that may cause compression of the transplant because the transplant usually is placed in the retroperitoneal space. Post-transplant lymphoceles are another common cause of early transplant dysfunction. Lymph drainage from transected lymph vessels accumulates in the perivascular and periureteral space and can cause ureteral obstruction or lower extremity swelling from iliac vein compression.

SURGICAL COMPLICATIONS

Urinary Problems

Urinary Obstruction

After implantation of a living donor kidney transplant, urine output begins immediately or within minutes. (See Chapter 27 for a more complete discussion of urinary problems.) The same is not generally true of cadaver donor kidneys, in which urine output may not be apparent for 1 hour or more after implantation and may be sluggish or nonexistent for days if the kidney has been injured (DGF) by donor factors or preservation. If a kidney that was formerly making urine slows down or stops and does not respond to fluid administration, urinary obstruction has to be considered in the differential diagnosis. The initial evaluation is to check the patient's vital signs and central venous pressure to ensure adequate hydration and to check that the Foley catheter is functioning correctly. Obstruction of the Foley catheter by blood clots may occur easily and can be cleared by gentle irrigation. If these problems are not present, renal transplant ultrasound is the fastest, most accurate, and least expensive method to assess the renal pelvis for obstruction. Pelvicaliceal dilation seen by ultrasound implies distal obstruction. If the bladder is collapsed rather than full, the problem is likely to be ureteral obstruction. Treatment should be immediate decompression of the renal transplant pelvis by percutaneous insertion of a nephrostomy tube. Subsequently (usually 1 or 2 days later to allow blood and edema to clear after nephrostomy tube placement), a nephrogram can be obtained to evaluate the ureter for stenosis or obstruction. The diagnosis is confirmed by a decline in the serum creatinine level after decompression of the renal pelvis.

After the Foley catheter is removed, the most common cause of urinary obstruction is not ureteral stenosis, but rather bladder dysfunction. This cause is particularly common in diabetic patients with neurogenic bladders. Initial management is replacement of the Foley catheter and a trial of an α-blocker, such as doxazosin or terazosin. If bladder dysfunction persists after one or two such trials, it may be necessary to start intermittent self-catheterization. In rare instances in which bladder dysmotility is severe and urinary tract infections are common, it may be preferable to drain the transplant ureter into an ileal conduit to the anterior abdominal wall. Ideally, a patient with a neurogenic bladder should have been evaluated before transplantation with urodynamic studies, and a decision should have been made about management at that time (see Chapters 4 and 12).

During the first 1 or 2 weeks after transplantation, obstruction usually is due to a technical problem related to surgery (see Chapter 27). If a ureteral stent was placed at the time of surgery, it is highly unusual to have obstruction. Possible explanations for obstruction are a twisted ureter or anastomotic narrowing. Generally, obstructions appear several weeks postoperatively, after the stent has been removed, and occur most frequently at the anastomosis between ureter and bladder.[24] Usually, these obstructions can be crossed by a guidewire and dilated percutaneously by an interventional radiologist (Fig. 14-1). If the the nephrostogram shows a long (>2 cm) stricture, especially a proximal or midureteral stricture, it is likely that the problem is not amenable to balloon dilation and that surgical repair is necessary (Fig. 14-2). The operation of choice for a long stricture or one that has failed balloon dilation is ureteroureterostomy or ureteropyelostomy using the ipsilateral native ureter. The spatulated ends of the transplant and native ureters are anastomosed using running 5-0 absorbable suture. This anastomosis can be done over a 7F double-J stent, which is left in place for 4 to 6 weeks. If no ipsilateral ureter is available, it may be necessary to use the contralateral ureter. If neither the ipsilateral ureter nor the contralateral ureter is available, alternatives include bringing the bladder closer to the kidney using a psoas hitch or

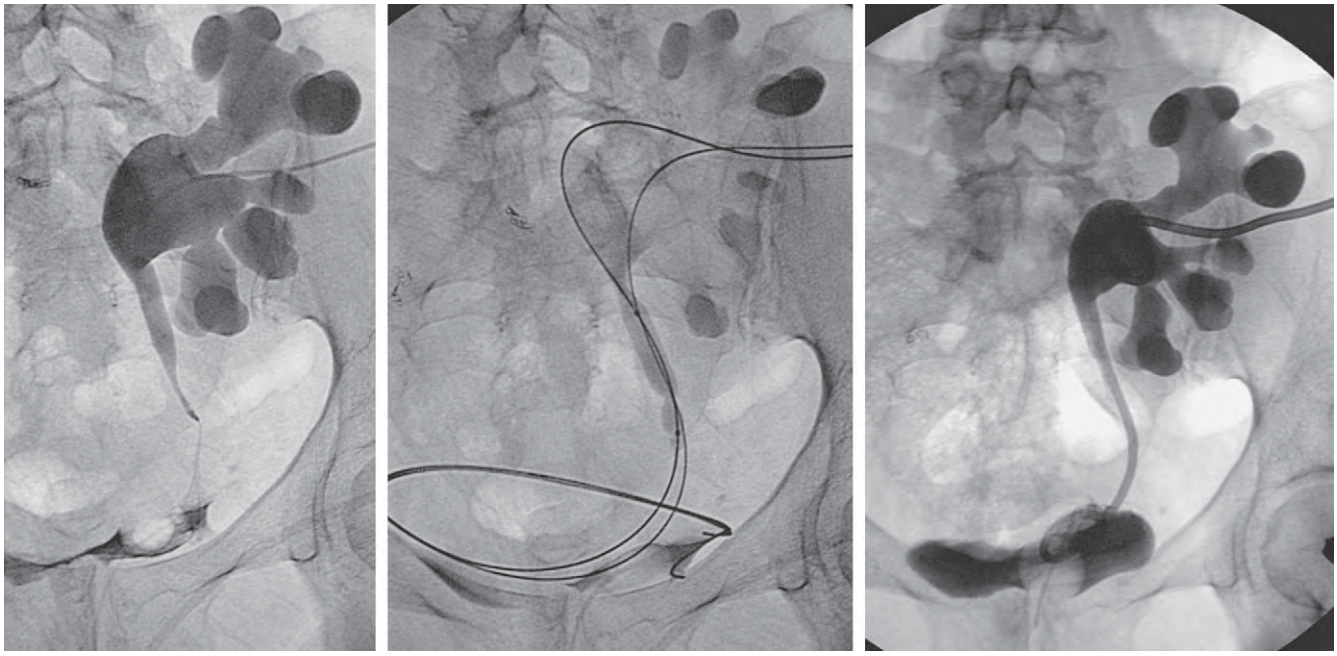


Figure 14–1 This patient presented with an elevated creatinine level. Ultrasound showed pelvicaliceal dilation. **A**, A percutaneous nephrostomy tube was placed, and the following day a nephrostogram was obtained. **B**, The midureteral stenosis was crossed successfully with a guidewire, and the ureter was dilated with a balloon (the waist of the dilated balloon corresponds to the stricture). **C**, Subsequently, a double-J stent was placed from the renal pelvis into the bladder across the dilated stricture.

fashioning a Boari flap,[18] but these measures are rarely necessary. Another method is endoureterotomy[20]; experience with this method is growing.[29]

Even if urinary obstruction is clinically silent (i.e., the patient is asymptomatic with a normal creatinine value), urinary obstruction manifested by dilation of the pelvis and calices on ultrasound should be treated because it ultimately leads to thinning of the renal cortex and loss of renal function. Urinary obstruction should be treated immediately to minimize damage to the transplanted kidney.

Bleeding into the Urinary System

Gross hematuria is common immediately postoperatively because of surgical manipulation of the bladder. The Leadbetter-Politano procedure for ureteroneocystostomy is associated with more hematuria compared with the extravesical approach typified by the Lich technique or the technique described by us (see Chapter 11).[28] The advantage of this technique is that it effectively prevents reflux and can be done with excellent long-term results. Occasionally, continuous bladder irrigation is necessary if gross hematuria is associated with clots, although intermittent manual irrigation usually is adequate. Obstruction of the bladder outlet by a blood clot is an emergency; vigilant nursing care is required to ensure that it does not occur. It is preferable not to distend the bladder in the immediate postoperative period to avoid disrupting the bladder sutures or causing a leak, and continuous bladder irrigation and cystoscopy ideally are avoided. Minor hematuria without clots is common in the first 1 or 2 days regardless of the surgical method of ureteroneocystostomy and does not require treatment; it resolves over time without specific treatment.

Urine Leak

A leak of urine from the transplanted kidney in the early postoperative period may be clinically obvious if the patient presents with abdominal pain, an increasing creatinine level, and a decrease in urine output. Urine in the peritoneal cavity causes peritonitis and pain. More commonly, assuming that the kidney was placed in the retroperitoneal position, a urinoma collects around the kidney and bladder and causes a bulge in the wound and pain with direct displacement of adjacent viscera, including the bladder. The diagnosis should be suspected if the serum creatinine level is increasing (or not decreasing appropriately). Adjunctive tests to help make the diagnosis of urine leak, if it is not obvious clinically, include a renal scan, which would show urine in the retroperitoneal space surrounding the bladder or around loops of bowel, or an ultrasound, which would show a fluid collection outside the bladder and which when aspirated has a high creatinine level. Urine leak generally is due to a surgical problem with the ureteroneocytostomy or ischemic necrosis of the distal ureter. This leak should be immediately repaired surgically because the risk of wound infection increases with delay in treating this complication.

Vascular Problems

Arterial Stenosis

Transplant renal artery stenosis may manifest in the early postoperative period by (1) fluid retention, (2) elevated creatinine levels, and (3) hypertension.[21,57] (See Chapters 26 and 28 for a more complete discussion of vascular problems.)

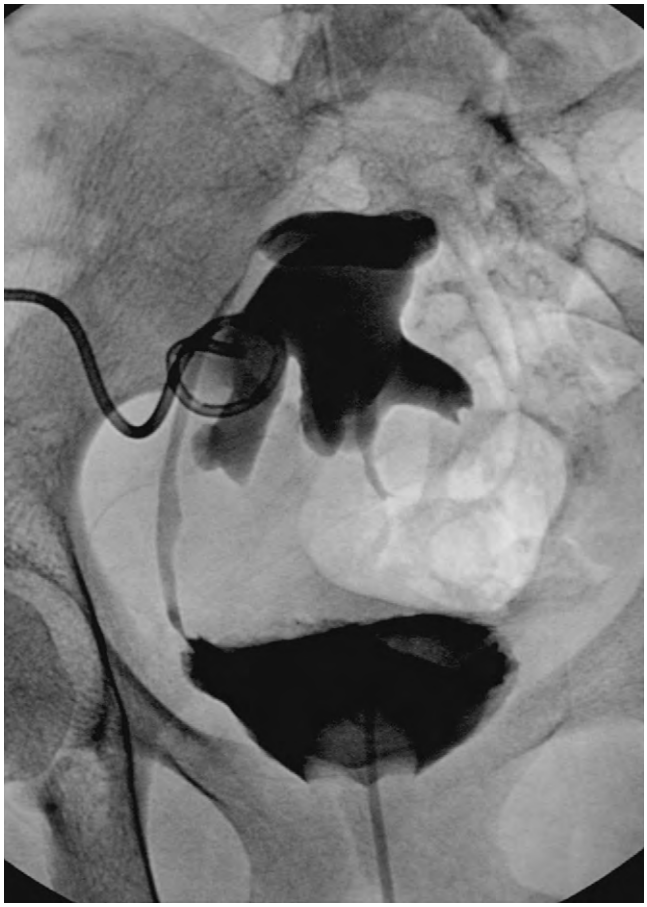


Figure 14–2 This intra-abdominal kidney transplant was found by ultrasound to be obstructed. A nephrostomy tube was placed, and a nephrostogram was obtained the following day. The kidney had rotated medially and twisted the ureter proximally. The patient was managed operatively by placing the kidney laterally in a retroperitoneal pocket and performing ureteroureterostomy using the ipsilateral native ureter.

Commonly, the patient does not tolerate cyclosporine or tacrolimus because these drugs exacerbate the already existing ischemia at the glomerular arteriolar level. The aforementioned triad of clinical findings need not all be present, and the diagnosis should be suspected for any one of the three clinical signs. Cytomegalovirus infection and DGF have been described as risk factors for transplant renal artery stenosis.[4] If the creatinine level is greater than 2 mg/dL, renal arteriography is best avoided because of the nephrotoxicity of the contrast dye. Magnetic resonance imaging angiography usually can give an accurate delineation of the arterial anatomy. Ultrasound also is safe, but not particularly discriminating, and is helpful only if jetting of flow is seen.

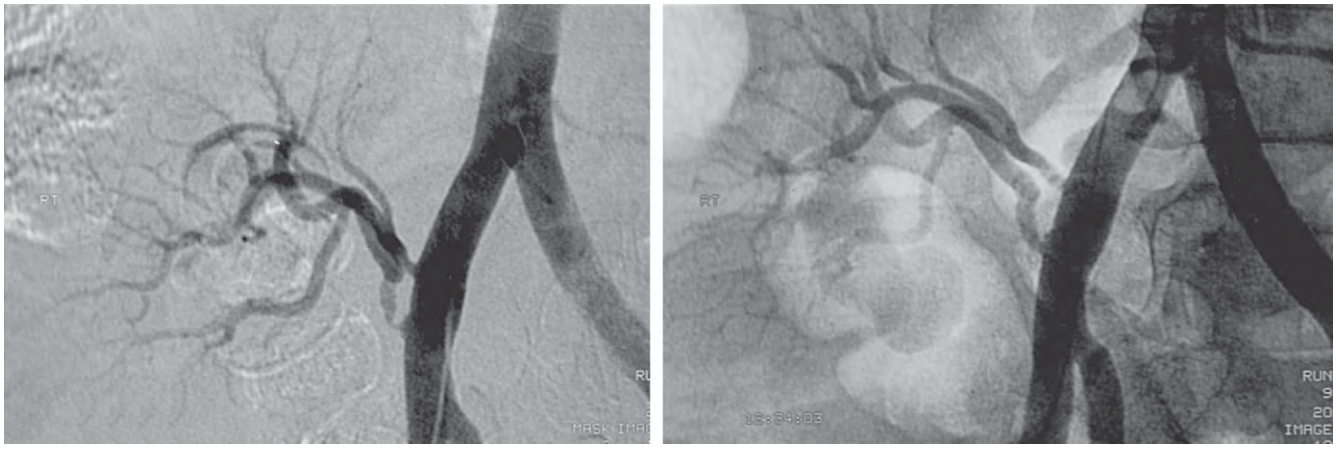
As the population of renal transplant recipients has become older and includes more diabetic patients and patients with vascular disease, transplant renal artery pseudostenosis has become increasingly common. Pseudostenosis refers to arterial stenosis in the iliac artery proximal to the implantation of the transplant renal artery. Although the anastomosis and renal artery may be completely normal, the problem is high renin output by the transplanted kidney, resulting from its hypoperfusion.

Treatment of transplant renal artery stenosis and pseudostenosis includes balloon dilation and surgery. Generally, ostial stenosis, long areas of stenosis, and stenosis in tortuous arteries difficult to access radiographically are not treated as successfully with balloon dilation as with surgery. Stenoses within smaller branches of the renal artery may be treatable only by angioplasty. Iliac artery disease causing pseudostenosis may be treated by angioplasty, but the risk is present of embolization or dissection causing thrombosis or further ischemia. Surgical options include bypass of the stenosis using autologous saphenous vein, a prosthetic graft, or an allogeneic arterial graft procured from a deceased donor. The risk of the procedure has to be weighed against the potential benefit of improving renal transplant blood flow. In addition to the serum creatinine determination, a biopsy may be useful to assess the quality of the renal parenchyma. In advanced chronic rejection with a creatinine value greater than 2.5 mg/dL for more than 1 month, it may not be prudent to repair such arteries. Figure 14-3 shows a renal artery stenosis in the lower pole artery that was managed successfully by balloon angioplasty.

Arterial Thrombosis

Renal transplant arterial thrombosis usually occurs early (within 30 days) in the post-transplant period,[43] but should be a rare event because it is generally due to a technical error at the time of surgery. It usually is related to an intimal injury to the donor kidney during procurement or to anastomotic narrowing or iliac artery injury during implantation. Kidneys from donors younger than 5 years old have been associated with a higher risk of thrombosis.[53] The kidney tolerates only 30 to 60 minutes of warm ischemia before it is irreversibly injured, making it difficult to diagnose and correct this problem before it is too late to salvage the kidney. The diagnosis should be suspected in a patient who has had a transplant hours to days before and has had a good urine output but who suddenly has a decrease in urine output to zero. A high degree of suspicion has to be present, and the patient should be returned to the operating room promptly. If the patient had urine output preoperatively from the native kidneys, the diagnosis is hard to make in a timely manner because urine output may continue after the renal transplant has thrombosed. The advantage of diagnostic ultrasound has to be weighed against the disadvantage of delaying a return to the operating room. Almost all kidney transplants with arterial thrombosis are lost because of ischemic injury.

In cases of more than one renal transplant artery in which arterial reconstruction is performed at implantation, there may be increased risk of thrombosis of one or more arteries. This increased risk particularly is a concern if there is a small accessory renal artery supplying the lower pole of the kidney and providing the ureteral blood supply. Thrombosis of a branch artery may manifest as an increase in serum creatinine levels associated with increased hypertension. Angiography shows partial thrombosis and loss of perfusion of a wedge-shaped section of renal parenchyma. The risk of this situation, in addition to potential long-term hypertension, is caliceal infarction and urine leak in the early postoperative period. Such kidneys, with partial infarction, generally can be salvaged. Urine leaks occurring through the outer cortex of the kidney after partial infarction may be managed by nephrostomy tube placement for urinary drainage and placement of another drain adjacent to the kidney to prevent urinoma. When the transplant ureter necroses as a result of



A B

Figure 14–3 This patient presented with fluid retention, hypertension, and an elevated creatinine level. **A**, An arteriogram showed that the artery to the lower pole arising from a common aortic patch was stenotic proximally. **B**, This stenosis was successfully treated with balloon angioplasty with resolution of the patient's symptoms.

arterial ischemia, alternative urinary drainage needs to be provided surgically; this would be managed most often by ureteropyelostomy using the ipsilateral native ureter.

Renal Vein Thrombosis

Renal vein thrombosis may occur when the donor renal vein was narrowed by repair of an injury or when the vein was twisted or compressed externally, but it may occur in the absence of a technical complication. The diagnosis is indicated by sudden onset of gross hematuria and decrease in urine output, associated with pain and swelling over the graft. Ultrasound shows absence of flow in the renal vein, diastolic reversal of flow in the renal artery (Fig. 14-4), and an enlarged kidney often with surrounding blood. Ultrasound can point to this diagnosis definitively. Only if it is immediately recognized and repaired can this problem be reversed. Immediate surgical repair of the vein and control of bleeding are required, and it is generally necessary to remove the kidney and revise the venous anastomosis. Bleeding from the swollen and cracked kidney surface usually can be controlled with hemostatic agents.

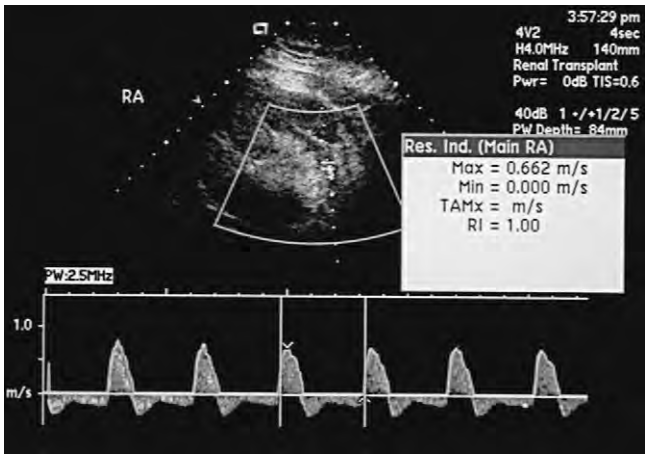


Figure 14–4 Ultrasound shows absence of flow in the renal vein and reversal of diastolic flow in the renal artery. This kidney was enlarged to 14 cm in length with a surrounding fluid collection that represented blood. These ultrasound findings were pathognomonic of transplant renal vein thrombosis. The condition was treated surgically with excision of the kidney, placement of a venous extension graft using donor iliac vein obtained from a third-party donor, and reimplantation of the kidney. Three weeks later, the patient had a normally functioning kidney transplant.

Postoperative Bleeding

As with all surgery, postoperative bleeding may complicate renal transplant outcomes. Bleeding generally occurs during the first 24 to 48 hours after transplantation and is diagnosed by a decreasing hematocrit, swelling over the graft with a bulging incision, or significant blood seepage from the incision. Most often, bleeding occurs in patients taking anticoagulation agents for other medical problems. Patients treated with clopidogrel for underlying cardiac disease are at significant risk for postoperative bleeding; this class of medications should be avoided or discontinued 1 week before renal transplantation if acceptable from a cardiac perspective.[19] If the hematoma is not clinically obvious, an ultrasound or computed tomographic scan can define its size and help determine whether or not surgical evacuation is appropriate. Treatment includes immediate surgery and blood transfusions as necessary.

REJECTION DURING THE EARLY POSTOPERATIVE PERIOD

Hyperacute Rejection

If a renal transplant is performed in the setting of ABO mismatch or a positive lymphocytotoxic crossmatch, the risk of hyperacute rejection is 85% (see Chapter 22). The incidence is not 100%, presumably because some antibodies have lower affinity or do not bind complement. There is no effective treatment for hyperacute rejection. It may be possible to prevent it by plasmapheresis to remove preformed antibodies, but variable results have been reported. Cases of blood type A_2 donors being transplanted to type O recipients have

been reported because type A_2 expresses less of the putative antigen, but this strategy also has increased risk of graft loss.[25] In almost all transplant centers, a crossmatch-negative, ABO-compatible recipient can be identified, or the kidney can be shipped to a center that has such a patient awaiting a kidney. A hyperacutely rejected kidney has no perfusion on renal scan (because of microvascular thrombosis) and needs to be removed.

Accelerated Vascular Rejection

Despite a negative T cell crossmatch test preoperatively, some patients may develop an early aggressive form of rejection, termed accelerated vascular rejection. This rejection is seen most often in sensitized patients with a high level of a panel-reactive antibody and in patients with a previous transplant. The time course of this type of rejection is typically within 2 to 5 days of the transplant procedure, and it tends to be poorly responsive to steroids and occasionally resistant to all forms of antirejection therapy. Histologically, such patients have fibrin deposition evident in the renal transplant biopsy specimen and endothelitis. Although successful prophylaxis of rejection has been described using intravenous immunoglobulin, rituximab, plasmapheresis, or thymoglobulin in highly sensitized patients,[26] when this form of rejection has started there is no standard treatment. We use plasmapheresis in this setting because of the likely contribution of the humoral immune response.

Acute Rejection

The most common form of immunological rejection in the early post-transplant period is acute cellular rejection, mediated predominantly by host lymphocytes responding to the allogeneic donor kidney. Acute rejection typically occurs 5 to 7 days after transplantation, but it can occur at virtually any time after this. The highest incidence of acute rejection is within the first 3 months, and overall rates of rejection vary from 5% to 50% within the first 6 months, depending on HLA matching and the immunosuppressive protocol. The clinical harbingers of acute rejection include an increasing creatinine level, weight gain, fever, and graft tenderness. Since the introduction of cyclosporine and tacrolimus, fever and graft tenderness are seldom present. The diagnostic "gold standard" is kidney biopsy, which can be performed safely under local anesthesia with light sedation. An 18-gauge biopsy needle is introduced under ultrasound guidance and removes a core of tissue that can be evaluated immediately for histological criteria of rejection (see Chapter 24). These criteria include tubulitis (invasion of tubules by lymphocytes) and arteritis.[48]

First-line treatment of acute cellular rejection is bolus steroid therapy with methylprednisolone sodium succinate (Solu-Medrol). Many regimens are used successfully, but a typical dose and duration are 500 mg intravenously, followed by 250 mg the following day, then a daily taper by 30-mg increments. Another commonly used regimen is three intravenous boluses of 0.5 g or 1 g of methylprednisolone 24 hours apart. About 85% to 90% of acute cellular rejection episodes are steroid responsive. If the patient's serum creatinine level has not begun to decrease by day 4 of therapy, alternative treatment must be considered, such as antilymphocytic globulin, alemtuzumab (Campath), or rituximab (anti-CD20) as lymphocytotoxic therapy. Antibody-depleting therapies may be associated, however, with an increase in infectious complications when used to treat rejection compared with when used for induction.[34] Rejection that does not respond to treatment with steroids or antibody therapy occurs in less than 5% of patients, although more frequently in sensitized patients or repeat transplants.

Patients who experience acute cellular rejection while taking cyclosporine or tacrolimus should have their calcineurin phosphatase inhibitor withheld during treatment of rejection because the increase in creatinine level makes them more susceptible to nephrotoxicity from these drugs, and there is generally no need for them to be taking cyclosporine while they are taking high-dose steroids or antilymphocyte therapy. This measure eliminates the possibility that a further increase of creatinine is due to cyclosporine or tacrolimus nephrotoxicity.

The impact of acute cellular rejection on graft survival depends on the response to treatment. Whether or not an early rejection episode predisposes the kidney to chronic rejection is controversial.

Graft Loss

During the early post-transplant period, if a renal transplant loses perfusion because of thrombosis or because of hyperacute, acute, or accelerated vascular rejection, it must be removed. Otherwise, the systemic toxicity of a necrotic kidney may cause fever, graft swelling or tenderness, and generalized malaise. Loss of perfusion can be assessed by nuclear scan or Doppler ultrasound. The technically easiest way to perform a transplant nephrectomy depends on how long the kidney has been in place. If nephrectomy is performed within 4 weeks, there are minimal adhesions, and the vessels are exposed easily for ligation and transplant nephrectomy. At later times, it is usually easiest to reopen the transplant incision and enter the subcapsular plane around the kidney. The kidney is dissected free in the subcapsular space, and a large vascular clamp is placed across the hilum. The kidney is amputated above the clamp, and 3-0 polypropylene (Prolene) is used to oversew the hilar vessels. The ureter also is oversewn (see Chapter 11).

MEDICAL COMPLICATIONS

Delayed Graft Function

DGF is the earliest and most frequent post-transplant complication. DGF is an important post-transplant complication because its occurrence has early and long-term consequences for allograft survival. The mechanism and cellular events that may cause DGF include donor factors, such as age, cause of death of the donor, and postischemic reperfusion injury with subsequent injury and activation of the immune system leading to an increased incidence of acute rejection.[31]

DGF is one of the main predictors of poor graft survival in cadaver donor renal transplantation. DGF typically is defined as the need for dialysis during the first week after transplantation. The incidence of DGF is significantly higher in cadaver versus living donor transplants and is less common in patients receiving first cadaver donor grafts than in patients undergoing repeat transplantation. An analysis of 107,787 cadaver donor kidney transplants reported to the

United Network for Organ Sharing Scientific Renal Transplant Registry between October 1987 and 2001 showed an incidence of approximately 23% for standard criteria donors versus 34% for expanded criteria donors.[9] An increase in DGF has been noted with advancing donor age. Young donors have a lower incidence of DGF (approximately 20%) compared with donors older than age 55 years (38%).[52] Prolonged cold ischemia time, at least 30 hours, does not seem to have a significant impact on the incidence of DGF, unless there is an episode of rejection. Although overall rejection rates have declined, recipients of expanded criteria donor kidneys are more likely to receive treatment for rejection, which may be a consequence of an increased incidence of DGF.[10]

The diagnosis of DGF is apparent during the first 24 hours after transplantation. Although some kidneys may make urine initially, a decline in urine output unresponsive to fluid challenge is the most common clinical scenario indicating DGF. The major differential diagnostic consideration in a patient with decreasing or absent urine output is an acute vascular or urological complication. Other conditions that can mimic DGF are antibody-mediated rejection[47] and recurrent focal glomerulosclerosis. This differential diagnosis can be determined easily with urgent ultrasound or radionuclide renal scanning. Typically, a transplant with DGF shows good renal perfusion and good parenchymal uptake of orthoiodohippurate (^{123}I OIH) or mercaptoacetyltriglycine (^{99m}Tc MAG 3) with poor or no renal excretion. Kidney transplant biopsy is the gold standard for diagnosis. When the diagnosis of DGF is established, careful attention to fluid status is paramount to decrease the frequency and necessity for dialysis. The usual time course of DGF is 10 to 14 days, and patients may require supportive dialysis therapy for management of fluid and electrolyte disturbances.

The major concern for transplant recipients with DGF is the potential for early acute rejection. Data are accumulating that the development of DGF may lead to activation of the immune system with release of cytokine and adhesion molecules (see Chapter 24).[23,31] This situation may lead to an anti–major histocompatibility complex (MHC)–directed alloimmune response, leading to an increased frequency of acute rejection. The diagnosis of rejection in patients with DGF may be hindered because the primary clinical monitoring tool is a decrease in serum creatinine levels. For this reason, some centers use antilymphocyte therapy, such as thymoglobulin or Atgam, to prevent early acute rejection in patients with DGF. Alternatively, frequent biopsies in patients with DGF have been proposed as a way to detect early acute rejection episodes. Graft half-lives in patients with DGF are shortened with or without acute rejection. Graft half-lives in standard criteria donor recipients with DGF average 8.8 years compared with 13 years for patients without DGF or rejection. Graft half-lives in expanded criteria donor kidneys are 7.7 years without DGF and 6 years with DGF.[9] Prevention of DGF and early recognition of rejection are important goals to help improve early and long-term graft survival.

Nephrotoxicity from Calcineurin Inhibitors

Early institution of calcineurin inhibitors (cyclosporine and tacrolimus) after transplantation is important to prevent acute rejection episodes. Because of the potential for additive nephrotoxicity, however, some centers avoid instituting calcineurin inhibitors until there is adequate function of the transplanted kidney. Most centers that delay the onset of calcineurin inhibitors use some form of sequential antibody induction therapy with humanized or chimeric interleukin-2 receptor inhibitors such as daclizumab or basiliximab, polyclonal antibodies such as thymoglobulin or Atgam, or a monoclonal antibody such as OKT3. Other centers begin administering calcineurin inhibitors early in the post-transplant course whether or not the allograft is functioning well or in DGF. Both of the calcineurin inhibitors, cyclosporine and tacrolimus, are effective in preventing acute rejection episodes, but they can lead to nephrotoxicity primarily by decreasing renal blood flow in the afferent arteriole, leading to tubular injury.[32,44] Because of variability of intestinal absorption in the early transplant period, underdosing and overdosing of these agents is common, which can lead to rejection episodes or cyclosporine nephrotoxicity, or both occurring in the same patient. Although there are many clinical parameters that have been advocated to differentiate calcineurin inhibitor nephrotoxicity from rejection, most clinical parameters are of insufficient sensitivity to predict confidently the cause of the transplant dysfunction. In patients with DGF, it may be more difficult to diagnose acute rejection or calcineurin nephrotoxicity reliably. Monitoring cyclosporine and tacrolimus levels is valuable in preventing significant increases in blood levels, which may lead to nephrotoxicity. Some centers routinely use a high-dose calcineurin inhibitor protocol to prevent rejection and accept a certain level of nephrotoxicity as a consequence.

The most reliable way of differentiating calcineurin nephrotoxicity from rejection is percutaneous renal allograft biopsy. Generally, biopsies can be performed 3 to 5 days after transplantation using real-time ultrasound imaging and automated biopsy needle devices. The histological hallmarks of calcineurin nephrotoxicity vary. Early functional nephrotoxicity is manifested most often by evidence of tubular injury. In patients with established calcineurin nephrotoxicity, reducing the dose or temporary discontinuation of cyclosporine or tacrolimus can lead to reversal of the renal injury.

The avoidance of subclinical or clinical episodes of nephrotoxicity may be important in terms of long-term allograft histology.[54] A study that examined 2-year biopsy specimens of tacrolimus-treated and cyclosporine-treated recipients showed that chronic transplant nephropathy and fibrosis strongly correlate with episodes of early clinical nephrotoxicity from these agents. This study led to a re-examination of calcineurin-sparing protocols, and clinical studies are now in progress.

Prerenal Azotemia and Volume Contraction

Prerenal azotemia or volume contraction often may lead to allograft deterioration during the immediate postoperative period. Excessive use of diuretics and uncontrolled blood glucose are two of the most common causes for the development of prerenal azotemia from volume contraction. Because most of these patients already are receiving calcineurin inhibitors, which decrease renal blood flow, the concomitant insult of volume contraction may lead to elevated blood urea nitrogen and serum creatinine levels, which may be difficult to distinguish from an episode of acute rejection. Careful attention to daily weights and intake

and output and assessment of orthostatic blood pressure changes can diagnose volume contraction as a contributing factor for renal allograft dysfunction. Volume repletion with intravenous or oral fluids is indicated.

Other Drug Toxicity

Transplant patients often have complex pharmacological regimens at the time of transplantation, which may include nephrotoxic medications or medications that may cause concomitant nephrotoxicity with calcineurin inhibitors.[30,56] Examples of the former include nonsteroidal anti-inflammatory drugs and nephrotoxic antibiotics such as amphotericin and aminoglycosides. Drugs that may interact with the metabolism of calcineurin inhibitors include calcium channel blockers such as diltiazem and verapamil, ketoconazole, erythromycin, and fluconazole. Tacrolimus and cyclosporine are metabolized in the cytochrome P-450-3A4 system, and all of these agents may increase the blood levels of tacrolimus or cyclosporine. Grapefruit juice also has been shown to increase the gastrointestinal absorption of cyclosporine (see Chapters 16 and 17).

Routine drug level monitoring is paramount when drugs that are metabolized in the cytochrome P-450-3A4 system are used. Adjustment in the daily dose of cyclosporine and tacrolimus to attain therapeutic blood levels may help prevent episodes of nephrotoxicity from the concomitant use of these agents. Avoidance of concomitant medications that interfere with drug metabolism is desirable. Selective serotonin reuptake inhibitor antidepressants are another class of pharmacological agents that need to be used with care. In particular, nefazodone and fluvoxamine are metabolized in the cytochrome P-450-3A4 system and may increase calcineurin blood levels.

Recurrent Disease

Most causes of renal failure do not recur in the transplanted kidney; when they do, it is usually later in the post-transplant course. (See also Chapters 4 and 24 for further discussion of recurrent disease.) Two diseases may occur in the immediate post-transplant period and lead to significant graft dysfunction or graft loss if not treated aggressively. Focal glomerulosclerosis is the most common glomerulonephritis that can recur in the immediate postoperative period.[2,3] Presumably, a serum factor is present that causes glomerular injury and massive early proteinuria.[50] It is uncommon but may occur immediately after transplantation. The diagnosis is established by the development of a nephrotic range of proteinuria in a patient with a pretransplant diagnosis of focal segmental glomerulosclerosis and is confirmed on biopsy. Electron microscopy shows diffuse foot process effacement, which is diagnostic in this setting. Various strategies have been used to treat recurrent focal segmental glomerulosclerosis, including high-dose calcineurin inhibitors, prednisone, and plasmapheresis. Currently, plasmapheresis seems to be most effective in the treatment of recurrent focal segmental glomerulosclerosis; however, some patients may have only a partial remission or may not respond to this modality.[3] The usual course of therapy is 9 to 10 plasmapheresis treatments over several weeks. In some cases, plasma exchange may need to be repeated if there is an initial response and subsequent relapse. If patients do not have any response, it is unlikely that additional plasmapheresis therapy will be effective.

The other recurrent disease of concern in the immediate postoperative period is thrombotic microangiopathy, which can result from recurrent disease, endothelial injury from calcineurin inhibitors, hypercoagulable disorders, or antibody-mediated rejection.[11] Thrombotic microangiopathy is multifactorial in origin. It is characterized clinically by a decrease in hematocrit or platelet count, or both, with evidence of a microangiopathic process on peripheral blood smear, increased lactate dehydrogenase levels, and transplant allograft dysfunction. Kidney biopsy specimens show fibrin clot in the small arterioles of the kidney. Thrombotic microangiopathy has been noted to be induced by tacrolimus or cyclosporine. Discontinuation of the calcineurin inhibitor and plasmapheresis[27] have been beneficial in some series. The use of anticoagulants and aspirin is of uncertain benefit.

Infection

In the immediate postoperative period, most infections are related to the surgical procedure and usually involve wound infection, bacteremia from a central line, urinary tract infection, or pneumonia.[49] (See Chapter 29 for a complete discussion of infection.) Prevention of these infections involves meticulous surgical technique, careful line care and use, removal of the Foley catheter as soon as possible, and early mobilization of the patient to prevent atelectasis or pneumonia. Most opportunistic infections do not occur until after the first 30 days. Of the opportunistic infections, cytomegalovirus is still common after transplantation, particularly in recipients who are seronegative for cytomegalovirus and who receive seropositive organs. Epstein-Barr virus infection may occur early after transplantation and usually is related to heightened immunosuppression in a previously seronegative patient. In the past, *Pneumocystis carinii* pneumonia was a frequent complication of transplantation; however, most centers now use routine prophylaxis with trimethoprim/sulfamethoxazole, which has nearly eliminated the occurrence of this infection in transplant patients. Other prophylactic strategies that have been used include intravenous ganciclovir in the immediate postoperative period followed by high-dose oral acyclovir or oral valganciclovir for at least 3 months.[41] The antiviral agents are effective at reducing the incidence and severity of cytomegalovirus infection (particularly oral ganciclovir); however, after stopping ganciclovir, cytomegalovirus may still occur. Other prophylactic agents include antifungal agents, such as fluconazole or clotrimazole troches, which can reduce the risk of mucosal *Candida* superinfection.

Highly resistant organisms have been detected with increasing frequency in transplant patients. Vancomycin-resistant *Enterococcus*[37,38,40] and *Candida*[35,42,46] infections are becoming significant causes of morbidity in hospitalized transplant patients. Risk factors for vancomycin-resistant *Enterococcus* include prolonged hospitalization in the intensive care unit, extensive surgical procedures, and intra-abdominal infection. Treatment options for this infection are limited. Quinupristin/dalfopristin (Synercid), linezolid (Zyvox), and daptomycin (Cubicin) may be useful for control of serious vancomycin-resistant *Enterococcus* infections. The increase in *Candida* infection seems to be due to the routine use of clotrimazole or fluconazole to prevent *Candida* infection. Intravenous antibiotic use predisposes patients to fungal infection after transplantation.

Table 14–2 **Advantages and Potential Side Effects of Antihypertensive Agents in Transplant Recipients**

Class	Advantages/Indications	Side Effects
Diuretics	Salt-sensitive hypertension	Hyperuricemia Volume depletion
β-Blockers	Large selection Selective agents preferred	Adverse effect on lipids Relative contraindication with asthma, CHF, diabetes, or peripheral vascular disease
α-Blockers	Useful with prostatic hypertrophy	Postural hypotension (first dose)
Central α-agonists	Clonidine useful in diabetic patients Clonidine available as transdermal patch	Dry mouth Rebound hypertension Fatigue
Calcium channel blockers	Improve renal blood flow May ameliorate cyclosporine nephrotoxicity	Drug interaction with cyclosporine (verapamil and diltiazem)
ACE inhibitors	Native kidney hypertension	May cause renal insufficiency Hyperkalemia
Angiotensin II receptor antagonists	Proteinuria	Anemia

ACE, angiotensin-converting enzyme; CHF, congestive heart failure.

When an infection has occurred, aggressive management is indicated. This management may include removal of central venous catheters or Foley catheters. Any intra-abdominal fluid collections should be aspirated and drained if found to be infected. Urinary tract infections should be treated promptly, preferably after the Foley catheter and ureteral stent have been removed.

Hypertension

Hypertension develops in nearly 80% of renal transplant patients after transplantation.[13,14,22,45,59] (See also Chapter 28.) In kidney transplant recipients, hypertension may be due to intrinsic problems with the allograft (DGF, rejection, cyclosporine nephrotoxicity, or donor allograft nephropathy) or due to extrinsic causes (hypertension from the native kidneys or familial hypertension). Because multiple causes may be present in the same patient, it often is difficult to ascertain the specific cause of hypertension after transplantation.

For some patients, hypertension is associated with immunosuppression. Cyclosporine, tacrolimus, and corticosteroids all may contribute to the development of hypertension. Cyclosporine and tacrolimus cause afferent arteriole vasoconstriction, which may stimulate the release of endothelin. Hypertension may ensue as a result of the activation of the renin-angiotensin system. Patients with significant hypertension should be treated aggressively. Most centers prefer the use of calcium channel blockers and β-blockers as first-line agents, although angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists are being used more frequently. The major issue with the use of angiotensin-converting enzyme inhibitors or angiotensin II receptor inhibitors is anemia, which can be a problem in patients treated with calcineurin inhibitors. Patients who do not respond readily to antihypertensive therapy or have new-onset hypertension need to be evaluated. Hypertension may be the result of renal artery or iliac artery stenosis, which may be compromising renal blood flow to the kidney and causing hypertension.[6,51] A patient with a bruit over the transplant with poorly controlled hypertension and fluid retention needs to be evaluated carefully for renal artery or iliac artery stenosis. Table 14-2 presents the advantages and potential side effects of antihypertensive agents in transplant recipients.

Management of Graft Dysfunction

The diagnosis and treatment of graft dysfunction are integral components of successful long-term management of the renal transplant recipient. Early diagnosis and directed therapy are crucial in the early post-transplant period to initiate appropriate therapy and avoid potential overimmunosuppression. Evaluation of graft dysfunction should start with a careful history to see if there is a potential for nephrotoxicity from drugs or if there is any likelihood of volume contraction contributing to the elevation of serum creatinine levels. A vigorous search for potential infection should follow, and if there is no obvious cause for deterioration in graft function, an ultrasound followed by a renal biopsy should be performed. If there is any clinical suspicion of renal artery or iliac artery stenosis, a magnetic resonance angiogram or arteriogram should be performed. The differentiation of calcineurin nephrotoxicity or rejection is ascertained most easily with percutaneous renal biopsy. Nephrotic range proteinuria in a patient whose original disease was focal segmental glomerulosclerosis or thrombotic microangiopathy should prompt an immediate biopsy for diagnosis and potential treatment with plasmapheresis.

SUMMARY

Optimization of outcomes after renal transplantation depends on rapid diagnosis and treatment of surgical and medical complications. In view of the invasiveness of the transplant procedure itself, the complexity of medical problems in this patient population, and the side effects of nonspecific immunosuppressive therapy, close attention to the problems outlined in this chapter is crucial to avoid graft loss and patient death. Because the frequency of complications is greatest during the early post-transplant period, this is the time when vigilance should be highest.

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Chapter 15
Azathioprine and Steroids

Simon R. Knight • Peter J. Morris

Azathioprine and steroids were the backbone of immunosuppression in renal transplantation for many years and the only form of immunosuppression from the early 1960s to the early 1980s, when cyclosporine first became available. After the introduction of cyclosporine, azathioprine and steroids were used in combination with cyclosporine or often after cessation of cyclosporine in so-called conversion protocols (see Chapter 16). One might wonder whether in the sixth edition of this book there still needs to be a chapter on azathioprine and steroids, bearing in mind the introduction of mycophenolate and sirolimus, both of which are antiproliferative agents, but with different mechanisms of action. Mycophenolate has largely replaced azathioprine in developed countries as a standard therapy with a calcineurin inhibitor and steroids (see Chapters 16, 17, and 18). Azathioprine is an inexpensive agent, however, and it is expected to continue to have a role in transplantation not only in the Western world in combination with cyclosporine but also, in particular, in developing countries where the cost of immunosuppression is a major factor in determining immunosuppressive protocols.

Although steroids are expected to continue to have a place in the prevention and treatment of rejection, the introduction of more powerful immunosuppressive agents is allowing steroid-sparing protocols to be developed. As outlined later in this chapter, the complications of steroids are considerable, and a major aim of current immunosuppressive protocols and trials is to diminish the use of steroids or to avoid their use altogether.

Mercaptopurine was developed by Elion and Hitchings at Burroughs Wellcome as an anticancer agent in the 1950s.[30,31] Subsequently, mercaptopurine was shown to be an immunosuppressive agent by Schwartz and Dameshek[127,128]; it suppressed the humoral response to a foreign protein and prolonged the survival of skin allografts in rabbits. The key publication by Schwartz and Dameshek on drug-induced immunological tolerance was noted by Calne in the United Kingdom and Hume in the United States, and these investigators independently showed that mercaptopurine could delay or prevent rejection of renal allografts in dogs. In the original paper of Calne,[14] only two dogs survived the renal transplant operation for a short time, but when the dogs died from pneumonia at a little more than 1 month after transplantation, there was no histological evidence of rejection whatsoever, which was a unique finding at that time. Similar results in a much larger series of dog renal transplants in Hume's unit in Richmond, Virginia, were published at the same time.[153] Soon after that, Elion and colleagues[32,33] produced azathioprine, an imidazolyl derivative of mercaptopurine, and this drug seemed to be less toxic than mercaptopurine.[15] Azathioprine was first used in the clinic at the Peter Bent Brigham Hospital, Boston in 1961.[99,100] Soon thereafter, azathioprine was introduced into renal transplantation in a rapidly increasing number of renal transplant units throughout the world.

Steroids first were used to treat rejection in patients on azathioprine,[41] but then were added to azathioprine by Starzl and coworkers[137] to prevent rejection from the time of transplantation because rejection seemed inevitable. From the beginning of this so-called azathioprine era, arbitrarily large doses of steroids were given from the time of transplantation with a gradual reduction over 6 to 12 months to maintenance levels. The high doses of steroids used with azathioprine were responsible for most of the morbidity of transplantation (discussed later). It was not until the 1970s that a series of randomized trials and observational studies led slowly to the realization that low-dose steroids were as effective as high-dose steroids in preventing rejection and that there was a major reduction in steroid complications of transplantation with low-dose regimens. By the late 1970s, azathioprine and low-dose steroids, sometimes used together with an antilymphocyte serum or globulin for induction (particularly in North America), were the standard immunosuppressive therapy until the introduction of cyclosporine in the early 1980s.

AZATHIOPRINE

Mechanism of Action

Azathioprine and mercaptopurine are thiopurines, and azathioprine is an imidazolyl derivative of mercaptopurine.

Azathioprine is metabolized in the liver before becoming active. One metabolic pathway is through its conversion to mercaptopurine, the active metabolite of mercaptopurine being 6-thioinosinic acid. Azathioprine also is metabolized by other pathways independent of mercaptopurine. Azathioprine inhibits DNA and RNA synthesis by preventing interconversion among the precursors of purine synthesis and suppressing de novo purine synthesis. Azathioprine and mercaptopurine block lymphocyte proliferation in vitro and the production of interleukin-2, which is probably an important aspect of its antiproliferative activity.[5] Xanthine oxidase has an important role in the catabolism of mercaptopurine, and if allopurinol is used with azathioprine, it is mandatory to reduce the dosage of azathioprine significantly because the allopurinol inhibits the xanthine oxidase pathway.[33] This inhibition increases not only the immunosuppressive potency but also the major side effect of azathioprine—bone marrow depression. Although the metabolites are excreted in the urine, they are inactive, and no reduction in dosage is required in the presence of a nonfunctioning kidney.[4] Polymorphisms in the thiopurine S-methyltransferase enzyme, which catalyzes the S-methylation of mercaptopurine and azathioprine, may be associated with an increased likelihood of myelotoxicity and leukopenia.[34,81]

Dosage

Azathioprine is given as a single daily dose; if used with steroids alone, a suitable dose is 2.5 mg/kg/day. Careful monitoring of the leukocyte count is required, particularly in the early weeks after transplantation, when the dosage is reduced only in the presence of leukopenia. Although the dose of azathioprine may be reduced with time, a maintenance dose, particularly in the presence of low-dose steroids, should not be less than 2 mg/kg/day. An important multicenter randomized trial was done in Australia to test low-dose versus high-dose steroids used with azathioprine after transplantation. The trial failed to show that low-dose steroids were as effective as high-dose steroids (in contrast to earlier but smaller trials), until it was realized that the poorer outcome with low-dose steroids was confined to units using low-dose azathioprine (i.e., <2 mg/kg/day).[22] A more recent analysis of data from the Collaborative Transplant Study also suggested that long-term graft survival was related to the dose of azathioprine that patients were receiving for maintenance. Patients receiving azathioprine and steroids only, who were receiving greater than 1.5 mg/kg, had better graft survival than patients receiving a lower maintenance dose of azathioprine.[105]

When azathioprine is used with cyclosporine and steroids (triple therapy), lower doses are given. A fairly standard dose of azathioprine in a triple-therapy protocol is 1.5 mg/kg, or 100 mg/day (see Chapter 16). At this level, hematological toxicity is uncommon except in the presence of cytomegalovirus infection. There is some evidence in experimental models that azathioprine and cyclosporine are synergistic in terms of immunosuppression,[135] but there is no evidence of this in clinical studies.

In a randomized trial, low-risk patients received azathioprine and steroids or cyclosporine, azathioprine, and steroids. All patients received antilymphocytic globulin induction. Patient and graft survival were the same at 12-year follow-up, as was the incidence of rejection, but renal function was better in the patients not given cyclosporine.[46]

Side Effects

The major complication of azathioprine therapy is bone marrow aplasia most commonly evident as leukopenia, although in cases of more severe marrow depression, anemia and thrombocytopenia may be present. Regular monitoring of the leukocyte count is an important aspect of azathioprine therapy, and if the leukocyte count decreases to less than 3×10^9/L, the azathioprine dose should be reduced. Megaloblastic anemia has been described in association with the use of azathioprine. As already mentioned, if allopurinol is required for the prevention of gout, the azathioprine dose should be reduced to 25% of the previous dose.

Hepatotoxicity has been attributed to azathioprine for many years, and although undoubtedly azathioprine is associated with hepatic dysfunction, this is probably rare (see Chapter 30). Other causes of hepatic dysfunction in the presence of azathioprine need to be sought energetically before attributing it to azathioprine. Hair loss is a common side effect of azathioprine when used in therapeutic doses. Early observations attributed an increased incidence of squamous cell cancer in transplant patients to azathioprine. There does not seem to be any evidence, however, that squamous cell cancers have a greater incidence in patients treated with azathioprine and steroids compared with patients treated with other immunosuppressive protocols, such as cyclosporine and steroids. The major factor in the increased incidence of squamous cell cancer in immunosuppressed patients is the overall immunosuppressive load, rather than any specific drug activity (see Chapters 32 and 33).

Monitoring of Azathioprine Therapy

Blood levels of azathioprine or its metabolites are not routinely monitored in clinical practice. As already suggested, the leukocyte count is monitored, and the dose is adjusted if leukopenia arises. It has been noted, however, that leukopenia also can result from viral infection, leading to the suggestion that erythrocyte 6-thioguanine nucleotide levels may be a better indicator of azathioprine activity in transplant patients.[126]

Numerous genetic variations in the thiopurine methyltransferase gene have been identified, which have been related to azathioprine-induced myelotoxicity.[34,81] Genotyping for this polymorphism before starting azathioprine might allow the appropriate azathioprine dosage to be determined for an individual patient.[122]

Azathioprine and Mycophenolate Mofetil

Three classic randomized controlled trials comparing azathioprine or placebo with two doses of mycophenolate mofetil (MMF) in a triple-therapy protocol with cyclosporine (Sandimmune) and steroids were done in the early 1990s (see Chapter 18). These three trials showed a significant reduction in the incidence of acute rejection, although patient survival and graft survival were not different at 1 year. Gradually, azathioprine was replaced with MMF in most modern immunosuppressive protocols. In recent years, there has been some doubt cast on the superior efficacy of MMF over azathioprine, however, especially in the era of microemulsion formulations of cyclosporine. Remuzzi and colleagues[118] conducted a trial comparing azathioprine with

MMF, in which Neoral was used instead of Sandimmune, together with steroids. They found no difference in rejection rates or in graft survival. This result was attributed to the superior absorption of the Neoral formulation compared with Sandimmune. Remuzzi and colleagues[118] also pointed out that the cost of MMF was some 15 times more than azathioprine. Another cohort study from UK Transplant compared the long-term outcome of cadaver donor kidneys in which one kidney went to a recipient who received azathioprine and the paired kidney went to a patient given MMF.[130] In this paired kidney analysis, there was no difference in patient or graft survival, but increased rejection rates were noted in the MMF group.

Another small trial compared MMF with azathioprine in combination with tacrolimus and steroids and found no difference in outcome.[98] A large randomized trial comparing tacrolimus, MMF, and steroids with tacrolimus, azathioprine, and steroids or cyclosporine (Neoral), MMF, and steroids showed that at 3 years all three regimens were safe and efficacious, but the best overall results were with the tacrolimus, MMF, and steroid combination.[40] Finally, an analysis of 49,666 primary renal allograft recipients reported to the United States Renal Data System suggested that continued therapy with MMF was associated with a protective effect against declining renal function at 1 year compared with azathioprine.[96]

Cyclosporine Conversion to Azathioprine

Conversion of cyclosporine to azathioprine can be successfully achieved at 3 to 12 months after transplantation, with a resulting improvement in renal function, albeit with an increased risk of acute rejection; this is well documented in Chapter 16.

Azathioprine Conversion to Mycophenolate Mofetil

There have been numerous studies in patients with chronic allograft nephropathy receiving a calcineurin inhibitor with azathioprine and steroids in whom azathioprine has been switched to MMF, and the calcineurin inhibitor dosage has been either reduced or eliminated. Generally, most of these studies, but not all, showed either stabilization or an improvement in renal function.[38,86,138]

Tacrolimus and Azathioprine

Several studies of tacrolimus with or without azathioprine suggest that azathioprine does not add anything to the immunosuppressive potency provided by tacrolimus. One large randomized trial in Europe involving nearly 500 patients showed no difference in outcome at 3 years with regard to patient survival, graft survival, and acute or chronic rejection.[110] (See Chapter 17.)

STEROIDS

Mechanism of Action

Steroids are administered as prednisone or prednisolone. These agents are absorbed rapidly from the gut, and peak plasma concentrations occur 1 to 3 hours after administration. The mechanism of action of steroids is extremely complex and is still not understood fully.[21,35,119] Steroids are anti-inflammatory and immunosuppressive. It was first noted by Billingham and colleagues[9] that cortisone would produce a modest prolongation of the life of skin allografts in the rabbit. In the treatment of acute rejection, the anti-inflammatory activity probably produces the immediate response, whereas when used prophylactically the immunosuppressive activity is predominant. A small randomized trial comparing prednisolone with a nonsteroidal anti-inflammatory drug (ibuprofen) showed a higher rate of rejection in the patients receiving the nonsteroidal agent, suggesting that the major role of steroids in renal transplantation is not their anti-inflammatory effect.[79]

Steroids are metabolized in the liver, where prednisone is converted to prednisolone. Although it has been estimated that the bioavailability of prednisone is approximately 80% of that achieved by prednisolone, no evidence exists in practice that there is a difference in outcome between prednisone (used most commonly in the United States) or prednisolone (used most commonly in Europe).[13,37] The half-life of steroids is short—about 60 minutes for prednisone and 200 minutes for prednisolone. These half-lives are increased substantially in the presence of hepatic dysfunction and are shorter in the presence of drugs such as phenytoin and rifampicin that induce hepatic enzymes. There is no evidence that these interactions have produced significant problems in clinical practice. It also has been shown that the clearance of prednisolone is slower in patients receiving cyclosporine compared with patients receiving azathioprine.[108] A later study suggested, however, that cyclosporine did not influence the metabolism of methylprednisolone, but the authors noted a considerable variation of the metabolism of methylprednisolone among patients.[144] The time-dependent and dose-dependent induction of uridine diphosphate glucuronosyltransferase activity by steroids may increase the clearance of mycophenolic acid, reducing exposure to mycophenolate. Cattaneo and coworkers have shown that as steroids are tapered over the postoperative period, the mycophenolic acid area under the curve increases.[16] The pharmacokinetics of prednisolone during sirolimus therapy also have been studied, with some evidence for a minor interaction between sirolimus and prednisolone in some patients.[70]

Steroids do have a significant effect in vitro on T cell proliferation, blocking interleukin-2 production.[103] A variety of other actions may augment their immunosuppressive activity (e.g., preventing the induction of interleukin-1 and interleukin-6 genes in macrophages).[77,152] The anti-inflammatory activity perhaps is mediated by the inhibition of migration of monocytes to areas of inflammation,[35] and this same anti-inflammatory activity has a marked deleterious effect on wound healing.

Steroid Resistance

The sensitivity of individuals to steroid therapy varies. A study in healthy volunteers showed a wide interindividual variation in the inhibition of lymphocyte proliferation by steroids.[53] Steroid resistance is seen frequently in patients with inflammatory conditions and has been shown to correlate well with in vitro measurements of lymphocyte steroid sensitivity in patients with rheumatoid arthritis,[76] ulcerative colitis,[52] asthma,[20] and systemic lupus erythmatosus.[129]

In vitro studies of lymphocyte steroid sensitivity have shown a higher incidence of resistance in patients with chronic renal failure than in healthy volunteers (52.9% versus 3.8%).[72] In renal transplant recipients, Langhoff and colleagues[82] showed that pretransplant in vitro measurements of lymphocyte sensitivity are predictive of graft survival at 1 year in patients coadministered azathioprine, but less so in patients receiving cyclosporine. These results have been confirmed in vivo, with significantly higher sensitivity to methylprednisolone seen in patients with graft function at 6 months compared with patients with graft failure.[83] This difference in sensitivity is smaller in cyclosporine-treated patients than in patients receiving azathioprine, suggesting that the effect is partly offset by the use of cyclosporine. A more recent study from Hirano and colleagues[55] has shown an increased risk of acute allograft rejection after renal transplantation in patients with low pretransplant lymphocyte steroid sensitivity cotreated with cyclosporine and prednisolone. The variability in pretransplant lymphocyte response was higher with prednisolone than with methylprednisolone, suggesting a role for methylprednisolone in prednisolone-resistant recipients. Reduced lymphocyte prednisolone sensitivity correlates with impaired sensitivity to cyclosporine and tacrolimus, which may play a role in the high risk of allograft rejection in these patients.[72]

Numerous potential mechanisms for this resistance to steroids have been suggested.[123] Administration of glucocorticoid receptor (GR) agonists is capable of downregulating GR expression in human lymphocytes, although the mechanism for this homologous downregulation is poorly understood.[124] Studies have shown no correlation, however, between GR density or affinity and resistance to steroids, pointing to a postreceptor mechanism.[53,55]

Alternate splicing of human GR pre-mRNA generates two isoforms, hGRα and hGRβ; hGRβ is capable of acting as an inhibitor of hGRα-mediated transcription.[6] It has been suggested that differences in the ratio of the two isoforms may result in relative steroid resistance. Proinflammatory cytokines are capable of inducing hGRβ expression,[85,139] and increases in hGRβ-positive lymphocytes have been identified in many inflammatory conditions, including ulcerative colitis[57] and asthma.[50,85] More hGRβ-positive cells are seen in glucocorticoid-resistant than in glucocorticoid-sensitive individuals in these conditions.

The hGRα isoform is capable of suppressing the activity of the proinflammatory transcription factor nuclear factor κB (NFκB), and this suppression seems to be mutual.[95] NFκB activity is upregulated by several proinflammatory signals (e.g., tumor necrosis factor-α, lipopolysaccharide), providing a potential mechanism for the decreased steroid sensitivity seen in inflammatory conditions. Expression of the NFκB p65 subunit is increased in ulcerative colitis patients before treatment and is decreased by the administration of steroids in steroid-sensitive patients but not in steroid-resistant patients.[88]

More recent studies have concentrated on the role of interleukin-2 in glucocorticoid resistance. Interleukin-2 and anti-CD28 have been shown to reduce dexamethasone-mediated suppression of CD4 cell proliferation.[146] Interleukin-2 and CD28 signals are transduced via the mitogen-activated protein kinase and extracellular signal-regulated kinase pathway. Blockade of this signal transduction pathway abolishes the costimulation-induced resistance to dexamethasone. Inhibitors of this pathway may have a role to play in the therapy of steroid resistance. Steroid resistance is a complex phenomenon and must be relevant to the occurrence of so-called steroid-resistant rejection (discussed later).

Dosage

Steroids have been used since the introduction of azathioprine to prevent and to treat rejection. When used prophylactically, steroids were used initially in high doses (e.g., 100 mg/day), reducing to a maintenance dose of 20 mg/day over 6 to 9 months. As mentioned earlier, a maintenance dose of steroids in association with azathioprine requires a therapeutic dose of azathioprine in most instances—at least 2 mg/kg/day of azathioprine. McGeown and coworkers[94] consistently reported excellent graft survival from Belfast with a low incidence of steroid-related complications using a dose of prednisolone of 20 mg/day given orally as a single morning dose, with a further reduction occurring at 6 months to a baseline maintenance dose of 10 mg/day. Because most of the Belfast patients had bilateral nephrectomies, and all had more than 100 blood transfusions before transplantation, it was unclear whether the excellent results were related to the low dosage of steroids or to a transfusion effect, which was recognized widely as an important factor in improving graft outcome in the azathioprine era.

Initially, trials of low-dose versus high-dose steroids were performed in Oxford, then in many other centers, all of which showed not only that low-dose steroids were as effective as high-dose steroids in preventing rejection but also that there was a significant reduction in steroid-related complications in patients receiving low-dose steroids.[12,17,18,24,64,66,97,109,136] The results of these trials led quickly to the wide adoption of low-dose steroid regimens with azathioprine. In contrast, a study from Helsinki suggested that an initial high dose of methylprednisolone resulted in significantly better graft survival at 1 year.[51] The results of the large multicenter trial reported by d'Apice and associates,[22] already referred to, showed that low-dose steroids are only equally effective as high-dose steroids in preventing rejection if therapeutic doses of azathioprine are used (i.e., at least 2 mg/kg/day).

With the introduction of cyclosporine, steroids remained in use with or without azathioprine. Generally, low-dose steroid protocols were continued, although there was a tendency, particularly in North America, to go back toward higher steroid dosage regimens in the first few weeks after transplantation. This was a relatively transient practice, and now with modern immunosuppressive protocols, low-dose steroids are the norm, and discontinuation of steroids is becoming increasingly possible, not only after 1 year in the case of triple therapy (see Chapter 16) but also as early as 7 days after transplantation with more potent immunosuppressive protocols (see Chapters 17 and 20).

Whether steroids should be given as a single daily dose in the morning or in divided doses has not been resolved. Because of the short half-life of prednisone and prednisolone, divided doses may be more rational, but it could be argued that a single morning daily dose would be more appropriate taking into account the diurnal rhythm of glucocorticoid metabolism.[42,102] There is no clinical evidence that one or the other protocol is more effective or less likely to produce side effects.

For many years, maintenance dosages of prednisone or prednisolone of 10 mg/day were standard therapy in association with azathioprine. In patients with long-surviving grafts with good function, steroid dosages have been reduced to 5 or 6 mg/day. It is unlikely, however, that many patients who are taking azathioprine and steroids long term would be able to have their steroid dosage reduced to much less than 5 mg/day. Previous attempts to withdraw steroids have often led to the onset of rejection when dosages of less than 5 mg/day are reached; this is important to note because there are many long-surviving patients still taking azathioprine and steroids. When patients have been on steroids for many years, their adrenocortical function may not recover from the long-standing suppression as the steroid dose is reduced, and this may produce clinical features of adrenocortical insufficiency.[101]

Alternate-day steroid therapy for maintenance also has been used widely, especially in children in an attempt to reduce side effects, particularly growth retardation.[11,25,27,84,93,115] In children, alternate-day therapy may be associated with a greater incidence of rejection, but this is probably not the case in adults. A small randomized trial of alternate-day therapy failed to show any benefit over daily steroids, however.[93] Alternate-day therapy may lead to greater problems with respect to compliance, in contrast to a daily regimen of steroids. It has been and still is common practice to administer a bolus of methylprednisolone prophylactically during the transplant operation with the aim of increasing immunosuppression and perhaps preventing delayed graft function, but a randomized prospective trial of bolus methylprednisolone versus placebo at the time of surgery did not show any benefit of the high perioperative intravenous dose of methylprednisolone.[74] Nevertheless, it remains standard practice whatever the immunosuppressive regimen is to be.

Treatment of Acute Rejection

High-dose steroids are the first approach to the treatment of an acute rejection episode. In some units in the early days of azathioprine, especially at the Necker Hospital in Paris, steroids were not administered prophylactically to prevent rejection; they were administered only if rejection occurred. In the case of HLA-identical sibling transplants, many patients never required steroids, but in cadaver transplantation, most patients had rejection and had to be treated with steroids.[78] Using steroids prophylactically with azathioprine from the time of transplantation became the standard practice.

Early approaches to the treatment of an acute rejection episode involved either increasing the oral dosage of steroids to high levels (e.g., 200 mg/day for 3 days), with a rapid reduction over 10 days to the dosage levels of steroids being given before the acute rejection episode, or giving boluses of intravenous methylprednisolone (e.g., 0.5 to 1 g/day for 3 to 5 days). Probably both approaches are equally effective. In an early randomized prospective trial in Oxford, high intravenous doses were as effective as high oral doses in reversing rejection, but there was a definite suggestion that steroid-related complications were reduced in patients who received intravenous therapy.[43] In a randomized study in children, a high intravenous dosage of methylprednisolone (600 mg/m^2 daily for 3 days) was no more effective than low oral doses of prednisolone, reversing rejection in 70% as opposed to 72% of episodes.[107]

The most common form of high-dose intravenous therapy to treat acute rejection has been 1 g of methylprednisolone given intravenously as a single bolus daily for 3 days. The intravenous bolus should be administered slowly over 5 minutes because the sudden injection of the bolus can lead to cardiac arrhythmias.[143] It is probable that 1 g of methylprednisolone is a much greater dose than required; we have used 0.5 g of methylprednisolone daily intravenously for 3 days in Oxford for many years, whereas the Stockholm unit has used 0.25 g daily intravenously for 3 days. The lower intravenous doses do not seem to be associated with any greater incidence of steroid-resistant rejection, as originally suggested by a prospective trial of high-dose versus low-dose intravenous steroids to treat rejection.[75] Similarly, in a small double-blind, randomized trial, Stromstad and associates[140] failed to show any therapeutic benefit of a 30 mg/kg bolus over a 3 mg/kg bolus, and Lui and coworkers[89] failed to show any benefit of a bolus of 15 mg/kg body weight over a bolus of 3 mg/kg.

The concept of steroid-resistant rejection as a surrogate marker of inadequate immunosuppression has become part of the analysis of efficacy of all new immunosuppressive protocols. Treatment generally requires use of a lymphocyte-depleting agent. As discussed earlier, steroid resistance is a complex phenomenon, however, and has been studied extensively in autoimmune disorders. Perhaps not enough attention has been paid to this phenomenon in organ transplantation.

Side Effects

The side effects of continuous steroid therapy are numerous (Table 15-1). High-dose steroids were responsible for most complications of renal transplantation in the azathioprine era, especially as experience with azathioprine led to its use in lower doses. With the widespread use of low-dose steroids, the incidence of serious side effects has been reduced markedly, but side effects still are a problem. Efforts to develop protocols that allow the withdrawal of steroids or, ideally, avoid their use entirely have been carried out or are in progress with a variety of new immunosuppressive protocols. In a study of the cost of steroid side effects over 10 years in a cohort of 50 patients, the additional cost per patient attributable to a steroid complication was assessed at $5300 (U.S. dollars).[149]

Table 15–1 **Side Effects of Steroids after Renal Transplantation**

Cushingoid facies
Poor wound healing
Growth retardation
Diabetes
Hyperlipidemia
Bone disease
Obesity
Hypertension
Psychiatric disturbance
Cataracts
Pancreatitis
Skin changes
Peptic ulceration

Cushingoid Facies

Cushingoid facies used to be the hallmark of a renal transplant patient—a moon face, buffalo hump, acne, obese torso, and thin, easily bruised skin, all representing the cumulative effect of high-dose steroids. With lower dose steroids, cushingoid facies is seen much less often, although most patients show modest changes in their facies in the early months after transplantation, particularly in association with the brutalization of the face that may be associated with cyclosporine therapy. Most patients taking low-dose steroids, which are the normal practice now with cyclosporine, have relatively minimal facial changes related to steroids.

Wound Healing

The anti-inflammatory activity of steroids leads to poor wound healing. In the days of high-dose steroids, poor wound healing was a major problem, affecting the healing not only of the incision but also of the ureterovesical reconstruction. With low-dose steroids, poor wound healing is no longer a major problem, but nevertheless skin sutures are left in situ for at least 14 days.

Growth Retardation

Growth retardation is of particular concern in children after renal transplantation. A major advantage of cyclosporine is that it allows lower doses of steroids to be used in children, and growth retardation is less of a problem.[120] As discussed in Chapter 35, however, growth retardation in children requiring transplants is still a problem because retardation resulting from renal failure already is present, and protocols for immunosuppression that might allow catch-up growth are favored. Such a protocol requires the use of low-dose steroids or alternate-day steroids, or preferably no steroids. The use of growth hormone has had a significant impact on growth rates after transplantation.[36]

Diabetes

Glycosuria and insulin-dependent and non–insulin-dependent diabetes are common after transplantation. The occurrence of diabetes is related partly to steroid use,[67] but it has become more common with the concomitant use of cyclosporine and tacrolimus, both of which can induce diabetes independently of steroids. In the presence of these two agents, the use of steroids augments the potential for diabetes, and often patients who become diabetic on cyclosporine or tacrolimus have a regression of the diabetes when steroid therapy is discontinued.

Hyperlipidemia

Hypercholesterolemia and hypertriglyceridemia are associated with steroid use, as was evident in the azathioprine and steroid era. Hyperlipidemia has become a greater problem in the cyclosporine era because cyclosporine also leads to an increased incidence of hyperlipidemia (see Chapter 28).[26,73,92] Withdrawal of steroids leads to improvements in the lipid profile.[56,106,116]

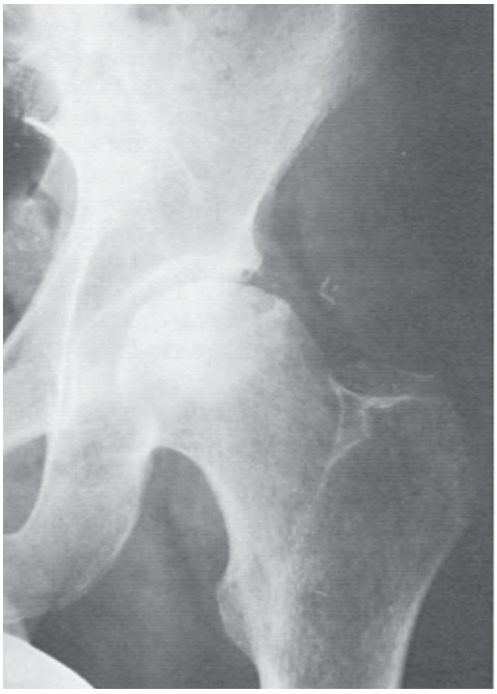
Bone Disease

Bone disease (osteopenia, osteoporosis) is a common and major problem after transplantation, especially in postmenopausal women.[1,47,48,58,69,151] This problem is not entirely due to steroids; more space is devoted to it here, however, because it is not discussed in detail elsewhere in this book. In the days of high-dose steroid therapy after transplantation, avascular necrosis of bones, particularly of the head of the femur, was common, occurring with an incidence of approximately 10% to 15% within 2 years of transplantation (Fig. 15-1). All of the evidence suggests that this incidence was due to a cumulative effect of steroid dosage. As low-dose steroid protocols were introduced, the incidence of avascular necrosis decreased dramatically. The cumulative dose of steroids received by a patient on a high-dose steroid regimen, as opposed to a low-dose regimen, is not that much higher after 6 months, however. Avascular necrosis of the hip should be treated by hip replacement early to enable full rehabilitation to occur. In patients requiring hip replacement, every attempt should be made to withdraw steroids if that seems feasible.

Osteoporosis is associated with steroid therapy. In randomized studies, Hollander and associates[56] and van den Ham and colleagues[147] showed that vertebral bone density was increased significantly in patients discontinuing steroids. Similar evidence was reported by Aroldi and coworkers[3] in a randomized study of three different immunosuppressive protocols and vertebral bone density. These investigators showed that lumbar bone density decreased significantly in patients receiving cyclosporine and steroids, but increased significantly in patients receiving cyclosporine alone without steroids. A more recent randomized controlled trial comparing a steroid-free regimen with a low-dose steroid regimen for 4 months after transplantation showed no important influence on bone density during the first year after renal transplantation.[142] The osteoporosis associated with steroids may be cumulative, as suggested by a study showing that patients on modern low-dose steroid regimens had only minimal loss of bone mineral density at 1 year after transplantation.[150]

Many patients who are to undergo renal transplantation have a degree of secondary hyperparathyroidism, and bone changes related to the hyperparathyroidism are enhanced by steroid therapy. Much more aggressive approaches to parathyroidectomy in patients with renal failure are being taken by most units now before transplantation. In post-transplantation patients with increased parathormone levels, early parathyroidectomy also should be considered.

Although there are no firm data in transplant patients, it has been generally thought that women who are postmenopausal should receive hormone replacement therapy in an attempt to diminish the overall likelihood of significant bone disease. The Million Women Study suggests, however, that hormone replacement therapy increases the risk of breast and ovarian cancer, which would seem to contraindicate this approach today.[7,8] The use of protocols that would allow low-dose steroids to be used or steroids to be discontinued is particularly important in these women. Another study has suggested that use of deflazacort instead of prednisone is associated with a decreased loss of total skeleton and lumbar spine density and improving the lipid profile.[87] Data now suggest that vitamin D and calcium supplements or the use of bisphosphonates may prevent the loss of bone density in adult and pediatric kidney transplant recipients.[28,29,49,68,145] A randomized trial in postmenopausal nontransplanted women has shown a significant reduction in the risk of fractures following



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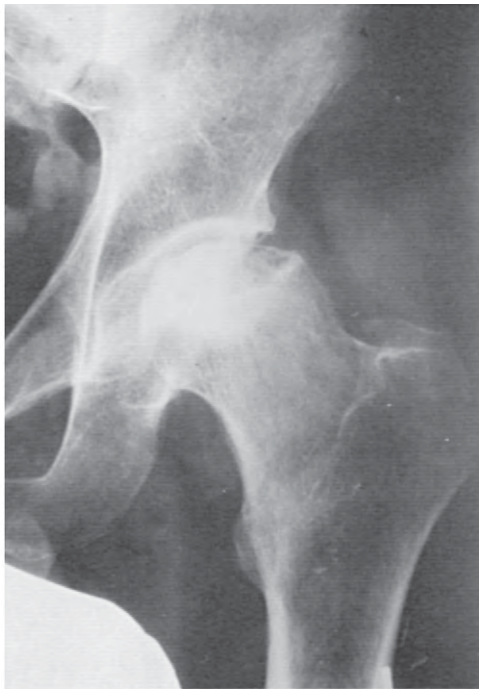

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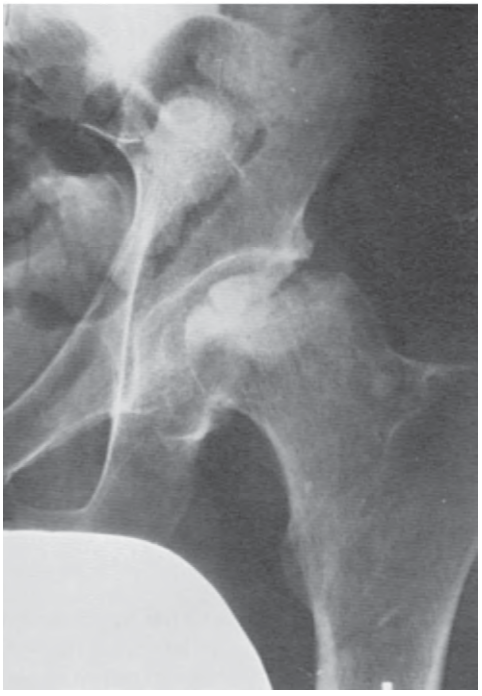

C

Figure 15–1 **A-C**, The progression of avascular necrosis of the head of the femur. **A,** Normal radiograph on first complaint of pain 1 year post-transplantation, 5 months later (**B**), and 20 months later (**C**). At this time, a hip replacement was performed.

a once-yearly infusion of zoledronic acid.[10] A strong case can be made for the administration of bisphosphonates and vitamin D and calcium in renal transplant patients of middle age or older, especially in postmenopausal women. More randomized controlled trials are required in this area.

Obesity

Steroid therapy leads to a marked increase in appetite, and without any dietary restrictions after transplantation, all patients tend to gain weight, which is in addition to a weight increase resulting from salt and water retention. Many patients become obese (body mass index >30), and this adds to the risks of poor survival. Every attempt should be made to advise patients from the time of transplantation to restrict calorie intake carefully because after patients have gained weight in the presence of steroid therapy, it is extremely difficult for them to reduce their weight.

Hypertension

Hypertension after transplantation is common and is related partly to steroids, but in the cyclosporine era hypertension also is due to cyclosporine (see Chapter 28). In steroid withdrawal protocols, hypertension improves after steroids are discontinued.[116]

Psychiatric Disturbance

Psychiatric disturbance is evident in patients on steroids in two ways. In the early days after transplantation, particularly with the need for high-dose steroids to treat rejection, significant psychiatric mood changes may be observed. Later, when steroids are being withdrawn or reduced to low doses, psychiatric mood changes, especially depression, also may occur.

Cataracts

Steroid-related cataracts are common after renal transplantation, occurring in approximately 25% of patients.[132]

Pancreatitis

Acute pancreatitis occurs with a much greater incidence after renal transplantation than would be expected. Azathioprine and steroids have been associated with acute pancreatitis. The pancreatitis is probably related to overall immunosuppression and is often severe.[134] The clinical features of acute pancreatitis can be masked to some extent by steroids.

Skin Changes

Long-term steroids produce typical skin changes in renal transplant patients—the skin being thin, atrophic, easily bruised, and susceptible to knocks (see Chapter 32). A syndrome known as transplant leg is associated with long-term steroid use; this occurs when a patient bumps into a chair or a table (a trivial injury), and a flap of skin is stripped or elevated from the lower leg.

Peptic Ulceration

Although it is debatable whether steroids lead to development of peptic ulceration, most units use prophylactic H_2 antagonists or proton-pump inhibitors in the early months after transplantation, when steroid doses are at their highest. The advent of low-dose steroid therapy has been associated with a dramatic diminution in the incidence of peptic ulceration after transplantation.

Acute Abdomen

In all renal transplant patients who present with an acute abdomen, steroids may mask the symptoms noted by the patient. If this fact is not remembered, diagnosis of diverticulitis or a perforated peptic ulcer may be delayed, with disastrous results.

Steroid Withdrawal

As a result of the numerous complications associated with midterm and long-term steroid therapy in renal transplant patients, many attempts have been made to reduce the cumulative dose of steroids after transplantation and to withdraw steroids altogether. In the azathioprine era, reducing or withdrawing steroids was impossible, but with the advent of cyclosporine there was renewed interest in reducing the dosage of steroids and withdrawing steroids from immunosuppressive protocols. The availability of additional potent immunosuppressive agents, such as sirolimus, tacrolimus, and MMF, together with monoclonal antibodies used for induction, has allowed further steroid-sparing protocols to be developed.

Steroid Withdrawal in the Azathioprine Era

As discussed earlier, the side effects of steroids were improved by the use of alternate-day regimens. Attempts to withdraw steroids, mostly anecdotal, were generally associated with rejection, however. In patients receiving azathioprine and steroids, there seems to be a crucial dosage level, below which there are likely to be problems with rejection.[101] This crucial dosage level is possibly about 5 to 6 mg of prednisolone per day. In one study from Edinburgh, patients with long-term surviving grafts were receiving azathioprine and 10 mg of prednisolone per day, a protocol that allowed for a slow reduction in the prednisolone dosage; many patients developed rejection when the daily steroid dose was reduced to less than 6 mg/day. As a result, the study directed at weaning patients with long-surviving transplants off steroids was abandoned.[2] Today there still are many long-surviving patients on azathioprine and prednisolone. If such patients are stable, no attempt should be made to alter this regimen.

Steroid Withdrawal in the Cyclosporine Era

Controversy still exists as to whether cyclosporine is best used with or without steroids (see Chapter 16). It would seem that if a high dose of cyclosporine is used, monotherapy may be satisfactory in many patients, but with a greater risk of acute rejection often requiring the addition of steroids and perhaps with a greater risk of nephrotoxicity.[23,54,114,125] The use of steroids possibly allows the use of lower doses of cyclosporine, and steroids may decrease the incidence of nephrotoxicity in the early weeks after transplantation, but there is no firm evidence for either of these suggestions.[45]

The timing of steroid withdrawal has been shown to be a risk factor for the failure of withdrawal, with cessation of steroids before 6 months after transplantation increasing the risk of acute rejection.[62] An early meta-analysis from Hricik and colleagues[63] included seven randomized controlled trials of steroid avoidance or withdrawal in patients receiving cyclosporine-based protocols, six of which involved withdrawal in the first 3 months after transplantation. The results of this meta-analysis suggested that avoidance of steroids or early withdrawal increased the risk of acute rejection but did not affect patient or graft survival adversely. Only one of the studies included had a follow-up period of longer than 2 years. This was the Canadian Multicentre Cyclosporine Trial, which showed a superior longer term graft survival in the patients who continued taking steroids compared with patients in whom steroids were withdrawn at 3 months.[133] This finding emphasizes the importance of long-term follow-up in such studies.

Later withdrawal of steroids may improve the outcome from such protocols. In a large retrospective analysis of data from the Collaborative Transplant Study, Opelz[104] found that patients with a functioning graft at 1 year who had steroids withdrawn had better graft and patient survival thereafter than patients remaining on steroids. The initial criticism of this study was that patients still on steroids at 1 year represented those with poorer function as a result of

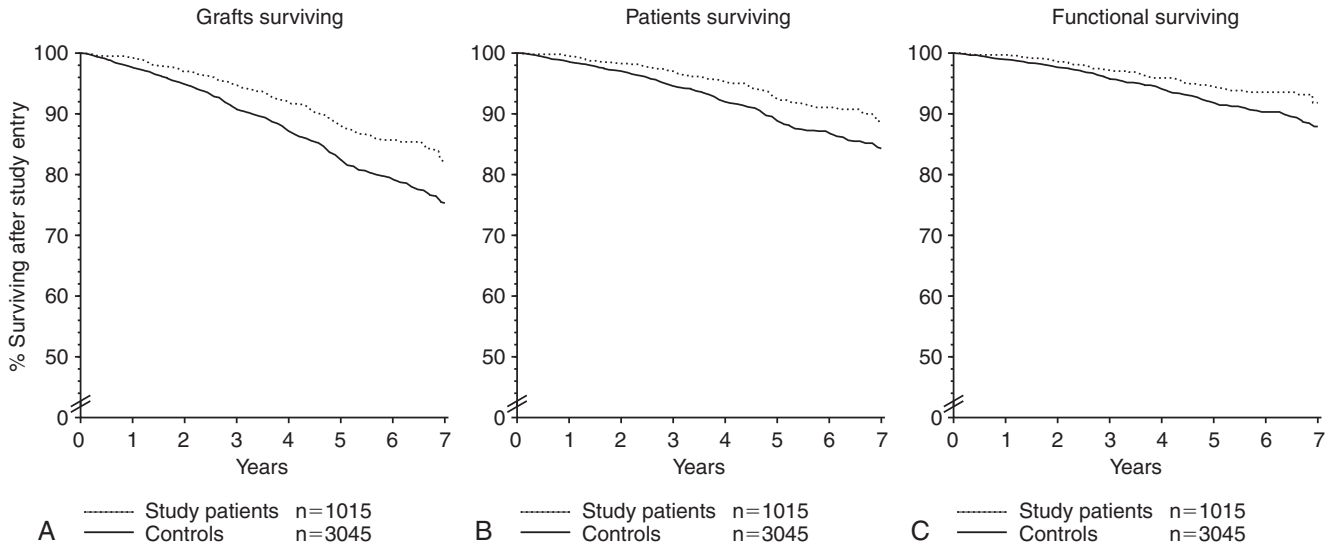

Figure 15–2 **A-C**, Seven-year graft (**A**), patient (**B**), and functional graft (**C**) survival in renal transplant recipients after steroid withdrawal (study patients) or steroid continuation (matched controls). (From Opelz G, Dohler B, Laux G: Long-term prospective study of steroid withdrawal in kidney and heart transplant recipients. Am J Transplant 5[4 Pt 1]:720-728, 2005.)

rejection episodes or delayed graft function, but a subsequent analysis examining outcome only in patients with satisfactory renal function at 1 year found the same results. A more recent prospective study from the same group has confirmed a benefit in graft and patient survival in renal and cardiac transplant patients in whom steroids are withdrawn more than 6 months after transplantation, with no increase in incidence of acute rejection (Fig. 15-2).[106] Benefits also were seen in cardiovascular parameters in the withdrawal group, with significantly fewer patients showing elevated cholesterol levels. Although the number of patients and length of follow-up in this study is impressive (7 years), a criticism is a lack of a randomized design, with the control cohort being retrospectively matched patients from the Collaborative Transplant Study registry.

Further, more recent data from randomized prospective trials support the suggestion that steroids may be withdrawn with a relative degree of safety and a low incidence of rejection in patients with stable graft function at about 1 year after transplantation. In a trial from Oxford, patients receiving triple therapy (cyclosporine, azathioprine, and steroids) with stable function at least 1 year after transplantation were randomly assigned to have steroids withdrawn over several months or continued at a maintenance level of 10 mg/day.[116] Although steroid withdrawal was successful in most patients, there was 10% deterioration in renal function at 1 year, with a further modest deterioration during the second year. Thereafter, renal function seemed to be stable (Fig. 15-3). This decrease in renal function, evidenced in the serum creatinine level and the creatinine clearance, was concerning, but there is no evidence that further deterioration of function was occurring in a longer follow-up of patients in the trial (unpublished observations). As already described in Opelz's observations, benefits were seen in terms of a decline in blood pressure (although not sustained in all patients) and a 1 mmol/L decrease in total cholesterol in the patients in the withdrawal group. The standard triple-therapy protocol (cyclosporine, azathioprine, and steroids) in Oxford thereafter included cessation of steroids at 1 year after transplantation.

Another randomized trial from Holland, with a protocol similar to the Oxford study, enrolled patients with stable renal function at 1 year or longer.[56] Steroids were withdrawn successfully in two thirds of patients, with acute rejection as the major cause of withdrawal failure. No grafts were lost from rejection, however, and significant benefits from withdrawal were seen with respect to hypertension, hypercholesterolemia, hyperglycemia, and appearance. The authors concluded that steroids could be withdrawn safely 1 year after transplantation provided that careful follow-up was maintained.

Favorable changes associated with steroid withdrawal have been documented by other groups, including cholesterol levels, glucose tolerance, and growth in children.[59-61,64,67] The risk of acute rejection and graft loss after steroid withdrawal in children is much greater than in adults, however, and despite the potential growth benefits it has not been recommended.[65,117,121,141]

Steroid Withdrawal with Newer Immunosuppressant Agents

In many transplant centers, azathioprine has now been replaced with MMF for use in conjunction with either cyclosporine or tacrolimus. A considerable amount of data, mostly from observational studies, suggest that tacrolimus is more steroid sparing than cyclosporine. In units using tacrolimus, many patients can have steroids withdrawn during the first year after transplantation. In one report from Pittsburgh, Shapiro and colleagues,[131] who had noted previously that steroids could be discontinued in 70% of renal transplant patients receiving tacrolimus, reported a further longer term follow-up of approximately 289 patients not receiving steroids. The patients in the steroid withdrawal group had impressive 1-year and 3-year graft survivals of 98% and 94% compared with 90 patients in whom steroids had not been withdrawn, who had 1-year and 3-year graft

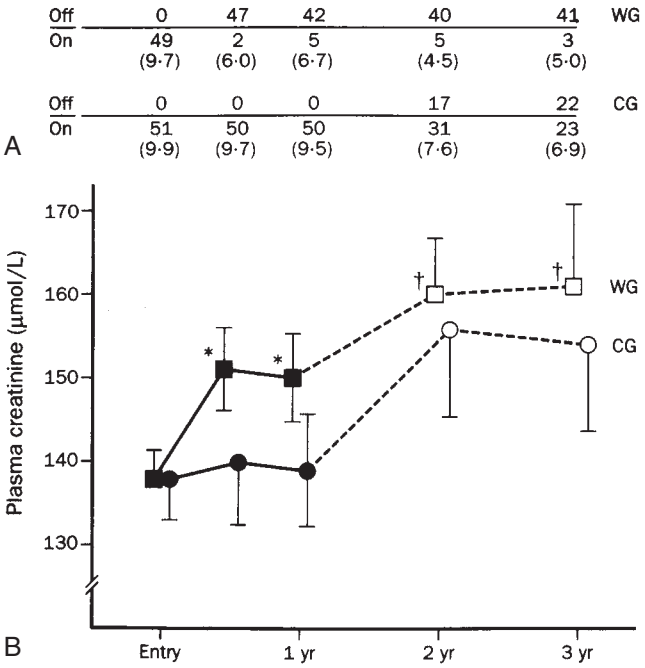

Figure 15–3 Changes in creatinine during the trial period and after 2 years of further follow-up. **A**, Numbers at the top are number of patients in each of the original groups who were not taking (off) or taking (on) prednisolone, together with the mean daily dose of prednisolone in the patients taking steroids. **B**, Mean plasma creatinine in each group at the trial end points (*solid symbols*) and subsequent follow-up (*open symbols*) for each group assigned by intention to treat (*squares*, withdrawal group; *circles*, control group). *Bars* indicate standard errors. Statistical comparison, with entry values by Student two-tailed paired *t*-test. **P* <.001. †*P* <.05. CG, control group; WG, withdrawal group. (From Ratcliffe PJ, Dudley CR, Higgins RM, et al: Randomised controlled trial of steroid withdrawal in renal transplant recipients receiving triple immunosuppression. Lancet 348:643-648, 1996.)

survivals of 77% and 50%. Although the authors stated that there was no difference between the two groups in terms of the proportion of living and cadaver donors, HLA matching, recipient sex, race, or sensitization, the patients in whom steroids were not withdrawn were generally patients who had delayed graft function or experienced acute rejection, both of which are factors having a significant deleterious impact on graft outcome (see Chapter 37). This type of observational study suggested that steroid withdrawal in renal transplant patients receiving tacrolimus-based immunosuppression is possible most of the time and is reasonably safe in short-term and medium-term follow-up after transplantation. Renal function remains stable in patients in whom steroids have been withdrawn, at least in the medium term. Another small, prospective, observational study of patients receiving tacrolimus, MMF, and steroids, in whom steroids were withdrawn at 1 week, showed an incidence of acute rejection of about 25%, but no grafts were lost.[44]

Following these encouraging observational studies, numerous randomized trials have now been performed investigating steroid withdrawal in patients receiving these newer immunosuppressive regimens. Pascual and colleagues[111] performed a meta-analysis of six such trials, four with MMF and cyclosporine and two with MMF and tacrolimus. Although the risk of acute rejection was increased slightly more than twofold when steroids were withdrawn, there was no significant difference in the incidence of graft failure. This meta-analysis did not differentiate the relative steroid-sparing potential of cyclosporine and tacrolimus. A more recent randomized trial from the European Tacrolimus/MMF Renal Transplantation Study Group randomly assigned immunologically low-risk patients who had undergone transplantation 3 months earlier to continue triple therapy (tacrolimus, MMF, and steroids), withdraw steroids, or withdraw MMF.[148] The incidence of acute rejection at 6 months was similar in all three groups, supporting the earlier observations that tacrolimus enables more effective steroid sparing than cyclosporine. Reductions in total cholesterol and low-density lipoprotein cholesterol were greater in the steroid-free group.

Patients from this study have now been followed for 3 years, with only 23.3% of patients randomly assigned to stop steroids having restarted steroid therapy at this time.[112] Graft and patient survival and the incidence of acute rejection were similar between groups at 3 years, and serum creatinine levels remained stable. The reduction in total cholesterol and low-density lipoprotein cholesterol seen at 6 months was maintained, with a lower mean systolic blood pressure. There was no difference seen in adverse events, such as malignancy, infection, and fractures. It would seem that in a tacrolimus and MMF–based regimen, steroids can be withdrawn without long-term detriment to graft function or survival and with a reduction in cardiovascular risk factors.

Pescovitz and colleagues[113] first noted that sirolimus may aid in the withdrawal of steroids from a calcineurin-based regimen. Further observational studies seem to support this, and the use of sirolimus may allow steroid withdrawal alongside reduction in the exposure to calcineurin inhibitors.[19,71,90,91]

The use of newer immunosuppressive agents, such as MMF and sirolimus, also may allow the safe withdrawal of steroids earlier than previously seen with cyclosporine-based regimens. Kumar and coworkers[80] reported a 3-year analysis of a large trial of 300 patients receiving basiliximab induction, a calcineurin inhibitor, and MMF or sirolimus in which patients were randomly assigned either to have steroids withdrawn on day 2 or to continue steroids. There was no difference in graft function, patient and graft survival, biopsy-proven acute rejection, or chronic allograft nephropathy. The incidence of new-onset diabetes was lower in the steroid-free group.

Gelens and colleagues[39] attempted to combine early steroid withdrawal with a calcineurin inhibitor–free maintenance regimen. Patients were randomly assigned to receive tacrolimus and sirolimus, tacrolimus and MMF, or daclizumab induction, sirolimus, and MMF. Steroids were withdrawn after 2 days in all patients. The trial was halted after an interim analysis showed an unacceptably high incidence of acute rejection in the calcineurin-free group. It would seem that even with modern immunosuppressant agents and antibody induction, it is impossible to combine the complete withdrawal of calcineurin inhibitors with steroid withdrawal.

Conclusions

In cyclosporine-based protocols such as triple therapy, early steroid withdrawal is associated with a significant increase in acute rejection. Late steroid withdrawal is feasible in these protocols in most patients with stable graft function with demonstrable metabolic benefits. The use of the newer immunosuppressant agents tacrolimus, MMF, and sirolimus has allowed further development of these steroid-sparing

protocols with the possibility of earlier steroid withdrawal. Although 3-year follow-up results from studies have now been reported, long-term follow-up of these protocols to at least 5 years is required in light of the results from the Canadian Multicentre Study.[133]

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Chapter 16
Cyclosporine

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Cyclosporine is a powerful immunosuppressive drug and has proved to be a potent agent in a wide variety of experimental models of tissue transplantation and in clinical organ transplantation. When it became available in the early 1980s, it revolutionized kidney transplantation (as well as liver and heart transplantation) primarily by markedly reducing the loss of kidneys from acute irreversible rejection in the first 3 months after transplantation. A new spectrum of drug-specific side effects appeared, however, not the least of which was nephrotoxicity. Over the next 20 years, protocols designed to reduce the side effects while maintaining this potent new immunosuppression were developed and tested. The development of another potent calcineurin inhibitor, tacrolimus (see Chapter 17), in the 1990s with a slightly different spectrum of side effects has led gradually to the use of tacrolimus as a first-line immunosuppressive agent in an increasing number of renal and liver transplant units in the Western world. Because most young clinicians are less familiar with cyclosporine and in particular the history of its introduction to the clinic, which was an exciting development in transplantation, we have retained much of this material in this chapter because there is still much that can be learned in this current era of immunosuppression, and perhaps all that is new is not better.

Cyclosporine was first isolated from two strains of imperfect fungi (*Cylindrocarpon lucidum Booth* and *Trichoderma polysporum Rifai*) from soil samples by the Department of Microbiology at Sandoz (Basel, Switzerland) as an antifungal agent of limited activity.[88] The latter, from which cyclosporine now is produced, is known more correctly as *Tolypocladium inflatum Gams* and was shown by Borel and colleagues[33,34,36] to have potent immunosuppressive activity in a variety of in vitro and in vivo experiments. The drug has a molecular weight of 1200 kD and comprises 11 amino acids, one of which is unique, and most of which are hydrophobic. Cyclosporine is soluble only in lipids or organic solvents.

After Borel's initial description of the immunosuppressive properties of cyclosporine, it was shown to suppress rejection of vascularized organ allografts in rats, dogs, and rabbits[46,121,150,188] and skin allografts in rabbits.[120] Similar observations in various models of vascularized organ allografts in many species followed quickly.[226] Clinical trials of the drug in renal transplantation began in Cambridge in 1978[47]; by the early 1980s, cyclosporine was licensed for use in renal transplantation, first in Europe and then in the United States.

Cyclosporine-based protocols rapidly became standard therapy in renal transplantation, unless restricted by cost, and until more recently represented the conventional therapy against which new immunosuppressive agents were compared. Now tacrolimus is considered the first-choice calcineurin inhibitor in many units. Because cyclosporine made a significant impact not only in renal transplantation but also in liver and heart transplantation and because it is still widely used, especially in developing countries,

we review the early clinical experience in this chapter. For information regarding the early experimental work done with cyclosporine, the reader is referred to the 5th edition of this book.

MECHANISM OF ACTION

It was apparent from many early in vivo experiments that cyclosporine exerts its effect at an early stage after exposure of the recipient to a tissue allograft. This situation was illustrated in the rat renal allograft model (Table 16-1),[150,228] showing that cyclosporine is ineffective in this model if given after induction of the immune response has taken place or before the recipient animal has been exposed to the allogeneic histocompatibility antigens of the transplanted kidney.

In vitro experiments correlated well with these in vivo observations. In several species and in humans, cyclosporine has been shown to inhibit the proliferative response of lymphocytes to concanavalin A, phytohemagglutinin, and pokeweed mitogen in vitro.[35,42,198,200,353,357] If cyclosporine is added 48 hours after the addition of mitogen to a culture, there is no inhibition of proliferation, and the effect is reversed by washing the lymphocytes and re-exposing them to the mitogen.[357] Complete inhibition of the mixed lymphocyte reaction by cyclosporine has been shown in several species, including humans.[139,152,178,179,200,333] The generation of cytotoxic lymphocytes in the mixed lymphocyte reaction is prevented by cyclosporine, but when these lymphocytes are generated, cyclosporine has no effect on their cytotoxic activity.[40,139,140,152,179] Cyclosporine also has been shown to reduce markedly the generation of cytotoxic T lymphocytes in the blood of patients with renal transplants compared with patients receiving azathioprine and prednisolone.[180] Theoretically, cyclosporine might be expected to be less effective in preventing graft rejection in sensitized animals.[149] Although it does not inhibit the secondary mixed lymphocyte reaction response or the generation of cytotoxic T lymphocytes in such a secondary reaction,[139,200] it does inhibit interleukin (IL)-2 production significantly,[17,141] suggesting that cyclosporine could have some efficacy in sensitized recipients or in the treatment of ongoing rejection. Cyclosporine also has been shown to inhibit the induction of major histocompatibility complex (MHC) class II antigen expression in the transplanted kidney[224] and to a considerable extent in humans.[107] Cyclosporine not only inhibits the generation of cytotoxic T cells but also may reduce the antigenicity of the target organ.

The predominant action of cyclosporine is directed against $CD4^+$ T (T helper) lymphocytes.[33,35,42,51,117,168,194] This effect on the $CD4^+$ T cell prevents the production of lymphokines, especially IL-2,[40,196,198,256] which inhibits the further proliferation of $CD4^+$ T cells and the generation of cytotoxic T cells from the cytotoxic T cell precursor.

It was not until the late 1980s and early 1990s that the mechanism of action of cyclosporine was described at a molecular level within the cell.[289,290,302] Cyclosporine binds within the cytosol to cyclophilin, a *cis-trans*-peptidyl-prolyl isomerase that has an important role in folding proteins and peptides into their native conformation.[96] Cyclophilin has been found in a wide variety of cell types and organisms other than lymphocytes. The inhibition of this isomerase activity was thought initially to be responsible for the immunosuppressive activity of cyclosporine.[95,322] There is a family of cyclophilins to which cyclosporine binds, although most of the drug binds to cyclophilin A, a 12-kD molecule. It has been shown that mice who are deficient in cyclophilin A are resistant to the immunosuppressive effects of cyclosporine.[72] The cyclophilins belong to a larger family of immunophilins (proteins that bind immunosuppressive agents), FK-binding protein being another member of that family to which tacrolimus and rapamycin (sirolimus) bind. Tacrolimus and cyclosporine seem to have an identical mechanism of action that is quite different from that of rapamycin (see Chapters 17 and 19).

The complex of cyclosporine and cyclophilin is the immunosuppressive molecule, cyclosporine being a prodrug and not by itself immunosuppressive. This complex of the drug and its immunophilin binds to a calcium-dependent and calmodulin-dependent phosphatase, calcineurin.[104,209] Calcineurin plays a crucial role in the transduction of the calcium-dependent signal that leads to the activation of the enhancer region of the IL-2 gene[70,245] because it dephosphorylates the cytosolic form of the nuclear factor of activated T cells (NFATc), which is necessary for its translocation into the nucleus as NFATn, which activates the enhancer region of the IL-2 gene leading to its transcription.[119] Other transcription factors, such as NFIL-2 A and B, also are inhibited by cyclosporine.[221] This cyclosporine-immunophilin complex, which binds to calcineurin and blocks the dephosphorylation

Table 16–1 **Effects of Cyclosporine on Rejection of Dark Agouti Renal Allografts in Lewis Rats Depending on the Time of Administration***

Treatment Period (Days)	Dose of Cyclosporine (mg/kg)	No. Rats	Median Survival and Range (Days)
—	0	9	11 (10-11)
–14-–1	10	7	12 (10-13)
–2-0	10	6	12 (12-14)
0-2	10	5	22 (15->100)
0-4	10	5	28 (20->100)
0-14	10	7	>100 (all >100)
4-14	5	5	13 (11-14)
4-14	10	5	11 (10-11)
4-14	25	4	9 (9-10)

*Cyclosporine was given orally. An orthotopic renal transplant was done on day 0 with removal of the remaining kidney on day 7.
Adapted from Homan WP, Fabre JW, Williams KA, et al: Studies on the immunosuppressive properties of cyclosporin A in rats receiving renal allografts. Transplantation 29:361-366, 1980.

of NFATc and its translocation into the nucleus, prevents the transcription of the IL-2 gene.[97,118] There is a second pathway of activation (the so-called second signal), however, mediated by CD28 with distinct signal pathways, including protein kinase C.[335] Activation of this pathway also leads to IL-2 production and IL-2 receptor expression, and this pathway is resistant to cyclosporine.[142]

Although the reduction of IL-2 production and IL-2 receptor expression and the resultant reduction of T cell activation is the main pathway by which cyclosporine induces immunosuppression, it has other mechanisms of action that are thought to contribute to its immunosuppressive effects. Cyclosporine enhances transforming growth factor (TGF)-β mRNA expression in normal human T cells and constrains new DNA synthesis through a TGF-β–dependent mechanism.[182,209] It increases the production of TGF-β by activated T cells,[5] and kidney transplant recipients being treated with cyclosporine have been found to have higher levels of circulating TGF-β than patients on other immunosuppressive agents.[68,219] TGF-β has been found to inhibit IL-2–dependent T cell proliferation and inhibit IL-2 receptor upregulation by IL-2[176] and suppress antigen-specific T cell proliferation.[332] It also has been shown to promote the expansion of regulatory T cells.[154] It has been suggested that TGF-β may have an immunoregulatory role and may be an immunosuppressive cytokine in its own right,[56,344] and more recently it has been suggested that TGF-β mediates in part the immunosuppressive properties of cyclosporine. As discussed later, TGF-β may play a role in the development of fibrosis, a characteristic feature of chronic rejection.[183]

The role of dendritic cells in antigen presentation is pivotal (see Chapter 2). More recent data suggest that dendritic cells are a target for cyclosporine and T cells, and this may play a role in the immunosuppressive properties of cyclosporine. Cyclosporine has been shown to alter the migratory abilities of dendritic cells[64] and to inhibit the maturation of circulating dendritic cells.[321] It also is thought that cyclosporine can alter the antigen presentation capabilities of dendritic cells and have an effect on their ability to activate T cells.[67,202,220]

Although cyclosporine generally has not been thought to inhibit the function of B lymphocytes,[34,42,117] there is some evidence to the contrary in humans[255,265] and mice.[38,184,194] O'Garra and colleagues[244] showed a cyclosporine-sensitive subpopulation of T cell–independent B lymphocytes in mice and showed that cyclosporine can inhibit the production of murine B cell–derived lymphokines. It has been shown that cyclosporine and tacrolimus can inhibit B cell activation when the stimulating factor triggers an increase in intracellular calcium.[356] Venkataraman and coworkers[339] suggested that cyclosporine may inhibit B cell function in the same manner as it inhibits T cell function, by inhibiting NFAT. They showed that NFAT also is present in B cells, and that it is inhibited within B cells by cyclosporine. See Chapter 2 for discussion of the role of B cells in rejection.

Unraveling the molecular mechanism of action of the calcineurin inhibitors (cyclosporine and tacrolimus) and the newer immunosuppressive agents, such as mycophenolate mofetil (MMF) and sirolimus, has led to a much more detailed understanding of the nature of signal transduction after the T cell receptor has recognized alloantigen. This understanding should allow drugs with more specific actions to be designed. This design also may be enhanced by the description of the three-dimensional structure of the cyclosporine-cyclophilin complex, which at a crystallographic level appears to be a pentamer with the two pentamers forming a sandwich with the cyclophilin pentamers on the outside and the cyclosporine molecules inside the sandwich.[263,327]

The major mechanism of action of cyclosporine is still thought to be due to inhibition of T cell activation as a result of the blocking of IL-2 production. It is now clear, however, that T cell inhibition is not the only pathway by which cyclosporine produces immunosuppression.

EARLY EXPERIENCE

Cyclosporine first was used in renal transplantation by Calne and colleagues[47,48] in Cambridge. Initially, it was used with other drugs, such as prednisolone or Asta 036.5122 (cytimum, an analogue of cyclophosphamide), but this proved a dangerous combination, with many patients dying of infection. For the first time, it became apparent that cyclosporine was nephrotoxic in humans; this was not a feature of the extensive experimental use of cyclosporine in animal models at that time, although it became apparent later.[23,354,355] Three lymphomas were seen in these early patients, which caused considerable alarm. A new policy was adopted at Cambridge whereby cyclosporine was used only in patients whose grafts were diuresing, and it was used alone, with high-dose methylprednisolone given to treat acute rejection. If more than 6 g of methylprednisolone was required, patients were converted to azathioprine and prednisolone. Following this policy, 60 cadaver grafts were performed in 59 patients, all but 1 of whom had been transfused previously. Actuarial graft survival at 1 year was 82%. Six deaths occurred, five from infection, and 10 patients were converted to azathioprine and prednisolone because rejection was not controlled with cyclosporine.[49] Many of these patients were not receiving steroids; many had never received any steroids.

This early experience in Cambridge prompted many controlled trials of cyclosporine—single-center trials in Minneapolis, Oxford, Pittsburgh, and Sydney; multicenter trials in Europe and Canada; and uncontrolled studies in Denver, Pittsburgh, Stockholm, and Boston. In the European multicenter trial, only patients who were given grafts that were diuresing 6 hours after surgery were randomly assigned to receive cyclosporine alone or conventional treatment with azathioprine and prednisolone, according to the custom of the unit. The trial was closed at the end of 1981 after 1 year, when slightly more than 200 patients had been entered. Actual survival at 1 year was 72% in the cyclosporine group and 52% in the control group,[13,14] although many patients were converted to azathioprine and steroids because of apparent rejection on cyclosporine. At 5 years, there was a marked difference (but not as great) in graft survival in favor of cyclosporine—55% versus 40% in the control group[50]—and at 10 years, the difference was 35% versus 29%, with stable renal function in the cyclosporine group, although at a higher serum creatinine level than in the control group (Fig. 16-1).[16] Many of the grafts considered to be in a process of rejection in this trial likely had nephrotoxicity in retrospect, an ever-present problem that is discussed later. Actual survival in the patients excluded from the trial and treated with conventional immunosuppression was similar to that of the control group within the trial.

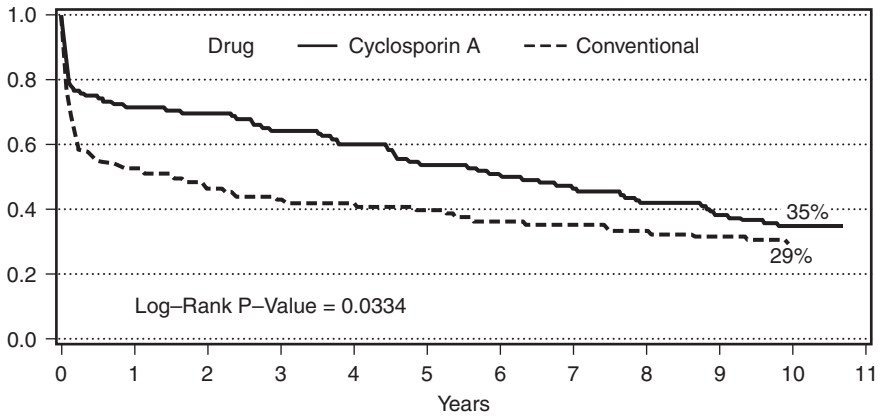

Figure 16–1 Graph showing actual graft survival with cyclosporine compared with azathioprine and steroids, one of the first randomized controlled trials of cyclosporine versus azathioprine and steroids.[16]

An excellent randomized multicenter trial was conducted in Canada, in which cyclosporine and prednisolone therapy was compared with standard therapy based on azathioprine and prednisolone in 209 cadaver renal allograft recipients.[54] In this first analysis, actuarial graft survival at 1 year was 84% in the cyclosporine group compared with 67% in patients receiving standard therapy, with patient survivals of 97% and 90%, respectively, in the two groups. At 3 years, graft survival was 69% in the cyclosporine-treated group and 58% in the control group, a less striking difference than in the initial analysis.[15] Patient survival was 90% in the cyclosporine group and 82% in the control group. A detrimental effect on graft survival was seen in cyclosporine-treated patients if they received kidneys that had been preserved for longer than 24 hours or if the surgical anastomosis time took longer than 45 minutes, suggesting that cyclosporine nephrotoxicity is more likely to occur in kidneys that have some ischemic damage.

In Minneapolis, all HLA-mismatched living or cadaver donor transplants were eligible for a trial in which cyclosporine plus prednisolone was compared with conventional therapy of azathioprine, steroids, and antilymphocyte globulin.[91,235,236] All patients had had a splenectomy and at least 5 U of blood before transplantation. The trial comprised 230 patients and included cadaver and living related transplants and diabetic and nondiabetic recipients. Overall graft survival rates at 2 years were 82% in the cyclosporine group and 77% in the control group, and patient survival was 88% and 91%, respectively. In the living related transplants, graft survival at 2 years was 87% in the cyclosporine group and 83% in the control group, whereas the 2-year graft survival figures in the cadaver transplants were 78% and 73%, respectively. These differences in survival were not significant, but the cumulative incidence of rejection episodes in the first year after transplantation in the cyclosporine group was half that in the control group, as was the incidence of infection.

Similarly, Starzl and colleagues,[313,314] first at Denver (where treatment was not standardized) and then at Pittsburgh, reported impressive results with cyclosporine and prednisolone (at a maintenance dose of 20 mg/day after a burst of high-dose prednisolone) in primary and secondary cadaver transplants. Graft survival was about 90% at 1 year in primary cadaver transplants. In 26 patients who received 27 cadaver second transplants, 1-year graft survival was 78%. After that initial experience, virtually all contraindications to the use of cyclosporine in renal transplantation were disregarded, and in 96 primary cadaver grafts, patient survival at 1 year was predicted as 90%, and graft survival was predicted as 80%.[314] Early anuria was not considered a contraindication to cyclosporine, which was sometimes considered to be the result of rejection or nephrotoxicity, or both, although it did cause diagnostic problems in the management of patients.

In the Sydney controlled trial of cyclosporine versus azathioprine, prednisolone, and antilymphocyte globulin, 60 patients receiving first cadaver grafts were entered, and graft survival of 70% at 1 year was similar in both groups. Persistent anuria after transplantation was a major problem in the cyclosporine group.[297] In the Oxford trials, all patients were started on cyclosporine, but were randomly assigned at 3 months either to azathioprine and prednisolone or to remain on cyclosporine. The objective was to reduce nephrotoxicity.[227,229] This approach is discussed later in this chapter.

This early experience with cyclosporine in prospective controlled trials and in uncontrolled observational studies indicated that cyclosporine was a major advance in immunosuppressive therapy, as was evident in the Collaborative Transplant Study, which had data from more than 200 transplant centers and several thousand renal transplants.[249] Many side effects had become evident, the major one being nephrotoxicity, and so subsequent protocols were designed to obtain the same improved immunosuppression achieved with cyclosporine, but with a reduction in side effects resulting from lower doses of cyclosporine (Table 16-2).

CYCLOSPORINE WITH OR WITHOUT STEROIDS

The initial use of cyclosporine in Europe was based on the experimental data and the early Cambridge experience, using a high dose of cyclosporine alone (monotherapy), whereas in North America cyclosporine was used with steroids. Gradually, most units added steroids to their cyclosporine protocols, but not with any convincing evidence that steroids were necessary. In the United States, there had been a tendency to use high-dose steroids, at least in the early weeks after transplantation. Four prospective controlled trials comparing cyclosporine alone with cyclosporine and steroids were performed.[83,123,160,210,316]

Table 16–2 **Cyclosporine-Based Protocols That Have Been Used or Are in Use in Renal Transplantation**

Cyclosporine (monotherapy)
Cyclosporine + prednisolone
Cyclosporine + azathioprine (dual therapy)
Cyclosporine + azathioprine + prednisolone (triple therapy)
ALG/OKT3 + azathioprine + prednisolone → cyclosporine + azathioprine + prednisolone (sequential therapy)
Cyclosporine + prednisolone → azathioprine + prednisolone (conversion therapy)
ALG/OKT3 + cyclosporine + azathioprine + prednisolone (quadruple therapy)
New Therapies
Cyclosporine + MMF + prednisolone
Cyclosporine + sirolimus/everolimus + prednisolone
Anti–IL-2R antibody + cyclosporine + MMF/azathioprine + prednisolone
Anti-CD52 antibody + cyclosporine + MMF ± prednisolone
Anti–IL-2R antibody + cyclosporine + CTLA-4 immunoglobulin + MMF + prednisolone
Cyclosporine conversion and reduction protocols*

*See section on cyclosporine reduction.
ALG, antilymphocyte globulin; IL, interleukin; MMF, mycophenolate mofetil.

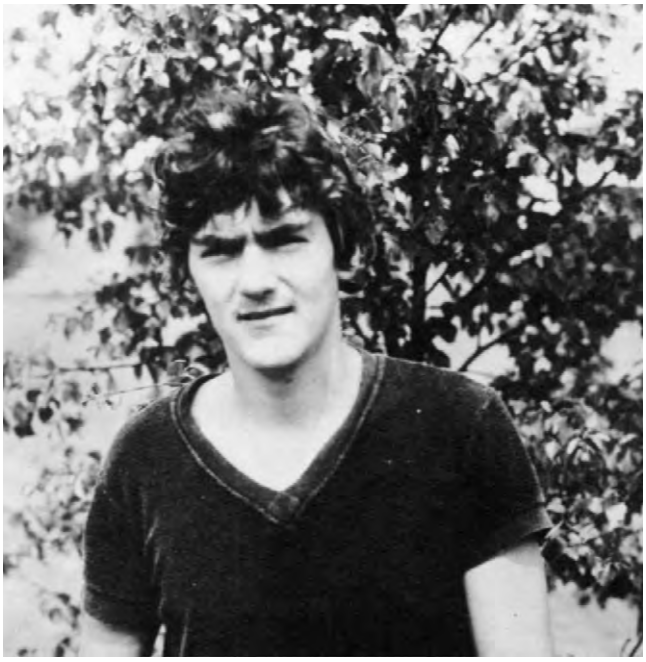

Figure 16–2 Patient in the cyclosporine era with the lack of cushingoid facies so typical of the azathioprine steroids era.

None was able to confirm an additive effect of steroids in terms of immunosuppression. Two groups found an increased incidence of infection with steroids, although Griffin and coworkers[123] suggested that steroids reduced the incidence of cyclosporine nephrotoxicity. Schmidt and colleagues,[287] in a morphological study of renal biopsy specimens 1 year after renal transplantation, found no difference in biopsy specimens from patients treated with cyclosporine and steroids compared with those treated with cyclosporine without steroids. In these studies, steroids were used for the treatment of rejection, and in many patients with recurrent rejection steroids were added to the cyclosporine regimen.

Many patients can be managed without steroids or weaned off steroids early, although the long-term outcome of the Canadian trial suggested that the withdrawal of steroids was associated with a poorer graft outcome.[304] Patients who have not had steroids present an entirely different facies to that which clinicians had become used to in the precyclosporine era (Fig. 16-2). The need for steroid maintenance therapy and the length of time for which such therapy is required after transplantation remain unresolved and are discussed in more detail in Chapter 15. Most patients are likely to need high-dose methylprednisolone for the treatment of acute rejection episodes. Most units have used steroids with cyclosporine from the time of transplantation. Two types of protocols were used. In North America, the tendency was to use high oral doses of prednisolone (e.g., 100 mg/day) from the time of transplantation, reducing rapidly to lower doses over the first 2 to 3 weeks, whereas in Europe, the trend was to use low doses from the time of transplantation (e.g., 20 to 30 mg/day), reducing to maintenance doses over the first 3 to 6 months. There is no evidence that one approach was better than the other, and on general principles the low-dose steroid protocol should be favored if steroids are to be used routinely with cyclosporine.

Cyclosporine alone has been used in the past at what would now be considered high doses (17.5 mg/kg/day), and many of the reported side effects of cyclosporine, most of which are dose related, can be attributed to these high doses. In subsequent years, cyclosporine doses gradually were reduced based on the maintenance of adequate trough blood levels (200 to 400 ng/mL in the early months and 100 to 200 ng/mL thereafter), and although this led to a reduction in nephrotoxicity and other side effects, it has not led to the disappearance of nephrotoxicity, which remains a major side effect. It is possible that the concurrent use of steroids with these lower doses of cyclosporine is important for adequate immunosuppression; that is the view held by most clinicians. The trials mentioned previously all used higher doses of cyclosporine. A randomized prospective trial comparing cyclosporine monotherapy with cyclosporine, azathioprine, and prednisolone triple therapy did not show any difference in graft survival, but more severe rejections were seen in the monotherapy group, which required more high-dose steroid rejection treatments. Cyclosporine nephrotoxicity also was more common in the monotherapy group, which was started on 15 mg/kg/day of cyclosporine, than in the triple-therapy group, which received 8 mg/kg/day.[324]

Cyclosporine is administered as a single daily dose or twice-daily dose with the older formulation (Sandimmune) or as two 12-hourly doses with the newer microemulsion formulation (Neoral). After administration of Sandimmune, trough levels (C_0) are reached at 12 to 18 hours, whereas with Neoral, which is much better absorbed and has an increased bioavailability, trough levels are achieved at 10 to 12 hours. Sandimmune can be given as a single daily dose, whereas Neoral probably needs to be given twice daily (every 12 hours). There is increasing concern that nephrotoxicity may be related more to high peak levels rather than high trough levels, and although there is no evidence that twice-daily doses

of Sandimmune produced better immunosuppression, it was suggested that there was a greater incidence of nephrotoxicity.[286] There is more recent evidence, however (discussed later), that low peak levels may predict rejection better than low trough levels.

TRIPLE THERAPY

In an attempt to maintain the improved immunosuppression provided by cyclosporine and to reduce the incidence of cyclosporine side effects, especially nephrotoxicity, triple therapy with low doses of cyclosporine, azathioprine, and steroids was introduced by several groups.[105,155,303,306] Data in experimental models suggested that azathioprine and cyclosporine might be synergistic in their immunosuppressive activity.[312]

The results of triple therapy by the late 1980s were excellent,[106,163] with 1-year first cadaver graft survival rates of about 80% reported in most instances and with many patients having no rejection. In the Oxford experience, 38% of patients with cadaver grafts had no clinical or histological rejection episodes in the first 3 months after transplantation.[163] There did not seem to be an increase in the incidence of infection despite the possible enhancement of immunosuppression achieved with the triple therapy. This form of triple therapy soon became the most commonly used immunosuppressive therapy in renal transplantation (as well as liver and cardiac transplantation), although today azathioprine has been replaced largely by MMF. Despite low doses of cyclosporine, renal function remained suboptimal and did not seem to be much improved over that seen in the Oxford unit in our earlier experience using high doses of cyclosporine alone. Although triple therapy is a potent immunosuppressive regimen, it did not seem to be any more effective than some of the other cyclosporine protocols described in this section.[129] Its ease of use made triple therapy an increasingly popular protocol in many units, with acceptable results that continue to improve despite the increased acceptance of older recipients with other comorbidities (Fig. 16-3).

An attempt to resolve the problem of efficacy was reported first from Milan[266,324] in a randomized controlled trial of triple therapy versus high-dose cyclosporine and steroids. Although patient and graft survival rates were similar in the two groups, there were more rejection episodes in the triple-therapy group but evidence of greater renal impairment and infection in the high-dose cyclosporine group. The second report was of a multicenter prospective trial from Australia comparing triple therapy with cyclosporine and prednisolone and with cyclosporine and azathioprine.[129] Approximately 140 patients were entered into each arm of the trial, which included nondiabetic transfused recipients of first cadaver grafts. Patient and graft survival rates were excellent in all three groups—91% and 85%, respectively, at 1 year. Of patients receiving cyclosporine and azathioprine, however, 36% required long-term prednisolone treatment to control rejection. The investigators suggested that optimal therapy might involve the initial use of cyclosporine plus one other agent, with the possible addition of a third if required.

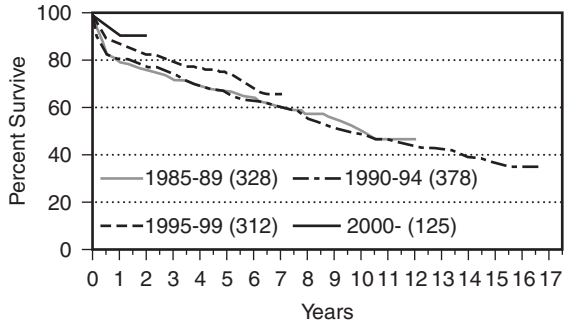

Figure 16–3 Graph showing cadaver donor graft survival in the cyclosporine era in the Oxford unit in 5-year cohorts from 1985. All patients received triple therapy (cyclosporine, azathioprine, and steroids).

Many units have explored the possibility of dropping one of the three drugs, most commonly steroids, after several months of triple therapy. It seems that this can be done safely in most patients. In a trial from Finland, patients on triple therapy were randomly assigned to drop one of the three drugs after 3 months.[133] The early experience of this trial suggested that any one of the three drugs can be discontinued safely with excellent graft survival being maintained in all three groups. Subsequently a randomized prospective trial of steroid withdrawal in patients on triple therapy with stable renal function after 1 year was reported from Oxford.[271] Complete steroid withdrawal was possible in most patients with significant improvement in cardiovascular risk factors (i.e., serum cholesterol and blood pressure) and in bone mineral metabolism. A modest reduction in graft function, of uncertain origin, was common, but was not progressive, at least in the medium term. Withdrawal of steroids in patients on triple therapy has been shown to improve significantly the management of patients with post-transplant diabetes mellitus.[153] It seems reasonable to attempt withdrawal of steroids in all patients on triple therapy who have stable renal function. Whether this steroid withdrawal can be done earlier than 1 year after transplantation is uncertain, but it may be possible (see Chapter 15).

As mentioned earlier, triple therapy can be undertaken with MMF replacing azathioprine, which results in a significant reduction in the incidence of acute rejection episodes in the first 6 months after transplantation.[17,309] This reduction in the incidence of acute rejection was not reflected by better graft survival. The ability of MMF to reduce acute rejection rates was reinforced by a study of the United States Renal Data System. When 47,693 patients were analyzed, it was shown that treatment with MMF reduces the incidence of acute rejections beyond 1 year post-transplantation compared with azathioprine. In a systematic review of MMF versus azathioprine published in 2004, the authors showed a reduced incidence of acute rejection at 6 and 12 months with MMF.[347] More recently, a randomized controlled trial comparing MMF with azathioprine when combined with the Neoral preparation of cyclosporine did not show any benefit with MMF, however, compared with azathioprine.[273] An analysis of the United Kingdom transplant database between 1999 and 2002 comparing paired kidneys in which one kidney went to a patient treated with cyclosporine and MMF and one went to a patient treated with cyclosporine and azathioprine showed no difference in graft or patient survival rate, but a significant reduction in acute rejections in the patients treated with azathioprine (44% versus 31%; $P < .01$).[295] There also is a significant cost implication with treating patients with MMF compared

with azathioprine. In the study by Remuzzi and colleagues,[273] the cost of 1 day of treatment with MMF was $15.30, whereas the cost of azathioprine for 1 day was $1.10. In the United Kingdom, the cost of 125 mg of azathioprine is £0.62 compared with £6.99 for 2 g of MMF (prices obtained from the British National Formulary).

QUADRUPLE THERAPY

Adding a prophylactic course of heterologous antilymphocyte globulin or OKT3 or, more recently, a monoclonal antibody against the IL-2 receptor (basiliximab or daclizumab) to triple therapy has been advocated by some groups, with delay of the administration of cyclosporine in patients with delayed primary function.[303] Although induction with an antilymphocyte agent is common practice in the United States, these are potent agents (antilymphocyte globulin or OKT3) associated with an increased risk of lymphoproliferative disease and infection and are not necessary in most patients receiving renal transplants.[251] Their use for induction in quadruple-therapy protocols possibly should be restricted to highly sensitized patients and patients with delayed graft function. The use of OKT3 as an induction agent has diminished, and OKT3 has been replaced for the most part by either thymoglobulin or other monoclonal antibodies such as basiliximab and daclizumab (anti–IL-2 receptor antibodies). The use of humanized or chimeric monoclonal antibodies against the IL-2 receptor has resulted in less rejection without an apparent increase in infection or lymphoproliferative disease (see Chapter 20),[237,341] and the tendency is to use these agents routinely as induction therapy. New, more potent monoclonal antibodies also are available, including the anti-CD52 monoclonal antibody, alemtuzumab, which produces a profound and lasting lymphopenia. It seems to be a good induction agent and may allow sparing of steroids or calcineurin inhibitors as suggested in a systematic review.[230] Rituximab, an anti-CD20 monoclonal antibody, also currently is being evaluated as an induction agent for use in sensitized patients (see Chapter 20 for more details).

SEQUENTIAL THERAPY

Sequential therapy has been used routinely by many units that previously gave antilymphocyte globulin or OKT3 with azathioprine and steroids.[24,93,192,298] Cyclosporine is not started until renal function has reached an acceptable level. The simplest approach used by the Basel group is to administer antilymphocyte globulin alone for the first 5 days before starting cyclosporine.[326] Generally, the more common approach is to give antilymphocyte globulin with low-dose azathioprine and prednisolone, starting cyclosporine after 7 or 14 days.[85,310] Although there is no firm evidence that this type of protocol is better than others, the graft survival figures from units using this approach were impressive at the time. In one prospective trial from Brussels, comparing sequential therapy using OKT3 with triple therapy, the graft survival rate was improved significantly—83% at 1 year in the OKT3-treated group versus 75% in the control group.[2] A subsequent report of a randomized prospective multicenter trial from the United States, in which sequential therapy using OKT3 for 14 days with the addition of cyclosporine on day 11 was compared with triple therapy, showed significantly fewer rejections in the OKT3 patients (51% versus 66%); 2-year patient and graft survival rates were 95% and 84%, respectively, in the OKT3 group and 94% and 75%, respectively, in the triple-therapy group.[241] Although no increased morbidity was associated with OKT3 in this trial, the routine use of antilymphocyte globulin or OKT3 induction therapy in low-risk renal transplant recipients seems to expose patients to more potent immunosuppression than generally is required and is not justified in most patients. More recently, a trial of sequential therapy using an anti–IL-2 receptor antibody for induction, with patients being randomly assigned to start cyclosporine therapy early, day 0, or late, day 6, showed that there was no difference in acute rejection or renal function or incidence of delayed graft function between the two groups, and there was no difference in patient or graft survival.[172]

CYCLOSPORINE IN HIGH-RISK PATIENTS

High-risk patients include a miscellaneous group of patients who may be at high risk for immunological or medical reasons. The following groups probably represent the patients who have benefited most from the use of cyclosporine.

1. Older patients. Cyclosporine has allowed transplantation to be offered to patients older than age 55 years with end-stage renal failure, patients who would have been excluded from transplantation by most units in the azathioprine-prednisolone era because the risks of the procedure and immunosuppression were considered unacceptable. In that earlier era, however, the Stockholm group[252] had shown that transplantation represented the most satisfactory solution to end-stage renal failure in the elderly patient. Similar data were reported from Dallas using cyclosporine immunosuppression.[338] Similarly in Oxford, using triple-therapy immunosuppression, renal transplantation was shown to be a safe procedure in most patients older than age 55. Although loss of grafts from rejection is rare in this older group, graft survival is lower because of a greater death rate with a functioning graft, usually as a result of cardiovascular disease. In Oxford, in patients younger than age 55, patient and graft survival are 96% and 87%, respectively, at 1 year compared with 84% and 74%, respectively, in patients older than age 55. At 5 years, the corresponding figures are 90% and 74%, respectively, in the younger cohort and 68% and 56%, respectively, in the older cohort. The pharmacokinetics of cyclosporine in elderly patients do not seem to be different, the main problem being interaction with other medications that are eliminated by the same metabolic mechanisms, such as cytochrome P-450 and P-glycoprotein.[191] Because elderly patients require less immunosuppression, attention should be paid to reducing cyclosporine levels to the lowest acceptable level. It is important in this group to withdraw steroids as early as possible, not later than 9 to 12 months after transplantation and probably earlier.
2. Very young patients. As described in detail in Chapter 35, cyclosporine has made transplantation an acceptable approach to renal failure in infants and young children.

3. Diabetic patients. Patients with diabetes have done much better after renal transplantation with cyclosporine protocols than previously. Cadaver renal transplantation has now become the treatment of choice for diabetics with chronic renal failure and in many instances is accompanied by a pancreas transplant (see Chapter 34).
4. Sensitized patients. Sensitized patients, particularly patients having a second graft, show much improved graft survival rates with cyclosporine protocols than seen before with azathioprine and prednisolone.[41,314] This improvement has been particularly evident at Oxford, most likely owing to the sophisticated analysis of the antibody status of sensitized recipients and the crossmatch between donor and recipient, rather than cyclosporine (see Chapter 10).

LIVING RELATED TRANSPLANTS

HLA-Identical Transplants

Although cyclosporine has had an impact on living related transplantation, its use for HLA-identical transplants is controversial because patient and graft survival are excellent with azathioprine and steroids, and most patients can be weaned off steroids after about 1 year. Flechner and coworkers[100] always had considered cyclosporine as the preferred therapy in these patients, however. A thought-provoking analysis of renal transplantation between HLA-identical siblings treated with azathioprine and prednisone or cyclosporine and prednisone has been reported from New York.[319] Patient and graft survival rates were 100% and 97%, respectively, in the cyclosporine group at 1 year compared with 91% and 85%, respectively, in the azathioprine group. Although renal function remained stable in the azathioprine group, there was a progressive deterioration in renal function in the cyclosporine group, a cause for considerable concern. At the Cleveland Clinic, a group treated with azathioprine and prednisone was compared with a group treated with cyclosporine and prednisone. Five-year patient survival was 100% versus 96%, and graft survival was 92% versus 83%. A nonsignificant increase in the serum creatinine level was noted in the cyclosporine patients (1.7 mg/dL) compared with azathioprine patients (1.3 mg/dL).[114] The question of whether cyclosporine is required for immunosuppression in HLA-identical living related transplants remains unresolved. A case can be made for the use of azathioprine and steroids or conversion from cyclosporine to azathioprine or MMF at 3 months in HLA-identical sibling transplants.

Non–HLA-Identical Transplants

The use of donor-specific transfusions in the early 1980s in patients with non–HLA-identical transplants led to a dramatic improvement in graft survival, approaching that of HLA-identical siblings.[284] Many patients become sensitized against the donor as a result of the transfusions, however, even with the concurrent administration of azathioprine.[8] Kahan[169] and Groth[125] first advocated that donor-specific transfusions be abandoned because equally good results in this group could be obtained with cyclosporine.[98] The concurrent use of cyclosporine with donor-specific transfusions was explored by Hillis and associates[146] and Cheigh and coworkers.[63] There were still some instances of sensitization, and it was unclear that results of the subsequent transplants were superior to the results of transplants in patients given donor-specific transfusions alone. The use of cyclosporine without donor-specific transfusions before transplantation simplifies the whole procedure and became the protocol followed by the Oxford unit for non–HLA-identical living related transplants for many years. Nevertheless, the long-term outcome of the non–HLA-identical transplants pretreated with donor-specific blood under azathioprine cover remains impressive in the St. Louis experience.[9] The role of prior donor-specific transfusion in this type of transplant is unresolved, but today there are other reasons for avoiding blood transfusions, and deliberate transfusions in nontransfused recipients are avoided in most units today.

LIVING UNRELATED TRANSPLANTS

The improved results that were obtained with cyclosporine have led many groups to embark on living unrelated transplants between highly motivated donors and recipients, usually spouses. The results of the Madison unit have been excellent. A protocol of donor-specific transfusions under azathioprine cover was followed by quadruple therapy with delayed administration of cyclosporine after transplantation.[22] A smaller study from Norway without donor-specific transfusions but using cyclosporine, either with prednisolone or azathioprine and prednisolone, also reported good early results for living unrelated transplantations.[308]

These early results have led to an increasing number of living unrelated transplants throughout the world, mostly with spouse donors. Registry results from the United Network for Organ Sharing and from the Collaborative Transplant Study have confirmed the excellent outcome of these transplants, results being equivalent to that of one haplotype–disparate living related transplants.[250,325] With living unrelated transplants, outcome still is related to the degree of fortuitous matching between donor and recipient.[250] The use of paired donations for ABO-incompatible or highly sensitized recipients is becoming more common, although this presents considerable logistic problems (see Chapter 22).

CONVERSION TO CYCLOSPORINE

Conversion to cyclosporine from azathioprine and steroids may be considered for side effects of azathioprine and steroid therapy in an ever-decreasing cohort of long surviving patients or for steroid-resistant acute or chronic rejection. In a phase I study at Oxford, nine patients with long-standing stable renal function were converted to cyclosporine because of steroid side effects. Although the early experience was encouraging,[330] the longer term follow-up was unsatisfactory: Only four patients remained on cyclosporine. Although the steroid side effects resolved, other problems arose: Two kidneys were lost, two patients died of sepsis, renal function declined in all grafts, and one patient developed recurrent squamous cell carcinoma of the skin; hypertrichosis and gout were less serious problems. A more favorable experience was reported after conversion to cyclosporine for steroid side effects from Basel and Odense,[137,326] but follow-up in these patients was short, which may be relevant, considering the initial favorable impression of conversion at Oxford. Although conversion of

long-term renal allograft recipients to cyclosporine for steroid side effects does not place the graft at risk for rejection, problems may occur from cyclosporine nephrotoxicity and other side effects. Before adopting this approach in patients with severe steroid side effects, one should be aware of the potential problems, and only patients with excellent renal function should be considered as candidates for conversion. Otherwise, conversion to MMF may be a more satisfactory path to follow.

Conversion to cyclosporine from tacrolimus may be considered when patients have side effects associated with tacrolimus. In their systematic review comparing cyclosporine with tacrolimus, Webster and colleagues[352] showed that post-transplant diabetes is more commonly associated with tacrolimus. In kidney transplant patients who have developed post-transplant diabetes with tacrolimus therapy, the conversion to cyclosporine has been shown to improve glucose metabolism and in some cases resolve the diabetes.[37,247] Conversion from tacrolimus to cyclosporine is not associated with increased risk of rejection during conversion.[144]

CYCLOSPORINE COST REDUCTION

Numerous studies have investigated the use of drugs to slow the metabolism of cyclosporine to reduce the dose and the cost of immunosuppression. Such strategies are of particular relevance to the care of transplant patients in developing countries or uninsured transplant patients in some areas of the Western world.

Cyclosporine is metabolized by isoenzymes of the cytochrome P-450 system. Ketoconazole, a broad-spectrum antifungal agent, inhibits this enzyme system in vitro and in vivo. Cyclosporine toxicity has been reported in the presence of ketoconazole owing to high blood or serum levels of the drug.[79,92,115] First and colleagues[94] at Cincinnati first proposed that ketoconazole might be used to decrease the dose of cyclosporine required for adequate blood levels, reducing the cost of the drug. Using a ketoconazole dose of 200 mg/day, retrospective and prospective studies support a reduction in the cyclosporine dose of 60% to 85%, with an associated cost reduction of 60% to 79%.[55,112] There was no associated increase in acute rejection episodes or hepatotoxicity. These findings were replicated in a more recent randomized controlled trial administering 100 mg/day of ketoconazole, with a dose reduction of 65% after 10 years.[90] This latter study also reported a significant decrease in chronic allograft nephrotoxicity, with no difference in metabolic complications. A further randomized trial showed a cost reduction of 42% using a smaller ketoconazole dose of 50 mg/day.[1]

One caveat to this application of ketoconazole is that all of the aforementioned studies monitored cyclosporine using trough levels. More recent, albeit small, studies have suggested that the use of ketoconazole alters the pharmacodynamic profile of cyclosporine microemulsion, meaning that newer 2-hour (C_2) monitoring strategies may be invalid.[87,340,361] Analysis of cyclosporine pharmacokinetic profiles has shown a flattening of the absorption curve with increased variability and longer elimination half-life with the addition of ketoconazole. In this situation, the trough level and the level at 4 hours after dosing are better predictors of area under the curve (AUC) than C_2. A small retrospective study from Chile has suggested that the use of ketoconazole in conjunction with C_2 monitoring may increase the risk of hepatotoxicity.[340]

Numerous trials have investigated the use of other metabolic inhibitors in reducing the cost of cyclosporine treatment.[217] These include other antifungal preparations such as fluconazole and itraconazole, calcium channel blockers, and the macrolide antibiotics. The dose reduction achieved with these drugs is generally less than with ketoconazole, with reductions of 20% to 50% seen with coadministration of diltiazem. It also has been suggested that addition of diltiazem or verapamil can improve clinical outcomes, with reductions in the severity of rejection episodes and improvements in renal function.[66,82,211]

CYCLOSPORINE FORMULATIONS

Sandimmune

The original, oil-based formulation of cyclosporine (Sandimmune; Novartis Basel, Switzerland) was introduced in 1983. Although a significant advance in immunosuppressive therapy, this formulation had numerous problems. Absorption was slow and showed a great deal of intrapatient and interpatient variability, making dosing difficult and increasing the risk of chronic rejection.[171,189]

Neoral

In 1995, Neoral (Sandimmune Neoral; Novartis Basel, Switzerland), a microemulsion formulation of cyclosporine, was approved for use by the Food and Drug Administration. This new formulation improved bioavailability with more rapid absorption and less variability in de novo and stable transplant patients.[189,190] Since its introduction, numerous randomized and nonrandomized studies have been performed to ascertain whether this new formulation improved clinical outcomes in transplant recipients. Shah and colleagues[294] collected the results of these trials in a thorough meta-analysis. Rates of graft loss and renal function do not differ when the two formulations are compared. The investigators found that in de novo renal, liver, and cardiac transplant recipients, acute rejection rates are lower in patients treated with Neoral. In stable patients, no difference in acute rejection rates is seen. Generally, adverse event rates are similar between the two formulations, with an increase in adverse events in Sandimmune-treated de novo liver recipients. When only randomized controlled trials were considered, lower rejection rates were seen in de novo and stable patients treated with Neoral. The tradeoff was an increase in adverse events seen in these stable patients in randomized, blinded trials.

More recently, longer term outcomes have been reported. Goel and colleagues[116] showed in a retrospective analysis that graft and patient survivals do not differ at 5 years. Although chronic rejection rates and renal function do not differ, use of Neoral leads to significantly more patients free of acute rejection at 5 years. Another longer term study has suggested that the increase in bioavailability of the microemulsion formulation of cyclosporine may lead to increased rates of Kaposi's sarcoma.[58] A review of pharmacoeconomic studies from Europe and Canada in renal and liver transplant patients has suggested that the overall costs of treating patients with the microemulsion formulation is

marginally less than the original formulation, but this does not reach statistical significance.[74]

Generic Formulations

In recent years, numerous generic microemulsion formulations have been approved for use, and many others are in use in developing countries (e.g., India). Although these have been shown to be bioequivalent in healthy male volunteers, questions have arisen as to how appropriate such testing is in the field of transplantation. Transplanted patients have considerable differences in drug absorption and availability compared with healthy individuals, meaning that testing bioavailability of these drugs in healthy individuals may be invalid.[162,267]

Pharmacological studies of generic formulations in transplant recipients give conflicting results. Many studies have shown bioequivalence between Neoral and various generic formulations in stable renal patients.[81,143,218,278] Despite such suggestions of equivalence, Qazi and coworkers[269] showed that when switching from Neoral to the generic formulation Gengraf, nearly 20% of patients required dosage changes to maintain trough blood levels.

Numerous studies also have assessed the clinical outcomes in patients treated with generic formulations, with varied results. Sharma and associates[296] prospectively treated 37 de novo renal transplant recipients with either Neoral or the ArpimuneME (RPG Life Sciences, Mumbai, India) formulation, showing equivalent clinical outcomes at 6 months. Taber and colleagues[320] showed increased rates of acute rejection with the use of Gengraf in a retrospective review of de novo renal recipients. An analysis of data from the Collaborative Transplant Study shows significantly worse 1-year survival in patients treated with generic formulations,[73] but this has not been confirmed by Opelz in more recent data (Opelz, personal communication, 2007). Generic cyclosporine also is used widely in developing countries with no obvious deleterious effects (see Chapter 36).

When a switch to the use of generic cyclosporine formulations is being considered, patients must be closely monitored for the need for dosage adjustments. More prospective clinical data are required to confirm the impact of such formulations on long-term clinical outcomes.

CYCLOSPORINE VERSUS TACROLIMUS

Tacrolimus, a calcineurin inhibitor similar to cyclosporine, was introduced into transplantation in the early 1990s. There have been numerous clinical trials to try to determine whether tacrolimus is a better immunosuppressive agent than cyclosporine and to ascertain if the side-effect profiles are comparable. It is beyond the scope of this chapter to review all of the trials comparing cyclosporine with tacrolimus (see Chapter 17). The Cochrane Renal group produced a thorough systematic review and meta-analysis on this subject in 2005 that showed tacrolimus may provide significant improvements in graft survival and acute rejection rates over cyclosporine.[352] It was shown that graft loss with tacrolimus is reduced by 44% at 6 months and 29% at 3 years compared with cyclosporine. The data available for 5 years showed no significant benefit, however, in terms of graft survival with tacrolimus. Tacrolimus seemed to reduce acute rejection rates beyond 3 months and the rate of steroid-resistant acute rejections. Tacrolimus also was shown to be less nephrotoxic than cyclosporine as determined by serum creatinine levels. Tacrolimus did pose a significantly greater risk, however, with respect to post-transplant diabetes, with an incidence twice that of cyclosporine.

CYCLOSPORINE SPARING

During the early experience with cyclosporine in Cambridge, the side effect of nephrotoxicity was discovered.[47] This side effect had not been seen in the early animal models. The knowledge of this side effect and the resultant concern for the long-term effects on kidney grafts resulted in the cyclosporine-sparing protocols being introduced early after the introduction of cyclosporine. The first such protocol was developed in Oxford, where patients in a small phase I trial were randomly assigned to treatment with cyclosporine alone with conversion to azathioprine and prednisolone at 3 months or to the conventional treatment at that time of azathioprine and prednisolone.[227] There were 35 patients entered into the trial; 21 were randomly assigned to the conversion protocol, and 14 were assigned to the conventional treatment. The results of this study showed that the treatment with cyclosporine and conversion to azathioprine and prednisolone was satisfactory and that the renal function of those who were converted showed improvement after conversion from cyclosporine. A second, larger randomized trial from the same center produced similar results with good long-term graft survival, but there was a 25% incidence of acute rejection episodes after conversion, all of which responded to treatment or conversion back to cyclosporine.[229]

Other centers have adopted similar protocols of conversion to azathioprine. Some long-term results of these trials have been published more recently. Bakker and coworkers[19] published 15-year results of a randomized trial with conversion from cyclosporine and prednisolone to azathioprine and prednisolone at 3 months. There was no difference in patient survival over the 15 years, but there was worse graft survival in the patients remaining on cyclosporine, and the risk of chronic allograft nephropathy was greater in that group; patients who were converted to azathioprine had better renal function and required less antihypertensive medication.

Gallagher and colleagues[108] also published 15-year follow-up data on an Australian trial in which patients were randomly assigned to one of three arms: azathioprine and prednisolone, cyclosporine and prednisolone, or initial treatment with cyclosporine alone followed by conversion to azathioprine and prednisolone at 3 months. Their study showed no difference in patient survival or graft survival between the groups, but there was a benefit in graft function seen in the group converted to azathioprine.

A similar trial in Glasgow, in which patients were converted from cyclosporine and prednisolone to azathioprine and prednisolone at 1 year or continued on cyclosporine and prednisolone, showed that there was no difference in graft or patient survival at 15 years.[165] Graft function was improved in the conversion group at 2, 5, and 10 years, but by 15 years the improvement in renal function in the azathioprine group was no longer significant. The investigators also showed that conversion to azathioprine carried an increased risk of rejection; this was not seen or reported in the other trials, except from Oxford.

Although these trials seemed to have satisfactory short-term and long-term results, conversion protocols never became standard practice. More recently, sirolimus and MMF have been used to replace cyclosporine in patients with evidence of worsening graft function.[89,351] Both trials showed that conversion from cyclosporine to sirolimus[351] or MMF[89] can improve graft function without a resultant increase in acute rejection or graft survival in the short-term.

Other protocols have been considered in an attempt to reduce the nephrotoxic effects of cyclosporine. The protocols fall into four main categories: (1) replacement of cyclosporine by another agent (as mentioned earlier), (2) withdrawal of cyclosporine without addition of another immunosuppressive agent, (3) cyclosporine-free protocols (i.e., the patient never receives any cyclosporine at any point), and (4) reduction of the cyclosporine dose compared with normal.

The large Rapamune Maintenance Regimen (RMR) study, which looked at withdrawal of cyclosporine at 3 months from treatment with cyclosporine, sirolimus, and prednisolone, reported 4-year results that showed the withdrawal of cyclosporine resulted in graft survival of 91.5% at 4 years compared with 84.2% in the cyclosporine continuation arm ($P = .024$) and significantly better renal function with cyclosporine withdrawal.[246] No difference in acute rejection rates was seen. In a meta-analysis of calcineurin inhibitor (cyclosporine or tacrolimus) withdrawal from sirolimus-based therapy, Mulay and coworkers[232] looked at six trials, including the RMR trial, and found no benefit to graft survival with calcineurin inhibitor withdrawal. There was a significant increase, however, in acute rejection rate with calcineurin inhibitor withdrawal, although better renal function was seen with calcineurin inhibitor withdrawal.

Withdrawal protocols also have been used in regimens with MMF.[3,134,288,307] These trials all have a follow-up of at least 1 year and show no change in graft or patient survival between the cyclosporine continuation arm and the cyclosporine withdrawal arm. All four trials showed an increase in acute rejection rates in the cyclosporine withdrawal arm, but only two of the trials showed a benefit in renal function with cyclosporine withdrawal.[134,288]

Kasiske and associates[174] published a meta-analysis of trials of cyclosporine withdrawal from protocols with azathioprine. This meta-analysis showed a picture similar to the more recent meta-analysis by Mulay and coworkers,[232] mentioned previously. There was a significant increase in acute rejections in the cyclosporine withdrawal arm, but no difference in graft or patient survival. The meta-analysis did not comment on graft function.

Removing the risk of cyclosporine nephrotoxicity completely by using cyclosporine-free immunosuppressive regimens has been tried by using sirolimus instead of cyclosporine along with prednisolone and MMF or azathioprine.[101,126,193] These three trials showed similar results in that there was no difference in patient or graft survival or acute rejection rates, but the cyclosporine-free arms had better renal function. Different side-effect profiles were encountered with sirolimus, including poor wound healing, thrombocytopenia, hypercholesterolemia, and hypertriglyceridemia.

Another approach to reducing the nephrotoxic effects of cyclosporine, while maintaining its immunosuppressive properties, is to reduce the doses of cyclosporine used. Cyclosporine reduction has been tried in regimens containing conventional immunosuppressive agents such as azathioprine and MMF and in more novel protocols with the use of newer induction agents, such as alemtuzumab.

Cyclosporine reduction 1 year after transplantation in combination with azathioprine and prednisolone resulted in no change in graft or patient survival compared with full-dose cyclosporine, but there was a reduced rate of cancers, at the expense of an increase in acute rejection at 6 years after transplantation.[80] A similar study with cyclosporine reduction from the time of transplantation showed no difference in graft or patient survival or renal function at 7 years after transplantation, but the article did not report acute rejection rates; no difference in cancer incidence was seen.[225] Cyclosporine reduction in combination with MMF and prednisolone was shown to be effective in a study in which cyclosporine was reduced by 50% at 1 year after transplantation. Six-month results show a benefit in renal function over full-dose cyclosporine without any increase in acute rejection rate or change in graft survival.[259]

Alemtuzumab, a powerful lymphocytic monoclonal antibody, also has been used for induction to try to reduce exposure to cyclosporine. In a small randomized study, 20 patients who received alemtuzumab and low-dose cyclosporine monotherapy were compared with 10 control patients who received conventional triple therapy. At 6 months, there was no difference in acute rejection or graft and patient survival, but approximately 80% of patients with a functioning graft who received alemtuzumab were no longer taking steroids.[337]

There seems to be a general trend in most trials to try to reduce the nephrotoxic side effects of cyclosporine by reducing the patient's exposure to the drug in that often an improvement in renal function is observed, but reduction also may be associated with an increase in the incidence of acute rejection. This is a dilemma in that renal function at 1 year and acute rejection rates have been shown to be surrogate markers of long-term graft survival.[131] Does the improvement in renal function outweigh the increased risk of rejection? If one looks at the long-term results on conversion of cyclosporine to azathioprine from Glasgow,[165] although the study showed an improvement in renal function when converting to azathioprine, it also showed an increase in the incidence of acute rejection. There was no detrimental effect on long-term graft survival, however, by converting to azathioprine. One could conclude that the benefit of improved renal function outweighs the increased risk of acute rejection with regard to long-term graft survival. In these early trials, the dose of cyclosporine was higher than is currently used in triple-therapy regimens. A very strong case can be made for cyclosporine (or tacrolimus) conversion protocols, with the expectation that renal function will improve. A conversion protocol should be considered, however, only if regular and frequent follow-up is possible for at least 6 months after conversion.

MONITORING OF CYCLOSPORINE

Close monitoring of cyclosporine is essential to control the tradeoff between immunosuppression and the nephrotoxicity associated with long-term use. Cyclosporine levels are particularly valuable in the first 2 weeks after transplantation for detecting patients who are not absorbing the drug

adequately, and later on for detecting lack of compliance or adherence. Many drugs interact with cyclosporine, and measuring cyclosporine levels is valuable in monitoring these interactions (see Table 16-5).

Trough Monitoring

Traditionally, cyclosporine levels have been monitored at their trough, before administering the next dose (C_0 levels) (Fig. 16-4). Table 16-3 shows commonly used target levels. Although C_0 monitoring is convenient, allowing a relatively wide time window in which samples can be taken, there are questions as to how effective it is. The most accurate method of monitoring cyclosporine levels is calculating the AUC using multiple blood samples to give an estimate of total drug exposure. For the original and microemulsion formulations of cyclosporine, C_0 levels are shown to correlate poorly with AUC.[122,156,212] The relationship between C_0 levels and nephrotoxicity is not linear,[148,170] and C_0 levels correlate poorly with episodes of acute rejection.[212]

Area under the Curve

The inability of C_0 levels to predict important clinical outcomes accurately has fueled interest in finding a more effective monitoring strategy. Although an AUC measurement for the 12 hours following a dose correlates well with clinical outcomes, it is largely impractical outside of the research setting, requiring blood samples at multiple time points.[207,291] The microemulsion formulation of cyclosporine has the advantage of more rapid, more consistent absorption, which allows accurate monitoring with fewer blood samples. Gaspari and colleagues[111] showed a good correlation with the full AUC using samples taken at 1, 5, 8, and 11 hours, but 0, 1, and 3 hours also correlated well. Mahalati and others[177,212,213] showed that most variability in Neoral absorption occurs in the first 4 hours after administration, leading to the suggestion of a monitoring strategy measuring AUC for the first 4 hours after dosing ($AUC_{0\text{-}4h}$). Although $AUC_{0\text{-}4h}$ correlates well with clinical outcomes, it still requires multiple blood samples, making it impractical for everyday use. The ideal strategy is a single time point surrogate that correlates well with AUC and clinical outcomes, and a blood sample 2 hours after the ingestion of Neoral (C_2) is considered as the ideal surrogate marker.

Two-Hour Monitoring

The role of C_2 monitoring in organ transplantation has been systematically reviewed[186]; only the highlights of this review with respect to renal transplantation are presented here. Results from the International Neoral Renal Transplantation Study Group show that a blood sample taken 2 hours after intake of Neoral (C_2) is the most accurate one-point predictor for $AUC_{0\text{-}4h}$ and shows less variability than either C_0 or C_1. In clinical studies, retrospective analysis shows C_2 levels to correlate well with acute rejection in de novo renal transplant patients.[177,262] The Canadian Neoral Renal Transplant Study Group showed in retrospective analysis a significantly lower acute rejection rate in patients in whom C_2 levels were maintained at greater than 1500 μg/L in the 2 weeks after transplantation.[177] Dose adjustments in this study were based on trough levels.

The evidence from prospective studies with dose adjustment according to C_2 levels is less convincing. Commonly used target ranges in these studies are shown in Table 16-3. A group from Helsinki randomly assigned de novo renal transplant recipients to either C_0 or C_2 monitoring for the first 3 weeks after transplantation.[195] There was no significant difference in the rates of acute rejection between groups. Patients monitored by C_2 levels had difficulty reaching target levels, and the mean cyclosporine dose was 56% higher over the first 20 days. Although this difference in dose did not cause impairment in renal function over the short period of this study, over a longer time course this higher dose may have detrimental effects. A further randomized study from China contradicts these results, showing a significantly higher acute rejection rate in C_0 monitored patients.[348] The authors do not specify the C_0 target range or mean levels, however, and it is possible that a difference in target ranges could account for these differences. Many nonrandomized studies have failed to show a beneficial effect on

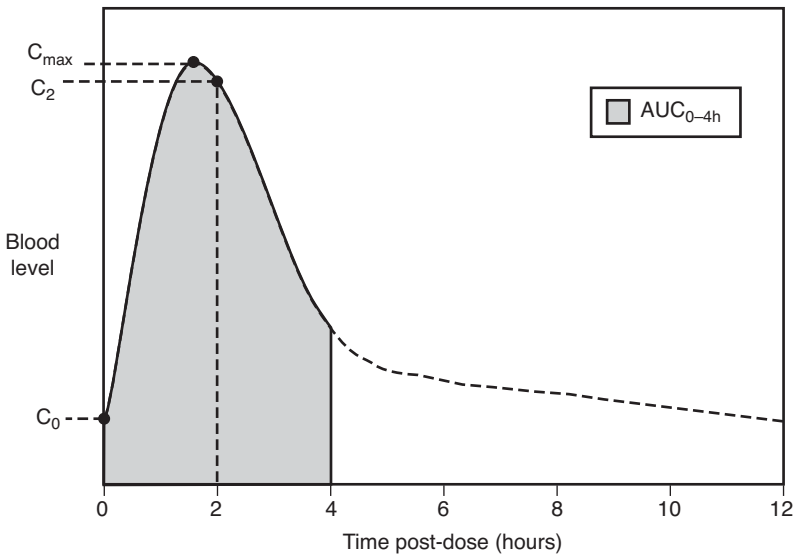

Figure 16–4 Cyclosporine absorption curve. C_0, trough (predose) level; C_2, 2-hour postdose level; C_{max}, maximal blood level; $AUC_{0\text{-}4h}$, area under the concentration-time curve from 0 to 4 hours.

Table 16–3 Commonly Used Cyclosporine Target Ranges from Prospective Trials*

	Time Post-Transplant (mo)		
	0-1	1-6	6-12
Target C_0 level (μg/L)	200-300	150-250	100-200
Target C_2 level (μg/L)	1500-1800	1000-1500	600-1000

*Guidelines based on prospective trials in published literature. Actual target range depends on concurrent immunosuppression.

acute rejection rates,[28,130,214,268,285] and some studies support the finding from the Helsinki study that the mean cyclosporine dose in the early period in C_2 monitored patients is significantly higher than the dose in patients monitored by C_0.[28,214,285] Most of these nonrandomized studies show no difference in renal function between groups.

Less evidence is available in stable renal transplant recipients. The only randomized trial to date involved patients more than 3 months post-transplantation, who were randomly assigned to continue monitoring by C_0 levels or switched to C_2 monitoring. Although there was no difference in acute rejection rates between the cohorts, the switch to C_2 monitoring allowed a dose reduction in 34% of patients compared with reductions in 14.3% of patients monitored by C_0. Observations from before and after studies in which cohorts of stable patients are switched from C_0 to C_2 monitoring support these findings of significant cyclosporine dose reductions with no increase in acute rejection rates.[69,71,305] Despite the dose reductions, none of these studies show an improvement in renal function during follow-up periods of 40 months.

When considering the role of C_2 monitoring in patient management, logistical aspects must be addressed. Blood samples for C_2 levels are taken during a more dynamic phase of cyclosporine absorption than samples for trough levels, making accurate timing of sampling essential. Consensus guidelines suggest that there is a 15-minute "window of opportunity" before and after the 2-hour point in which samples should be taken.[204] More recent evidence suggests that this window may be 10 minutes to give an acceptable (±20%) error around the true value.[279] Although such strict timing requirements may be adhered to in the context of a clinical trial, it is likely that problems will arise in the setting of a busy outpatient clinic.

Indirect evidence suggests an advantage of C_2 monitoring over trough levels. Retrospective analysis shows that the risk of acute rejection is reduced in patients in whom a certain threshold for C_2 is exceeded.[177] In prospective studies, these advantages are not substantiated. In the Helsinki study, 45% of C_2 monitored patients failed to reach the target levels by day 5 post-transplantation compared with 2.5% of C_0 monitored patients.[195] This difficulty in reaching target levels may partially explain why the theoretical benefit of C_2 monitoring in the early post-transplant period is not borne out. It can be argued that if such difficulty is met trying to reach target levels in the controlled environment of a clinical trial, it would be even more difficult to implement such a strategy in a nontrial population. For this reason, more prospective evidence, particularly in the early postoperative period, is required before adoption of C_2 monitoring can be recommended. For the moment, trough levels (C_0) remain the standard despite the inherent poor correlation with outcomes.

Cyclosporine Assays

Regardless of the sampling points used, the laboratory measurement of cyclosporine has been the subject of much interest over the years.[161] The reference "gold standard" is often regarded as high-performance liquid chromatography because of its specificity and ability to separate the parent compound from metabolites. High-performance liquid chromatography can lead to poor precision with difficulty identifying low plasma concentrations of the drug, however, and does not have a short enough turnaround time for the busy transplant clinic.[207]

Many nonspecific and specific immunoassays are available (Table 16-4). Although the nonspecific assays show a poor relationship to clinical events, the specific assays are much more clinically useful[207] and tend to be the most commonly used. Novartis, the manufacturer of Neoral, recommends high-performance liquid chromatography as the reference method but reports the specific immunoassays as sensitive, convenient, and reproducible alternatives (Neoral product literature).

Even the newer specific immunoassays have drawbacks. There is still cross-reactivity of the antibodies used in these assays with inactive metabolites of cyclosporine leading to overestimation of blood levels, the magnitude of which cannot be easily predicted.[315] The immunoassays have limited analytical ranges with an inability to detect potentially significant low levels of cyclosporine, while requiring dilution for the measurement of high blood concentrations adding a potential source of error. For this reason, the laboratory in Oxford has now adopted the use of a rapid liquid chromatography–mass spectrometry method to enable the accurate and rapid detection of cyclosporine blood levels over a wide concentration range. This method gives good agreement with the existing enzyme multiplied immunoassay technique.[175]

DRUG INTERACTIONS

Cyclosporine is metabolized almost entirely in the liver, mostly through the cytochrome P-450 system. Most of the drug is excreted in the bile, with only trace amounts being

Table 16–4 Assays Available for Monitoring of Cyclosporine

High-performance liquid chromatography
Rapid liquid chromatography–tandem mass spectrometry
Nonspecific polyclonal immunoassays
  Abbott TD_x NS (Abbott Laboratories, Abbott Park, Ill., USA)
Nonspecific monoclonal immunoassays
  DiaSorin Cyclo-Trac-SP (RIA NS, Diasorin S.p.A., Vercelli, Italy)
Specific monoclonal immunoassays
  Abbott TDx (Abbott Laboratories, Abbott Park, Ill., USA)
  Abbott AxSYM (Abbott Laboratories, Abbott Park, Ill., USA)
  DiaSorin Cylo-Trac-SP (RIA) (Diasorin S.p.A., Vercelli, Italy)
  ADIVA Centaur (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA)

excreted in the urine. Drugs that induce hepatic enzymes, such as rifampicin, increase the rate of metabolism of cyclosporine and decrease blood levels of the parent compound. Other drugs that are potentially nephrotoxic, such as gentamicin, have an additive effect with cyclosporine on nephrotoxicity. It is important to be aware of known drug interactions and to keep in mind the possibility of other, but as yet unconfirmed, interactions. The measurement of levels is important in detecting such interactions and in the monitoring of levels at which drugs with known interactions have to be used. Table 16-5 lists well-known interactions. All known interactions are noted, and the relevant citations to the literature are given in the "Sandimmune Drug Interactions and Neoral Drug Interactions," available from the Novartis Medical Information Department (Basel, Switzerland); these are continually updated and are available on the Internet.

Other drugs or dietary products that can affect cyclosporine levels include atorvastatin, which has been shown to increase cyclosporine trough concentration by 25%,[274] and grapefruit juice, which can increase cyclosporine AUC by 37%.[29] Lopinavir and ritonavir (antiretroviral protease inhibitors) have been shown to increase levels of cyclosporine such that doses could be reduced to 5% to 20% of the initial dose to maintain AUC concentrations.[342] Isoniazid has been shown to decrease cyclosporine levels,[76] as has more recently vitamin C and vitamin E supplementation.[30,84]

Cyclosporine itself alters the plasma levels of other drugs. It can increase the levels of methotrexate and reduce the clearance of digoxin, colchicine, prednisolone, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors.

Table 16–5 **Drugs That Interact with Cyclosporine**

Drugs That May Potentiate Renal Dysfunction
Amphotericin B
Cimetidine
Ciprofloxacin
Diclofenac
Gentamicin
Ketoconazole
Melphalan
Naproxen
Ranitidine
Sirolimus
Tacrolimus
Trimethoprim/sulfamethoxazole
Vancomycin
Drugs That May Increase Cyclosporine Concentrations
Allopurinol
Amiodarone
Atorvastatin
Bromocriptine
Colchicine
Diltiazem
Fluconazole
Itraconazole
Ketoconazole
Lopinavir and Ritonavir
Macrolide antibiotics (e.g., erythromycin)
Methylprednisolone
Metoclopramide
Nicardipine
Verapamil
Drugs That May Decrease Cyclosporine Concentrations
Carbamazepine
Isoniazid
Nafcillin
Octreotide
Phenobarbitone
Phenytoin
Rifampicin

Data from Novartis Neoral/Sandimmune prescribing information, Novartis, Basel, Switzerland, August 2005.

SIDE EFFECTS OF CYCLOSPORINE

Renal Effects

Nephrotoxicity is the most worrying side effect of cyclosporine (and similarly tacrolimus) and is of particular concern in renal transplantation, in which it has to be distinguished from acute or chronic rejection as a cause of deteriorating renal function. In the early rat and dog models of transplantation, nephrotoxicity was not noted. Nephrotoxicity became evident soon after initial clinical use,[47] however, and the investigators advocated the use of cyclosporine only in patients whose kidneys were diuresing after transplantation.[49] Nephrotoxicity subsequently was shown in animal models using larger doses and more sophisticated evaluation of renal function, and some morphological changes attributed to nephrotoxicity in humans were observed.[354,355]

Three clinical types of nephrotoxicity are observed with cyclosporine. The first occurs immediately after transplantation, usually in a kidney already damaged by ischemia and perhaps associated with the use of intravenous cyclosporine. The nephrotoxic effect of cyclosporine in experimental models of ischemia of the kidney has been controversial in that one of the first attempts to show this interaction in dogs failed to do so.[151] Since then, the susceptibility of the ischemic kidney to damage by cyclosporine has been well documented in rat models.[65,158,173] In humans, the incidence of delayed function after renal transplantation has tended to be higher in patients treated with cyclosporine than in patients given azathioprine and steroids,[14,297] although there is no general agreement about this.[99] The implications of the possible additive effects of cyclosporine nephrotoxicity on an ischemic kidney are important because they suggest that protocols that delay the administration of cyclosporine until adequate renal function is established are more appropriate. Because intravenous cyclosporine is rarely used now, however, this type of toxicity is not seen as commonly as the acute and chronic cyclosporine nephrotoxicity seen with oral cyclosporine.

Acute Cyclosporine Nephrotoxicity

The second type of nephrotoxicity is seen any time after the first 2 or 3 weeks and is associated with deteriorating renal function, usually but not always associated with high trough blood levels of cyclosporine, and responds to a reduction in cyclosporine dosage. This type of nephrotoxicity has to be differentiated from an acute rejection episode. As pointed out in Chapter 14, this differentiation often is difficult because the florid signs of acute rejection previously seen in patients taking azathioprine and prednisolone (i.e., fever, graft tenderness and swelling, oliguria, and rapidly increasing serum creatinine levels) are much less evident in patients treated with cyclosporine. Although high blood trough levels

often are associated with nephrotoxicity and low levels are associated with rejection, there are numerous exceptions to this.[148] If the serum creatinine level has increased to greater than 300 μmol/L, it suggests rejection, and treatment for rejection should be started (e.g., 0.5 g of methylprednisolone intravenously daily for 3 days) while awaiting the results of the obligatory graft biopsy; this should be followed by an improvement in renal function.[103] At lower levels of serum creatinine, without any other clinical evidence of rejection, a significant reduction in the dose of cyclosporine (e.g., by 30%) should be implemented, and an improvement in renal function should follow rapidly if true nephrotoxicity is present; if not, a biopsy should be performed.

Percutaneous biopsy or fine-needle aspiration of the kidney can be valuable in helping to make the correct diagnosis; at Oxford, both approaches have been used in cases of acute renal dysfunction in which the distinction between rejection and nephrotoxicity is unclear. The development of an automated percutaneous needle biopsy technique has made frequent biopsies quick, easy, and safe. There are no definite morphological changes in biopsy specimens that implicate cyclosporine nephrotoxicity; the diagnosis still tends to be one of exclusion.[78,239] A simple technique for measuring the intrarenal pressure has been described by Salaman and Griffin[280]; they claimed it distinguishes rejection (pressures >40 mm Hg) from nephrotoxicity (pressures <40 mm Hg) with a high degree of accuracy.[281] With the simplicity of ultrasound-guided biopsy today, however, this technique no longer has a place, as is also the case with fine-needle aspiration biopsy. This type of nephrotoxicity recovers rapidly with a cyclosporine dosage reduction or conversion to azathioprine, MMF, and prednisolone.[61]

Cyclosporine-induced acute nephrotoxicity is caused by functional changes that result in a reduction in renal blood flow, an increase in renal vascular resistance, and a decrease in glomerular filtration rate.[261] The metabolites of cyclosporine have a similar effect.[276] These changes are reversible on reduction or withdrawal of cyclosporine, resulting in improvement in renal function usually within 1 week. The mechanisms involved that result in the changes to the renal vasculature that cause the nephrotoxicity are likely multifactorial and interdependent. They include an increase in the vasoactive substance endothelin I, the activation of the renin-angiotensin system resulting in increased levels of angiotensin II, and a decrease in the synthesis of nitric oxide (NO).

The vasoactive peptide, endothelin, potentially may contribute to the hemodynamic alterations caused by cyclosporine.[261] Endothelin release is increased from smooth muscle cells in culture on exposure to cyclosporine and in patients on cyclosporine who have received bone marrow transplants.[132] This increase in endothelin subsequently has been shown in kidney transplant recipients[59] and heart transplant recipients.[203] The fact that the use of endothelin receptor blockers in animals has been shown to reduce cyclosporine-mediated vasoconstriction of afferent arterioles[197] and that endothelin receptors are upregulated in rats with cyclosporine-induced nephrotoxicity[260] suggests that endothelin may have a role in acute cyclosporine nephrotoxicity.

The renin-angiotensin system is believed to play an important role in acute nephrotoxicity because it has been shown experimentally that cyclosporine increases plasma renin activity[201,233] and that cyclosporine causes hyperplasia of the juxtaglomerular apparatus, where renin is synthesized.[201,240] Increased levels of renin also have been shown in non–renal transplant patients treated with cyclosporine.[166] An increase in renin alters renal hemodynamics, resulting in a decrease in renal function. The blockade of the renin-angiotensin system by angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers has provided some evidence of improved renal hemodynamics in the presence of cyclosporine.[43,52,277,299]

NO is a powerful vasodilator, and it has been implicated in acute cyclosporine nephrotoxicity, although there seems to be some confusion as to its role. There is some debate as to whether cyclosporine increases or decreases NO production. It has been shown in healthy volunteers to increase NO production, but in recipients of renal transplants it has been shown that basal and stimulated levels of NO are reduced,[231] and in rat models cyclosporine has been shown to increase, reduce, and not alter NO production.[44] The effect of NO on blood vessels also has been tested, and it has been shown that cyclosporine reduces endogenous epithelium-dependent vasodilation mediated by NO.[39,109,215] Cyclosporine has been shown not only to enhance endothelial NO synthetase activity, causing an increase in NO production, but also to decrease acetylcholine-induced NO production.[238,318]

The role of NO is unclear; it is likely multifactorial, and whether NO is causative in cyclosporine-induced nephrotoxicity, or the changes in NO production are an effect of cyclosporine nephrotoxicity is unknown. It is suggested that changes in NO production may have a causative role to play because blocking NO production can induce similar histopathological changes as seen in cyclosporine administration.[31,32] Also, promoting NO production by the administration of L-arginine, a substrate for NO synthetase, protects against the effects of NO blockade and the changes seen with cyclosporine administration.[10,359] This protection has been shown experimentally, but L-arginine has not been proved to show any protective effect in clinical trials.[187]

Other factors that may have a role in acute nephrotoxicity are the increase in thromboxane, the production of free radicals, and the increase in sympathetic tone, all of which may be attributed to cyclosporine.[44,45,53,57] Another possible uncommon manifestation of acute cyclosporine nephrotoxicity is a hemolytic-uremic syndrome–like condition that occurs in the first week after transplantation. A biopsy specimen shows striking arteriolopathy and thrombosis. Despite the striking nature of the histological findings, a return of renal function was noted with cessation of cyclosporine or the use of streptokinase and heparin.[181,272,310]

Chronic Cyclosporine Nephrotoxicity

Chronic cyclosporine nephrotoxicity is a condition in which there is a slow, steady deterioration in renal function, and the histology of the kidney may reveal severe interstitial fibrosis (see Chapter 25). This type of nephrotoxicity shows some improvement in renal function with a decrease in the cyclosporine dosage, but this improvement tends to be short-lived. It is likely that many of the changes observed result from chronic immunological damage on which some element of cyclosporine nephrotoxicity is superimposed. That chronic changes of cyclosporine nephrotoxicity do occur is unquestioned in view of the striking morphological changes of interstitial fibrosis and tubular atrophy observed in the native kidneys of patients with uveitis treated

with cyclosporine.[257] After cardiac transplantation, this steady deterioration in renal function of patients receiving cyclosporine resulted in some patients requiring hemodialysis[234]; this remains a problem in cardiac transplantation and is seen also in liver transplant patients. Chronic nephrotoxicity probably is a cumulative effect of initial ischemic damage to the kidney in association with high early doses of cyclosporine. A hypothesis was put forward by Salomon,[282,283] however, in which he postulates that the deterioration of renal function in patients on cyclosporine was not due to cyclosporine nephrotoxicity but represented chronic rejection resulting from underimmunosuppression as doses of cyclosporine are reduced with time. There is no evidence to support this hypothesis, intriguing as it was at the time.

TGF-β type 1 has been suggested to play a role in chronic cyclosporine nephrotoxicity. TGF-β type 1 is a prosclerotic cytokine. Detectable levels have been found in the plasma of transplant recipients but not in healthy controls or patients with membranous nephropathy. There was no difference in levels among patients with differing renal function, time since transplantation, or cyclosporine trough levels.[75] In isolated human renal proximal tubular cells, increasing concentrations of cyclosporine caused an increase in the production by the tubular cells of TGF-β and platelet-derived growth factor, both fibrogenic cytokines.[159] In a study of renal biopsy specimens, TGF-β content correlated with cyclosporine toxicity, as opposed to acute tubular necrosis. TGF-β also was expressed in biopsy specimens from patients with acute rejection, however, with more expression in patients with more severe rejection.[258] Another study on renal biopsy specimens showed that nonrejected kidneys lacked TGF-β expression, whereas biopsy specimens from kidneys with acute rejection, chronic allograft nephropathy, or acute cyclosporine toxicity showed high levels of TGF-β expression.[254]

In rats receiving cyclosporine on a low-sodium diet, a model for cyclosporine toxicity that gives similar histological appearances to those of chronic cyclosporine toxicity in humans, mRNA expression of TGF-β was increased. Peripheral renin activity also was increased.[300] Human mesangial cells and renal fibroblasts in culture also produced more collagen III on exposure to cyclosporine.[113] Cyclosporine also has been shown to increase the expression of TGF-β and its receptors in mesangial cells and the production of fibronectin and plasminogen activator inhibitor by mesangial cells.[343]

Studies have shown that some of the changes seen in chronic cyclosporine nephrotoxicity can be prevented by the use of anti–TGF-β antibodies,[157,208] strengthening the argument for cyclosporine-induced increase in TGF-β having a role in the development of chronic cyclosporine nephrotoxicity. It is uncertain, however, what therapeutic benefit would result from reducing TGF-β because, as has been previously mentioned in this chapter (and in Chapter 2), TGF-β also may have a positive role to play in immunomodulation and preventing acute rejection.

As mentioned earlier in the rat model of chronic cyclosporine nephrotoxicity, renin is increased as well as TGF-β. There is evidence to support the fact that the renin-angiotensin system also may play a part in chronic cyclosporine nephrotoxicity. Cyclosporine has been shown to stimulate the renin-angiotensin system in many studies.[43,110,222] The increase in activation of the renin-angiotensin system has been linked with the morphological changes that occur in chronic cyclosporine nephrotoxicity by experimental studies in which angiotensin II receptor blockers have been shown to reduce these changes.[264,300,360]

The current mechanism by which cyclosporine stimulates the renin-angiotensin system is unknown. It is generally accepted that it increases renin release from the juxtaglomerular apparatus and that the mechanism is probably multifactorial.[199,205] Other mechanisms that are thought to be involved in chronic cyclosporine nephrotoxicity are the decrease in renal NO production caused by cyclosporine; the upregulation of osteopontin by cyclosporine, which is a chemotactic factor for macrophages resulting in macrophage infiltration and fibrosis; the induction of apoptosis of some renal cells by cyclosporine; and the activation of nuclear factor κB and activator protein 1 (AP-1), which are transcription factors thought to have a role in chronic nephrotoxicity.[57,205]

Hepatic Effects

Hepatotoxicity has been observed in patients receiving cyclosporine after renal, cardiac, and bone marrow transplantation (see Chapter 30).[136,185,253] Generally, this hepatotoxicity has not been more than a temporary elevation of liver enzymes on function tests that regressed on dosage reduction. These biochemical changes are uncommon with the lower doses of cyclosporine used today. No histological changes have been described in association with these biochemical changes, but high doses of cyclosporine in rats produce ultrastructural changes and a deterioration in liver function.[331] Cyclosporine may be contraindicated in patients with abnormal liver function tests before renal transplantation because there is a risk of the development of frank cirrhosis in such patients.[145] Because cyclosporine is metabolized in the liver, depressed liver function may alter blood levels of the drug, and careful attention must be paid to cyclosporine levels in such patients.

Neoplastic Effects

An apparent increased incidence of lymphomas in the early patients with a renal allograft receiving cyclosporine caused considerable alarm.[26,27,328] As time has passed, however, this increased incidence of lymphoma in renal and cardiac allograft recipients is no greater than that expected in recipients treated with azathioprine and steroid therapy. Most patients who developed lymphomas received other drugs as well, such as prednisolone and antilymphocyte agents, suggesting that the occurrence of lymphoma is due to excessive immunosuppression, rather than specifically to cyclosporine. The pathogenesis and incidence of lymphomas are described in detail in Chapter 33. Skin cancer, a major complication after transplantation in countries such as Australia with heavy sun exposure, appears just as commonly with cyclosporine immunosuppression, and this too is described in detail in Chapters 32 and 33. It has been observed that cyclosporine produces striking morphological changes in vitro, including increased cell motility, and in vivo, enhancing tumor growth in immunodeficient SCID-beige mice. These effects seem to be mediated by TGF-β. Cyclosporine potentially can promote tumor progression independent of its effect on the immune response.[147]

More recently, the protumor effects of cyclosporine have been linked to its promotion of tumor angiogenesis by a vascular endothelial growth factor–dependent mechanism.[128] It has been shown that cyclosporine can increase vascular endothelial growth factor.[301] Cyclosporine also may promote tumor growth independent of its effect on the immune system because it has been shown to increase IL-6 in Epstein-Barr virus–infected B cells, and IL-6 is capable of promoting B cell growth and possible progression to post-transplantation lymphoproliferative disorder.[323,345] The DNA repair capabilities of cells also may be disrupted by cyclosporine, with it affecting DNA repair in a dose-dependent fashion.[138]

In contrast, there is some evidence that cyclosporine has antitumor activity. It was previously suggested that cyclosporine may inhibit drug resistance in cancer cells,[334] and more recently it has been used in combination with cytotoxic drugs to reverse the tumor resistance to those drugs.[206]

Dermatological Effects

Dermatological problems, of which hypertrichosis is the most worrisome, are discussed in detail in Chapter 32. In children, facial dysmorphism may be striking. This feature is not evident in children receiving azathioprine and steroids.[77]

Gastrointestinal Effects

The development of a gelatin capsule for cyclosporine was welcomed by most patients. The capsules are large and difficult to swallow, however, and some patients prefer to take the liquid form. Cyclosporine in the liquid form is unpleasant to take and is not disguised adequately even when taken with flavored drinks, such as orange juice or chocolate milk. The unpalatability of cyclosporine causes nausea and anorexia in some patients, particularly with large doses, but in general this is less of a problem with current low doses.

Metabolic Effects

Hyperkalemia is common in patients taking cyclosporine[4,102] and is reversible with reduction of the dose or cessation of the drug.[62] The mechanism is unclear, but the decreased potassium excretion may be due to decreased serum aldosterone levels[4,20] or to a primary tubular defect.[20]

Renal handling of uric acid is affected by the use of cyclosporine, leading to higher serum urate levels in cyclosporine-treated patients after correction for elevated serum creatinine levels.[61] The high urate levels return to normal slowly over several weeks after discontinuing the drug and probably reflect a tubular defect associated with cyclosporine nephrotoxicity. Gout occasionally occurs as a by-product of the hyperuricemia, and urate levels may need to be reduced with allopurinol, remembering that the leukocyte count needs to be monitored carefully if the patient is taking azathioprine and cyclosporine.

Hypomagnesemia is due to an increased magnesium clearance in patients taking cyclosporine and usually is associated with high blood levels of the drug.[6,167] Hypomagnesemia reflects another manifestation of cyclosporine nephrotoxicity. Convulsions, which may be another manifestation of cyclosporine toxicity (as discussed in the next section and in Chapter 31), have been attributed to hypomagnesemia.

Glycosuria may occur in patients taking cyclosporine and is often associated with an increased blood glucose level. Glycosuria is a manifestation of nephrotoxicity,[60] but hyperglycemia may reflect a toxic effect of cyclosporine on beta cells of the islets of Langerhans. This condition seems to be reversible. There is evidence in rats that cyclosporine produces glucose intolerance, probably through the inhibition of insulin secretion.[358]

Neurological Effects

A variety of neurological complications have been reported with the use of cyclosporine, including tremor, convulsions, various paresthesias of the limbs, mania, and depression.[18,21,124,329,346] These complications are discussed in detail in Chapter 31. Although neurological syndromes are not always clearly caused by cyclosporine, there is sufficient evidence that such syndromes can be attributed to cyclosporine toxicity in many instances because they seem to be associated with high serum and blood levels. The syndrome is reversible with dosage reduction. These problems have become infrequent with current low doses of cyclosporine. Some evidence suggests that cyclosporine-induced hypomagnesemia may be the cause of these neurological complications, especially the convulsions, as already mentioned.

Cardiovascular Effects

Hypertension and hyperlipidemia are associated with the use of cyclosporine and are discussed in detail in Chapter 28. Cyclosporine seems to have complex effects on intravascular coagulation, and there have been reports of an increased incidence of renal artery and vein thrombosis[14,164,223,275] and an increase in the incidence of deep venous thrombosis,[336] which was not confirmed at Oxford (see Chapter 26).[7] Although it is tempting to attribute these complications, including microangiopathy and hemolytic-uremic syndrome, to the effect of cyclosporine on the arachidonic acid metabolic pathway as discussed earlier, the evidence is too uncertain to draw any firm conclusions. Raynaud's phenomenon seems to be another uncommon complication of cyclosporine therapy,[86] and one such case has occurred at Oxford.

Dental Effects

Gingival hypertrophy (see Fig. 32-3) is associated with poor dental hygiene and high doses of cyclosporine[293] and is discussed in detail in Chapter 32.

Hematological Effects

ABO autoimmune hemolytic anemia may occur after renal transplantation when a blood group O kidney is placed in a blood group A or B recipient. Several such cases have been reported,[25,216,242] although ABO autoimmune hemolytic anemia is more common after liver transplantation.[270] The occurrence of this complication, a form of graft-versus-host reaction, reflects the better immunosuppression achieved with cyclosporine.

Genotoxicity and Breast-Feeding

Experimental animal and human data so far indicate that cyclosporine is unlikely to be genotoxic.[248] In studies reported so far, there has been no increase in congenital anomalies or genetic disease. Monitoring should be continued, however, to increase the sample size.

Cyclosporine concentrations in breast milk were similar to concentrations in blood, but they were below detection limits in breast-fed infants. No change in creatinine levels of the infants occurred over 12 months of continued breast-feeding.[243]

Skeletal Effects

Although the major culprit for osteopenia after kidney transplantation is the use of steroids in immunosuppression protocols, it may be that cyclosporine also contributes to the loss of bone mass. Animal studies have shown that cyclosporine has adverse effects on bone and mineral metabolism, with a resulting loss in bone volume, although most clinical studies do not show these toxic effects of cyclosporine on bone, including studies in which cyclosporine is used without steroids.[135,311]

Antiviral Effects

Cyclosporine may possess anti–human immunodeficiency virus (HIV) and anti–hepatitis C virus (HCV) properties. It has been shown that cyclophilin A (the intracellular protein with which cyclosporine binds) is involved in the maturation and replication of HIV-1, and that by cyclosporine binding to cyclophilin A this process can be altered.[317] The use of cyclosporine in HIV-1-infected individuals has been shown to increase the CD4 count and to reverse HIV-associated lymphadenopathy.[11] The effect on CD4 T cells may be due to the ability of cyclosporine to prevent HIV-related activation–induced T cell apoptosis.[127] Cyclosporine also seems to slow the progression of HIV infection to AIDS. In a review of cases of transplant patients who contracted HIV either through their transplant or through blood transfusions at the time of transplant, the 5-year cumulative incidence of AIDS was 31% in patients who were taking cyclosporine compared with 90% ($P = .001$) in patients who were not taking cyclosporine.[292]

Cyclosporine also seems to have the ability to alter HCV viral replication through a mechanism similar to that of HIV. Cyclophilin B is a cellular replication cofactor of the HCV genome, and by binding cyclophilin B, cyclosporine shows anti-HCV properties.[349,350]

CONCLUSION

Cyclosporine, the first calcineurin inhibitor, represented a major advance in immunosuppression when it became available in the early 1980s, the first new immunosuppressive drug since the advent of azathioprine 2 decades earlier. The striking effect of cyclosporine was the reduction in the rate of acute irreversible rejection in the first 3 months after transplantation, with a resultant increase in 1-year graft survival by 15% to 20% compared with that achieved previously with azathioprine and steroids. However, side effects, mostly dose related, soon became evident, the most serious of which was nephrotoxicity. As a result, the decline in graft survival after 1 year was not altered, the major benefits being obtained in that first year—actually in the first few months—after transplantation. In recent years, considerable efforts have been directed at protocols that allow cyclosporine sparing or withdrawal to diminish the nephrotoxicity and other side effects. Nevertheless, calcineurin inhibitors have a major role in renal transplantation and are likely to remain in use for some time.

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Chapter 17

Tacrolimus in Renal Transplantation

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Tacrolimus is an immunosuppressive agent that has improved clinical outcomes in liver and kidney transplant recipients.[39] The phase III trials leading to Food and Drug Administration (FDA) approval of tacrolimus (in 1994) were conducted first in liver rather than in kidney transplant recipients, in contrast to other immunosuppressive agents. Subsequent clinical trials in kidney transplantation led to FDA approval for kidney transplantation in 1997. By 2003, 67% of all new kidney transplant recipients and 89% of all new liver transplant recipients were receiving tacrolimus as maintenance immunosuppressive therapy before discharge[116]; these percentages have continued to increase over time.

Tacrolimus (FK506, Prograf) was isolated in 1984 from the fermentation broth of *Streptomyces tsukubaensis*, a soil organism found at the foot of Mount Tsukuba near Tokyo. This compound was developed by researchers at the Chiba University of Japan. In the first clinical (rescue) trial, tacrolimus was administered to patients who were taking standard immunosuppressive therapy but who faced retransplantation because of ongoing organ rejection, or who had undesirable drug toxicities.[39] The initial clinical trial of tacrolimus as a primary immunosuppressive agent for the prophylaxis of rejection in liver transplant recipients began in the spring of 1990 at the University of Pittsburgh.[125] This work led eventually to multicenter randomized trials in liver and kidney transplantation.[86,99] Patients treated with tacrolimus had significantly fewer and less severe episodes of acute rejection than did patients given cyclosporine therapy. Tacrolimus also has shown efficacy as a rescue agent and as a primary maintenance immunosuppressive agent in heart, lung, pancreas, and small bowel transplantation[44,58,76,89,104,123] and was approved for heart transplantation in 2006.

MECHANISM OF ACTION

Tacrolimus inhibits T lymphocyte activation by binding to FK BP-12, an intracellular protein. A complex is then formed of tacrolimus–FK BP-12, calcium, calmodulin, and calcineurin, which inhibits the phosphatase activity of calcineurin. This complex prevents the dephosphorylation and subsequent translocation of the nuclear factor of activated T cells (NF-AT), a nuclear component that initiates gene transcription for the formation of interleukin-2 (Fig. 17-1). As a result, T lymphocyte activation is inhibited.[39] Tacrolimus is 10 to 100 times more potent than cyclosporine in its immunosuppressive effects.[100] Tacrolimus inhibits nitric oxide synthetase activation; it also inhibits apoptosis and potentiates the action of corticosteroids in the inhibition of apoptosis (see Chapter 16).[65,90]

PHARMACOKINETIC PROPERTIES

The pharmacokinetic characteristics of tacrolimus show high interindividual and intraindividual variability, and the drug has a narrow therapeutic index; therapeutic drug monitoring is necessary to optimize treatment. Because 90% of the drug is partitioned in the cellular components of blood, whole blood concentrations correlate better with drug exposure (area under the curve) than do plasma concentrations.[23]

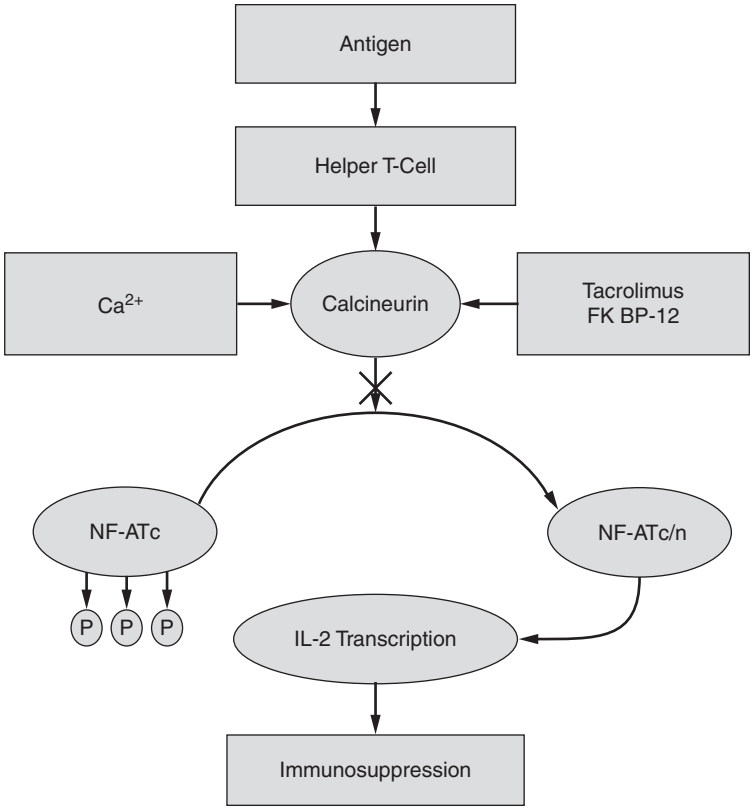

Figure 17–1 Mechanism of action of tacrolimus. A complex is formed of tacrolimus–FK BP-12, calcium, calmodulin, and calcineurin, which inhibits the phosphatase activity of calcineurin. This prevents the dephosphorylation and subsequent translocation of nuclear factor of activated T cells (NF-AT), a nuclear component that initiates gene transcription for the formation of IL-2. C, Cytoplasm; n, nucleus, P, phosphate. (From Fung JJ: Tacrolimus and transplantation: a decade in review. Transplantation 77:S41, 2004.)

Therapeutic drug monitoring of tacrolimus can be achieved using whole-blood trough concentrations to individualize dose requirements and reduce drug-related toxicity.[12]

ABSORPTION AND DISTRIBUTION

Tacrolimus is rapidly, but incompletely, absorbed in the gastrointestinal tract, and peak tacrolimus concentrations in whole blood are attained 1 to 2 hours after oral administration.[131] Tacrolimus has low oral bioavailability (average 25%; range 4% to 93%).[131] The mean oral bioavailability of tacrolimus is comparable in adult (25%) and pediatric (31%) transplant recipients. The rate and extent of absorption of tacrolimus is reduced in the presence of food, with the peak concentration in whole blood compared with the fasting state decreased by approximately 50% to 75%, and the area under the curve decreased by 25% to 40% when the drug is taken after a meal.[100] Tacrolimus is highly bound to erythrocytes, in a concentration-dependent manner, with reduced ratios at higher drug concentrations related to binding saturation. Plasma protein binding may be 99%, with most of the drug bound to α_1-acid glycoprotein and albumin. Tacrolimus is widely distributed in most tissues, including the lungs, spleen, heart, kidney, pancreas, brain, muscle, and liver; tacrolimus crosses the placenta, with umbilical cord plasma concentrations one third of those in maternal plasma.[100,131] Tacrolimus also is present in breast milk, but at extremely low levels (<2.5 ng/mL).

METABOLISM AND ELIMINATION

Tacrolimus is metabolized extensively in the liver and, to a much lesser extent, in the intestinal mucosa, with metabolism mediated at both sites by cytochrome P-450 (CYP) 3A4 isoenzymes.[100,131] Tacrolimus is converted by hydroxylation and demethylation to at least 15 metabolites, with the main metabolite being 13-*O*-dimethyl-tacrolimus. The mean clearance after intravenous administration of tacrolimus is as follows: 0.040 L/hr/kg in healthy volunteers, 0.083 L/hr/kg in adult kidney transplant patients, 0.053 L/hr/kg in adult liver transplant patients, and 0.051 L/hr/kg in adult heart transplant patients. When administered orally, fecal elimination accounts for 92.6 ± 3.07% and urinary elimination accounts for 2.3 ± 1.1% of the administered dose in healthy volunteers.[5]

The main drugs that interact with tacrolimus when administered simultaneously are either inducers or inhibitors of CYP3A4. Although CYP3A4 inhibitors potentially increase whole-blood tacrolimus concentrations, CYP3A4 inducers decrease tacrolimus concentrations (Table 17-1; see Chapter 16).

SPECIAL PATIENT POPULATIONS

Three percent of patients require higher dosages (>0.4 mg/kg/day) to reach therapeutic tacrolimus concentrations; this is a reflection of the low bioavailability and, to a lesser extent, the high clearance of the drug.[131] In a nonblinded, parallel-group study, the bioavailability of tacrolimus was significantly (P=.01) lower in African-American (11.9%) and Latin-American (14.4%) patients than in white patients (18.8%).[82] A retrospective study in renal transplant recipients showed that African-American recipients required higher dosages of tacrolimus on a milligram-per-kilogram basis.[131]

Children typically require higher tacrolimus dosages on a milligram-per-kilogram basis than adult patients, most

Table 17–1 **Drug Interactions Associated with Tacrolimus**

Drugs Increasing Tacrolimus Concentration (Cytochrome P-450 3A4 Inhibitors)
Calcium channel blockers—diltiazem, nicardipine, nifedipine, verapamil
Imidazole antifungal agents—clotrimazole, fluconazole, itraconazole, ketoconazole
Macrolide antibiotics—clarithromycin, erythromycin
Prokinetic agents—cisapride, metoclopramide
Other drugs—bromocriptine, cimetidine, corticosteroids, danazol, protease inhibitors
Grapefruit juice
Drugs Decreasing Tacrolimus Concentration (Cytochrome P-450 3A4 Inducers)
Anticonvulsants—carbamazepine, phenobarbital, phenytoin
Rifabutin/rifampicin, isoniazid
St. John's wort

likely reflecting the higher mean total body clearance and volume of distribution in children. Clinically relevant differences do not exist between adults and children, however, in terms of the time taken to reach maximal blood concentrations (2.1 hours in children versus 2 hours in adults), bioavailability (31% versus 25%), and mean terminal elimination half-life (11.5 hours versus 12 hours).[108] The mean clearance of tacrolimus in patients with renal dysfunction was similar to that in normal volunteers; tacrolimus pharmacokinetics after a single intravenous administration was similar in seven patients not receiving dialysis and five receiving dialysis.[5]

The mean clearance of tacrolimus in patients with mild hepatic dysfunction (mean Pugh score of 6.2) was not substantially different from that in normal volunteers after a single intravenous and oral dose. The mean clearance was substantially lower in patients with severe hepatic dysfunction (mean Pugh score >10), regardless of the route of administration.[5]

CLINICAL STUDIES IN KIDNEY TRANSPLANTATION

Rescue Therapy in Adults

The efficacy of tacrolimus in kidney transplantation was first shown in recipients with refractory rejection. Tacrolimus showed remarkable efficacy in the therapy of refractory rejection[74]; the first article on tacrolimus rescue was by Jordan and colleagues,[64] and the first multicenter trial was reported by Woodle and coworkers.[147] Refractory rejection episodes in cyclosporine-treated patients could be reversed by replacing cyclosporine with tacrolimus as the maintenance immunosuppressive agent. In contrast to antilymphocyte antibody preparations (e.g., OKT3 and polyclonal antibody preparations) that induce long-term suppression of T cell responses, the immunosuppressive effects of tacrolimus could be titrated on a daily basis by following drug levels.[74]

An early large experience with tacrolimus in treating refractory acute renal allograft rejection in 77 patients receiving cyclosporine-based immunosuppressive therapy was reported from Pittsburgh.[64] Several conclusions were drawn from this study, as follows: (1) Tacrolimus provided effective therapy for acute renal allograft rejection, (2) tacrolimus often provided effective therapy for vascular rejection in kidney transplants, and (3) the success of tacrolimus therapy for refractory acute renal allograft rejection was related to the severity and duration of rejection.

The 5-year follow-up of the Pittsburgh experience showed good long-term renal allograft function in patients undergoing tacrolimus rescue therapy.[62,63] A total of 169 patients were converted from cyclosporine to tacrolimus for refractory rejection, with a 74% success rate and a mean serum creatinine value of 2.3 ± 1.1 mg/dL (202 μmol/L). Of the patients receiving dialysis at the time of tacrolimus initiation, 46% were salvaged, with a mean serum creatinine level of 2.2 ± 0.4 mg/dL (189 μmol/L). Corticosteroid withdrawal was achieved in 22% of patients after conversion to tacrolimus, and the mean prednisone dose was reduced from 28 ± 1.1 mg/day to 8.5 ± 4.1 mg/day.

A prospective, randomized, multicenter comparative trial has confirmed the efficacy of tacrolimus-based rescue therapy in patients with acute renal transplant rejection.[31] Rescue therapy with tacrolimus-based regimens reduced the incidence of recurrent acute rejection to 8.8% versus 34.1% ($P = .002$) in patients who remained on cyclosporine-based immunosuppression. Three-month Kaplan-Meier estimates for freedom from a second biopsy-proven acute rejection were 89.1% versus 61.4% ($P = .002$) in the tacrolimus-rescue and the cyclosporine-continuation groups, respectively. Freedom from treatment failure was 72.6% versus 43% ($P = .005$), with treatment failure being defined as graft loss, second acute rejection, or withdrawal from treatment.

In a large European study on tacrolimus conversion for cyclosporine-induced toxicities, 73% of patients with cyclosporine-induced gingival hyperplasia ($n = 32$) showed significant resolution of hyperplasia, and recipients with cyclosporine-induced hypertrichosis ($n = 116$) showed marked improvement. The mean serum low-density lipoprotein (LDL) level decreased from 138 mg/dL to 120 mg/dL, and the high-density lipoprotein levels remained unchanged in patients with cyclosporine-induced hyperlipidemia ($n = 78$). Finally, hypertension had markedly or completely resolved in 25% of patients ($n = 75$).[101]

Antibody-Mediated Rejection

Antibody-mediated rejection often occurs within the first 2 weeks after transplantation and is associated with oliguria, graft tenderness, fever, leukocytosis, and circulating antidonor antibodies. Before the introduction of tacrolimus, combinations of bolus corticosteroids, plasmapheresis, and antilymphocyte antibody preparations were used to treat acute humoral rejection, with inconsistent and unsatisfactory response rates. Tacrolimus-based regimens were developed for acute humoral rejection in renal transplant recipients, based on clinical experiences with tacrolimus in treating liver and heart transplants with acute humoral rejection.[98,144,145] Experimental evidence also supported the potential of tacrolimus in limiting antibody responses.[137,138]

Tacrolimus-based regimens for treating acute humoral rejection are based on the removal of circulating antibody at the time of the rejection episode (by plasmapheresis), suppressing the formation of new antidonor antibody

with high-dose tacrolimus, and monitoring kidney allograft histology with protocol biopsies. Tacrolimus-based regimens were shown to reverse antibody-mediated rejection in renal allograft recipients.[143,146] In one series, all four patients had aggressive rejection episodes confirmed by immunohistopathology. These rejections were treated successfully with daily plasmapheresis for 5 days and high-dose tacrolimus (initial target levels 20 to 25 ng/mL) that resulted in reversal of rejection and allowed long-term graft survival. This regimen was not associated with life-threatening opportunistic infections or post-transplant diabetes mellitus (PTDM), despite high tacrolimus trough levels. The efficacy of tacrolimus in acute humoral rejection preceded the use of plasmapheresis and intravenous immunoglobulin regimens in the management of humoral rejection and highly sensitized patients (see Chapter 22).[143,146,148]

Maintenance Immunosuppression

The outcomes of kidney transplantation have improved with the advent of powerful immunosuppressive agents such as tacrolimus and the use of tacrolimus as part of highly effective immunosuppressive regimens. Several studies have addressed short-term outcomes of immunosuppression, including rates of acute rejection and patient and graft survival. Studies also have addressed medium-term outcomes with tacrolimus-based immunosuppression, including 5-year patient and graft survival, renal function, cardiovascular events, and post-transplant diabetes mellitus.

Comparison of Tacrolimus-Based and Cyclosporine-Based Regimens

The phase III U.S. multicenter clinical trial compared the efficacy and safety of tacrolimus with that of the original formulation of cyclosporine.[99] At 1 year post-transplantation, 30.7% of tacrolimus-treated patients had experienced acute rejection compared with 46.4% of cyclosporine-treated patients ($P = .001$). The incidence of moderate-to-severe rejection was 10.8% in the tacrolimus-treated group compared with 26.5% in the cyclosporine-treated group. Intent-to-treat analysis revealed that the 1-year patient survival was 95.6% and 96.6% for the tacrolimus-treated and cyclosporine-treated patients, respectively (P = nonsignificant [NS]). The 1-year graft survival rate was 91.2% and 87.9% for the tacrolimus-treated and cyclosporine-treated patients, respectively (P = NS). The intent-to-treat analysis showed no significant differences in 5-year patient or graft survival between the tacrolimus-treated and the cyclosporine-treated patients. When crossover because of rejection was counted as graft failure, a statistically significant increase in graft survival was found in the tacrolimus group at 5 years (63.8% versus 53.8%; $P = .014$).[132] There also was a significant difference in the serum creatinine level between the tacrolimus-treated and cyclosporine-treated patients and in the number of patients who had a serum creatinine value greater than 1.5 mg/dL (tacrolimus 40.4% versus cyclosporine 62%; $P = .0017$). The patients treated with tacrolimus had a lower incidence of hirsutism and gingival hyperplasia, but a higher incidence of alopecia than patients treated with cyclosporine.

Racial differences also were evaluated for acute rejection in the U.S. phase III multicenter clinical trial.[96] Among African-Americans, 23.2% of patients in the tacrolimus-treated group developed acute rejection compared with 47.9% of patients in the cyclosporine-treated group ($P = .012$). When crossover because of rejection was counted as graft failure, there was a significant increase in the 5-year graft survival in African-American patients in the tacrolimus-treated group (65.4% versus 42.6%; $P = .013$) compared with the cyclosporine-treated group.[133]

The U.S. multicenter study that compared the efficacy and tolerability of tacrolimus versus cyclosporine also revealed that significantly fewer kidney transplant recipients required antihypertensive treatment in the tacrolimus-treated group compared with the cyclosporine-treated group.[59] In this 3-year multicenter comparative study, tacrolimus was associated with a significantly lower incidence of hypercholesterolemia than was cyclosporine (24% versus 38%; $P = .007$), and the need for lipid-lowering agents was significantly lower in the tacrolimus-treated patients (14% versus 38%; $P < .001$).[59] The projected graft half-life evaluated by the European Multicenter Renal Transplant Study also favored tacrolimus over cyclosporine (15.8 years versus 10.8 years).[85]

All adult kidney transplants from 1995 to 2000 reported to United Network for Organ Sharing/Organ Procurement and Transplantation Network were analyzed by discharge immunosuppression.[13] The 1-, 3-, and 5-year adjusted actuarial graft survival rates with the tacrolimus-based regimens were 91.8%, 81.1%, and 69.8%, and for the cyclosporine-based regimens, these rates were 90.3%, 79.9%, and 67.5% ($P < .0001$).

A single-center report studied the effects of immunosuppression on African-American recipients undergoing kidney transplantation between 1995 and 2001.[37] The 1-year and 5-year graft survival rates for African-Americans were 89% and 79% with tacrolimus-based therapy and 85% and 60% with cyclosporine-based therapy ($P = .006$).

Cadaver donors reported to the Scientific Registry of Transplant Recipients Database between 1995 and 2002 were included in a study analyzing paired kidneys in which one kidney was allocated to a patient who was treated with cyclosporine microemulsion and the other kidney was allocated to a patient receiving tacrolimus therapy.[67] There was no difference in 5-year patient or graft survival. Renal function was superior in the tacrolimus-treated group at all time points. The 6-month inverse creatinine levels were significantly worse in the microemulsion cyclosporine arm compared with the tacrolimus arm ($P < .0001$).

In normal, healthy subjects, treatment with cyclosporine increased baseline creatinine level and blood pressure and reduced renal plasma flow and glomerular filtration rate in otherwise normal kidneys. In contrast, treating normal human subjects with tacrolimus did not influence renal hemodynamic parameters, and the mean arterial blood pressure remained unchanged.[72]

A multicenter trial evaluated the effect of tacrolimus as secondary intervention in patients being treated with cyclosporine for 3 or more months after transplantation who had one of the following risk factors for chronic renal allograft failure: serum creatinine 2 mg/dL or greater for men and 1.7 mg/dL or greater for women, or a greater than 30% increase in the nadir post-transplant serum creatinine level.

The trial randomly assigned 197 patients to convert to tacrolimus or remain on cyclosporine.[136] At 24 months, 56.8% of the patients in the tacrolimus-treated group and 87.5% in the cyclosporine-treated group had a serum creatinine level 2 mg/dL or greater ($P = .002$). Significantly fewer patients who were converted from cyclosporine to tacrolimus experienced a cardiovascular event compared with patients who continued treatment with cyclosporine (5.6% versus 24.3%; $P = .002$). Median serum cholesterol and LDL cholesterol levels were significantly lower in the tacrolimus-treated group compared with the cyclosporine-treated group. Therapeutic intervention with tacrolimus resulted in improved renal function, better lipid profiles, and fewer cardiovascular events in patients who were at risk for developing chronic renal allograft failure.[136]

Patients who have an acute rejection episode and hypercholesterolemia have a more than twofold greater risk of graft loss.[133] These combined risk factors were significantly different between treatment arms (tacrolimus 4.7% versus cyclosporine 17.4%; $P = .0008$). In another study, tacrolimus therapy was associated with a significantly reduced requirement for medications to control hypertension and hyperlipidemia. A 6-month study of 560 patients in the European Tacrolimus versus Cyclosporine Microemulsion Renal Transplantation Study Group showed that patients treated with cyclosporine had significantly higher rates of hypertension (23.2% versus 15.7%; $P = .032$) and hypercholesterolemia (8.9% versus 4.2%; $P = .037$) compared with the tacrolimus-treated group.[84]

Blood pressure and lipid profiles were measured in stable renal transplant recipients during initial treatment with cyclosporine and again after 4 weeks of treatment with tacrolimus.[81] Antihypertensive drugs were stopped at least 3 weeks before the study. After patients were switched to tacrolimus, the mean daytime blood pressure decreased from 149 ± 12 mm Hg/95 ± 8 mm Hg to 138 ± 13 mm Hg/87 ± 9 mm Hg ($P < .001$). Total and LDL cholesterol levels also decreased from 6.1 ± 0.7 mmol/L and 3.84 ± 0.79 mmol/L to 5.1 ± 0.8 mmol/L and 2.98 ± 0.75 mmol/L ($P < .001$).[81]

A randomized, prospective study was done to compare the clinical and economic outcomes of tacrolimus versus cyclosporine in a regimen consisting of antithymocyte globulin (ATG) (Thymoglobulin) induction, an antimetabolite, and prednisone.[51] At 1 year, acute rejection, patient survival, graft survival, and the rate of cytomegalovirus infection were similar. Creatinine levels were lower in the tacrolimus-treated group compared with the cyclosporine-treated group. The requirement for dyslipidemia treatment was statistically similar at 12 months after transplant (30% tacrolimus versus 35% cyclosporine). Total 12-month medication costs were similar ($17,723 ± $11,647 tacrolimus versus $16,515 ± $10,189 cyclosporine).[51]

A clinical study conducted in the early 1990s that compared treatment with cyclosporine versus tacrolimus found that significantly more patients who received tacrolimus-based immunosuppression developed PTDM.[132] A more recent study that compared treatment with tacrolimus versus cyclosporine found a similar incidence of PTDM for both regimens, however.[128] The decrease in insulin secretion caused by treatment with tacrolimus was dose-dependent and reversible. PTDM was reversible when tacrolimus blood levels were reduced (see Chapter 16).

Comparison of Tacrolimus/Azathioprine and Tacrolimus/Mycophenolate Mofetil Regimens

A randomized, prospective three-arm study compared the impact of immunosuppressive protocols using tacrolimus/azathioprine ($n = 76$), cyclosporine microemulsion/mycophenolate mofetil (MMF) ($n = 75$), and tacrolimus/MMF ($n = 72$).[60] At 1 year, although there were no significant differences in overall rejection rates, there were significant differences in the total number of patients who required antilymphocyte antibody treatment (4.2% in the tacrolimus/MMF arm compared with 10.7% in the cyclosporine/MMF arm and 11.8% in the tacrolimus/azathioprine arm; $P = .05$). There were no significant differences among the three groups in patient or graft survival at 1, 2, and 3 years.[2,41,60] In patients with delayed graft function, there was a trend toward improved graft survival in the tacrolimus-based treatment group at 1 year. This trend became significant when the tacrolimus/MMF arm was compared with the cyclosporine/MMF arm at 2 and 3 years. At 3 years, the serum creatinine level was significantly lower in the tacrolimus-treated patients than in the cyclosporine-treated patients.[41]

Comparison of Tacrolimus/Mycophenolate Mofetil and Tacrolimus/Sirolimus Regimens

Long-term post-transplant renal function is influenced by the incidence of acute rejection episodes, chronic allograft nephropathy, age of the kidney donor, and the use of calcineurin inhibitors.[132] Analysis of registry data examining the rate of change of creatinine clearance for patients who received kidney transplants between 1990 and 2000 showed that renal function improved in transplants performed after 1997. A more stable creatinine clearance was associated with tacrolimus versus cyclosporine therapy and with MMF versus azathioprine therapy.[43]

A randomized study comparing the combination of sirolimus or MMF with tacrolimus-based immunosuppression showed no significant differences in the incidence of biopsy-confirmed acute rejection (13% tacrolimus/sirolimus [$n = 185$] versus 11.4% tacrolimus/MMF [$n = 176$]; $P = .64$).[42] Graft survival and patient survival were not significantly different between the groups at 6 months after transplantation. Significantly more recipients discontinued treatment with sirolimus (21.1% versus 10.8%; $P = .0008$). Renal function was significantly better in the tacrolimus/MMF group (serum creatinine 1.44 ± 0.45 mg/dL versus 1.77 ± 1.42 mg/dL; $P = .018$). The combination of tacrolimus and MMF was superior to tacrolimus and sirolimus in terms of improved renal function and a lower risk of hypertension and hyperlipidemia.[42]

The incidence of acute rejection was significantly higher in the cyclosporine/sirolimus arm (21% versus 4% for tacrolimus/sirolimus and 4% for tacrolimus/MMF; $P = .013$) in a randomized trial comparing these three regimens in renal transplantation.[91] At 12 months after transplantation, the mean serum creatinine level was 1.48 mg/dL in the tacrolimus/sirolimus treatment group, 1.29 mg/dL in the tacrolimus/MMF treatment group, and 1.69 mg/dL in the cyclosporine/sirolimus treatment group ($P < .05$).

Analysis at 3 years showed similar patient and graft survival rates among the three groups. There was a trend toward better graft function, fewer endocrine disorders, and fewer acute rejection episodes in the tacrolimus/MMF group versus the tacrolimus/sirolimus or cyclosporine/sirolimus groups.[25]

Several publications have reported on the comparison of the efficacy of tacrolimus and MMF with that of tacrolimus and sirolimus. The course of 97 kidney transplant patients treated with sirolimus and reduced-dose tacrolimus was reviewed. The outcomes of 19 patients who were converted to a tacrolimus/MMF protocol for various nonrenal side effects were compared with 78 patients who remained on a tacrolimus/sirolimus protocol. Tacrolimus levels were increased in patients who were converted. Conversions from tacrolimus/sirolimus to tacrolimus/MMF led to improved renal function, however, despite increased tacrolimus exposure after conversion.[6]

A prospective study compared the safety and efficacy of steroid avoidance in tacrolimus/MMF ($n = 75$) and tacrolimus/sirolimus ($n = 75$) in kidney transplantation. The primary end point was acute rejection. Surveillance biopsies were done to analyze subclinical acute rejection and chronic allograft nephropathy. Clinical acute rejection and subclinical acute rejection were treated with methylprednisolone.[75] Two-year patient and graft survival, renal function, and adverse effects were monitored. Steroid avoidance under tacrolimus-based immunosuppression with MMF or sirolimus provided equivalent 2-year patient and graft survival, with a low incidence of acute rejection and new-onset diabetes mellitus. Subclinical acute rejection and chronic allograft nephropathy were lower in the tacrolimus/sirolimus group than in the tacrolimus/MMF group.

These optimistic findings were countered by an analysis of 44,915 adult renal transplants in the Scientific Renal Transplant Registry from 2000 to 2004. A total of 3524 (7.8%) patients received a baseline immunosuppressive regimen of tacrolimus/sirolimus, with an inferior overall survival ($P < .001$) and death-censored graft survival ($P < .001$) compared with tacrolimus/MMF ($n = 27{,}007$). In multivariate Cox models, the adjusted hazard ratio for overall graft loss with tacrolimus/sirolimus was 1.47 and with cyclosporine/sirolimus was 1.38 relative to tacrolimus/MMF. These effects were most apparent in high-risk transplants. Six-month acute rejection rates were low and did not differ among groups.[88] These data have to be interpreted in the context of the limitations of any retrospective database analysis.

The efficacy of combining tacrolimus and two different dosages of sirolimus was compared with a tacrolimus/MMF regimen.[135] In addition to tacrolimus, 325 patients received 2 mg/day of sirolimus (tacrolimus-sirolimus 2 mg), 325 patients received 0.5 mg/day of sirolimus (tacrolimus-sirolimus 0.5 mg), and 327 patients received 1 g/day of MMF (tacrolimus-MMF). Steroid dosing was identical in all groups. The incidence of biopsy-proven acute rejection was lower in the tacrolimus-sirolimus 2 mg group compared with the tacrolimus-sirolimus 0.5 mg and tacrolimus-MMF groups. Graft and patient survival were similar among the three groups. Combining 2 mg/day of sirolimus with tacrolimus resulted in reduced rates of acute rejection, but a greater incidence of adverse events, including hyperlipidemia, hypertension, lymphoceles, and new-onset diabetes mellitus.

Comparison of Tacrolimus-Based Dual versus Triple Immunosuppression Therapy

Dual immunosuppression therapy refers to the use of tacrolimus with a second agent, such as a corticosteroid. Triple immunosuppression therapy refers to the use of tacrolimus and a corticosteroid with a third agent, such as azathioprine or MMF.

Dual therapy with tacrolimus-based immunosuppression provided similar efficacy to tacrolimus-based triple therapy for 36 months.[17,22,40,97,109,119] At 12 months, patient survival rates in the dual-therapy groups were ≥96% compared with ≥94% with triple therapy, with graft survival rates of ≥90% (dual-therapy groups) and ≥91% (triple-therapy groups). Three-year follow-up data are available from the Italian and Spanish trial, and graft survival was 87% in dual-therapy and triple-therapy groups. A similar percentage of patients experienced an acute rejection episode with dual-therapy or triple-therapy tacrolimus-based immunosuppressive regimens. Most of these episodes occurred in the first year after transplantation, with a 10-fold to 15-fold reduction in the incidence of rejection over the next 2 years.[40,97] In one study, the addition of MMF to tacrolimus plus corticosteroid therapy significantly ($P = .007$) reduced the incidence of rejection at 9 months.[119]

A prospective, randomized trial was performed to compare FK506/prednisone with FK506/azathioprine/prednisone from August 1, 1991, to October 11, 1992. With a mean follow-up of 9 ± 4 months, the 1-year actuarial patient survival in the two-drug group was 95%, and for the three-drug group it was 91% (P = NS). One-year actuarial graft survival in the two-drug group was 90%, whereas in the three-drug group it was 82% (P = NS).[113] In another prospective, randomized trial reported from the same center, the combination of tacrolimus and prednisone was compared with tacrolimus, MMF, and prednisone in renal transplant recipients.[114] The combination of tacrolimus, steroids, and MMF was associated with excellent patient and graft survival and a lower incidence of rejection than occurred with the combination of tacrolimus and steroids.

Role of Tacrolimus and Corticosteroids in the Development of Hypertension and Hyperglycemia

Steroid dosing may play an important part in the development of complications after transplantation (see Chapter 15). In one study, patients were evaluated 4 months after kidney transplantation; twice as many patients treated with tacrolimus and high-dose prednisone developed hypertension compared with patients treated with tacrolimus and low-dose prednisone (63% versus 32%; $P < .05$).[30]

Corticosteroids may promote the development of PTDM by inducing insulin resistance, decreasing insulin receptor number and affinity, impairing endogenous glucose production, and impairing glucose uptake by muscle.[107] Reducing or withdrawing corticosteroids reduces hyperglycemia and can reduce the incidence of PTDM; however, it also can increase the risk of acute rejection.[55]

A study was done to assess the relative role of tacrolimus and corticosteroids in the development of glucose metabolic disorders.[10] Corticosteroid withdrawal in patients receiving tacrolimus-based immunosuppression led to a 22% decrease

in fasting C-peptide levels ($P = .0009$). Fasting insulin levels and the insulin-to-glucose ratio decreased (P = NS). Steroid withdrawal also led to a reduction in lipid levels. Tacrolimus trough level reduction from 9.5 ng/mL to 6.4 ng/mL resulted in a 36% increase in pancreatic beta cell secretion ($P = .04$), and insulin secretion increased by a similar rate. Hemoglobin A_{1c} improved from 5.9% to 5.3% ($P = .002$), although lipid levels did not change after trough level reduction.[10] Corticosteroid withdrawal resulted in a decrease in insulin resistance and a reduction in lipid levels; reduction of tacrolimus trough levels also improved glucose metabolism.

Early Corticosteroid Withdrawal Regimens

The safety of early corticosteroid withdrawal (see Chapter 15) was evaluated by a prospective, randomized, multicenter, double-blind study of early (7 days post-transplantation) corticosteroid cessation versus long-term maintenance of corticosteroids along with tacrolimus, MMF, and antibody induction in primary renal transplant patients.[142] Patient and graft survivals at 1 year were 98% and 96%, respectively. Biopsy-proven acute rejection occurred in 9.8% of patients, and 4% were treated empirically for rejection. Interim analysis suggested that early withdrawal of corticosteroids was safe, resulting in excellent patient and graft survival, low acute rejection rates, and no graft loss to rejection.[142]

A prospective, randomized study was done to determine the ideal long-term maintenance immunosuppressive regimen after discontinuation of prednisone on day 5.[66] Patients were randomly assigned to receive cyclosporine/MMF ($n = 85$), low-dose sirolimus/high-dose tacrolimus ($n = 72$), or high-dose sirolimus/low-dose tacrolimus ($n = 82$). No significant differences in patient or graft survival, acute rejection, or serum creatinine were noted; four patients developed PTDM (all in the tacrolimus-sirolimus groups). The incidence of wound complications was greater in the tacrolimus-sirolimus arms ($P = .02$), but the incidence decreased when the sirolimus loading dose was stopped.

A randomized, prospective trial of early steroid withdrawal versus low-dose steroids was performed in renal transplant recipients.[77] Serial protocol biopsies were done to assess efficacy and safety. Sixty patients were randomly assigned into two groups: Control patients ($n = 28$) received low doses of prednisone throughout, and study patients ($n = 32$) were withdrawn from steroids 7 days post-transplant. Immunosuppression consisted of rabbit ATG induction therapy, tacrolimus and MMF. Protocol biopsies were performed at 1, 6, and 12 months. Renal function was well maintained and was equivalent in both groups. The immunosuppressive combination of rabbit ATG, tacrolimus, and MMF prevented subclinical rejection and the need for high doses of steroids after transplantation. Serial protocol biopsy specimens showed increased allograft fibrosis over time in both groups, however, which was significant at 1 year in the steroid-withdrawal group.

In another study, 101 patients underwent renal transplantation with tacrolimus, MMF, and 7 days of corticosteroids.[11] Anti-CD25 monoclonal antibody was administered to 25 patients at higher immunological risk. After a median follow-up of 51 months (range 36 to 62 months), patient survival was 97%, and graft survival was 91%. The incidence of acute rejection at 12 months was 19%. Only three further episodes of rejection occurred beyond 12 months. Graft function was stable during the study, with a mean estimated creatinine clearance of 57 mL/min at the end of follow-up. This steroid avoidance regimen was associated with excellent medium-term patient and graft outcomes and a low incidence of side effects.

Corticosteroid-Free Immunosuppression Regimens

A 6-month, open-label, multicenter, parallel-group study included 538 renal patients randomly assigned (1:1) to a daclizumab/tacrolimus/MMF regimen ($n = 260$) or a tacrolimus/MMF/corticosteroid regimen ($n = 278$).[104] The incidence of biopsy-proven acute rejection was 16.5% in both treatment groups; the incidence of biopsy-proven corticosteroid-resistant acute rejection was 4.3% and 5% in the tacrolimus/MMF/corticosteroids and daclizumab/tacrolimus/MMF groups (P = NS). The median serum creatinine level at 6 months and overall safety profile were similar with both regimens. Compared with the tacrolimus/MMF/steroid regimen, a significantly lower incidence of new-onset insulin-dependent diabetes mellitus (5.4% versus 0.4%; $P = .003$) was found with the steroid-free regimen. Mean total cholesterol concentrations increased from baseline in the tacrolimus/MMF/corticosteroids group by 0.19 mmol/L; in the daclizumab/tacrolimus/MMF group, cholesterol concentrations decreased by 0.19 mmol/L.[104]

A single-center, nonrandomized, retrospective sequential study was used to evaluate outcomes in kidney transplant recipients given either alemtuzumab (Campath) ($n = 123$) or basiliximab ($n = 155$) in combination with a prednisone-free maintenance protocol using tacrolimus and MMF.[69] There was no significant difference in the 3-year graft and patient survival rates between the two groups. A lower rate of early (<3 months) rejection was observed in the alemtuzumab (4.1%) versus the basiliximab (11.6%) group, but rejection rates for both groups were equivalent at 1 year. Patient and graft survival and rejection rates were nearly identical between whites and African-Americans receiving alemtuzumab. The quality of renal function and the incidence of infectious complications were similar between the alemtuzumab and basiliximab groups.

Recipient pretreatment by lymphoid depletion using ATG or alemtuzumab combined with minimal post-transplant immunosuppression was used as an innovative approach to the management of kidney transplant recipients.[110] This treatment algorithm was derived from the notion that rejection and tolerance are stages of the same continuum.[120] The usually dominant host-versus-graft response can be reduced to a more easily deletable range by pretreatment with polyclonal ATG or the humanized monoclonal antibody, alemtuzumab. The aim of minimal post-transplant immunosuppression is to reduce further the clonal response with enough treatment to prevent irreversible immune damage to the graft, but not such heavy treatment that donor-specific clonal exhaustion-deletion is precluded (Fig. 17-2).[110]

Based on the aforementioned principles, 150 unselected renal transplant recipients with a mean age of 51 ± 15 years were pretreated with 5 mg/kg of rabbit ATG in the hours before transplantation, with two boluses of intravenous methylprednisolone to prevent cytokine reactions.[112] Minimal post-transplant immunosuppression was with

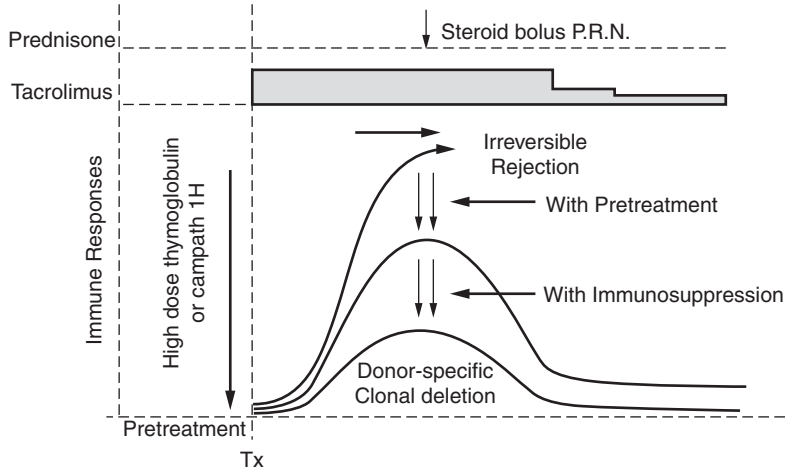

Figure 17–2 Mechanism of tolerogenic immunosuppression. Conversion of rejection (*arrows on the curve "irreversible rejection"*) to an immune response that can be exhausted and deleted by combination of pretreatment with high-dose antithymocyte globulin (Thymoglobulin) or alemtuzumab (Campath) and minimalistic post-transplant immunosuppression with tacrolimus. (From Starzl TE, Murase N, Abu-Elmagd K, et al: Tolerogenic immunosuppression for organ transplantation. Lancet 361:1502-1510, 2003.)

tacrolimus monotherapy to which steroids or other agents were added only for the treatment of rejection. Four months after transplantation, patients were consolidated to once-daily tacrolimus monotherapy; 2 or more months later, spaced weaning was carried out in stable patients. One-year patient and graft survivals were 97% and 92%, respectively. The incidence of early acute rejection was 37%; however, only 7% required prolonged treatment with agents other than tacrolimus. With a follow-up of 6 to 21 months, 94 (63%) of the 150 patients were receiving spaced doses of tacrolimus ranging from every other day to once a week.

The results in ATG-pretreated patients (n = 101) or alemtuzumab-pretreated patients (n = 90) were compared with the results in 152 conventionally immunosuppressed recipients in the immediately preceding era.[110] Spaced weaning was attempted in more than 90% of the kidney transplant recipients after pretreatment with either lymphoid-depleting agent. Although there was a much higher rate of acute rejection in the ATG-pretreated recipients than in the alemtuzumab-pretreated recipients, patient and graft survivals in both lymphoid depletion groups were at least equivalent to the survivals of historical control patients. Kidney transplantation after lymphoid depletion was readily accomplished under minimal immunosuppression, with less dependence on late maintenance immunosuppression, fewer viral complications, and less post-transplant diabetes. Alemtuzumab was the more effective agent for pretreatment.[110]

Two corticosteroid-free, tacrolimus-based regimens were compared with standard triple therapy in a 6-month, phase III, open-label, parallel-group multicenter study.[134] Four hundred fifty-one patients were randomly assigned (1:1:1) to receive tacrolimus/MMF/corticosteroids, tacrolimus/MMF, or tacrolimus monotherapy with basiliximab induction. The incidences of biopsy-proven acute rejection were 8.2% (triple therapy), 30.5% (tacrolimus/MMF), and 26.1% (basiliximab/tacrolimus) ($P < .001$). The incidences of corticosteroid-resistant acute rejection were similar among the groups (P = NS). Graft and patient survival rates were similar among the groups. Overall safety profiles were similar: Differences were noted for anemia (24.5% versus 12.6% versus 14.5%), diarrhea (12.9% versus 17.9% versus 5.9%), and leukopenia (7.5% versus 18.5% versus 5.9%) for the triple therapy, tacrolimus/MMF, and basiliximab/tacrolimus groups. Both corticosteroid-free regimens were equally effective in preventing acute rejection, with the basiliximab/tacrolimus regimen offering some safety benefits.[134]

A randomized clinical trial was done using three different induction agents in 90 first renal transplant recipients from cadaver donors: Group A received ATG, group B received alemtuzumab, and group C received daclizumab.[24] Maintenance immunosuppression included tacrolimus and MMF in all three arms, and methylprednisolone in groups A and C. Targeted trough levels of tacrolimus were 8 to 10 ng/mL in groups A and C, and the MMF dose was 1 g twice daily. The target tacrolimus trough levels in group B were 4 to 7 ng/mL to reduce nephrotoxicity, with 500 mg twice daily MMF and no steroid maintenance. At 15 months post-transplantation, no differences were noted among the groups in terms of patient and graft survival. Acute rejection at 1 year was equivalent in all three groups. In group B, there was slightly worse renal function at 1 month, but no difference at 1 year. Group B patients had more leukopenia, but a greater percentage of T regulatory cells and number of Fox-P3 RNA copies by flow cytometry and semiquantitative polymerase chain reaction analysis. In group B, 80% of patients remained steroid-free 1 year postoperatively with lower tacrolimus trough levels and no other adverse events.[24] At 18 months, although there were no differences in the incidences of acute rejection or infectious complications among the three groups, there was statistically worse graft survival, worse kidney function, and a higher incidence of chronic allograft nephropathy in the alemtuzumab group. The alemtuzumab group received less MMF because of a higher incidence of neutropenia, and the authors speculate that this may have accounted for the disparity in outcomes among the three groups.[27]

Comparison of Corticosteroid-Sparing Regimens Using Tacrolimus-Based and Cyclosporine-Based Immunosuppression

Studies of corticosteroid-sparing protocols in patients treated with cyclosporine and MMF showed acute rejection rates to be unacceptably high among African-American recipients.[1] A study examining corticosteroid withdrawal in 52 stable renal transplant recipients treated with tacrolimus and MMF showed a 98% patient survival and 92.3% graft survival.[14] The tacrolimus-based regimen was thought to

promote compliance by facilitating steroid withdrawal and reducing cosmetic complications (see Chapter 15).

Tacrolimus Avoidance Regimens

A prospective, randomized trial was performed in which 132 live donor renal allotransplant recipients were divided into two groups. Steroids and basiliximab induction were given to every patient.[50] Group A patients received tacrolimus/sirolimus as maintenance immunosuppression, whereas group B patients received MMF and sirolimus. No difference was noted in 1-year patient or graft survival between the two groups. The incidence of biopsy-proven acute rejection was slightly less in group B (P = NS). In addition, significantly better renal function was noted in group B patients 2 years after transplantation. One-year protocol biopsy specimens showed no significant differences in the chronic allograft damage index between groups.

A prospective, randomized trial in renal transplantation compared sirolimus/MMF/prednisone (n = 81) with tacrolimus/MMF/prednisone (n = 84). The mean follow-up was 33 months. There was no difference in patient survival, graft survival, or the incidence of clinical acute rejection between the two groups. There also was no difference in the mean glomerular filtration rate measured by iothalamate clearance between the tacrolimus and sirolimus groups at 1 or 2 years.[79] At 1 year, chronicity using the Banff schema showed no difference in interstitial, tubular, or glomerular changes, but fewer chronic vascular changes in the sirolimus group. This study suggests that many of the promises of calcineurin inhibitor–free immunosuppression have perhaps not been achieved with short-term follow-up. The question of improved safety and efficacy in the longer term with calcineurin inhibitor–free immunosuppression has to be subjected to longer term follow-up of the aforementioned study and similar studies.[87]

Many previous studies of complete calcineurin inhibitor avoidance have used cyclosporine as the comparator drug. In one study, the mean corrected iothalamate clearance was 60.6 mL/min/1.73 m^2 in sirolimus-treated patients, and the mean iothalamate clearance for cyclosporine-treated patients at 2 years was 49.2 mL/min/1.73 m^2.[36] The iothalamate clearances in the sirolimus versus tacrolimus study[79] were comparable to the mean corrected iothalamate clearance of 60.6 mL/min/1.73 m^2 at 2 years in the sirolimus-treated patients in the study reported by Flechner and colleagues.[36] The major difference between the results of the two studies[36,79] is that the tacrolimus-treated patients had better renal function at 2 years compared with the cyclosporine-treated patients.

A pilot study was done in 22 renal transplant recipients using alemtuzumab induction and maintenance immunosuppression with MMF (500 mg twice daily) and sirolimus (concentration controlled at 8 to 12 ng/mL). The results of this study showed reasonable composite end points, with most recipients calcineurin inhibitor–free and steroid-free at 1 year.[35] A higher than expected rate of acute rejection, leukopenia, and possibly pulmonary toxicity was noted, however. It is possible that an initial period of calcineurin inhibitor use could be considered in this steroid avoidance regimen.[35] It would be premature today to administer calcineurin inhibitor–avoidance protocols to most kidney transplant recipients (see Chapter 19).[141]

Pediatric Renal Transplantation (see Chapter 35)

The efficacy of tacrolimus as an immunosuppressive agent in pediatric renal transplantation has been shown in single-center experiences and in multicenter trials. A retrospective cohort study of 986 pediatric renal transplant recipients in the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) database (index renal transplant 1997 through 2000), who were treated with either cyclosporine/MMF/steroids (n = 766) or tacrolimus/MMF/steroids (n = 220), was performed to examine differences in outcome between these two groups.[95] In this analysis, tacrolimus and cyclosporine, in combination with MMF and steroids, were associated with similar rejection rates and graft survival in pediatric renal transplant recipients. Tacrolimus was associated with improved graft function at 1 year and 2 years after transplantation.

A 6-month, randomized, prospective, open, parallel group study with an open extension phase was conducted in 18 centers from nine European countries to compare the efficacy and safety of tacrolimus with cyclosporine in pediatric renal transplant recipients.[34] The study randomly assigned (1:1) 196 pediatric patients (<18 years old) to receive either tacrolimus (n = 103) or cyclosporine microemulsion (n = 93), administered concomitantly with azathioprine and corticosteroids. The primary end point was incidence and time to first acute rejection. At 1 year, tacrolimus therapy resulted in a significantly lower incidence of acute rejection (36.9%) compared with cyclosporine (59.1%; P = .003). At 4 years, patient survival was similar, but graft survival significantly favored tacrolimus over cyclosporine (86% versus 69%; P = .025). At 1, 2, 3, and 4 years, the mean glomerular filtration rate was significantly better in the tacrolimus group than in the cyclosporine group. Three patients in each arm developed post-transplant lymphoproliferative disorder, and the incidence of diabetes mellitus was similar in the two groups. Tacrolimus was significantly more effective than cyclosporine in preventing acute rejection in pediatric renal transplant recipients. Renal function and graft survival also were superior with tacrolimus.[34] This study represents the only randomized trial in pediatric renal transplantation to show significantly improved graft survival with tacrolimus compared with cyclosporine.

The effect of corticosteroids on the epiphyseal growth plates is well recognized and results in irreversible growth stunting. Experience with steroid withdrawal in pediatric kidney transplant recipients receiving cyclosporine has shown limited success. Late rejection episodes and graft dysfunction occurred in 68.8% of pediatric kidney transplant recipients after steroid withdrawal under primary immunosuppression with cyclosporine.[103] Experience with corticosteroid withdrawal under tacrolimus therapy in pediatric patients has been associated with favorable outcomes. Two thirds of the pediatric kidney transplant recipients were withdrawn successfully from corticosteroids, with a low incidence of graft dysfunction or acute rejection (23%).[115] Many of these patients had remarkable catch-up growth.

Changes in kidney function, mixed lymphocyte culture, cell-mediated lympholysis, cytotoxic antibodies, lymphocyte populations, and cytokine response were studied in 14 pediatric renal transplant recipients with chronic rejection who were converted to tacrolimus. Serum creatinine levels

decreased, creatinine clearance increased, and urinary protein excretion decreased after 6 months, and these values were maintained after 2 years under tacrolimus treatment.[32]

In adult renal transplant recipients, coadministration of tacrolimus and sirolimus resulted in reduced exposure to tacrolimus at sirolimus doses of 2 mg/day. Eight pediatric renal transplant recipients (median age at transplant 2 years; range 1.2 to 12.9 years) were converted to tacrolimus-based and sirolimus-based immunosuppression as rescue therapy. All patients had biopsy-proven chronic allograft nephropathy. After the addition of sirolimus, the median dose required to keep tacrolimus blood trough concentrations within the target range increased by 71.2% (range 21.9% to 245.4%), and the dose-normalized tacrolimus exposure (area under the curve) decreased to 67.1%. Adding sirolimus to tacrolimus-based immunosuppression in young pediatric renal transplant recipients resulted in a significant decrease in tacrolimus exposure.[33]

Heavy post-transplant immunosuppression can contribute to long-term immunosuppression dependence by subverting tolerogenic mechanisms. Two therapeutic principles were employed to achieve a degree of acquired tolerance: (1) pretransplant lymphoid depletion and (2) minimal post-transplant immunosuppression with tacrolimus monotherapy.[111] Lymphoid depletion in 17 unselected pediatric recipients of live ($n = 14$) or cadaver donor kidneys ($n = 3$) was carried out with ATG ($n = 8$) or alemtuzumab ($n = 9$). Tacrolimus was started after transplantation with eventual lengthening of intervals between doses (spaced weaning). After 16 to 31 months' follow-up (mean 22 months), patient and graft survivals were 100% and 94%, respectively. The only graft loss occurred in a nonweaned, noncompliant recipient. In the other 16 recipients, the mean serum creatinine level was 0.85 ± 0.35 mg/dL, and the calculated creatinine clearance was 90.8 ± 22.1 mL/min/1.73 m^2. All 16 patients were on monotherapy (15 tacrolimus, 1 sirolimus), whereas 14 were dosed every other day or three times per week. This strategy of lymphoid depletion and minimal post-transplant immunosuppression seemed safe and effective for pediatric kidney recipients, although more follow-up is needed to establish its long-term efficiency.

CLINICAL STUDIES IN KIDNEY-PANCREAS TRANSPLANTATION (see Chapter 34)

The increase in the number of pancreas transplants has been made possible by technical improvements and improved immunosuppressive regimens. Treatment with tacrolimus-based immunosuppression has been associated with lower rejection rates, higher graft survival rates, and less nephrotoxicity compared with treatment with cyclosporine.[47,52]

Simultaneous Pancreas-Kidney (SPK) Transplantation

In an analysis of 1194 pancreas transplantations performed at the University of Minnesota, the results were divided into five time periods ("eras") based on the technique and immunosuppressive regimen used.[121] In era II, the immunosuppressive regimen consisted of Minnesota antilymphocytic globulin (MALG) or muromonab-CD3 (OKT3) for induction and a combination of cyclosporine, azathioprine, and prednisone for maintenance. Duct management in eras II and III was by bladder drainage. In era III, tacrolimus was used for pancreas transplantation as soon as it was approved by the FDA in 1994. Induction was with equine antithymocyte globulin (Atgam), and OKT3 was used for treatment of rejection episodes. When MMF was approved a year later, it was added to the maintenance immunosuppressive regimen. In era IV, which began in March 1998, daclizumab, alone or in combination with the polyclonal anti–T cell antibody (Atgam or ATG), was added to the induction regimen. Enteric drainage was the principal exocrine drainage technique. In patients with primary simultaneous pancreas-kidney (SPK) transplantation, pancreas and kidney graft survival rates were significantly higher in eras III and IV than in era II. In eras III and IV combined, 1-year patient, pancreas, and kidney survival rates were 92%, 79%, and 88%, respectively; at 5 years, the corresponding figures were 88%, 73%, and 81%, respectively.[121]

The rate of acute rejection in SPK transplantation has been decreasing over the past decade at the University of Miami Medical Center, from nearly 100% to less than 10% in the first year after transplantation.[15] In a prospective, randomized trial, 42 SPK recipients received ATG and daclizumab induction, with tacrolimus and steroids as baseline immunosuppression. Twenty-two patients were randomly assigned to receive MMF, and 20 patients received sirolimus in addition to tacrolimus and steroids. Actuarial patient, kidney, and pancreas allograft survivals were 100%, 100%, and 95%, respectively, at 6 months in the sirolimus group and 100%, 100%, and 100%, respectively, in the MMF group. The incidence of acute rejection was less than 10% and was limited to instances in which recipient immunosuppression was reduced.[15]

A prospective study of combined tacrolimus, MMF, and steroids without antibody induction was done on 17 SPK transplant patients at Miami. Low-dose intravenous tacrolimus was used as induction therapy. Clinical and biopsy-proven rejection occurred in four (23%) patients. Patients who developed rejection had low tacrolimus levels or had had discontinuation of MMF because of leukopenia, gastroparesis, or gastrointestinal side effects.[26] All rejection episodes responded to steroids.

Immunosuppression for SPK transplantation at Northwestern University was divided into four eras over an 8.5-year period.[70] In era I (March 1993 to February 1997), three immunosuppression combinations were used: cyclosporine/azathioprine/steroids ($n = 28$), cyclosporine/MMF/steroids ($n = 8$), or tacrolimus/MMF/steroids ($n = 10$); bladder drainage was used. In era II (July 1995 to February 1998), the combination of tacrolimus, MMF, and corticosteroids was used, with bladder drainage. In era III, combinations of tacrolimus (12-hour trough concentrations 10 to 12 ng/mL) and MMF (3 g/day) were used along with corticosteroids for maintenance immunosuppression; enteric drainage was used. In era IV, steroids were eliminated within 6 days of transplantation, and tacrolimus was combined with either MMF ($n = 20$) or sirolimus ($n = 38$); enteric drainage was used.

In eras I and II, all recipients received induction therapy with Atgam for 7 to 14 days after transplantation. In era III, for induction therapy, 17 patients were randomly assigned to a noninduction therapy arm, and 37 patients were randomly assigned to an anti–interleukin-2 receptor monoclonal antibody (daclizumab, $n = 35$; basiliximab, $n = 2$).

Induction therapy in era IV consisted of rabbit ATG, 1 mg/kg intraoperatively and on postoperative days 1, 2, 4, 6, 8, 10, 12, and 14. One-year actuarial patient survival rates in eras III and IV were 96.3% and 100%, respectively; 1-year actuarial kidney survival rates in eras III and IV were 94.4% and 97.7%, respectively, and the 1-year actuarial pancreas survival rates were 88.9% and 100%, respectively. The 1-year rejection-free rate was 87.1% for era III and 96.6% for era IV. Compared with era I, kidney function significantly improved over the three eras. Rapid elimination of corticosteroids was successful in all recipients in era IV, with higher patient and graft survival rates than in the previous three eras. Rejection rates decreased further in era IV. The Northwestern group concluded that corticosteroids could be rapidly eliminated prospectively in all recipients without a decrease in graft survival rates or an increase in the rate of rejection.[70]

The combination of tacrolimus, MMF, and steroids with ATG induction was associated with an incidence of acute rejection of 33% compared with an incidence of 73% using ATG induction followed by cyclosporine, azathioprine, and steroids, in a randomized trial reported from Ruhr University, Germany.[140] The incidence of cytomegalovirus and malignancies was not higher using tacrolimus/MMF compared with the cyclosporine/azathioprine regimen, with 5 years' follow-up.

A multicenter trial was done to assess the effect of antibody induction in SPK transplant recipients receiving tacrolimus, MMF, and corticosteroids.[16] The trial randomly assigned 174 SPK transplant recipients to induction ($n = 87$) or noninduction ($n = 87$), and the recipients were followed for 3 years. Induction agents included T cell depleting or interleukin-2 receptor antibodies. At 3 years, actual patient (94.3% and 89.7%) and pancreas (75.9% and 75.9%) survival rates were similar in the induction and noninduction groups. Actual kidney survival was significantly better in the induction group compared with the noninduction group at 3 years (92% versus 82%; $P = .04$).[16]

The EuroSPK Study Group, which compared tacrolimus and cyclosporine in primary SPK transplantation, enrolled 205 patients.[78] After antilymphocyte globulin induction, patients were randomly assigned to receive either tacrolimus or cyclosporine microemulsion together with MMF and steroids. At 1 year after transplantation, patient and kidney survival rates were excellent in both treatment groups. There was a significant difference in pancreas graft survival: 94.2% for tacrolimus and 73.9% for cyclosporine ($P = .00048$). There were significantly fewer grade 2 and grade 3 rejections with tacrolimus-based therapy. The EuroSPK group also presented data showing that 34 patients were switched from cyclosporine to tacrolimus, but only 6 patients receiving tacrolimus required conversion to alternative therapy during the course of the study.[9] The mean doses of MMF at 1 year also were lower in the tacrolimus group (1.36 g/day versus 1.67 g/day; $P = .007$).

Steroid Withdrawal and Steroid-Free Protocols

Reduction of steroid use is extremely desirable in pancreas transplantation because long-term steroid use is associated with hypertension, hyperlipidemia, and glucose intolerance.[56] Complete steroid withdrawal was achieved in 58 (47%) of 124 patients transplanted at the University of Pittsburgh, with a mean time to steroid withdrawal of 15.2 ± 8 months.[61] Patient, pancreas, and kidney survival rates at 1 year were 100%, 100%, and 98%, respectively, (off steroids) versus 97%, 91%, and 96%, respectively, (on steroids, all P = NS). The cumulative risk of rejection was 74% for patients off steroids versus 76% for patients on steroids (these patients had not received antibody induction). The mean glycosylated hemoglobin levels were 5.2 ± 0.9% (off steroids) and 6.2 ± 2.1% (on steroids; $P = .02$). The Pittsburgh group concluded that steroid withdrawal could be achieved in pancreas transplant patients under tacrolimus-based immunosuppression and was associated with excellent patient and graft survival.[61] More recently, the Pittsburgh group has used alemtuzumab preconditioning with tacrolimus monotherapy in pancreas recipients,[124] with promising early results.

Based on experimental studies, it was found that preconditioning with a depleting antibody and low-dose post-transplant immunosuppression could lead to partial tolerance.[18] T lymphocyte depletion strategies using alemtuzumab[19] or ATG[120] administered as preconditioning agents are based on this principle. Fourteen patients received pancreatic allografts at the University of Pittsburgh, which were transplanted alone ($n = 4$) or with kidneys from the same donor ($n = 10$). Two of the 4 pancreas-alone recipients and 6 of the 10 pancreas-kidney recipients also had donor-specific bone marrow cell infusion.[120] The immunosuppressive regimen consisted of pretreatment with 5 mg/kg of ATG over several hours preceding transplantation; participants also received 1 to 2 g of intravenous methylprednisolone concomitantly to minimize cytokine release. Twice-daily monotherapy with tacrolimus was begun the day after transplantation, with a target trough concentration level of 10 ng/mL. Other agents, including prednisone, sirolimus, or muromonab-CD3, were added as necessary for control of rejection and for as brief a period as possible. At 4 months, patients receiving tacrolimus monotherapy were considered for consolidation to once-daily tacrolimus and eventual spaced weaning. Patient survival was 100% and pancreas graft survival was 86%, with 13 to 18 months' follow-up. Five of 12 patients with functioning pancreas grafts were receiving spaced doses of tacrolimus monotherapy ranging from every other day ($n = 1$), to three times a week ($n = 2$), and once a week ($n = 2$).[120]

The Minnesota Group reported a prospective trial of steroid withdrawal in pancreas transplantation.[48] Recipients with functioning grafts ≥6 and ≤36 months after SPK transplantation or pancreas after kidney (PAK) transplantation were enrolled. All patients received triple therapy for maintenance immunosuppression using tacrolimus and MMF, with the following inclusion criteria: (1) low maintenance steroid dose 0.075 mg/kg/day, (2) MMF dosed ≥750 mg orally twice a day, and (3) tacrolimus levels ≥8 ng/mL. Fifty-five patients (29 SPK, 26 PAK) were randomly assigned to remain on steroids or to steroid withdrawal after 4 to 8 weeks. The median follow-up was 27 months in the SPK category and 26 months in the PAK category, and from randomization, 10 months in both categories. Steroid withdrawal 6 months after a successful pancreas transplant was not associated with a decrease in patient or graft survival, and it was not associated with an increase in the incidence of rejection or in the rate of graft loss from rejection. There was a better quality of life and a reduction in serum cholesterol levels in the steroid withdrawal group.[48]

Rapid corticosteroid elimination was carried out in 40 SPK recipients from Northwestern University in Chicago.[71] ATG was used for induction; maintenance immunosuppression was with tacrolimus/MMF in 20 patients and tacrolimus/sirolimus in 20 patients. Patient and graft survival rates and rejection rates were compared with historical controls ($n = 86$). One-year actuarial patient, kidney, and pancreas survival rates in the rapid corticosteroid elimination group were 100%, 100%, and 100%, respectively, and in the historical control group rates were 97%, 93%, and 97%, respectively. The 1-year rejection-free survival rate was 97% in the rapid corticosteroid elimination recipients versus 80% in the historical controls. Serum creatinine levels remained stable in all groups at 6 and 12 months after transplantation.[71]

Steroid-free immunosuppression has been used with excellent short-term results in low-risk pancreas-kidney transplantation recipients at the University of California at San Francisco.[38] Forty patients underwent pancreas-kidney transplantation from November 2000 to July 2002. ATG induction was combined with MMF, tacrolimus, and sirolimus for maintenance immunosuppression. Steroids were used as pretreatment only, given with ATG and discontinued by the end of the first postoperative week. Patient, kidney, and pancreas survival rates were 95%, 92.5%, and 87.5%, respectively. Biopsy-proven pancreas rejection rates at 1 and 3 months after transplantation were 2.5%, and kidney rejection rates at 1 and 3 months were 2.5%.[38]

Pancreas after Kidney Transplantation

According to data from the International Pancreas Transplant Registry, the current, nearly uniform use of tacrolimus/MMF in PAK transplantation makes a comparison with other regimens difficult, although graft survival rates have been significantly better than in the preceding era, when cyclosporine/azathioprine was used.[45] In the overall analysis of tacrolimus/MMF-treated primary PAK transplant recipients, graft survival rates did not differ significantly whether or not antibody induction was given, although they tended to be numerically higher in PAK recipients given depleting or nondepleting antibody than in recipients not given antibody induction. In the PAK category, the relative risk of pancreas graft failure was reduced by the use of tacrolimus/MMF for immunosuppression.[45]

Between July 1, 1978, and April 30, 2002, 406 PAK transplants were performed at the University of Minnesota.[46] Immunosuppression was divided into eras. In era III, tacrolimus was used in combination with prednisone and initially azathioprine. MMF replaced azathioprine when it was approved by the FDA. Polyclonal antibody induction therapy with Atgam was used in 99%, and monoclonal antibody (OKT3) was used in 1% of patients; the median duration of antibody therapy was 5 days. In era IV, tacrolimus, MMF, and prednisone were the principal maintenance immunosuppressive agents. Daclizumab was used for induction either alone (21%) or in combination (79%) with a polyclonal antibody (Atgam or ATG). The median duration of antibody therapy was 3 days. Overall patient survival rates (cadaver and living donor) at 1 and 3 years were 97% and 90%, respectively, and at 1 year in era IV overall survival was 96%. Overall pancreas graft survival rates (cadaver and living donor) at 1 and 3 years in era III were 78% and 60%, respectively, and in era IV, at 1 year overall graft survival was 77%. Of technically successful transplants, pancreas graft loss rates to rejection in era III at 1 and 3 years were 10% and 19%, respectively; in era IV, at 1 year, it was 9%. PAK transplants now can be performed almost as successfully as SPK transplants; the introduction of tacrolimus and MMF in the mid-1990s contributed to this development.

Using tacrolimus-based and MMF-based immunosuppression, only 20% of recipients experiencing rejection episodes ultimately lost their pancreas graft to irreversible rejection. In eras III and IV, when tacrolimus was being used, there no longer existed a difference in outcome between primary transplants and second transplants. With the use of tacrolimus, the advantage of living donor PAK transplants over cadaver donor PAK transplants no longer existed.[46]

SIDE EFFECTS AND TOLERABILITY OF TACROLIMUS

The side-effect profile of tacrolimus is similar to that of cyclosporine (Table 17-2; see Chapter 16). The physiological effects, including reduction in renal blood flow and glomerular filtration, are similar between tacrolimus and cyclosporine. The pathological manifestations of tacrolimus and cyclosporine toxicity are similar in that they include tubular vacuolization and arteriolar nodular hyalinosis that are indistinguishable. Microvascular changes involving arterioles or glomerular capillaries sometimes predominate, displaying a wide spectrum of severity from apoptosis and vacuolization of smooth muscle cells to thrombotic microangiopathy.

A review of 21 patients with tacrolimus-associated thrombotic microangiopathy was published[126]; 17 of these occurred in kidney transplant recipients, whereas 2 cases occurred in liver transplant recipients and 1 each in heart and bone marrow transplant recipients. The mean time from transplantation to the onset of thrombotic microangiopathy was 9.3 ± 7.9 months. Clinical presentation varied from an absence of signs and symptoms of hemolysis to florid hemolytic anemia, thrombocytopenia, and azotemia. Renal biopsy specimens were obtained from the patients

Table 17–2 **Adverse Effects Associated with Tacrolimus Therapy**

Nephrotoxicity
Reduced renal blood flow, glomerular perfusion
Tubular and vascular toxicity
Neurotoxicity
Headaches, tremors, seizures, peripheral neuropathy, paresthesias
Metabolic disturbances
Hyperkalemic, hyperchloremic acidosis
Hypomagnesemia
Diabetes mellitus
Hyperuricemia
Hypercholesterolemia
Hypertension
Gastrointestinal disturbances
Diarrhea
Anorexia, nausea, and vomiting
Epigastric cramping
Cosmetic
Alopecia

with a kidney transplant and showed acute thrombi within the glomerular capillaries or arterioles, or both. Tacrolimus causes tissue ischemia by reducing the renal plasma flow and glomerular filtration rate, leading to endothelial cell injury. There are large circulating polymers of von Willebrand's factor in thrombotic microangiopathy, which increases the tendency for platelets to adhere to and aggregate on the subendothelium, resulting in thrombi and fibrin deposition. Treatment consists of reducing the dose of tacrolimus and substitution with cyclosporine or sirolimus. Other treatment modalities have included plasmapheresis, fresh frozen plasma exchange, and anticoagulation.[126]

Adverse events dictate the optimal dosage regimen of the drug. Decreasing the dosage of tacrolimus generally reduces its toxic effects, although some adverse effects are idiosyncratic and do not respond to such measures.[108] Tacrolimus treatment is associated with a higher incidence of diarrhea, disturbances in glucose metabolism, and some types of neurotoxicity but a lower incidence of hypertension and hypercholesterolemia than cyclosporine. Tacrolimus is only rarely associated with the cyclosporine-specific adverse effects of hirsutism, gum hyperplasia, and gingivitis, but it may cause alopecia and pruritus.

In a trial in renal transplant recipients, significantly fewer (all $P < .05$) tacrolimus recipients (compared with cyclosporine microemulsion recipients) experienced new-onset or worsening hypertension (15.7% versus 23.2%), urinary tract disorders (4.9% versus 9.2%), hypercholesterolemia (4.2% versus 8.9%), hyperbilirubinemia (0.3% versus 3.3%), gastrointestinal hemorrhage (0.3% versus 2.6%), cholestatic jaundice (0.3% versus 2.6%), hirsutism (0% versus 4.4%), and gum hyperplasia (0% versus 4.1%).[84] Tremor (12.2% versus 4.1% of patients), hypomagnesemia (6.6% versus 1.5%), thrombosis (4.5% versus 1.5%, mainly affecting the dialysis access vessels), and gastritis (3.1% versus 0.4%) were significantly (all $P < .05$) more common in the tacrolimus group.

Because tacrolimus and cyclosporine cause acute and chronic nephrotoxicity, concomitant use of these two agents is contraindicated. Nephrotoxicity related to tacrolimus treatment is dose related and responds to dosage reduction.[117] Mean or median serum creatinine levels in renal transplant recipients were lower in tacrolimus-treated patients, with 5 years' follow-up, than in patients treated with cyclosporine microemulsion (or standard formulation).[49,133] Administration of other nephrotoxic agents simultaneously can exacerbate the adverse effects of tacrolimus. Examples of such agents include aminoglycosides, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, amphotericin, and nonsteroidal anti-inflammatory drugs.

Cardiovascular Adverse Effects

Hyperlipidemia occurs commonly after transplantation and is a risk factor for cardiovascular disease. Immunosuppression with tacrolimus-based regimens is associated with better lipid profiles than is immunosuppression with cyclosporine-based regimens.[4,83]

Analysis of the United States Renal Data System database showed that fewer tacrolimus (than cyclosporine) recipients had at least one new-onset hyperlipidemia code during the first year of treatment (11% versus 16%; $P = .0001$); a multivariate analysis showed that the risk of new-onset hypertension after transplantation was reduced by 35% under tacrolimus-based immunosuppression.[106] The 5-year follow-up results from a U.S. randomized trial indicated that significantly fewer tacrolimus than cyclosporine recipients were receiving antihypertensive treatment (80.9% versus 91.3%; $P < .05$).[133]

Concentric increases in left ventricular posterior wall and interventricular septum thickness can occur with tacrolimus immunosuppression in 0.1% of patients.[28] This condition is reversible after dosage reduction or discontinuation of the drug.

Post-Transplant Diabetes Mellitus

PTDM is a serious adverse effect of tacrolimus treatment; the complications of diabetes mellitus can result in decreased patient and graft survival.[93] PTDM is defined as insulin use for more than 30 consecutive days in the absence of preexisting diabetes. The incidence of PTDM was significantly higher among tacrolimus-treated patients than cyclosporine-treated patients (9.8% versus 2.7%), according to a more recent meta-analysis.[53] In corticosteroid minimization trials, the 6-month incidence of PTDM (use of insulin >30 days) in the corticosteroid-free arm ranged from 0.4% to 1.4%.[21,73] Tacrolimus target trough levels have tended to be lower and more rapidly tapered in recent years; this also has led to a decrease in the incidence of PTDM.[92] The introduction of MMF and sirolimus and the use of combinations of these agents with tacrolimus has led to a reduction in acute rejection rates and a reduction in corticosteroid treatment for acute rejection episodes in the first year after transplantation; this also has resulted in a reduced incidence of PTDM in recent years.

A study from Cleveland compared the outcomes of 56 African-American adult primary kidney transplant recipients treated with corticosteroids, sirolimus, and tacrolimus, targeted to low trough blood levels, with 65 white patients treated with steroids, MMF, and tacrolimus, targeted to higher blood levels. There were no significant differences in the actuarial 2-year patient, graft, and rejection-free graft survival rates between the two groups. PTDM occurred in 36% of the African-American patients, however, despite similar doses of corticosteroids and lower trough levels of tacrolimus, compared with 15% of white patients ($P = .024$).[54]

Recipient-related risk factors for PTDM include an underlying glucose metabolic disorder (e.g., family history of diabetes mellitus, older recipient age, nonwhite ethnicity, sedentary lifestyle, higher body mass index) and hepatitis C virus positivity. Transplantation-related risk factors include acute rejection during first post-transplant year, high doses of corticosteroids, and high tacrolimus trough levels.

The risk of developing PTDM is highest in the first few months after transplantation, after which the incidence increases more slowly. The European multicenter trial found a 6-month PTDM incidence of 4.5% and an additional incidence of 0.4% from months 7 through 12.[84]

Many patients with PTDM can have reversal of diabetes mellitus, with eventual discontinuation of insulin. In the European trial, the 1-year cumulative incidence of PTDM with tacrolimus was 8.3%, whereas the prevalence at 1 year was 5.5%.[86] In a U.S. trial combining tacrolimus with MMF and corticosteroids, the 1-year incidence was 6.5%, and the 1-year prevalence was 2.2%.[60]

High levels of FK BP-12 are present in pancreatic beta cells, and this is associated with a decrease in insulin mRNA transcription and reduced insulin production in rats.[122] In the clinical setting, tacrolimus affects insulin secretion but does not affect insulin resistance. In addition, PTDM is probably not a separate entity but a consequence of an underlying glucose metabolic disorder that is uncovered by immunosuppression.[105] The effects of tacrolimus on insulin release are reversible, and after the early post-transplant period, tacrolimus and cyclosporine are equivalent in terms of their effect on glucose metabolism.[129]

Compared with cyclosporine-treated patients, the relative risk for developing PTDM in tacrolimus-treated patients was 1.53 ($P < .001$).[68] Compared with cyclosporine-treated patients, tacrolimus therapy was associated with a reduced risk of death (relative risk 0.65; $P < .001$) and graft failure (relative risk 0.70; $P < .001$). Under tacrolimus-based immunosuppression, the positive effects of lower blood pressure, less hypercholesterolemia, better renal function, and lower fibrinogen[7] offset the negative effect of PTDM.

The main principles in the management of PTDM are as follows:[130] Therapeutic trough levels of tacrolimus are maintained in month 1—between 15 ng/mL and 20 ng/mL in the first 2 weeks and between 10 ng/mL and 15 ng/mL in the latter 2 weeks. Trough levels of tacrolimus are reduced to 4 to 7 ng/mL by month 3. Corticosteroid cessation is begun as soon as possible. Corticosteroids are to be reduced to 10 mg/day at month 1, after which they should be withdrawn. In patients who are at high risk for rejection, corticosteroids are administered at 5 mg/day. The aforementioned measures led to the resolution of PTDM in most instances. In cases in which impaired glycemic control does not resolve, the degree of insulin resistance should be determined. Underlying infection or obesity should be treated when appropriate. Use of the thiazolidinediones should be considered.[8] In patients with low insulin output, insulin treatment or conversion to calcineurin-free immunosuppression is recommended. Evidence from a meta-analysis suggests that targeting tacrolimus concentrations to less than 10 ng/mL minimizes graft loss and reduces the risk of diabetes mellitus without increasing the risk of acute rejection.[139]

Malignancies (see Chapters 32 and 33)

The use of immunosuppressive agents increases the risk of malignancies developing, the most common being malignancies of the skin and lymphoma. All agents increase these risks, and the risk is related to the intensity and duration of treatment. Epstein-Barr virus–related post-transplant lymphoproliferative disorder is associated with immunosuppressive treatment, with a lower risk in adults than in children. In the European Multicenter Renal Study, the incidence of post-transplant lymphoproliferative disorder at 1-year follow-up was 1% in the tacrolimus group and 0.7% in the cyclosporine microemulsion group.[86] The incidence of post-transplant lymphoproliferative disorder in pediatric renal transplant patients with tacrolimus immunosuppression was 0.96% based on an analysis of the NAPRTCS database.[29]

Other Side Effects

Tacrolimus-treated patients are more likely to have alopecia, tremor, headache, insomnia, dyspepsia, vomiting, diarrhea, and hypomagnesemia than cyclosporine-treated patients.[139] Cyclosporine-treated patients are more likely to have constipation, hirsutism, and gingival hyperplasia.

Special Patient Populations

In a large, randomized multicenter trial involving pediatric renal transplant recipients (children and adolescents), the most common (3% of patients) adverse events associated with tacrolimus-based primary immunosuppression were hypertension, infections, hypomagnesemia, increased mean serum creatinine, diarrhea, PTDM, and tremor.[127] Significantly more tacrolimus recipients experienced hypomagnesemia ($P = .001$) and diarrhea ($P < .05$) than did cyclosporine recipients, and significantly fewer tacrolimus recipients experienced hypertrichosis ($P = .005$), flu syndrome ($P < .05$), and gum hyperplasia ($P < .05$).

The risks of tacrolimus during pregnancy are similar to the risks associated with cyclosporine. Data from the U.S. National Transplantation Pregnancy Registry were used to compare outcomes in 19 tacrolimus recipients (24 pregnancies) with outcomes in 56 cyclosporine microemulsion recipients (71 pregnancies). Seventy-one percent of pregnancies resulted in live births in the tacrolimus group versus 80% of pregnancies in the cyclosporine microemulsion group; the mean gestational age was lower in the tacrolimus group than the cyclosporine group (32.9 weeks versus 35.8 weeks; $P = .0035$).[3] There were no other statistically significant differences in outcomes.

A single-center analysis was performed on 13 kidney transplant recipients and 2 SPK recipients who became pregnant under tacrolimus-based immunosuppression.[57] The 13 mothers after kidney transplantation delivered 19 infants, whereas the 2 mothers after SPK transplantation delivered 3 infants. All mothers survived the pregnancy. One infant was stillborn. Forty-one percent of the infants were either preterm or premature, and 27% of the infants were delivered by cesarean section. Toxemia of pregnancy or preeclampsia was seen in 23% of these pregnancies. None of the mothers experienced rejection during their pregnancy.

CONCLUSION

The studies discussed in this chapter have consolidated the place of tacrolimus as an important agent for primary immunosuppression in adult and pediatric kidney and in adult kidney-pancreas transplantation. The key comparator for tacrolimus is cyclosporine microemulsion. Treating kidney transplant recipients with tacrolimus results in a 44% reduction in graft loss (censored for death) compared with cyclosporine-treated patients in the first 6 months after kidney transplantation.[139] On the basis of meta-analyses of data from randomized trials, treating 100 recipients at low risk (e.g., adult, well-matched, first transplants) with tacrolimus instead of cyclosporine would avoid 6 cases of acute rejection; this number increases to 17 cases if high-risk recipients are considered (e.g., sensitized recipients, second or third transplants, children). In contrast, treating with tacrolimus would lead to excess harm in an extra five recipients by causing them to develop insulin-dependent diabetes.[139] Both of the calcineurin inhibitors are nephrotoxic, and this can contribute to chronic allograft nephropathy

directly via drug toxicity or indirectly via hypertension and dyslipidemia.[20,80,94,118]

Tacrolimus is associated with less hypertension and less hypercholesterolemia than cyclosporine. Tacrolimus use has steadily increased, and it is now used in more than 67% of kidney recipients. Despite its side effects, the superior immunosuppressive efficacy of tacrolimus has led to its preferential use in kidney and kidney-pancreas recipients.

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Chapter 18

Mycophenolate Mofetil

Barry D. Kahan

Mycophenolic acid (MPA), a fermentation product of *Penicillium brevicompactum* and related fungi,[48] has been known to be an inhibitor of nucleic acid synthesis for 100 years.[40] The development of the drug for transplantation was based on the findings that inherited deletions in this pathway result in immunodeficiencies; children lacking adenosine deaminase show combined T cell and B cell deficits.[45] In contrast, subjects with absence of hypoxanthine-guanine phosphoribosyl transferase display essentially normal immune function,[4] showing that, in contrast to the de novo synthesis pathway, the purine salvage pathway is not preeminent for lymphocyte function. These observations suggested the potential utility of inhibition of nucleotide synthesis for immunosuppression,[37] a hypothesis that was confirmed by the activity of the relatively nonselective mercapto-analogue azathioprine. Despite its profound capacity to block lymphocyte proliferation by inhibition of inosine monophosphate dehydrogenase (IMPDH),[37] MPA shows only marginal antitumor effects[83] and modest antibiotic activity toward a variety of organisms, including gram-positive bacteria, *Candida albicans, Leishmania*, and other intracellular protozoans, as previously reviewed.[84] To augment the oral bioavailability of MPA, the mofetil analogue (mycophenolate mofetil [MMF]) was formulated as an ester product.[81]

MECHANISM OF ACTION

MPA acts as a rapid, reversible, noncompetitive inhibitor of IMPDH, a rate-limiting enzyme in the de novo synthesis of guanine (Fig. 18-1).[37] This effect arrests new DNA synthesis in proliferating cells at the G_1/S interface; guanosine triphosphate (GTP) levels decrease to 10% of those in unstimulated T cells.[3] Addition of guanosine or deoxyguanosine reverses the inhibition, documenting the IMPDH target. Of its two isozymes, IMPDH type II, which is fourfold more sensitive to MPA,[23] shows the greatest increase in stimulated lymphocytes, the cell type that is particularly sensitive to the drug.

Guanine and adenine nucleotides produce coordinated feedback inhibition of 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase in most human cells. A decrease in guanine potentially would override the IMPDH block; however, guanosine monophosphate seems to be necessary to activate PRPP in lymphocytes.[5] The adenosine triphosphate (ATP) content is reduced to levels less than 50% of unstimulated cells.[113] This effect markedly dampens the activity of ATP-dependent enzymes, including tyrosine kinases that mediate signal transduction. Rescue of ATP pools by salvage via hypoxanthine or by de novo synthesis via inosine monophosphate is impaired because catalysis by adenylsuccinate synthetase is GTP-dependent.[83] There is no increase in cytosine triphosphate (CTP) because CTP synthetase also is a GTP-dependent enzyme. The deficiency in CTP is homeostatically countered by upregulated synthesis of uridine nucleotides, producing an imbalance in pyrimidine pools. MPA affects pyrimidine and purine pools.

The action at the G_1/S interface is selective. Neither the production of interleukin-2 nor the expression of its receptor is affected, showing a lack of influence on signal 1 of lymphocyte activation (Fig. 18-2). MPA decreases neither the cytoplasmic intermediates of extracellular signal-related kinase 1 nor signal transducer and activators of transcription,[114] suggesting preservation of signal 3. MPA has been claimed to

Figure 18–1 Purine synthetic pathways showing site of inhibition by mycophenolic acid (MPA). ATP, adenosine triphosphate; DP, diphosphate; HGPRTase, hypoxanthine-guanine phosphoribosyl transferase; IMPDH, inosine monophosphate dehydrogenase; MP, monophosphate; PRPP, phosphoribosyl pyrophosphate; TP, triphosphate. (Adapted from Budde K, Glander P, Bauer S: Pharmacokinetics and Pharmacodynamics of Mycophenolic Acid. A CME Monograph. Berlin, Walter de Gruyter, 2004, pp 2-13.)

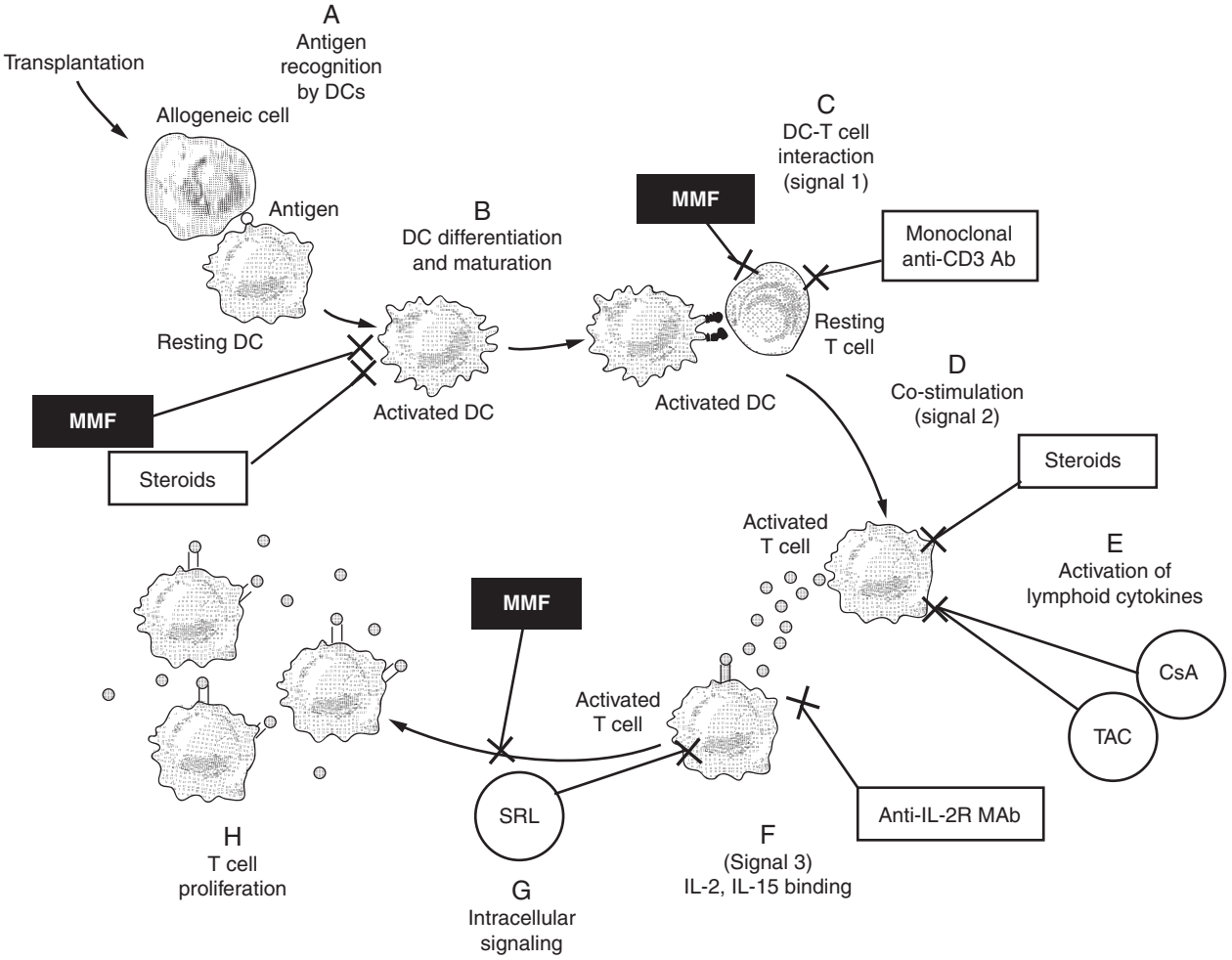

Figure 18–2 Steps in the activation of T cells, showing potential inhibition by mycophenolate mofetil (MMF) at sites *B, C,* and *H.* Ab, antibody; CsA, cyclosporine; DC, dendritic cell; IL-2R, interleukin-2 receptor; SRL, sirolimus; TAC, tacrolimus. (Adapted from Shaw LM, Korecka M, Venkataramanan R, et al: Mycophenolic acid pharmacodynamics and pharmacokinetics provide a basis for rational monitoring strategies. Am J Transplant 3:534, 2003.)

exert a modest proapoptotic effect, however, on lymphocytes responding to antigenic stimulation.[30] The primary antiproliferative action not only inhibits mixed lymphocyte responses but also retards induction of cytotoxic T cells.[37] Similarly, MPA seems to mitigate primary and ongoing B cell responses, presumably as a result of blockade of cell division.[51] Among patients in the U.S. pivotal trial, those who received MMF displayed a far lower incidence of production of xenoantibody toward rabbit antithymocyte globulin than the azathioprine cohort.[71] MPA treatment has been reported to blunt the synthesis of natural xenoantibodies after plasma exchange and splenectomy in rats.[38] Among the other elements of the immune response, the drug suppresses the maturation and allostimulation functions of dendritic cells in vitro.[31]

A distinct mechanism of drug action may relate to the need for GTP to activate fucose and mannose transfer as dolichol phosphate–linked oligosaccharides preparatory to glycoprotein synthesis. This effect is particularly relevant to transplantation because it would diminish the expression of adhesion molecules. α1,3-Fucosylated oligosaccharide ligands of L-selectin and vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 glycoproteins depend on GTP-sugar intermediates. This effect was proposed in a preliminary report,[5] which inferred that MPA-treated cells displayed reduced glycoprotein assembly based on an indirect index that suggested inhibited expression and limited incorporation of mannose into these macromolecules. Using an in vitro model, endothelial cells from rat donors treated with 20 mg/kg/day or 60 mg/kg/day of MMF showed reduced expression of mannose-containing glycoproteins and moderate protection of their cardiac allografts after storage for 2 hours at room temperature.[148] Theoretically, a deficiency of bound guanine nucleotides also could exert independent actions to disrupt cell membrane physiology. In aggregate, these effects, on the one hand, would interfere with leukocyte binding to the endothelium, but, on the other hand, would promote tumor cell metastasis.[17]

Although lymphocytes seem to be 10-fold more sensitive to the drug than other elements, vascular smooth muscle cells,[93] mesangial cells,[57] and myofibroblasts[12] also have been reported to display dampened proliferation, using in vitro and in vivo rodent models of chronic rejection, chronic allograft nephropathy, and atherosclerosis. In a subhuman primate model of orthotopic aortic allografts, intravascular ultrasound documented that four of six hosts showed dose-dependent inhibition of the progression of intimal volume changes owing to ongoing vasculopathy.[95] The effects were potentiated when MMF was combined with sirolimus,[64] although at least within synthetic vascular grafts, the effects of MMF to reduce intimal hyperplasia exceeded those of sirolimus.[158] The efficacy of MMF was confirmed using aortic allografts in another subhuman primate model[72] and in a rodent chronic rejection system.[11] Coadministration of MMF and sirolimus seemed to reduce transforming growth factor (TGF)-β_1 mRNA and protein levels, which stimulate the synthesis of extracellular matrix protein and inhibit extracellular matrix degradation.[132] TGF-β_1 is a presumed vector of long-term cyclosporine nephropathy. Coadministration of MMF decreased TGF-β despite the propensity of baseline sirolimus treatment to increase it.[131] Additional potential actions of MPA on endothelium in vitro include inhibition of nitric oxide synthase upregulation after combined stimulation with interferon-γ and tumor necrosis factor-α[128] and inhibition of prostaglandin E_2 release after activation by allogeneic cells, interferon-γ, or interleukin-1.[18]

MPA inhibits the proliferation of multiple cell types by direct effects on guanosine synthesis and indirect effects on the generation of other nucleotides. Therapeutic benefits on T cell and B cell adaptive immune responses may be augmented or counterbalanced (based on the tissue) by antiproliferative effects on other rapidly dividing cells. Postulated effects on glycoprotein synthesis or assembly await robust experimental and clinical data.

PHARMACOKINETICS

Drug Measurement

The MPA parent compound is readily measured in plasma by high-performance liquid chromatography owing to its high predose concentrations (C_0).[58] The parent drug (MMF) is not detected in plasma. In contrast, the widely available automated enzyme multiplier immunoassay technique[94] yields 15% to 20% higher results because its antibody reagent cross-reacts with the acyl-MPAG metabolite(Fig. 18-3), which has immunosuppressive and toxic effects.[151] In addition, this metabolite accumulates greatly in renal failure. Because of these properties, some workers have suggested that the enzyme multiplier immunoassay technique may be preferable, although it is currently not approved by the Food and Drug Administration (FDA) for clinical purposes.

Bioavailability

Orally delivered MMF is rapidly and almost completely absorbed in the stomach and upper intestine. Drug absorption as assessed by area under the curve (AUC) is not significantly altered by coadministered food, although the maximal concentration (C_{max}) is 40% lower in simultaneously fed renal transplant recipients (CellCept package insert). The time to maximal concentration (T_{max}) of less than 1 hour (CellCept package insert) is independent of hepatic or renal function. The T_{max} is slightly delayed, however, in the period immediately after transplantation (1.31 ± 0.76 hours) and in diabetic patients (1.59 ± 0.67 hours). In contrast, by 3 months, it is 0.90 ± 0.24 hours.

MMF is quickly metabolized to MPA, yielding 94% bioavailability. Over the range of 100 to 3000 mg/day, the MPA area under the concentration-time curve for 24 hours ($AUC_{0\text{-}24}$) is proportionate to dose.[21] The volume of distribution of MPA is about 4 L/kg in normal volunteers.

Metabolism

MPA is rapidly metabolized to an inactive glucuronide (MPAG) via one or more isoforms of the *UGT1* gene family of uridine diphosphate–glucuronosyl transferases in the gastrointestinal tract, liver, and possibly kidney. Two minor metabolites also are formed—the acyl glucuronide and the phenolic glucoside (see Fig. 18-3).

Clearance

The apparent elimination half-life of MPA in healthy volunteers is 17.9 hours—a clearance of 11.6 L/hr.[8] At 8 to 12 hours

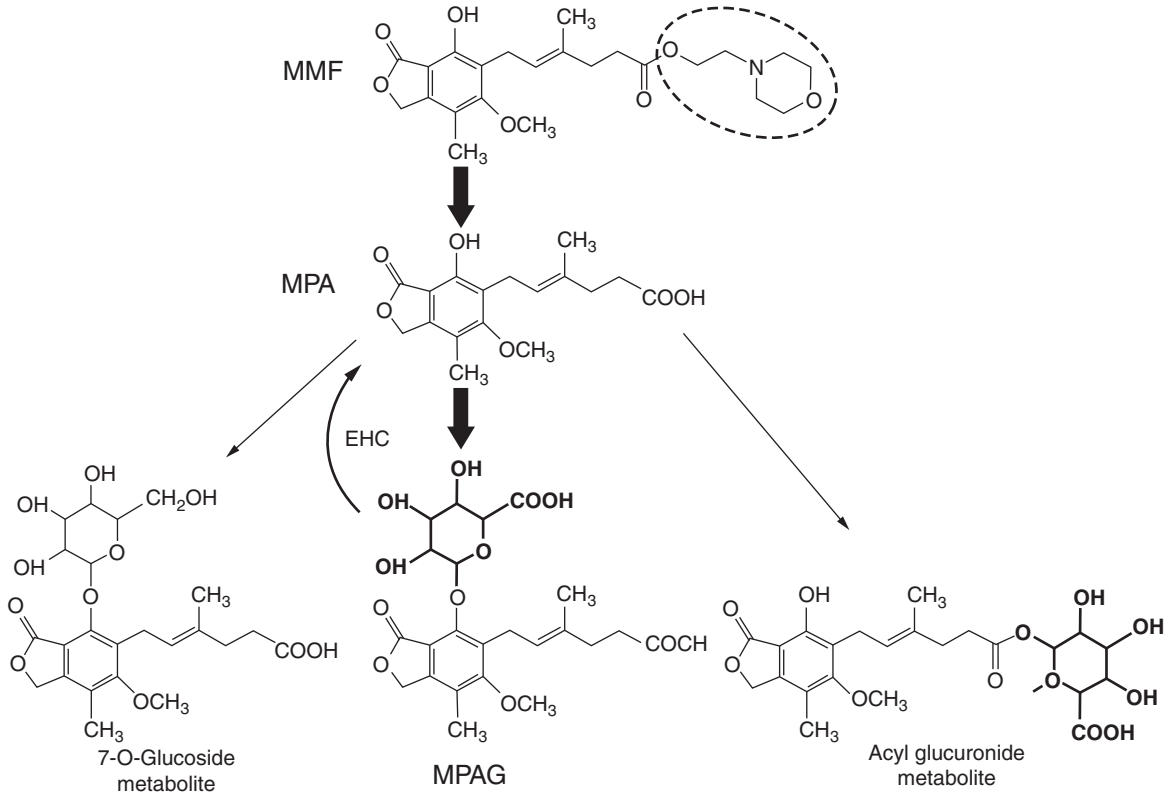

Figure 18–3 Metabolism of parent component mycophenolate mofetil (MMF) to mycophenolic acid (MPA) by cleavage of mofetil group (encircled) with primary metabolism to its glucuronide (MPAG), which may undergo enterohepatic recycling (EHC), and secondary acyl glucuronide and 7-O-glucoside metabolites. (Adapted from Shaw LM, Korecka M, Venkataramanan R, et al: Mycophenolic acid pharmacodynamics and pharmacokinetics provide a basis for rational monitoring strategies. Am J Transplant 3:534, 2003.)

after oral drug administration, 37% of patients (range 10% to 61%) display a secondary peak in plasma MPA concentrations representing enterohepatic recirculation. The additional peak results from excreted MPAG in bile undergoing deglucuronidation by intestinal bacteria with subsequent reabsorption of MPA.[21]

MPAG is the major urinary excretion product (93% of the radioactive parent compound); urinary excretion of MPA is negligible (<1%). Fecal excretion accounts for 6%. Neither hemodialysis nor peritoneal dialysis significantly affects MPA plasma concentrations, although in multiple-dose studies either dialysis method may remove some MPAG. With renal dysfunction, there is a moderate increase in plasma MPA and a marked accumulation of MPAG. MPA binds tightly and extensively (97%), but reversibly, to serum albumin, decreasing its ability to inhibit IMPDH.[101] The free fraction, constituting 1% to 3% of the total amount in the blood of stable patients, is cleared by biliary and renal routes. Hypoalbuminemia is associated with increased free MPA and greater MPA clearance. Renal dysfunction also increases the MPA free fraction because it decreases the binding of acidic drugs; in contrast, hepatic oxidative impairment produces no effect. Estimates of free MPA in ultrafiltrate samples have not been shown to offer any advantage over measurements of total MPA to predict therapeutic versus adverse reactions[8,77] except in the presence of impaired early renal function, wherein there are increased concentrations of MPAG and other metabolites.[130]

After the first few months following transplantation, drug clearance declines,[55] presumably reflecting MPA saturation of tissues. This decline may partially explain the 40% higher drug concentrations at these times compared with the concentrations observed within the first 40 days after transplantation. The increased levels are not consistently maintained after transplantation, however.

Drug-Drug Interactions

Recipients treated with cyclosporine in combination with MMF display lower MPA concentrations than do patients who either are not receiving cyclosporine or have been discontinued from the drug.[52] The reduction seems to be due to decreased biliary excretion of MPAG resulting from inhibition of multidrug resistance–associated protein-2, which is present in the canalicular membrane of hepatocytes.[73] Drug levels decreased by 40% also have been observed after coadministration of cholestyramine, which reduces the AUC of MPA, not by affecting the T_{max} but rather by affecting the absorption phase after 6 hours. Similarly, antibiotic therapy disrupting the gastrointestinal flora may interfere with deglucuronidation and enterohepatic recirculation, decreasing drug levels (CellCept package insert). Conversely, unexpectedly lower cyclosporine levels at 2 hours after oral delivery have been observed among pediatric patients concomitantly receiving MMF, possibly resulting from the diarrheal side effects of MPA.[106]

In contrast, coadministration with tacrolimus tends to increase MPA levels, primarily owing to the lack of the cyclosporine inhibitory effects, but also possibly to the inhibition of the uridine diphosphate–glucuronosyl transferase

that generates MPAG.[166] Sirolimus has neither positive nor negative effects, but the MPA levels are higher than levels observed with cyclosporine.[74] Finally, the steroid component of most regimens increases MPAG by inducing hepatic glucuronosyl transferase.[24,25] Because acyclovir and ganciclovir compete with MPAG for tubular secretion, they increase drug concentrations slightly, particularly among patients experiencing renal impairment.

PHASE I, II, AND III CLINICAL TRIALS ON PROPHYLAXIS OF ACUTE REJECTION EPISODES

Early dose-finding studies suggested beneficial therapeutic effects of MMF de novo at doses of 2 to 3 g/day in combination with cyclosporine[32] and in the treatment settings of an ongoing[138] or a steroid-resistant acute renal rejection episode.[137] Among the three pivotal trials in patients on a baseline cyclosporine-prednisone regimen, the European Mycophenolate Mofetil Study compared 2 g/day and 3 g/day doses of MMF versus placebo with no induction therapy.[98,112] The rates of biopsy-proven acute rejection episodes reported within 6 months were 17% and 13.8% versus 46.4%, respectively (Table 18-1). The U.S. Renal Transplant Study, which stipulated antithymocyte globulin antibody induction for all patients and included an azathioprine control arm, yielded corresponding acute rejection rates of 19.8%, 17.5%, and 38%, respectively.[136] The Tricontinental Study, which did not include antibody induction, but used an azathioprine comparator, yielded rates of 19.7% and 15.9% versus 35.5%, respectively.[146] All three pivotal trials documented the benefit of MMF compared with azathioprine (or placebo) to approximately halve the incidence of acute allograft rejection episodes within 6 months with equal graft and patient survivals at 12 months.

A 12-month analysis of combined data from the three studies, which included 1493 randomized subjects, confirmed the benefit of MMF on acute rejection episodes—19.8% and 16.5% versus 40.8%, yielding a relative risk ratio of 0.46.[56] Despite this benefit, however, the incidences of graft loss and death were 9.6%, 10.8%, and 12.4% (P = not significant), respectively. Longer term follow-up failed to suggest a benefit of MMF versus azathioprine or placebo in the U.S. Renal Transplant Study or Tricontinental Study, although

Table 18–1 **Efficacy of Oral Mycophenolate Mofetil in Prevention of Acute Rejection in Renal Transplantation in Randomized, Double-Blind Multicenter Trials***

Design	No. Patients	Treatment and Dosage	Time Point (mo)	Results (% of Patients) Biopsy-Proven Rejection†	All Graft Loss‡	All Graft Loss or Death
Comparisons with AZA						
U.S. Study§	167	MMF 2 g/day	6	19.8	1.8	5.5
			12		8	8.5
			36		13.4	18.9
	166	MMF 3 g/day	6	17.5	6.7	8.5
			12		11	11.5
			36		17	22.6
	166	AZA 1-2	6	38	8.6	10.4
		mg/kg/day	12		12	12.2
			36		17.1	25.3
Tri-Continental Study¶	173	MMF 2 g/day	6	19.7		
	171		12		8.8	11.7
			36		14.6	18.1
	164	MMF 3 g/day	6	15.9		
	164		12		8	11
			36		8.5	15.2
	166	AZA 100-150	6	35.5		
	162	mg/day	12		11.2	13.6
			36		15.4	19.8
Comparison with Placebo						
European Study§	165	MMF 2 g/day	6	17	4.3	6.7
			12		5.5	8.5
			36		8.7	15.2
	160	MMF 3 g/day	6	13.8	6.3	8.8
			12		7.6	10
			36		12.8	18.8
	166	Placebo	6	46.4	9	10.2
			12		9.1	11.4
			36		16	22

*All treatment regimens also included cyclosporine and corticosteroids.
†First biopsy-proven rejection (i.e., most occurred within the first 6 mo).
‡Defined as graft loss, death, or premature withdrawal from the study for any reason.
§First cadaver donor renal transplant; included induction with antithymocyte globulin.
¶First or second cadaver donor renal transplant.
AZA, azathioprine; MMF, mycophenolate mofetil.

data from the European trial revealed that the 2-g, but not the 3-g, dose of MMF reduced death-censored graft loss compared with placebo—8.7%, 12.8%, and 16%, respectively ($P = .03$).

An analysis of the outcomes of 66,774 renal recipients in the U.S. Renal Transplant Registry suggested that prescription of MMF yielded significantly better 4-year patient and graft survivals. The risk of late acute rejection episodes was reduced by 65%,[88] and the risk of chronic allograft nephropathy was reduced by 27%.[87,103] Owing to the incomplete database in this Registry, the authors had to assume a random distribution of all other nonreported factors that affect these outcomes, an assumption that has not yet been tested in multivariate fashion. This problem with Registry data markedly dilutes the observation. Further support for concern about the Registry conclusion is the observation that despite apparently widespread use of MMF, U.S.-wide renal allograft survivals have not improved, suggesting that any potential benefit has been counterbalanced by alterations in other immunosuppressants, increased recipient and donor ages, increased waiting times on dialysis, and emergence of BK viral nephropathy.

Because of the high cost of MMF relative to azathioprine, pharmacoeconomic analyses are of particular importance to determine whether the acquisition expense offsets the savings associated with a lower incidence and severity of rejection episodes. Although information from single-center analyses has been comprehensively reviewed,[163] the data from the randomized pivotal trials seem more relevant. An analysis of data from 1003 among the 1493 trial patients performed from the perspective of the French health insurance payers[82] suggested that the lower health care costs over the first 6 months of MMF treatment were substantially offset by the acquisition expense. An analysis of the U.S. multicenter trial[124] revealed similar costs and benefit for the azathioprine and the 2-g MMF cohorts, but a higher expense for the 3-g MMF dose. If one examined graft survival cost-effectiveness, the MMF combination was most effective at 1 year, but not at 10 years. At the later time, azathioprine or MMF regimens including antithymocyte globulin induction were most effective, suggesting that MMF may not be as useful in the longer term.[123] The economic implications of the Tricontinental Study for Canada were evaluated by comparing the 2-g dose of MMF with the azathioprine cohort, including drug acquisition costs. There was only a slight difference in the first year. Subsequently, the incremental costs per graft-year gained were $Can 14,268, and, per quality-adjusted life-year, $50,717, amounts that offset the acquisition fee.[68] A United States Renal Data System (USRDS) cost analysis, including an examination of the earlier-described limited database using multivariate regression tools, suggested that at 6.4 years, the average costs to Medicare with respect to graft loss were the same for MMF and azathioprine.[123] The benefit apparent at 4 years had eroded by 6.4 years.

De novo treatment with MMF augments the immunosuppressive effects of cyclosporine and steroid during the first 6 months. Thereafter, maintenance therapy with MMF is expensive. To date, the benefits have not been shown to outweigh the costs, although, as described subsequently, substantial reductions in the doses of concomitant medications in the immunosuppressive matrix may engender savings and improved outcomes. Longer term pharmacoeconomic analyses of various treatments by cooperative-group protocols with complete databases should resolve these cost-effectiveness issues.

TOXICITIES

The initial clinical trials of MMF suggested a lack of nephrotoxicity, neurotoxicity, or hepatotoxicity. The myelotoxicity and gastrointestinal side effects were reportedly "modest."[138] There was a consistent risk of an increased incidence of invasive cytomegalovirus (CMV) infections. To evaluate toxicity requires controlled clinical trials, however, recognizing that these studies tend to enroll patients in a more optimal condition than patients encountered in general transplant practice. In the pivotal trials, adverse events accounted for withdrawal of 14.7% of subjects in the MMF 3 g/day dose, 8.7% from MMF 2 g/day dose, and 5.2% from the comparator cohorts.

Gastrointestinal Adverse Reactions

The constellations of gastrointestinal symptoms are the most commonly reported adverse effects of MMF therapy, including diarrhea, indigestion with nausea and vomiting, abdominal pain, and gastroesophageal reflux. In the renal transplant setting, diarrhea can be due to a variety of causes other than immunosuppressant therapy, including preexisting diabetic or uremic conditions, intercurrent infectious diseases, and concurrent antibiotic treatment. The overall incidences of any gastrointestinal complaint were 52.5% and 45.5% versus 41.6% for the MMF 3-g and 2-g doses versus the azathioprine arms, respectively. Because diarrhea is the most frequently reported complication, colonic biopsies often have been performed; the biopsy specimens have shown apoptosis of intestinal gland epithelial cells[105] and atrophy of the intestinal villi, which in one patient was documented to disappear a few months after MMF withdrawal.[34]

Diarrhea that is persistent and not accompanied by fever may be associated with an erosive enterocolitis causing malabsorption of nutrients. Presumably reflecting the immunosuppressed state, 60% of 26 cases of diarrhea were due to an infectious origin—CMV, *Campylobacter*, or bacterial overgrowth,[84] or in another report, microsporidiosis.[54] In the other 40%, enterocolitis was characterized by faster colonic transit, crypt distortion, and focal inflammation, attributed in one study to a toxic action of the acyl MPAG metabolite on absorptive cells, leading to a predominance of goblet cells.[133] A review has suggested the potential gastrointestinal toxicity of *N*-(2-hydroxyethyl) morpholine, which is a de-esterification product of MMF that has local irritative effects on gastric mucosal cells.[68] Generally, the occurrence of these side effects more frequently has been linked to the MMF dose rather than to the plasma concentration of parent compound or its metabolites. The syndromes respond to MMF dose reduction. Although the presence of diarrhea, if anything, may reduce cyclosporine levels, it tends to enhance tacrolimus concentrations markedly.[147]

For the cohorts of MMF 2-g dose ($n = 336$), MMF 3-g dose ($n = 330$), or azathioprine ($n = 326$), the incidences of diarrhea were 31%, 36%, and 21%, respectively; of constipation, they were 23%, 19%, and 22%, respectively; of nausea, they were 20%, 24%, and 25% respectively; of dyspepsia,

they were 18%, 14%, and 14%, respectively; of vomiting, they were 13%, 14%, and 9% respectively; and for nausea, vomiting, and diarrhea the incidences were 10%, 10%, and 11%, respectively (CellCept package insert). Patients with preexisting gastrointestinal illnesses were excluded from participation in the pivotal trials. It is difficult to define precisely and categorize all gastrointestinal effects because they are of variable degree and may be delayed in onset. Although gastrointestinal effects are not life-threatening, they may seriously affect the quality of life.

An alternative, enteric-coated form of MPA—mycophenolate sodium (EC-MPS)—has been developed to mitigate the gastrointestinal toxicities. Compared with MMF, EC-MPS showed equivalent efficacy in animal models and in humans,[122] but only similar safety when administered de novo in randomized fashion or on conversion of stable patients.[19,20] Subsets of patients who displayed gastrointestinal intolerance on MMF administration required fewer dose changes of EC-MPS, however, and showed an apparently reduced symptom burden, better functioning, and improved health-related quality of life.[26] Although renal transplant patients, particularly patients experiencing these symptoms, seem to be able to be safely converted from MMF to EC-MPS, the clinician should be aware that the FDA U.S. Prescribing Information does not regard the two formulations as proved to be equivalent.

Myelosuppression

In addition to immunosuppressive drugs, the multiple causes of anemia in renal transplant patients include poor allograft function, iron deficiency, viral infections, and treatment with agents affecting angiotensin. An MMF dose-dependent occurrence of anemia was reported in a prospective pivotal trial. Patients receiving 3-g or 2-g doses of MMF versus placebo-treated control subjects showed 25.5% and 23.8% versus 13.3% incidences, respectively, of anemia or leukopenia.[98] In other pivotal trials, the bone marrow toxicity seemed to be slightly less frequent with MMF than with azathioprine.[136,146] This result may not solely reflect relative myelosuppression because the azathioprine dose is generally chosen to be the greatest one tolerable, which would predispose this cohort to cytopenia.

MMF dose and MPA predose (C_0) plasma concentrations have been correlated with decreased hemoglobin values among stable renal transplant recipients.[149] A more recent study suggested an even better correlation of anemia, however, with MPA metabolites—MPAG and acyl MPAG—than with MPA itself.[75,76] Compared with another antiproliferative agent, 87 sirolimus-treated renal allograft recipients in a single-center study experienced a greater incidence, severity, and resistance to treatment of anemia at 6 and 12 months after transplantation than did 127 patients on an MMF regimen. Similarly, sirolimus-treated patients displayed fewer instances of post-transplant erythrocytosis[10] and worse renal function.[9] These findings reinforce the major danger of combinations of sirolimus and MMF—profound anemia that is resistant to erythropoietin treatment.

Similar to other agents that interfere with cell division, MMF may produce leukopenia, which in some cases may be associated with markedly abnormal neutrophil morphology.[13] MPA plasma concentrations and particularly free MPA $AUC_{0\text{-}12hr}$ have been shown to be significantly related to severe infections and leukopenia.[59,160,161] The leukopenia has been associated with stomatitis,[44] particularly when combined with a sirolimus-based regimen, another hazard of this combination regimen.[152] In one case, failure of improvement after administration of granulocyte stimulation factor suggested to the authors that MMF produced a direct antiproliferative effect on the oral mucosa, which is subject to recurrent abrasions and to direct exposure to the orally administered MMF.[6] Similarly, the drug showed teratogenic effects at subclinical doses in animal studies,[33] suggesting caution in its use in pregnant women (U.S. FDA category C), although a successful outcome has been reported involving renal transplantation in the first trimester of pregnancy under a tacrolimus/MMF/steroid regimen.[110]

Infections

Compared with azathioprine, MMF therapy has been associated with greater incidences and severity of tissue-invasive CMV, herpes simplex and zoster, and BK virus infections. These effects may reflect its potency to impair the immune response, particularly when prescribed in combination with tacrolimus. BK infection rates of 10% have been reported from several centers and are held to be responsible for the emerging significance of this entity in renal transplant practice. Some workers have argued that MMF should be continued even during treatment of CMV disease because of its effects on critical viral enzymes. The primary antibiotics for treatment of CMV—acyclovir and related drugs—are metabolized to monophosphates and then triphosphates, which are incorporated into replicating viral DNA, irreversibly inactivating viral DNA polymerase. MPA has been suggested not only to enhance this phosphorylation of acyclovir but also to deplete the 2-deoxy-guanosine pool, inhibiting viral DNA polymerase.[100] These potential beneficial effects must be counterbalanced, however, against the hazards of unaltered immunosuppression and the general impression of a greater prevalence and severity of infection among patients treated with this drug.

MMF therapy has been reported to produce a significant increase in hepatitis C virus viremia among patients receiving concomitant cyclosporine.[120] In contrast, inception MMF seems to show no significant effect on hepatitis B virus viremia despite in vitro studies that suggested that it inhibited viral replication.[85] In contrast, an anti–*Pneumocystis carinii* effect has been noted among transplant recipients who do not require ongoing antibiotic prophylaxis.[104]

Neoplastic Diseases

Three pivotal trials involving almost 1500 patients followed for at least 1 year reported a numerical but nonsignificant increase in the incidence of lymphoma among the MMF arm compared with the placebo or azathioprine groups.[56] A large, prospective, observational cohort study that investigated this question failed to reveal an increased risk of lymphoma or malignancy associated with MMF compared with the other immunosuppressant regimens available at that time.[117] In contradistinction to the antilymphoma activity of sirolimus, however, MMF does not retard neoplastic cell division. Conversion from MMF to sirolimus is probably indicated for patients who are experiencing or are at risk for

neoplastic disease in conjunction with a regimen of minimal immunosuppression.[67]

Pulmonary Toxicity

In the refractory rejection study,[144] 5.2% of patients had to interrupt the drug because of noninfectious respiratory side effects. In a single-center experience, within 3 months after transplantation, 11.1% of deceased donor recipients experienced a nonproductive cough on administration of MMF in combination with cyclosporine and steroids.[36] In addition to cough, dyspnea and abundant sputum production, seemingly representing a bronchiectasis-like condition, have been reported to be associated with MMF treatment.[118] The findings have been attributed to impaired leukocyte recruitment leading to reduced pulmonary clearance of microorganisms. Conversion to alternative immunosuppressants seems to resolve the symptoms.

Another constellation of adverse reactions related to pulmonary fibrosis was reported in at least three cases by independent investigators. The disorder apparently is not associated with infection. Whether this condition is specific for MMF or typical of antiproliferative agents as a class is unclear. Severe diffuse pulmonary fibrosis has been known to be a reaction to drugs, including azathioprine as reported in the 1980s,[14] and sirolimus.[65]

Experimental Animal Models

Rats treated with MMF show reduced intestinal mucosal protection against invasive bacterial or toxic agents. The antiproliferative effects of MMF have been cited as the cause of impaired healing of left-sided colon anastomoses in rats,[165] a finding that has not been confirmed in humans, possibly because most surgeons would withdraw the drug in this clinical situation.

MMF treatment of Wistar rats (40 mg/kg × 21 days), using a dose equivalent to that which produces a high incidence of gastrointestinal side effects in humans, led to downregulation of four genes expressed in the liver, jejunum, ileum, and colon—polymeric immunoglobulin receptor (pIgR), major α-hemoglobin, CCAAT/enhancer protein (C/EBP-α), and catalase. PIgR, which mediates IgA and IgM transport into bodily fluids, may be a cause of diarrhea because downregulation of its expression would be expected to enhance host vulnerability to exogenous pathogens. The diminished catalase level suggests altered resistance to oxidative stress, which is known to play a role in the formation of gastric lesions and chronic ileitis and generally to predispose to gut cell apoptosis. The reduced C/EBP-α would exacerbate effects on the catalase gene because it plays an important role in the promoter region for the expression of this enzyme. Finally, the reduced expression of α-hemoglobin presumably reflects the systemic anemic state.[134] A report described the benefits of an herbal gastrointestinal relaxant to mitigate the diarrheal effects of MMF combined with the antibacterial levofloxacin: There was decreased fecal water content and bacterial flora.[159]

The appearance of gastrointestinal or myelosuppressive adverse effects generally demands progressive MMF dose reduction, seeking to determine the maximal amount acceptable to a given individual. Patients tolerating only modest, presumably subtherapeutic, doses of MMF may benefit from conversion to EC-MPS. The morbidity of the infectious and neoplastic complications depends on the aggregate intensity of the immunosuppressive regimen relative to the immunocompetence of the individual patient.

THERAPEUTIC DRUG MONITORING

Although fixed doses of MMF generally have been used in clinical therapy, there is a rationale to implement therapeutic drug monitoring for this drug.[15] Considerable interpatient pharmacokinetic variability of MMF has been documented to be due to differences in hepatic/renal functions, concurrent drug administration, and the presence of diarrhea but not to ethnicity[129] or gender.[111] At least some patients show a poor relationship between drug dose and measured C_0 plasma concentrations,[24] suggesting the benefit of AUC estimates. Finally, most available studies suggest that a concentration metric may be more useful to diagnose a rejection episode, and that the dose may correlate with adverse reactions.

Pharmacokinetic Therapeutic Drug Monitoring

A correlation between MPA AUC and the risk of early rejection has been reported to show a sensitivity of 83% and a specificity of 64%.[70,142,150] Combined with full exposure to cyclosporine, the apparent optimal MPA AUC is 30 to 60 mg/hr/L, and the most useful value of the less robust metric of the predose concentration (C_0) is 1 to 3.5 mg/L. Using a receiver operating characteristic analysis, the cutoff points for optimal benefit with MPA were AUC ($P = .001$) and C_0 ($P = .02$) values each 20% lower than those reported in previous studies.[109] Because full AUC monitoring with at least seven samples is impractical on a routine basis, abbreviated sampling strategies have been proposed (e.g., concentrations predose and at 0.67 and 2 hours after dosing). This estimate shows an r^2 correlation coefficient of 0.75, which is acceptable[42]; however, it is not sufficiently robust for routine clinical application. The most likely use of concentration monitoring is early after transplantation when absorption may be slow and incomplete, and clearance more rapid than at 3 months.

There seems to be a better relationship between adverse reactions and MMF dose rather than MPA concentrations (C_0, C_{max}, or AUC). Some investigators have claimed MPA AUC to correlate with hematologic and infectious side effects,[8] whereas others have noted predose C_0 values to be associated with anemia[24] or hematologic and gastrointestinal side effects.[133] The acyl-MPAG content has been proposed as a surrogate metric of gastrointestinal and anemic side effects.[75-77,133]

MMF has achieved a respectable position in the immunosuppressive armamentarium using uniform dosing regimens adjusted based on individual patient tolerance. Only extremely large clinical trials that show the utility of pharmacokinetic therapeutic drug monitoring are likely to change this general practice.

Pharmacodynamic Therapeutic Drug Monitoring

Global estimates of antiproliferative activity of immunosuppressants used since the azathioprine era have been, as expected, employed to assess MMF effects.[102] So many other

factors affect the proliferative activity of peripheral blood cells in this setting that their clinical utility is modest. IMPDH assays are technically demanding and difficult to reproduce; in addition, the whole blood matrix may not reflect the activated lymphocytes, which are the cells of interest. Estimates of IMPDH activity in isolated peripheral blood mononuclear cells, clearly the preferable matrix to whole blood, display considerable interindividual variability. The time course of IMPDH inhibition, as measured by the production of xanthine monophosphate by isolated mononuclear cells, parallels the MPA plasma concentration.[19,20] An alternative assay proposes measurement of inhibition of CEM cell proliferation by patient serum because this cell line is unaffected by calcineurin antagonists or steroids.[91] Patients who showed lower IMPDH levels before transplantation, suggesting a genetically determined susceptibility to the drug, more frequently underwent dosage reductions within 6 months.[114] It is unclear, however, whether levels or fluctuations in IMPDH activity can be used to predict acute rejection episodes or a tendency to drug toxicity.

Can pretransplant estimates of IMPDH activity be used to tailor MMF doses, or is there a relationship between MPA pharmacokinetic parameters and IMPDH inhibition? In the absence of a currently confirmed, robust benefit of MMF on long-term graft survival, and in view of the probably variable dose-concentration-effect relationships, one viable avenue to reform the drug regimen and achieve durable therapeutic benefits may be a rigorous pharmacokinetic and pharmacodynamic therapeutic drug monitoring approach.

IMMUNOSUPPRESSIVE DRUG COMBINATIONS WITH MYCOPHENOLATE MOFETIL

Cyclosporine (see Chapter 16)

Because the pivotal trials used the less effective oil-based form of cyclosporine, a European study employing the superior microemulsion cyclosporine formulation (Neoral) is of particular interest. This study showed only modest, insignificant reductions in acute rejection episodes with MMF compared with azathioprine, questioning the value of the highly significant 10-fold to 15-fold cost differential.[115] A European Collaborative Group described similar results among patients converted from MMF to azathioprine at 3 months compared with subjects maintained on MMF in combination with the microemulsion formulation of cyclosporine and steroids.[121] These findings in patients probably at low immunological risk should be extrapolated cautiously to other situations.

Because clinical trials generally are conducted within populations of ideal risk candidates to minimize the contributions of other factors, such as age and ethnicity, a variety of derivative analyses have been performed in these populations. Two reports have shown opposite outcomes of renal transplants among patients older than 55 years, a population putatively at reduced risk of rejection. One group[63] noted worse patient survival with MMF than with azathioprine, whereas other workers observed a significantly better outcome with MMF.[140] Critics have ascribed the findings in the latter study to the lower, probably subtherapeutic, doses of azathioprine that were prescribed for the control cohort.

A subgroup analysis of putatively higher immunological risk patients enrolled in the U.S. pivotal trial shows that the benefit for African-American versus white recipients was restricted to the MMF 3-g dose compared with the MMF 2-g dose or azathioprine cohorts—there were acute rejection rates of 12%, 32%, and 48%, respectively.[99] These findings were independently confirmed: MMF produced greater reduction in rejection risk among African-American (relative risk 0.88) than white (relative risk 0.35) recipients.[126]

MMF may have putative benefits to mitigate chronic allograft nephropathy. Despite full exposure to cyclosporine/steroid, the addition of MMF seemed to be associated with a lower incidence of chronic nephropathy than the cyclosporine/ azathioprine/prednisone cohort.[27] Patients in the USRDS registry who were maintained on MMF for at least 2 years were reported to show a 34% reduced risk of worsening renal function.[87,103] A Spanish study suggested that even when cyclosporine was not reduced,[50] MMF displayed a benefit for patients with chronic allograft nephropathy at 6 years' mean follow-up. Patients with established chronic nephropathy are unlikely to respond to MMF addition, however, without alteration of the overall regimen.[47]

Tacrolimus (see Chapter 17)

A single-center study evaluating the combination of MMF and tacrolimus plus steroids versus only the last two agents showed a decreased risk of an acute rejection episode from 44% to 27% at about 1 year.[27-29] A subsequent multicenter trial[92] reported the benefit of the MMF 2 g/day dose to reduce the incidence of acute rejection episodes compared with MMF 1 g/day dose or azathioprine treatment—8.6%, 32.2%, or 32.2%, respectively. The low acute rejection rate with the 2-g MMF dose suggested to the authors that a combination with tacrolimus produced superior results to cyclosporine. The incidences of opportunistic infections and malignances at 1 year seemed to be similar across the groups. In contrast, another study failed to show a significant benefit of tacrolimus versus cyclosporine in combination with MMF on the incidence of acute rejection episodes, graft survival, or patient survival at 2 years among the 223 enrolled North American subjects.[62] A follow-up investigation by the United Network for Organ Sharing registry of living donor renal transplantations performed in 1998 and 1999 reported in 2003 that at 2 years, there was a significantly greater risk of graft failure with the tacrolimus/MMF than with the cyclosporine/MMF combination.[22]

A single-center report described tacrolimus as obtaining superior results in combination with MMF rather than sirolimus,[27] findings that were confirmed using a steroid-free regimen.[89] This observation was confirmed in a randomized, multicenter clinical trial comparing patients treated with MMF (n = 176) versus sirolimus (n = 185) in association with tacrolimus and steroids. The MMF cohort displayed better renal function, less hypertension, and reduced hyperlipidemia.[49,89] Among patients on a tacrolimus-based regimen, improvements in renal function were observed in 19 patients converted from sirolimus to MMF compared with 78 recipients remaining on sirolimus.[9]

Sirolimus (see Chapter 19)

Based on a primate study suggesting a supra-additive antirejection effect[157] and a rat study supporting prophylaxis of chronic renal allograft rejection,[11] the combination of MMF

and sirolimus seemed to be useful, even though they are both antiproliferative agents. An initial clinical study suggested that the combination of MMF plus sirolimus was at least as effective to prevent acute renal allograft rejection as MMF plus cyclosporine.[74] Because the actual incidence of treated acute rejections was greater than 30% in both arms of this study, the addition of basiliximab to the regimen was investigated as a means to enhance the immunosuppression. The initially favorable results at a single center[39] were not confirmed in a multicenter trial, which had to be prematurely terminated because of a high incidence of acute rejection episodes. The two antiproliferative agents show overlapping toxicities—anemia and diarrhea—and prohibitively high acquisition costs of each component of the regimen. These problems argue against prescription of MMF/sirolimus except for special situations, such as delayed renal graft function or possibly documented calcineurin inhibitor–induced vasculopathy in a patient at high immunological risk.

Antibodies (see Chapter 20)

The two anti–interleukin-2 receptor monoclonal antibodies—humanized daclizumab and chimeric basiliximab—have been tested in conjunction with calcineurin inhibitor/MMF/steroid protocols. In a randomized trial, addition of basiliximab to a regimen of cyclosporine/MMF/steroid produced a trend toward a reduced incidence of acute rejection episodes without augmented toxicity.[80] In conjunction with cyclosporine/MMF/steroid, even a two-dose regimen of daclizumab seems to reduce the acute rejection rate. Compared with a historical control group who received OKT3 induction therapy, the 305 renal recipients treated with tacrolimus/MMF/steroids plus five doses of daclizumab displayed a lower acute rejection rate (2% versus 7%; $P = .01$) and fewer infections, but there was no difference in patient or graft survival.[28] The regimen of daclizumab/tacrolimus (C_0 8 to 12 ng/mL)/MMF (1 g twice a day)/steroids yielded similar results in putatively high-risk African-American renal recipients and in patients of other ethnicities.[29]

The sole administration of the humanized anti-CD52 monoclonal antibody at a dose of 0.3 mg/kg produces profound depletion of T lymphocytes and, to a lesser extent, B lymphocytes. Despite these effects, it is inadequate immunosuppression as monotherapy for renal transplantation, however. The reagent did display beneficial effects in 44 recipients when combined with MMF (500 mg twice a day) and tacrolimus (C_0 5 to 7 ng/mL) but no steroid, although these patients experienced severe leukopenia.[28]

USE OF MYCOPHENOLATE MOFETIL TO POTENTIATE, MINIMIZE, OR AVOID PRESCRIPTION OF OTHER IMMUNOSUPPRESSANTS

Reversal of Acute Rejection Episodes

To reverse acute rejection episodes among 150 subjects treated with cyclosporine/steroid, MMF was reported to achieve a 45% benefit compared with a steroid regimen including five daily intravenous boluses followed by a tapered oral steroid dosing, which was more effective than azathioprine/steroids.[96] Despite a promising initial result, a 3-year analysis of the outcomes of 221 patients failed to confirm any benefit of MMF in this setting.[137] A multicenter study suggested that inception of MMF may be useful to ameliorate steroid-resistant acute rejection episodes.[137]

Reduction in Calcineurin Inhibitor Exposure

The use of MMF to achieve cyclosporine or tacrolimus dosage reduction has been generally accepted as a means to moderate immunosuppression and decrease drug-related nephrotoxicity after 6 months post-transplantation. This maneuver resulted in improved serum creatinine values.[2,108] A controlled trial documented the benefit of a 50% reduction versus no reduction in cyclosporine C_0 in terms of improved creatinine clearance, uric acid, blood pressure, and triglyceride values.[35] Another publication observed that low MMF doses (500 to 1000 mg/day) were sufficient to facilitate calcineurin inhibitor reduction with consequent improvement in renal function and decreased TGF-β levels.[60] Under the cover of MMF coadministration after 3 months, modest-to-moderate reductions in calcineurin inhibitor exposure (cyclosporine C_0 100 to 150 ng/mL; tacrolimus C_0 5 to 7 ng/mL) seem to be generally well tolerated by patients who have been previously free of rejection episodes.

Withdrawal of Calcineurin Inhibitor after Transplantation

Some studies have sought to eliminate cyclosporine from the maintenance regimen to avert chronic nephrotoxicity. One multicenter study of patients with deteriorating renal function at a mean of 6 years after transplantation documented significantly improved renal function at 6 and 12 months after stepwise withdrawal of cyclosporine.[141] A single-center randomized prospective study initiated at a mean 7 years post-transplantation compared 20 patients in an MMF/calcineurin inhibitor continuation arm with 19 patients in an MMF/calcineurin inhibitor withdrawal arm, showing improved renal function and blood pressure without an episode of acute rejection.[135] Discontinuation of cyclosporine at 1 year after transplantation resulted in a significant improvement in mean serum creatinine values at 8 months thereafter for the azathioprine and the MMF cohorts, although the latter group experienced fewer acute rejection episodes.[135] Replacement of cyclosporine with MMF after 6 months was successful in 15 of 17 patients who tolerated the drug, and renal function improved.[126]

A multicenter study compared the 1-year outcomes of withdrawal at 3 months of either cyclosporine ($n = 44$) or MMF ($n = 40$) from a three-drug regimen including steroid therapy.[125] Withdrawal of cyclosporine was associated with better creatinine clearances, decreased blood pressures, and more favorable lipid profiles despite a twofold increase in acute rejection episodes. Although 5-year data are needed before one can judge the benefits versus hazards of calcineurin inhibitor withdrawal, provisionally one may conclude that the maneuver is relatively safe in stable patients at low immunological risk, offering benefits on creatinine and lipid levels.

De novo Avoidance of Calcineurin Antagonists

Although the initial multicenter trials documented the efficacy of MMF in combination with full doses of cyclosporine, the

adverse effect of progressive nephrotoxicity associated with calcineurin inhibitor therapy has led to studies to avoid cyclosporine de novo. Using a five-injection daclizumab induction protocol and an MMF/steroid maintenance regimen in 98 low-risk recipients of cadaver and living donor kidneys, acute rejection episodes were observed in 53% of recipients within 12 months, requiring institution of calcineurin antagonists.[155] A smaller study confined to recipients of living donor kidneys failed to observe a substantial benefit.[145] In contrast, a single-center report described 12 patients older than 50 years who received grafts from elderly donors who were successfully treated de novo with MMF and steroids and an induction regimen of rabbit antithymocyte globulin.[164]

For avoidance of calcineurin antagonists, MMF has been employed with steroids in conjunction with sirolimus,[74] basiliximab/sirolimus,[39] or LEA29 (Belatacept)/basiliximab,[156] yielding vastly different acute rejection rates during the first 6 months. Even when there was an early effect, however, the durability of the immunosuppressive protection is doubtful. In a Spanish study, 65% of MMF-treated patients remained on an avoidance regimen at 12 months, but only 36% were free of these drugs at 60 months.[53] Although a large randomized trial of the basiliximab/sirolimus/MMF/ prednisone regimen has been discontinued because of an excessive occurrence of acute rejection episodes, two pivotal studies of the LEA29/basiliximab/MMF/prednisone combination are ongoing. The use of sirolimus in addition to MMF as the base for de novo immunosuppressive therapy has been widely employed, although one retrospective analysis of the U.S. Renal Data System noted an increased incidence of delayed graft function with this regimen.[127] However it was unclear how patients were selected for the SRL-MMF combinations; it is likely that they were at increased risk for DGF based on extended donor criteria or operative findings.

Steroid Avoidance or Withdrawal

A large multicenter trial of steroid withdrawal at 3 months after transplantation using a cyclosporine/MMF/prednisone regimen documented an increased risk of rejection episodes resulting in early termination of the study.[107] In contrast, steroid withdrawal beginning at 3 months after transplantation showed no significant difference in rejection rates versus continued steroid therapy among European patients prescribed MMF/tacrolimus[139] or MMF/cyclosporine with antibody induction.[153] Although the superior results of the latter trial may be attributed to prescription of more potent baseline therapy, it is more likely due to the fact that the European cohorts generally show a low immunological risk compared with African-Americans, who displayed most of the failures in the original trial. An analysis of multiple studies in low-risk patients suggested a benefit of MMF to permit steroid withdrawal after 1 year.[79]

Early withdrawal at day 5 post-transplantation in conjunction with antithymocyte globulin induction treatment combined with an MMF/cyclosporine regimen yielded acceptable results at 3 years in a single-arm, single-center analysis.[69] A controlled trial of early withdrawal versus persistent therapy confirmed the safety of the approach using basiliximab as opposed to antithymocyte globulin induction.[154] There were no significant differences between the outcomes of withdrawal at 3 days versus 4 months using a daclizumab/MMF/cyclosporine regimen.[143]

Complete steroid avoidance has been reported in 100 renal recipients in conjunction with antithymocyte globulin induction plus cyclosporine/azathioprine. The cohort displayed a 13% incidence of acute rejection episodes and an 82% rate of graft survival at 4 years.[16] Similar findings were obtained using daclizumab induction with tacrolimus/MMF maintenance therapy: 89% of patients were steroid-free at 6 months.[119]

There seems to be less evidence for a unique benefit of steroids within the immunosuppressive matrix. Adjusting other therapeutic components readily compensates for their avoidance or for their early withdrawal.

Discontinuation of Cyclosporine and Prednisone

After a prolonged period of quiescence, withdrawal of cyclosporine and prednisone from a combination with MMF has been described in a pilot trial.[61,86] On the one hand, there was a 10.9% risk of acute rejection episodes; on the other hand, the maneuver resulted in improved serum creatinine and lipid levels within 1 year. Similar findings have been reported in a multicenter study.[116] At present, there is no longer term follow-up of patients on MMF monotherapy, however, beyond an anecdotal comment in a review.[79]

SUMMARY

Although MMF has been widely accepted as a component of de novo and maintenance therapy in renal transplantation, the drug has the potential for use in other settings of inflammatory disorders. MMF may mitigate the development of anti-HLA antibodies among transfused chronic kidney disease patients. In a parallel setting, administration of MMF (at concentrations therapeutic for heart transplant recipients) reduced the amounts and shortened the persistence of anti–HLA antibody responses by children receiving allografts for repair of congenital heart defects. MMF may have a role in suppressing the production of anti–blood type antibodies after renal transplantation across the ABO blood barrier.[69]

Far broader applications of MMF would be in autoimmune kidney diseases. Because many of these entities are either resistant to or relapse on initial treatment, intense salvage therapy is frequently necessary. The conventional regimen of intravenous cyclophosphamide and steroid boluses is associated with concomitant adverse effects of infertility, alopecia, bladder problems, and infections. Among the autoimmune diseases, systemic lupus erythematosus (SLE) is the one most often reported to display beneficial effects after MMF treatment. Based on results in a murine model[154] and single-center reports,[16,119,143] a multicenter, randomized U.S. trial is under way to compare MMF with a regimen of intravenous boluses of steroid and cyclophosphamide in 140 lupus nephritis patients with biopsy-proven diffuse proliferative disease. At 6 months, there were fewer treatment failures in the MMF arm.[78] If these findings are confirmed on extended follow-up, it may be useful to examine whether MMF benefits other renal diseases of putatively autoimmune etiology, as reviewed more recently.[78]

MMF has become a component of many immunosuppressive regimens in renal transplantation because of its ease

Table 18–2 **Potential Algorithms of Induction Regimens Using Mycophenolate Mofetil: 7- to 14-Day Treatments**

Host	Donor	MMF	Thymo	αIL-2	CNA	SRL	Steroid
High*	High†	3 g	+	0	0	High‡	Yes
	Low	2 g	+	0	Moderate§	0	Yes
Low	High†	2 g	0	+	0	Moderate‡	Yes
	Low	1-2 g	0	+	Low§	0	No

*High-risk recipient: retransplantation, African-American, or panel reactive antibody >25%.
†High-risk donor: >60 years old, hypertensive, and cerebrovascular disease as cause of death; storage >36 hours; or adverse procurement conditions, including oliguria or hypotension.
‡SRL exposure: High: 10-15 ng/mL.
§CNA exposure: moderate, tacrolimus C_0 7-10 ng/mL, cyclosporine C_0 200-250 ng/mL; low, tacrolimus C_0 2-5 ng/mL, cyclosporine C_0 100-150 ng/mL; moderate, 5-8 ng/mL.
αIL-2, anti-CD25 monoclonal antibody; CNA, calcineurin antagonist; MMF, mycophenolate mofetil; SRL, sirolimus; Thymo, rabbit antihuman immunoglobulin.

of oral administration (1- to 3-g doses daily) without mandatory monitoring of plasma concentrations. Because MMF seems to be an agent of moderate potency—less potent than calcineurin inhibitors or sirolimus (but more potent than azathioprine or steroids)—it has been used successfully more often in association with other immunosuppressants, particularly agents that do not produce overlapping side effects of gastrointestinal disturbances, anemia, or leukopenia. The increased incidence or severity of viral infections observed with regimens including MMF seems to relate largely to the coadministered immunosuppressants.

Table 18-2 shows some possible regimens for induction immunosuppression that include MMF. The regimens are classified according to the risk status of the host and of the donor graft. High immunological responders benefit from rabbit antithymocyte globulin treatment. In the event of likely, or of documented, delayed or slow graft function—high donor risk—sirolimus comedication should be considered to achieve adequate immunosuppression, despite the potential problems with wound healing. For recipients of kidneys at low risk of dysfunction, reduced doses of calcineurin antagonists are preferable to mitigate their nephrotoxicity. The low-immunological-risk patient is frequently induced with an anti–interleukin-2R monoclonal antibody, accompanied in the high donor risk situation with sirolimus or in the low donor risk setting with a low dose of calcineurin antagonist.

In the maintenance phase, inception or intensification of the MMF regimen may facilitate a reduction in or elimination of the more potent coadministered agents. Among weak immune responders free of rejection after transplantation, there is the potential for steroid withdrawal, with modest exposures to calcineurin antagonist or sirolimus, depending on the renal function (Table 18-3). MMF monotherapy may represent a useful option in special cases, including elderly recipients, patients free of rejection for years, HLA-identical matches, or subjects intolerant of any other drug.

The high immunological responder presents a greater challenge because of the need to maintain a robust level of immunosuppression. Calcineurin antagonists remain the central agent in this setting; although steroids are normally continued, they have been withdrawn particularly in patients experiencing post-transplant diabetes mellitus, osteopenia, or another profound side effect. Patients with impaired renal function usually associated with chronic allograft nephropathy are frequently intolerant of calcineurin antagonists, however. Inception of sirolimus therapy early in the course when the creatinine increase does not exceed 3.5 mg/dL represents a potential, albeit as yet unproved, strategy to stabilize or possibly ameliorate dysfunction.

Several areas remain to be explored further. First, more efficient methods of therapeutic drug monitoring than AUC to estimate parent compound or metabolite concentrations need to be developed and correlated with

Table 18–3 **Potential Algorithms for Maintenance Immunosuppressive Regimens Using Mycophenolate Mofetil**

Host Risk	Renal Function	MMF	CNA	SRL	Steroid
High†	≥50 mL/min	2 g	Moderate‡	0	Yes
	<50 mL/min	2 g	0	Full§	Yes
Low*	≥50 mL/min	1 g	Low‡	0	No
	<50 mL/min	2 g	0	Reduced§	No

*Refers to patients who did not experience a prior rejection episode.
†High-risk recipient: retransplantation, African-American, or panel reactive antibody >25%.
‡CNI exposure: moderate, tacrolimus 2-5 ng/mL, cyclosporine C_0 75-150 ng/mL; low, tacrolimus C_0 approximately 2 ng/mL, cyclosporine C_0 50-75 ng/mL.
§SRL exposure: full, C_0 8-12 ng/mL; reduced, C_0 3-5 ng/mL.
CNA, calcineurin antagonist; MMF, mycophenolate mofetil; SRL, sirolimus.

pharmacodynamic assays. Second, multicenter studies need to be performed to yield quantitative clinical data on the outcomes of various MMF-based drug combinations that are tailored to provide optimal effects at minimal exposures in various patient cohorts, including the elderly, the mixed ethnic, and retransplantations. Finally, long-term, randomized, biopsy-based trials must be designed to show the potential of MMF for protective effects against the progression of chronic allograft nephropathy. Applications of 21st century molecular tools in the clinical setting are likely to improve the already excellent renal allograft outcomes obtained with MMF.

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Chapter 19

mTOR Inhibitors: Sirolimus and Everolimus

Christopher J. E. Watson • J. Andrew Bradley

Sirolimus and everolimus are closely related members of a relatively new class of potent immunosuppressive agents that impair T cell proliferation by inhibiting the mammalian Target of Rapamycin (mTOR). Sirolimus and its newer analogue everolimus have undergone extensive clinical evaluation during which they have shown potency as immunosuppressive agents after kidney and other types of solid organ transplantation. Much has been learned about their efficacy in preventing acute rejection and their side effects. Because sirolimus and everolimus are similar in their mode of action and clinical efficacy, it is convenient to consider them collectively under the term mTOR inhibitors. This consideration inevitably biases discussion toward sirolimus rather than its newer alternative everolimus, however, because most of the published literature on mTOR inhibitors in renal transplantation relates to sirolimus. Despite the many similarities of sirolimus and everolimus, significant clinical differences between the two agents may emerge.

DISCOVERY

Sirolimus (AY-22989, rapamycin, Rapamune) is a fermentation product of *Streptomyces hygroscopicus*, a microorganism first isolated from soil samples taken from Easter Island, known locally as *Rapa Nui*.[53] The geographical origin of the microorganism led the Ayerst company (now incorporated into the Wyeth company) to name the drug rapamycin. It was first investigated for its potential as an antifungal agent[7,125,140] and was found to inhibit tumor cell growth[41,57] and to reduce lymphocyte proliferation.[97] Sirolimus was evaluated further as an immunosuppressive agent in animal models of transplantation, but was noted to cause a lethal vasculitis in the dog renal transplant model,[16] hitherto considered to be one of the most reliable preclinical models for evaluating immunosuppressive agents. Interestingly in the light of subsequent clinical findings, the same authors also found a high incidence of interstitial pneumonitis in pigs after kidney transplantation.[16] The findings of drug-induced vasculitis delayed further clinical evaluation of sirolimus. Tacrolimus, which shares a marked structural similarity with sirolimus (Fig. 19-1), also causes a vasculitis in the dog,[24] but when used in humans in 1989 there was no sign of such toxicity.[133] The promising early clinical results with tacrolimus helped lead to a resumption in the clinical evaluation of sirolimus.

As the potential of sirolimus as a clinical immunosuppressive agent became apparent, other companies looked for similar compounds. Everolimus (RAD001, SDZRAD, Certican) was synthesized by chemists at Novartis, who made a 2-hydroxyethyl chain substitution at position 40 of the sirolimus structure (see Fig. 19-1) and created a molecule with improved oral availability.[123]

MECHANISM OF ACTION

After entering into cells, mTOR inhibitors bind to one of a family of immunophilins called FK506-binding

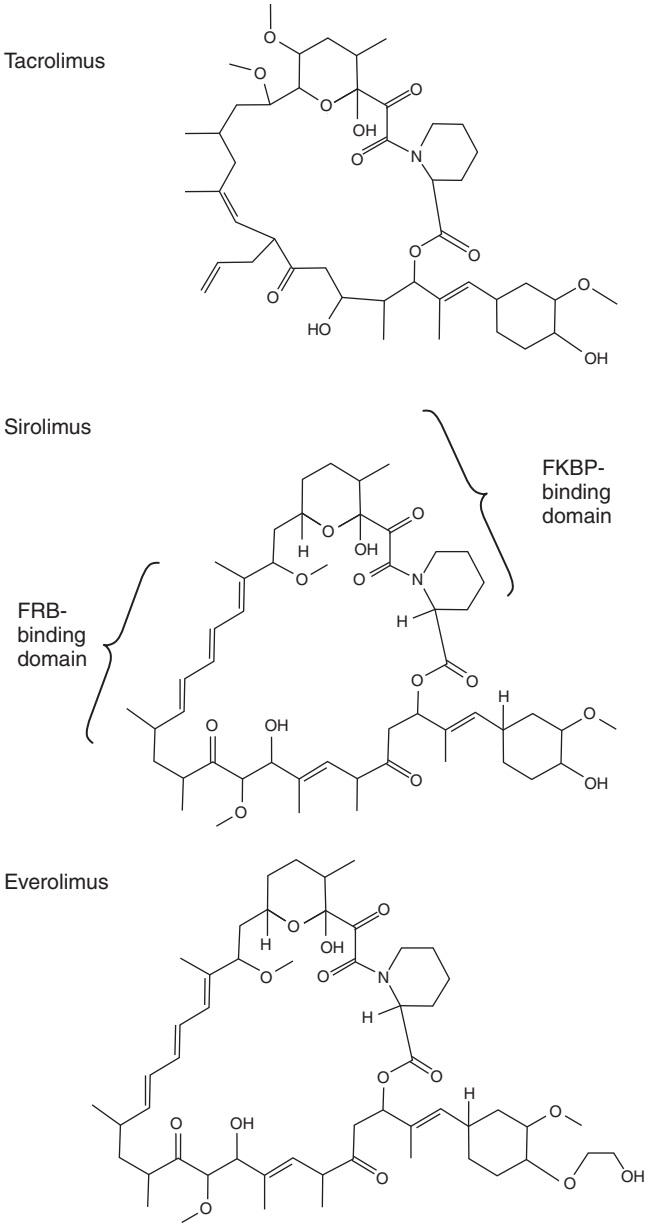


Figure 19–1 Structure of tacrolimus, sirolimus, and everolimus. Sirolimus and everolimus are macrocyclic lactones with structural similarity to tacrolimus (FK506, Prograf). Everolimus has a 2-hydroxyethyl chain substitution at position 40 of the sirolimus structure. All three molecules have a common area that binds to a family of intracellular carrier proteins, the FK506 binding proteins (FKBPs), in particular the 12-kD protein FKBP12.

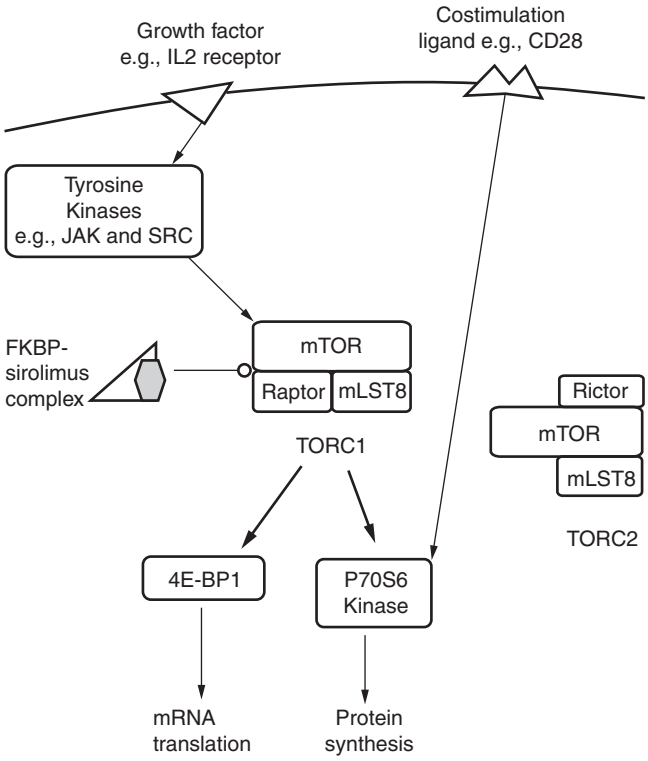


Figure 19–2 Highly simplified schematic representation of the mechanism of action of mTOR inhibitors. The mTOR inhibitors sirolimus and everolimus form an intracellular complex with FKBP12, and this complex inhibits the function of the TORC1 complex, possibly by preventing association of Raptor. TORC1 is important for cell proliferation in response to growth factor stimulation and regulates the S6K1 response to stimulation via the CD28 ligand in T cells. mTOR also forms the TORC2 complex, which is resistant to sirolimus and everolimus and is involved in cytoskeleton control.

proteins (FKBPs), particularly the 12-kD FKBP12 (Fig. 19-2). Immunophilins are protein chaperones with peptidylprolyl cis/trans isomerase activity. FKBPs are cytosolic proteins present in abundance in the cytoplasm.[46] The sirolimus-FKBP12 or everolimus-FKBP12 complexes with mTOR, previously known variously as FKBP-rapamycin associated protein, the Rapamycin and FK506 Target, and the sirolimus effector protein.[55] mTOR is a serine-threonine kinase that acts as a scaffold for the binding of other proteins and is a key component of the cell cycle regulatory signaling pathway.

Two mTOR complexes (TORC1 and TORC2) have been recognized, with different proteins binding to the TOR scaffold; only TORC1 is sensitive to sirolimus.[59,150] In mammals, TORC1 is involved in regulation of cell growth. TORC1 comprises mTOR, mLST8 (also known as GβL), and Raptor, and this complex controls activation of a p70 S6 kinase (S6K1), which is involved in regulating protein synthesis and mRNA translation, and eukaryotic initiation factor 4E binding protein 1, which also is necessary for translation and protein synthesis.[43,78,114] mTOR seems to be the catalytic subunit of the TORC1 complex, whereas Raptor is involved in substrate recognition. The role of TORC2 in mammalian cells is not clearly elucidated, but it is resistant to sirolimus and comprises mTOR, mLST8, and Rictor. It is probably involved in regulation of cell morphology and the cytoskeleton.

In mammalian cells, regulation of TORC1 signaling occurs in response to growth factors, cytokines, nutrients (especially amino acids), energy status (e.g., adenosine monophosphate-to-adenosine triphosphate ratio), and stress (e.g., hypoxia)—all factors that would be expected to regulate cell growth and proliferation. In lymphoid cells, the important signals originate from the cell surface and are generated by cytokine-receptor binding, such as the binding of interleukin-2 to the interleukin-2 receptor complex, or ligand binding to coreceptors such as CD28. When cell surface receptors are stimulated, kinases such as janus kinase 3 are activated, and the ensuing signaling cascade results in activation of TORC1. In the same way that calcineurin is

a rate-limiting step in gene transcription after activation of the T cell receptor complex, TORC1 is the rate-limiting step in the proliferative response to cytokine and coreceptor binding.

Sirolimus binds to the FKBP-rapamycin binding (FRB) domain on mTOR,[22] and binding to FRB is enhanced 2000-fold when sirolimus is complexed with FKBP12.[9] How binding of sirolimus to the FRB domain affects mTOR is unclear, but it may block binding of regulatory proteins such as Raptor, part of the TORC1 complex.[113] Blockade of mTOR results in inactivation of S6K1 and 4EBP and inhibition of CD28-mediated downregulation of IκBα, a regulatory protein that mediates upregulation of interleukin-2 transcription.[79] The result is cell cycle arrest in late G_1 phase.[138] In addition to its effects on lymphocytes, sirolimus may have a direct inhibitory effect on dendritic cells, inducing apoptosis through interaction with growth factor signaling.[105,149] It also may impair neutrophil responses by inhibiting neutrophil migration in response to chemoattractants.[50] Finally, sirolimus also inhibits cytokine-stimulated and growth factor–stimulated proliferation of smooth muscle cells, fibroblasts, and tumors in vitro and in animal tumor models.[3,19,41,57,98] These effects are of potential clinical importance in the context of chronic allograft nephropathy, in which arterial smooth muscle and fibroblast proliferation are major pathological features.

PHARMACOKINETICS

Sirolimus

Sirolimus is marketed in tablet form and as an oral solution. After absorption, it is extensively bound to blood cells, particularly erythrocytes, and less than 5% of the drug remains free in the plasma, where it is associated with the nonlipoprotein fraction.[151] It has a long half-life of about 60 hours in renal transplant patients (tacrolimus has a half-life of 18 to 20 hours), with rapid absorption time to maximal concentration at 1 to 2 hours) and exposure that is proportional to dose, but with a large intersubject coefficient of variance [CV] = 52%) and significant intrasubject variability (CV = 26%).[42,88] The pharmacokinetic profile of the tablet formulation and liquid formulations of sirolimus are similar apart from a lower maximal concentration with tablets.[70] With both formulations, the total drug exposure (area under the concentration-time curve [AUC]) correlates well with maximal concentration and trough concentration. Similar to cyclosporine and tacrolimus, the pharmacokinetics of sirolimus differ in different ethnic groups, with reduced oral bioavailability in African-Americans.[31]

Everolimus

Everolimus is more water-soluble than sirolimus, and this increases its bioavailability. In studies of single doses of everolimus capsules in renal transplant recipients, the drug was shown to have a much shorter half-life than sirolimus (16 to 19 hours), a rapid absorption (maximal concentration reached within 3 hours), and a good correlation between trough and AUC.[64] Similar to sirolimus, there is significant intersubject (85%) and intrasubject (41%) variability in AUC.[75] As with sirolimus, ethnicity affects everolimus pharmacokinetics, with a higher dose requirement in African-American patients.[74] Administration of everolimus does not seem to affect cyclosporine pharmacokinetics.[12,75]

Pharmacogenetics

Metabolism of sirolimus is by the cytochrome P-450 (CYP) 3A group of enzymes, particularly CYP3A4 and, to a lesser degree, CYP3A5. Polymorphisms of these enzymes are common and owing to linkage disequilibrium (the genes lie adjacent on chromosome 7q21) may occur together. Polymorphisms of CYP3A enzymes are associated with loss of function and seem to result in lower drug concentration-to-dose ratios in patients expressing the least common genotypes.[5,83] This genetic variability is an argument against fixed-dose administration of mTOR inhibitors and favors instead concentration-controlled dosing.

Drug Interactions

Similar to most immunosuppressive agents, and as noted previously, sirolimus and everolimus are metabolized primarily by CYP3A4, and their metabolism is altered by drugs that affect this enzyme pathway. Important among these are the calcineurin inhibitors, particularly cyclosporine, which can increase the concentration of sirolimus with a reciprocal increase in cyclosporine concentration; these effects are not observed in single-dose studies, but are apparent after multiple doses. This drug interaction is particularly noticeable when the time interval between sirolimus and cyclosporine ingestion varies, with cyclosporine markedly increasing the bioavailability of sirolimus.[152] It is important that patients receiving sirolimus and cyclosporine adhere to a standard pattern of medication and do not vary the interval between taking the two agents. Conversely, sirolimus reduces the exposure to tacrolimus when the two drugs are coadministered.[8] Other groups of drugs with important interactions with the CYP pathway are the antimicrobials (especially fluconazole and erythromycin) and the 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins), both of which are widely used in renal transplant recipients.

mTOR inhibitors also differ from cyclosporine in the way they interact with the other immunosuppressive agents. Patients taking sirolimus have a much higher exposure to mycophenolic acid, the active constituent of mycophenolate mofetil (MMF), than do patients taking cyclosporine and MMF.[11,38] It has been suggested that patients receiving 1 g twice daily of MMF while taking cyclosporine should have the dose of MMF reduced to 750 mg twice daily when they convert to sirolimus to maintain the same exposure to mycophenolic acid. A similar drug interaction is recognized with tacrolimus. Sirolimus also has been observed to cause a reduced exposure (lower AUC) to prednisolone compared with cyclosporine.[61]

USE OF mTOR INHIBITORS

mTOR inhibitors have been evaluated for use in renal transplantation as an addition to calcineurin inhibitor–based therapy and as a substitute for calcineurin inhibitors. mTOR inhibitors also have been used as de novo treatment from the time of renal transplantation, as a later addition to calcineurin inhibitors to enhance immunosuppression in response to acute rejection, and as a substitute for calcineurin

inhibitors to treat calcineurin inhibitor toxicity in the maintenance phase.

Early in vitro and in vivo studies suggested that mTOR inhibitors and calcineurin inhibitors when used together had a synergistic immunosuppressive effect.[62,71,135] Initially, it was envisaged that mTOR inhibitors might be best used along with calcineurin inhibitors to exploit this synergistic immunosuppressive effect, optimizing immunosuppression and minimizing agent-specific side effects. Evidence from rodent studies suggested, however, that sirolimus may exacerbate cyclosporine nephrotoxicity,[4] a finding that was subsequently confirmed in clinical studies.[66] Most of the initial work with sirolimus was done in conjunction with cyclosporine rather than tacrolimus because it was believed that competition for the FKBP12 immunophilin would preclude the coadministration of tacrolimus and sirolimus. It has become evident, however, that there is an abundance of FKBP12 in the cytoplasm, and in vitro studies suggest that less than 5% of the available FKBP needs to be bound to cause half-maximal immunosuppression.[33] Tacrolimus and sirolimus can be administered simultaneously at therapeutic doses in humans without significant competition for FKBP12.[143]

De novo Therapy with mTOR Inhibitors in the Absence of Calcineurin Inhibitors

Sirolimus has been investigated in numerous studies where it was the principal immunosuppressant. The first such studies were phase II trials conducted in Europe that examined sirolimus when used in a concentration-controlled manner, rather than when given at a fixed dose. When sirolimus was administered as a component of triple therapy with azathioprine and prednisolone, it was associated with a similar incidence of acute rejection to that observed in patients on the Sandimmune preparation of cyclosporine (41% versus 38% at 12 months).[51] A follow-up study substituted azathioprine with MMF and showed no significant difference in the incidence of acute rejection between sirolimus and cyclosporine, although there were numerically more acute rejection episodes in the sirolimus arm (27.5% versus 18.5%).[76] Patient and graft survival were similar in the two study groups, although the studies were insufficiently powered to detect small differences. Pooled data from both studies showed significantly higher glomerular filtration rates in patients receiving sirolimus.[106] These two early studies provided the first detailed insight into the toxicity profile of sirolimus in humans, and suggested side effects different from those associated with calcineurin inhibitors (Table 19-1).

Subsequent studies further explored the use of sirolimus with MMF, together with anti-CD25 monoclonal antibody induction therapy. Early data suggested that the combination of sirolimus and MMF was superior to a cyclosporine-based regimen.[44] A more recent randomized trial comparing sirolimus and tacrolimus (each given along with MMF and prednisolone) showed the two regimens to be comparable in terms of acute rejection rate and graft function.[82] A registry analysis suggested, however, that renal allograft recipients treated with a combination of sirolimus and MMF had a higher acute rejection rate and reduced allograft survival compared with recipients receiving alternative immunosuppressive regimens.[129] Later reports from two large-scale trials (ORION[129] and SYMPHONY[37a]) suggest that the combination of sirolimus and MMF is inferior to low-dose tacrolimus and MMF–based triple therapy. Finally, a systematic review of randomized trials in which mTOR inhibitors were used in place of calcineurin inhibitors as initial therapy after kidney transplantation (eight different trials with a total of 750 participants) revealed that there was no difference in the incidence of acute rejection at 1 year, but the level of serum creatinine (a possible surrogate end point for long-term graft survival) was lower in patients receiving mTOR inhibitors.[147]

De novo Combination Therapy with mTOR Inhibitors and Calcineurin Inhibitors

One of the first studies of sirolimus in renal transplantation to be performed was a dose-ranging study that combined different doses of sirolimus (given as a fixed dose) in conjunction with high-dose or low-dose Sandimmune cyclosporine (concentration controlled).[63] All groups received steroids but no azathioprine or MMF. Small numbers of patients in the study and an unequal distribution of African Americans between the six study groups meant that the results were difficult to interpret. Nevertheless, the study showed that the combination of sirolimus and cyclosporine was more potent than cyclosporine alone in the prevention of acute rejection and that half-dose cyclosporine and sirolimus was as efficacious as full-dose cyclosporine and sirolimus. The higher incidence of acute rejection seen in African Americans in this study also was observed in subsequent studies.[65] The other important finding to emerge from this study was a high incidence of *Pneumocystis* pneumonia in sirolimus-treated patients, mostly in patients from one center where routine prophylaxis against *Pneumocystis jirovecii* was not given.

Two large phase III studies of sirolimus followed shortly afterward, one conducted in the United States[65] and the second worldwide (Table 19-2).[89] Similar to the earlier studies, these studies used a fixed dose of sirolimus (2 mg/day or 5 mg/day) in combination with concentration-controlled cyclosporine. In the U.S. study, the two different doses of sirolimus were compared with azathioprine, and all groups received steroids but no induction therapy.[65] Only patients with functioning renal allografts were recruited, in contrast to the global study in which function of the graft was not a prerequisite for enrollment.[89] The other major difference between the U.S. and the global study was that the comparator in the global study was placebo rather than azathioprine. Both studies showed a clear benefit in terms of reduction in the acute rejection rate for patients receiving sirolimus, an effect that was more marked in patients receiving a higher dose of sirolimus. There was a difference in acute rejection rates, patient survival, and graft survival in the U.S. study compared with the global study in all treatment arms, which likely reflects the different enrollment requirements, with only recipients with functioning grafts being entered into the U.S. study.

These two pivotal studies in the development of sirolimus are the largest such studies to date and reveal much about how best to use sirolimus and its drawbacks. There was a high incidence of lymphocele formation (12% to 15% versus 3% in the azathioprine control in U.S. study) and wound infection compared with the control arm. Of particular

Table 19–1 Adverse Effects of Sirolimus Identified in Phase II Studies of Sirolimus Compared with Cyclosporine

	Sirolimus (*n* = 41 + 40)	Cyclosporine + Azathioprine (*n* = 42)	Cyclosporine + MMF (*n* = 38)
Metabolic			
Hypertriglyceridemia	21 + 29 = 50 (63%)	5 (12%)	19 (50%)
Hypercholesterolemia	18 + 26 = 44 (54%)	6 (14%)	17 (45)
Hyperglycemia	8 + 6 = 14 (17%)	3 (7%)	6 (16%)
IDDM	1 + 1 = 2 (2%)	1 (2%)	1 (3%)
ALT increase	8 + 8 = 16 (20%)	1 (2%)	3 (8%)
Hypokalemia	14 +8 = 22 (27%)	0	6 (16%)
Hypophosphatemia	6 + 6 = 12 (15%)	0	1 (3%)
Hyperuricemia	1 (3%)	—	7 (18%)
Hematological			
Thrombocytopenia	15 + 18 = 33 (41%)	0	3 (8%)
Leukopenia	16 + 11 = 27 (33%)	6 (14%)	7 (18%)
Anemia	15 + 17 = 32 (40%)	10 (24%)	11 (29%)
Infections			
CMV viremia	6 + 2 = 8 (10%)	5 (12%)	8 (21%)
Herpes simplex	10 + 6 = 16 (20%)	4 (10%)	6 (16%)
Herpes zoster	0 + 1 = 1 (1%)	1 (2%)	1 (3%)
Oral *Candida*	3 + 5 = 8 (10%)	0	3 (8%)
PCP	0 + 0	1 (2%)	0
Pyelonephritis/UTI	17 + 17 = 34 (42%)	12 (29%)	15 (39%)
Septicemia	6 + 2 = 8 (10%)	1 (2%)	1 (3%)
Pneumonia	7 + 6 = 13 (16%)	1 (2%)	2 (5%)
Wound infection	4 + 2 = 6 (7%)	2 (5%)	3 (8%)
Other			
Hypertension	7 + 16 = 23 (16%)	14 (33%)	18 (47%)
Arthralgia	8 (20%)	0	—
Tremor	1 + 2 = 3 (4%)	7 (14%)	8 (21%)
Gingival hyperplasia	0 + 0	4 (10%)	3 (8%)
Hirsutism	1 (3%)	—	4 (11%)
Diarrhea	15 (38%)	—	4 (11%)
Malignancies	0	2 (5%)	0

ALT, alanine aminotransferase; CMV, cytomegalovirus; IDDM, insulin-dependent diabetes mellitus; MMF, mycophenolate mofetil; PCP, *Pneumocystis carinii* pneumonia; UTI, urinary tract infection.

Data from Groth CG, Backman L, Morales JM, et al: Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. Transplantation 67:1036, 1999; and Kreis H, Cisterne JM, Land W, et al: Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. Transplantation 69:1252, 2000.

Table 19–2 Outcome of Two Phase III Sirolimus Adjuvant Therapy Studies

	U.S. Study (n = 719)			Global Study (n = 576)		
	Aza (*n* = 161)	SRL 2 mg (*n* = 284)	SRL 5 mg (*n* = 274)	Placebo (*n* = 130)	SRL 2 mg (*n* = 227)	SRL 5 mg (*n* = 219)
Acute rejection (%)	29.8	16.9*	12†	41.5	24.7‡	19.2§
Creatinine clearance (mL/min)	68.8	62.3	59.2	62.6		56.4
Graft survival (%)	94.4	94.3	92.7	87.7	89.9	90.9
Patient survival (%)	98.1	97.2	96	94.6	96.5	95

*P =.002 relative to the azathioprine arm.
†P <.001 relative to the azathioprine arm.
‡P =.003 relative to the placebo arm.
§P <.001 relative to the placebo arm.
Aza, azathioprine; SRL, sirolimus.
Note: Acute rejection incidence and creatinine clearance (Nankivell formula) are 6-month values; graft and patient survivals are 12-month values.
From MacDonald A; for the Rapamune Global Study Group: A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. Transplantation 71: 271, 2001.

importance was the observation that the renal function of patients on a combination of sirolimus and cyclosporine was worse than that of patients on cyclosporine alone, with a 12-month calculated creatinine clearance of 67.5 mL in the azathioprine control group compared with 62 mL/min and 55.5 mL/min in the 2-mg and 5-mg sirolimus groups ($P < .05$ and $P < .001$ compared with the azathioprine group). A similar effect was seen in the global study. The reason for the reduction in glomerular filtration rate in patients receiving sirolimus and cyclosporine is not well understood, but it is a concern because renal function in the shorter term is a surrogate for long-term graft survival.

The immunosuppressive synergy between cyclosporine and sirolimus in these studies was analyzed by median effect analysis of the pooled data.[66] This analysis showed that administration of sirolimus permitted a 2.2-fold reduction in cyclosporine exposure, and reciprocally cyclosporine permits a 5-fold reduction in sirolimus dose to achieve the same immunosuppressive efficacy. Experimental data also suggest that synergism accounts for the increased nephrotoxicity,[115] although this has not been proved clinically.

Sirolimus also has been evaluated in combination with tacrolimus after an initial report suggesting that the theoretical misgivings about the combination are not seen in clinical practice.[99] At the time of this writing, publication of two large-scale trials evaluating the combined use of sirolimus and tacrolimus are awaited. Other evidence suggests that there may be little to recommend the combination compared with tacrolimus plus MMF,[23,142] and registry data suggest poorer outcome in terms of graft survival for the sirolimus/tacrolimus combination.[103] One criticism of the clinical studies published to date is their use of fixed dose administration of sirolimus, in light of evidence that concentration-controlled dosing is more appropriate.

Everolimus also has been evaluated as an adjunct to cyclosporine in renal transplantation, again in fixed dose combinations. Phase III studies indicated that the combination of either 1.5 mg/day or 3 mg/day of everolimus was better than MMF in the prevention of acute renal allograft rejection when combined with cyclosporine and steroids after kidney transplantation, although the higher dose of everolimus was less well tolerated.[141] As with the combination of sirolimus and calcineurin inhibitors, the combination of everolimus with cyclosporine was associated with poorer renal function (creatinine clearance 52.9 mL/min on 1.5 mg, 49.3 on 3 mg, and 56.9 on MMF at 12 months; $P < .05$). These findings were echoed in a second phase III study.[86] Although mTOR inhibitors provide powerful immunosuppression when combined with calcineurin inhibitors, the increased nephrotoxicity observed suggests that this therapeutic combination might best be reserved for use in the early post-transplant period for patients at particularly high risk of rejection, or for patients who have steroid-resistant rejection.

Maintenance Therapy with mTOR Inhibitors

Although available data suggest that mTOR inhibitors are as efficacious in terms of immunosuppressive potency as calcineurin inhibitors when used as the principal immunosuppressive agent, their use immediately after renal transplantation may be undesirable because of their effects on wound healing and lymphocele formation. Because mTOR inhibitors when used in the absence of calcineurin inhibitors are not nephrotoxic, however, they are potentially attractive agents for use in the maintenance phase of the post-transplant course, especially in patients with calcineurin inhibitor–associated problems, including chronic allograft nephropathy. The first major study to examine the efficacy of mTOR inhibitors in this context used sirolimus combined with cyclosporine and steroids as initial therapy, with the cyclosporine being stopped at 3 months in half of the patients. Sirolimus was shown to provide sufficient immunosuppression during the maintenance phase, with a superior calculated creatinine clearance compared with patients remaining on sirolimus and cyclosporine. Although the acute rejection rate was slightly higher in the no-cyclosporine group, this did not translate into poorer renal function.[60] Longer follow-up confirmed the sustained benefit of sirolimus maintenance therapy.[77,112] This study had no standard control group, and the subsequent finding of enhanced nephrotoxicity when calcineurin inhibitors are combined with sirolimus casts a shadow over the results.[65,89]

Smaller studies also suggest a benefit of sirolimus over calcineurin inhibitors as maintenance therapy after renal transplantation. A dual-center randomized controlled trial from our own unit suggested that conversion to sirolimus in patients with impaired graft function results in a rapid improvement in measured glomerular filtration rate at 3 months, which was sustained to 2 years, whereas patients who remained on calcineurin inhibitors experienced deteriorating graft function.[145] Because of concerns about triggering acute rejection during the conversion from calcineurin inhibitors to sirolimus, some investigators have used a period of overlap of immunosuppression,[29] or covered the transition period with additional agents such as basiliximab,[137] but in patients who are greater than 6 months post-transplantation, it is unlikely that such manipulation is necessary.

Late conversion to sirolimus is associated with three dominant side effects that might limit its usefulness as a maintenance agent, in addition to the other side effects that are well recognized with sirolimus (see later). First, more than half of patients in some studies experience a rash, either an acneiform rash or a dermatitis-like rash affecting the hands and, in particular, the fingers. Second, the period of conversion to sirolimus is associated with the development of mouth ulcers, an occurrence that resolves within 4 weeks in most patients. If mouth ulcers do not resolve, herpes simplex should be considered. Finally, patients with suboptimal renal function, particularly patients with proteinuria, are prone to the development of marked proteinuria after conversion.[28,85] Whether this proteinuria reflects the increased glomerular filtration or difference in tubular response to protein is unclear,[122,136] although blockade of the angiotensin system may be useful to limit this phenomenon.

Despite the potential drawbacks in terms of side effects, evidence is accumulating that conversion from calcineurin inhibitors to mTOR inhibitors may be worthwhile in patients with chronic allograft nephropathy and, at least in the short term, may lead to improved graft function.[30] The optimal time for conversion in such patients is unclear, but early rather than late conversion is probably best, before the structural changes associated with chronic allograft nephropathy become extensive. Switching to mTOR inhibitors in patients who are experiencing other side effects

from calcineurin inhibitors, such as neurotoxicity and diabetes, also seems to be a reasonable option. Conversion from calcineurin inhibitors to mTOR inhibitors for patients who develop hemolytic-uremic syndrome also could be considered, although sirolimus itself has been identified as a cause of this condition.[10,121] Because mTOR inhibitors lead to an increased urinary excretion of uric acid, a further possible indication for use of mTOR inhibitors is in the management of severe gout in patients taking calcineurin inhibitors.

mTOR INHIBITORS AND MALIGNANCY

mTOR Inhibitors as Antitumor Agents

As noted earlier, mTOR inhibitors not only inhibit lymphocyte proliferation but also prevent tumor cell growth. The inhibitory effect of sirolimus on the in vitro growth of tumor cell lines and its inhibitory effect on transplanted tumors in rodent models have long been known, but the clinical potential of mTOR inhibitors as an important novel class of anticancer agents has been appreciated only more recently.[36] Although intuitively, the detrimental effects of immunosuppression after mTOR inhibition might be expected to outweigh any beneficial effect on limiting tumor cell growth in patients with malignancy, it seems that the anticancer activity of mTOR inhibitors is the dominant clinical effect in such patients. There is now intense interest in oncology in evaluating the role of mTOR inhibitors as therapeutic agents.

Many sirolimus derivatives have now been developed specifically for their use as antitumor agents. Temsirolimus (CC1-779), a sirolimus derivative formulated for intravenous administration, has now been used in many phase I/II clinical trials, either as monotherapy or as a component of combination chemotherapy in patients with a range of malignancies, including advanced renal cell carcinoma, breast cancer, prostatic cancer, pancreatic cancer, glioblastoma, and lymphoma. Although sometimes associated with serious side effects, there is evidence for potential clinical benefit, and currently phase III trials of mTOR inhibitors are under way in patients with renal cell carcinoma and patients with advanced or metastatic breast cancer.

mTOR Inhibitors and Post-Transplantation Malignancy

Because patients after renal transplantation are at increased risk of developing most types of malignancy, particularly lymphoma and skin cancer, the anticancer effects of mTOR inhibitors are of major relevance. Several more recent reports suggest that maintenance immunosuppression with mTOR inhibitors after renal transplantation may be associated with a reduced risk of post-transplant malignancy. A relatively low incidence of malignancy in patients receiving sirolimus-based maintenance immunosuppression has been reported from a center with extensive experience of mTOR inhibitor use.[67] A multivariate analysis of post-transplant malignancies in 33,249 renal allograft recipients in the United States revealed that the incidence rates of any type of post-transplant malignancy were 0.6% in patients taking mTOR inhibitors, 0.6% for patients taking mTOR inhibitors plus calcineurin inhibitors, and 1.8% for patients taking calcineurin inhibitors alone.[69] Similarly, the incidence of post-transplant malignancy in adults randomly assigned to remain on sirolimus and calcineurin inhibitors was found to be greater than that in subjects randomly assigned to early calcineurin inhibitor withdrawal and an increased dose of sirolimus.[18] Although these studies are encouraging, further long-term data on the potential for mTOR inhibitors to reduce the development of malignancy after renal transplantation are needed before firm conclusions can be drawn.

mTOR Inhibitors and Post-Transplantation Lymphoproliferative Disorder

Everolimus and sirolimus have been shown to inhibit markedly the growth of human post-transplantation lymphoproliferative disorder–derived cell lines and Epstein-Barr virus–transformed B lymphocytes in vitro and in vivo.[94,95,110] A renal transplant recipient in whom disseminated post-transplantation lymphoproliferative disorder resolved completely after conversion of immunosuppression to sirolimus also has been reported.[25] Sirolimus was not found to modify the risk of developing post-transplantation lymphoproliferative disorder, however, in an analysis of 25,127 patients (344 of whom developed post-transplantation lymphoproliferative disorder) who underwent renal transplantation in the United States.[15]

mTOR Inhibitors and Kaposi's Sarcoma

mTOR inhibitors may have a useful role in the treatment of renal transplant recipients who develop Kaposi's sarcoma associated with herpesvirus-8, especially if the disease is confined to the skin. In a study of 15 patients who developed cutaneous Kaposi's sarcoma after renal transplantation while taking cyclosporine, switching them to sirolimus led to complete, histologically confirmed remission in all patients for the duration of the study (6 months) with preservation of graft function.[132] Response varies, however, and may depend on the severity of disease. A retrospective analysis in which 14 renal transplant recipients with Kaposi's sarcoma (including several with visceral or advanced disease) were switched from calcineurin inhibitors to sirolimus showed that the switch was generally well tolerated. Complete remission was seen in two patients, and a partial response was seen in a further eight, although three of the partial responders with advanced disease relapsed after several months.[84] Further studies are needed to evaluate the role of mTOR inhibitors in treatment of Kaposi's sarcoma and to determine the optimal treatment schedule for patients with more advanced disease.

SAFETY AND SIDE EFFECTS OF mTOR INHIBITORS

It was not until the phase II studies of sirolimus by Groth and Kreis and their colleagues[51,76] that the sirolimus-specific side effects became clear because until then and in most of the subsequent studies sirolimus (and everolimus) was used in conjunction with calcineurin inhibitors. Table 19-1 shows the principal side effects found in these studies. In contrast to calcineurin inhibitors, it is notable that although mTOR inhibitors may cause a range of agent-specific side effects, these do not include nephrotoxicity, neurotoxicity, hypertension, or gingival hyperplasia.

Infection

The incidence and pattern of infections reported in patients receiving mTOR inhibitors is broadly similar to patients receiving calcineurin inhibitor–based immunosuppression. Neither the U.S. study nor the global studies of de novo sirolimus use (see Table 19-2) identified any particular problem with infection over and above that observed in the comparator groups, although the global study noted an increase in the incidence of mucosal lesions attributed (but without virological confirmation) to herpes simplex virus.[65,90] A meta-analysis of mTOR inhibitor use as primary immunosuppression after kidney transplantation confirmed the overall safety of mTOR inhibitors in terms of infection and noted that when mTOR inhibitors were substituted for antimetabolites, there was a reduction in the incidence of cytomegalovirus infection.[146] Some studies have suggested that the incidence of pneumonia may be greater in patients receiving mTOR inhibitors, but the evidence for this remains inconclusive and confounded by the occurrence of drug-induced pneumonitis.

Lipids

One of the most concerning long-term problems associated with mTOR inhibitors is their metabolic effect on lipid metabolism. Two thirds of patients may develop increased triglyceride levels, and half develop increased serum cholesterol levels. Fifty-three percent of sirolimus-treated patients required lipid-lowering agents compared with 24% in the cyclosporine groups combined. The full significance of the increased lipids associated with mTOR inhibitors is unclear, but it is a long-term concern. Lipids are implicated in the development of cardiovascular disease and in the genesis of chronic rejection.[100] What is unclear is whether these risks pertain in the presence of sirolimus. There is limited evidence in animal models that sirolimus inhibits graft vasculopathy,[58] an observation that has been confirmed with everolimus using intravascular ultrasound in heart transplant recipients.[37] Sirolimus also seems to be able to prevent the accelerated vascular disease seen in cholesterol-fed, apolipoprotein E–deficient mice despite a high cholesterol level.[40] Its effect on stabilizing the endothelial cell wall also underlies its beneficial effect when incorporated into intravascular stents. The occurrence of lipid abnormalities seems to be, at least in part, genetically determined with polymorphisms in apolipoprotein A implicated in at least one study.[96]

Pneumonitis

Although lipid abnormalities might be the most common side effect seen with mTOR inhibitor therapy, pneumonitis is the most feared. Pneumonitis may occur at any time after initiation of sirolimus treatment and manifests as progressive dyspnea, dry cough, fatigue, and fever,[21,54] and may progress to pulmonary failure. Imaging reveals bilateral pulmonary infiltrates (Fig. 19-3), and pulmonary function tests may show a restrictive pattern. Open lung biopsies have revealed granulomata in some cases, but not in others. The effect is reversible with discontinuation of sirolimus.

The true incidence of sirolimus-associated pneumonitis is unclear, and it is probably underrecognized and underreported. The first reports of sirolimus-associated pneumonitis were in 2000,[108,148] and these were followed by a disclosure from the U.S. Food and Drug Administration of 31 other cases of interstitial pneumonitis associated with sirolimus use.[128] Earlier studies had reported an increased incidence of pneumonia (see Table 19-1),[51] however, and one of the first studies reported an excess of *Pneumocystis* pneumonia[63]; it is possible that some of these were sirolimus-induced pneumonitis. Ten years previously, the complication had been noted as the principal cause of death in pigs undergoing renal transplantation with sirolimus.[16]

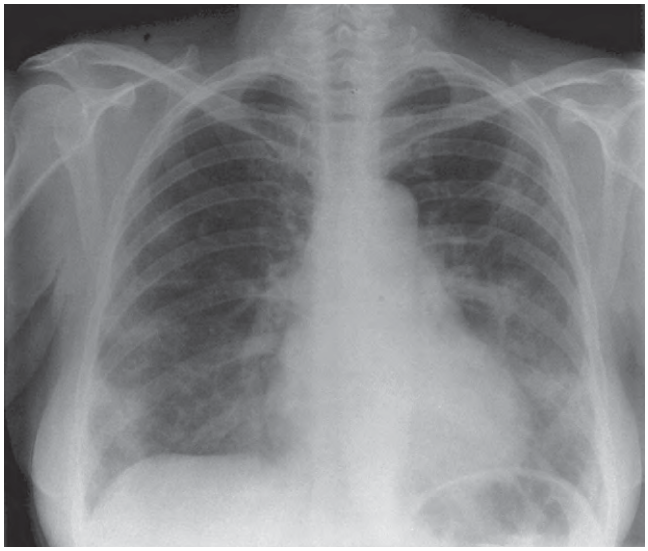
The etiology of sirolimus-associated pneumonitis is unclear. Reports suggest that it is more common in patients switching from a calcineurin inhibitor to sirolimus or having a calcineurin inhibitor withdrawn from a sirolimus/calcineurin inhibitor combination, and having high drug concentrations.[21,49,128] A mortality of 12% was noted in the Food and Drug Administration report, although early recognition of the problem, with immediate discontinuation of sirolimus, should reduce the mortality from this complication. One report suggests that conversion from sirolimus to everolimus is associated with recovery,[117] whereas another report also implicates the related antitumor drug temsirolimus in causing pneumonitis[34]; both observations would suggest that it is not mTOR blockade per se that is responsible for the complication, but that the lipophilic nature of sirolimus and temsirolimus also is important in its cause.

Hemolytic-Uremic Syndrome (Thrombotic Microangiopathy)

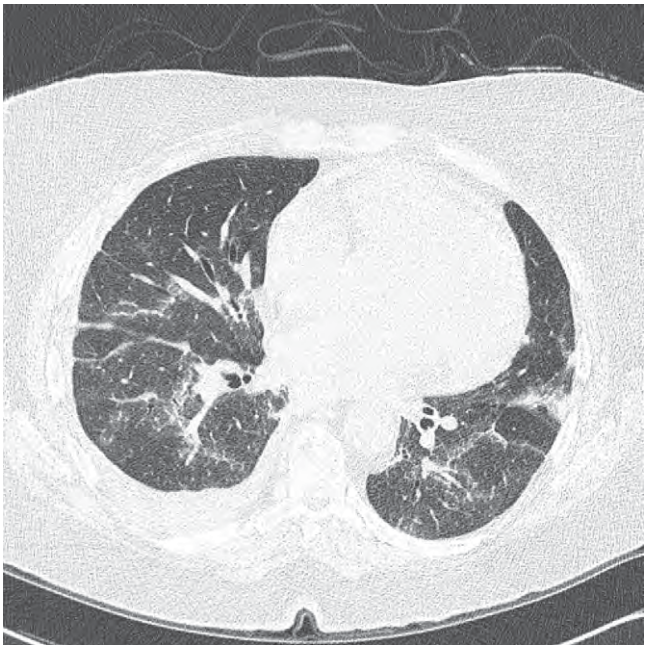
One of the main attractions of mTOR inhibitor therapy is its perceived lack of nephrotoxicity. Although sirolimus does not cause the typical changes associated with calcineurin inhibitor therapy, it is not entirely devoid of adverse effects on the kidney. The most dangerous of these is its association with hemolytic-uremic syndrome (thrombotic microangiopathy). Hemolytic-uremic syndrome was identified as a problem in patients taking cyclosporine and sirolimus,[80,118] but subsequent reports highlight it to be associated with sirolimus in the absence of calcineurin inhibitors.[10,121] It also occurs in the native kidneys of non–renal transplant recipients,[52] as is reported with everolimus, suggesting that it is a property of mTOR as a group.[86]

Proteinuria

Proteinuria is now recognized as a common manifestation of sirolimus toxicity in patients converted for renal impairment and has been noted in patients taking everolimus.[86] It is most common in patients who already have a degree of proteinuria at the time of conversion,[120] and it seems to be a direct sirolimus effect that occurs in adults and children.[14,85] The absence of proteinuria seems to be the best indicator of improvement in renal function after conversion.[13,28] The cause of the proteinuria is unclear. In one study of four patients who developed proteinuria, biopsy specimens revealed glomerulonephritis (membranoproliferative glomerulonephritis in one, membranous glomerulonephritis in another, and IgA nephropathy in the last two).[32] The proteinuria resolved when the patients were converted back to calcineurin inhibitors and the sirolimus was stopped. In a separate study involving patients who had liver transplants



A



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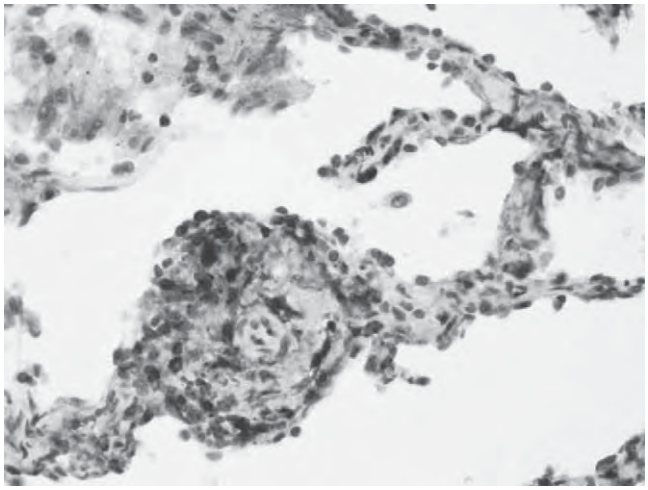

C

Figure 19–3 Sirolimus-induced pneumonitis. **A**, Plain chest radiograph shows bilateral interstitial infiltration. **B**, High-resolution computed tomography scan shows patchy ground-glass opacification and interstitial reticular change. **C**, Immunohistologic analysis of transbronchial lung biopsy specimen in sirolimus-induced pneumonitis shows heavy interstitial CD4 T cell infiltrate (immunoperoxidase, × 400). (**A** and **B** courtesy of Dr. A. Tasker; **C** courtesy of Dr. M. Griffiths.)

but developed renal impairment, no proteinuria was seen on conversion to sirolimus, suggesting that preexisting renal damage may be necessary before proteinuria manifests.[27] Proteinuria has been observed in patients undergoing islet transplantation and receiving sirolimus, however, in whom underlying diabetic nephropathy may have been contributory.[126]

Some authors have suggested that proteinuria may arise from the removal of arteriolar vasoconstriction afforded by calcineurin inhibitors, but such a mechanism cannot account for the observation that proteinuria occurs in patients treated from the outset on a sirolimus-based, calcineurin inhibitor–free protocol.[134] Other authors have suggested that an increased intraglomerular pressure might be causative,[122] whereas still others have suggested that a reduction in tubular protein reabsorption is responsible.[136]

Delayed Recovery from Ischemia-Reperfusion Injury

Delayed recovery of normal kidney function (delayed graft function) is a common manifestation of ischemia-reperfusion injury, most notable with kidneys donated after cardiac death, where warm ischemia and cold ischemia contribute to renal injury. Reduced exposure to calcineurin inhibitors in the early postoperative stage has been common practice in many transplant units, so the advent of a "non-nephrotoxic" agent, such as sirolimus, was an attractive alternative. Early experimental work in rats showed delayed recovery from ischemia-reperfusion injury,[48] and this has subsequently been observed in the clinic in small retrospective studies[101] and registry analyses.[127] The mechanism behind this observation presumably relates to the inhibition of cell proliferation

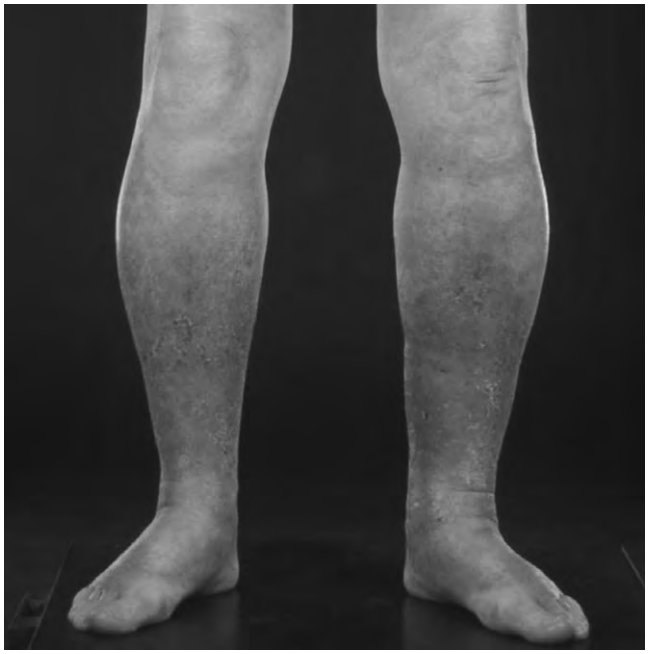

Figure 19–4 Sirolimus-induced erythema and limb edema. Acute erythema and swelling in the limbs associated with sirolimus. The symptoms resolved after administration of steroids.

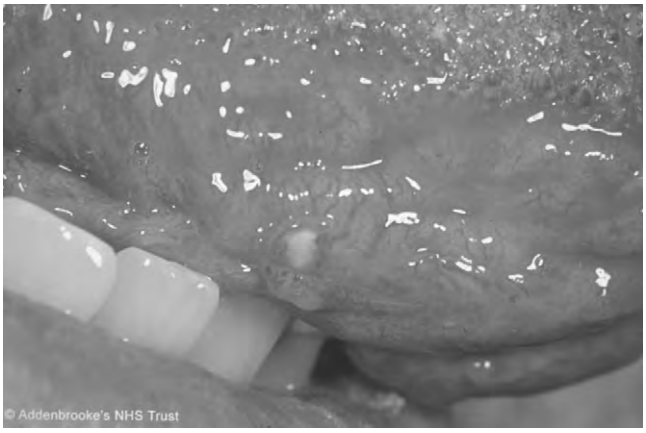

Figure 19–5 Sirolimus-induced oral ulceration. Solitary aphthous-type ulcer on the undersurface of the tongue occurring several days after late (>6 months) conversion from calcineurin inhibitor to sirolimus. Such lesions are often multiple and painful and can be distressing for the patient. They usually resolve rapidly after adjustment of sirolimus to the lower end of the target range of 5 to 10 ng/mL.

affecting tubular repair.[87] Although sirolimus is associated with a higher incidence of delayed graft function and prolonged recovery of function, the renal function in the long-term does not seem to suffer.[102,130]

Peripheral Edema

Although not widely recognized, the occurrence of edema in patients taking sirolimus and everolimus is well described. Most edema affects the lower limb[1] and may be unilateral (Fig. 19-4) or bilateral; it is not necessarily ipsilateral to the kidney transplant. Angioedema affecting the eyelids and tongue also has been described.[47,104,131,144] It typically resolves on discontinuation of the mTOR inhibitors. The cause of this complication is unknown.

Wound Healing and Lymphocele Formation

One of the most concerning complications of mTOR inhibitors is the potentially detrimental effect they have on the operative site. mTOR inhibitors not only impair wound healing but also are associated with a high incidence of lymphoceles after renal transplantation, although this seems partly center specific, suggesting a technical component, such as whether lymphatics were deliberately divided or ligated, or both. Wound problems include fluid collections around the graft and under the skin, superficial infections, and late hernias.[26] Anastomotic healing has not been reported as a problem after clinical renal transplantation, but poor healing of the airway anastomosis has been cited after lung transplantation,[35,72] and there is some evidence in the pig that ureteric anastomoses are not as strong.[68] The problems observed with wounds may relate to mTOR inhibition resulting in lack of fibroblast response to fibroblast growth factor and lack of neovascularization of wounds owing to blockade of vascular endothelial growth factor.

Mouth Ulcers

Oral ulceration (mucositis) manifesting as painful gingival or buccal mucosa leading to pain on eating is a well-documented and troublesome side effect of mTOR inhibitors (Fig. 19-5). The ulcers are usually small but multiple, and in many cases are probably related to herpes simplex virus infection. In the global phase III study of de novo treatment with rapamycin, ulceration of the oral mucosa was observed in 19% of patients randomly assigned to 5 mg/day of sirolimus, 10% of patients randomly assigned to 2 mg/day of sirolimus and 9% of patients in the placebo group.[90] The lesions all were mild and resolved spontaneously without discontinuation of sirolimus.[90]

Mouth ulcers also are common in patients converted to mTOR inhibitors. As is the case in de novo treatment, such ulcers usually resolve spontaneously, but they can be problematic. In one prospective randomized study in which renal transplant recipients were converted at 1 year from a steroid-free regimen of tacrolimus and MMF to sirolimus and MMF, oral ulceration occurred in 9 of 15 converted patients. The mucosal lesions healed within 2 weeks of discontinuing sirolimus, but the problem led to premature cessation of the study.[139] The authors postulated that the high incidence of oral ulceration may have been attributable to overimmunosuppression during conversion, the use of oral emulsion of sirolimus rather than tablets, and the lack of corticosteroids.[139] In a randomized study of conversion from calcineurin inhibitors to sirolimus after renal transplantation, aphthous-type mouth ulcers occurred in one third of patients during the first 2 weeks after conversion, although all resolved with adjustment of sirolimus to the lower end of the target range of 5 to 15 ng/mL.[145] The association between sirolimus and mucosal ulceration may be attributable predominantly to the detrimental effect of mTOR inhibitors on wound healing, rather than any direct effect in initiating ulcer formation.

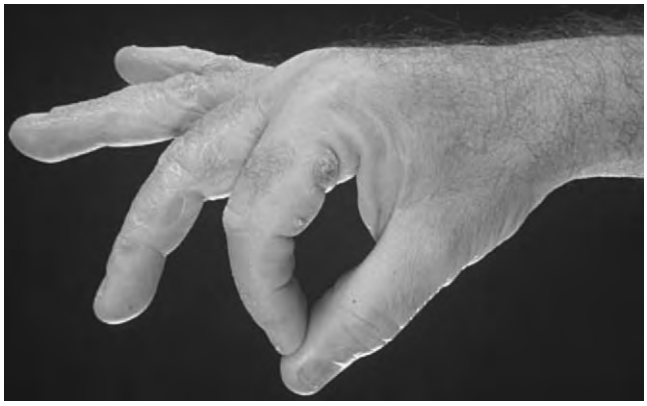

Figure 19–6 Sirolimus-induced rash. After conversion from calcineurin inhibitor to sirolimus, patients commonly develop skin problems that may take the form of a dermatitis-like rash affecting the hands and, in particular, the fingers.

Rash

As already noted, rash is a common complication of mTOR inhibitor therapy and most commonly takes the form of an inflammatory acneiform eruption[92] or a dermatitis-like rash affecting the hands and, in particular, the fingers (Fig. 19-6). This rash was apparent in both of the early multicenter randomized trials of de novo sirolimus treatment after renal transplantation. In the U.S. study, an acneiform rash was observed in 25% of recipients on 2 mg/day, 19% of recipients on 5 mg/day, and 11% in the azathioprine control group.[65] In the global phase III study, rash was observed in 14% of patients randomly assigned to 5 mg/day of sirolimus, 4% of patients randomly assigned to 2 mg/day of sirolimus, and 5% of patients in the placebo group.[90]

In studies in which an in-depth dermatological analysis has been undertaken, the incidence of dermatological side effects is considerably higher. A cross-sectional study of cutaneous adverse events in renal transplant recipients receiving long-term, sirolimus-based immunosuppression reported the presence of an acne-like eruption in 46%, scalp folliculitis in 26%, and hidradenitis suppurativa in 12% of patients.[91] In the absence of a control group, it is difficult to attribute side effects exclusively to mTOR inhibitors, but such studies indicate the high frequency of dermatological complications associated with mTOR inhibitors. Although rashes are usually mild, they may be a reason for discontinuing mTOR inhibitors. In the randomized trial of late conversion to sirolimus in our center, 68% of converted patients developed a rash, particularly acne, and 2 of the 19 converted patients discontinued sirolimus because of this.[145] The pathophysiology of rash in patients taking mTOR inhibitors is unclear, but may be attributable to the effect of mTOR inhibitors on the epidermal growth factor receptor, which is important in the differentiation and development of the hair follicle.[92]

Anemia, Thrombocytopenia, and Leukopenia

Anemia, thrombocytopenia, and leukopenia all are well-recognized side effects of use of mTOR inhibitors. Although thrombocytopenia attracted the most attention in early studies, anemia has emerged as the most significant clinical problem. Anemia is a common complication during the first 6 months after renal transplantation regardless of the immunosuppressive regimen,[2] but the incidence is increased with use of mTOR inhibitors. In the global study of primary use of sirolimus in renal allograft recipients, anemia was observed in 16% of recipients taking 2 mg/day and 27% of recipients taking 5 mg/day of sirolimus. The incidence of anemia in the 5 mg/day group was significantly higher than that in the placebo group (13%) receiving cyclosporine and steroids.[90] mTOR inhibitor dosage adjustment may be required, and in some patients administration of erythropoietin may be necessary.

Anemia after renal transplantation most often results from iron deficiency and defective erythropoietin production, but the mechanisms responsible for mTOR inhibitor–induced anemia are unclear. Sirolimus blocks the in vitro response of bone marrow cells to several hematopoietic cytokines, including granulocyte colony-stimulating factor, interleukin-3, and kit ligand.[116] Although mTOR inhibitor–induced suppression of nonerythroid bone marrow cells contributes to leukopenia and thrombocytopenia, the extent to which mTOR inhibitor–induced suppression of erythrocyte production leads to anemia is uncertain. A more recent study observed that sirolimus reduced hemoglobin levels but did not reduce the erythrocyte count in renal transplant recipients, arguing against a direct antiproliferative effect on erythroid bone marrow.[93] Instead, it was suggested that sirolimus had a direct effect on iron homeostasis.[93]

Thrombocytopenia was identified as a side effect of sirolimus in the global and U.S. phase III randomized trials of de novo sirolimus and seemed to be dose related.[65,90] In both studies, a few patients randomly assigned to the higher dose (5 mg/day) of sirolimus (6 of 208 [2.8%] in the global study and 3 of 274 [1.1%] in the U.S. study) had to have sirolimus discontinued because of thrombocytopenia, although none of the patients experienced severe thrombocytopenia or were reported to have had related hemorrhage. mTOR inhibitors may reduce circulating platelets as part of their inhibitory effect on hematopoietic cytokines. In addition, sirolimus has been shown to promote agonist-induced platelet aggregation in vitro,[6] and conceivably if increased platelet aggregation occurs in vivo, it may promote increased removal of platelets by the spleen.

Although it is now well recognized that mTOR inhibitors may lead to a decrease in the platelet count, this is not usually of clinical significance and is rarely a barrier to continued administration of mTOR inhibitors. Thrombocytopenia most often occurs within the first month of starting sirolimus, and its occurrence correlates with whole-blood trough levels of sirolimus that exceed 16 ng/mL. If the platelet count falls significantly, it usually responds well to dosage reduction without the need to withdraw mTOR inhibitors.[56] Finally, mTOR inhibitors may produce mild leukopenia, which is usually transient and dose related.

Gastrointestinal Symptoms

Gastrointestinal side effects include abdominal pain, nausea, and vomiting, but the most common symptom is diarrhea, which is usually mild, is dose related, and does not require mTOR inhibitor withdrawal. In the pivotal phase III studies

of de novo sirolimus use, mild diarrhea was observed in 27% to 32% of patients receiving 5 mg/day of sirolimus, 16% to 20% of patients receiving 2 mg/day of sirolimus, and 11% to 13% of patients in the control groups.[65,90] Mild diarrhea may be particularly common in patients receiving a combination of mTOR inhibitors and MMF.[76] The high incidence of diarrhea sometimes reported in patients receiving sirolimus and MMF may be related to pharmacokinetic interaction between the two agents[20]; concentration-controlled administration of MMF markedly reduces gastrointestinal symptoms.[45]

Thrombosis

Sirolimus, similar to calcineurin inhibitors, has been shown to increase platelet aggregation in vitro,[6] and it has been suggested that sirolimus, when used in combination with calcineurin inhibitors, may increase the risk of hepatic artery thrombosis after liver transplantation. Although there is no published trial evidence that mTOR inhibitors are associated with an increased risk of thromboembolic events after renal transplantation, it is recognized in the data sheets for sirolimus that thromboembolic events may be associated with its use. In a retrospective single-center analysis of deep vein thrombosis, graft thrombosis, and pulmonary embolism in renal transplant recipients, the addition of sirolimus in recipients taking cyclosporine did not increase the risk of postoperative thrombotic events.[81] A strong correlation between the development of deep vein thrombosis and lymphocele was observed, however, in patients receiving sirolimus,[81] and the increased risk of deep vein thrombosis in patients developing lymphocele should be kept in mind.

Renal Tubular Effects: Hypokalemia and Hypophosphatemia

mTOR inhibitors may contribute to hypokalemia after renal transplantation, and in the phase II and III trials of primary treatment with sirolimus, values of serum potassium less than the normal range were recorded during the first 3 months in about half of all patients.[106] Hypokalemia is usually mild, and only about 10% of patients required a period of potassium supplementation, which readily corrected the problem.[106] Hypokalemia may be partially related to the dose of mTOR inhibitors given and seems to be due to mTOR inhibitor–induced alterations in tubular function leading to increased tubular secretion of potassium.[107]

Hypophosphatemia also is common in the first few weeks after renal transplantation and is multifactorial in etiology. Although reduced serum phosphate levels may observed more often during the first 3 months in patients receiving mTOR inhibitors, this is rarely a clinically significant issue, and values return to normal with time or dosage adjustment.[106] The mechanisms underlying mTOR inhibitor–associated hypophosphatemia are not completely understood, but mTOR inhibitors may impair renal tubular phosphate reabsorption, prolonging the phosphate leak.[124]

Bone Effects

Arthralgia was identified as a side effect of mTOR inhibitors in the global phase III study of the sirolimus. It was observed in 27% of recipients on the higher dose (5 mg/day) of sirolimus compared with 16% and 13% of recipients on low-dose sirolimus or placebo.[89] Similar to the calcineurin inhibitor–induced pain syndrome, bone pain associated with sirolimus affects weight-bearing areas, particularly the feet, ankles, and knees, although the pain may be unrelated to weight bearing. It is generally bilateral and symmetrical. The problem is much less common when lower doses of mTOR inhibitors are used, and symptoms may improve after dosage reduction or respond to treatment with bisphosphonates or alfacalcidol. mTOR inhibitor–induced bone pain and calcineurin inhibitor–induced pain syndrome are likely due to a combination of increased adipocyte volume, reduced intraosseous perfusion, and marrow edema giving rise to a "bone compartment syndrome."[39] The diagnosis usually can be confirmed by radionuclide bone scan (Fig. 19-7) or magnetic resonance imaging scan that reveals hyperemia and marrow edema.

Osteoporosis and bone loss are common after renal transplantation, and there is evidence from preclinical and early clinical studies that mTOR inhibitors may have bone-sparing properties compared with calcineurin inhibitors. Although sirolimus is associated with bone remodeling, it does not result in a loss of trabecular bone volume in rat studies, in contrast to calcineurin inhibitors.[119] Similarly, everolimus inhibits osteoclast activity in vitro and reduces bone loss in an oophorectomized rat model.[73] Markers of bone turnover (serum osteocalcin and urinary N-telopeptides) also are significantly lower in renal transplant recipients taking de novo sirolimus compared with recipients taking cyclosporine.[17] Such studies suggest a possible advantage of mTOR inhibitors over calcineurin inhibitors, but more extensive clinical studies with extended follow-up are needed to confirm these early indications.

Liver Function Abnormalities

Sirolimus tends to cause increased levels of transaminases (alanine aminotransferase and aspartate aminotransferase) and lactate dehydrogenase. Whether this is clinically significant is unclear. There is a single case report of hepatotoxicity in a renal transplant recipient[111] and a series of 10 liver transplant recipients in whom sirolimus was thought to be responsible for abnormal liver function tests, with 2 of the patients having liver biopsy specimens with eosinophilia and sinusoidal congestion.[109] This latter group of 10 patients underwent transplantation for hepatitis C, which had reinfected their grafts, making a clear association with sirolimus difficult.

Amenorrhea

In a study of conversion from calcineurin inhibitors to sirolimus, it was noted that all three female patients younger than 40 years of age who were switched to sirolimus developed amenorrhea for a variable length of time and then resumed irregular menses.[145] Whether this finding is due to an effect of mTOR inhibitors on the hypothalamic-pituitary-gonadal axis or to a direct effect on the endometrium is unclear.

SUMMARY AND CONCLUSION

mTOR inhibitors have been undergoing clinical evaluation as immunosuppressive agents in renal transplantation for more than a decade, and much has been learned about their

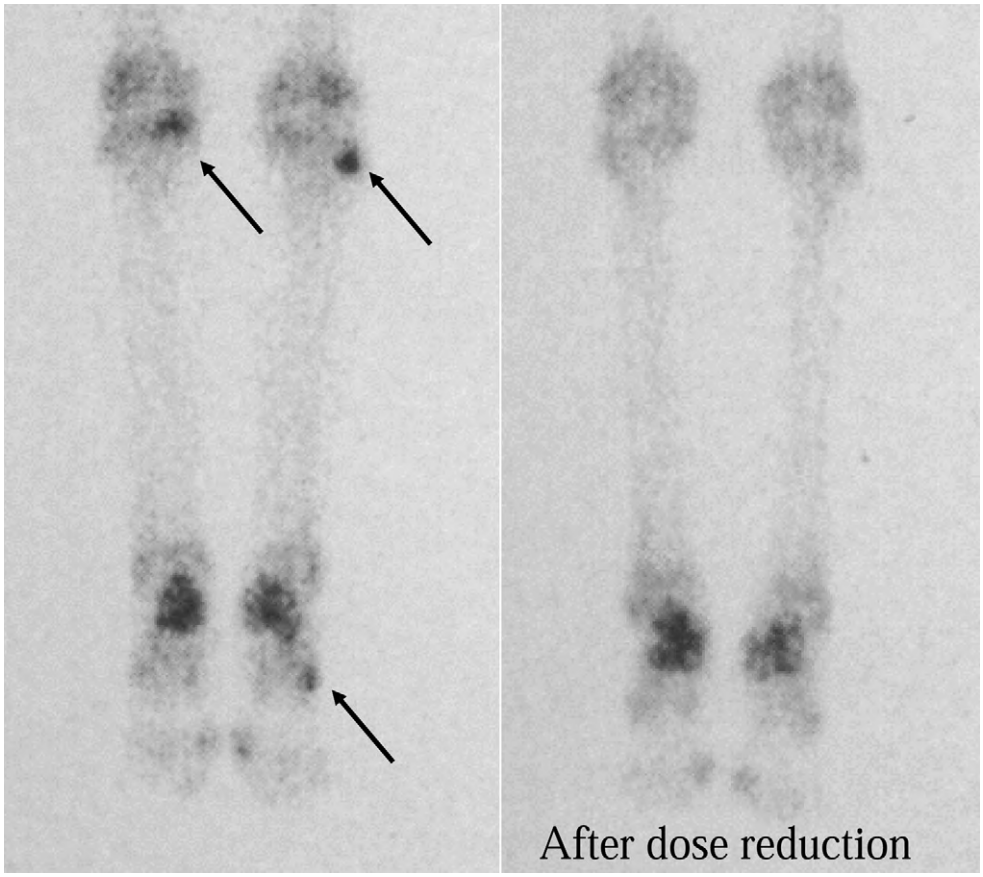

Figure 19–7 Radionuclide bone scan in a patient with sirolimus-induced bone pain. The patient complained of pain affecting the feet, ankles, and knees. The diagnosis was confirmed by radioisotope scanning that revealed areas of increased uptake in the knees and at the ankles (*arrows in left panel*). After sirolimus dosage reduction, the symptoms resolved, and the bone scan returned to normal (*right panel*).

efficacy and side effects. Sirolimus and more recently everolimus have been shown to be effective agents for preventing acute renal allograft rejection and preserving glomerular filtration rate, but their clinical niche remains to be clearly defined. The safety profile of mTOR inhibitors in terms of post-transplant infection is satisfactory and broadly comparable with that of patients receiving standard calcineurin inhibitor–based therapy.

The agent-specific side-effect profile of mTOR inhibitors also is now well established and has relatively little overlap with that of calcineurin inhibitors, making mTOR inhibitors an attractive alternative for patients who cannot tolerate calcineurin inhibitors. mTOR inhibitors are not nephrotoxic when given in the absence of calcineurin inhibitors, and there is some evidence that they may limit chronic allograft nephropathy. The problems of lymphocele formation and impaired wound healing seen with sirolimus argue against the immediate use of mTOR inhibitors after renal transplantation, however, and the adverse effect of mTOR inhibitors on the lipid profile and the significant, but ill-defined risk of life-threatening pneumonitis are significant concerns with long-term use.

The optimal timing for the introduction of mTOR inhibitors after renal transplantation needs to be determined, and ways to better manage the troublesome mucosal and dermatological complications that are commonly seen after conversion to mTOR inhibitors need to be found. Most importantly, long-term studies are needed to determine whether the early benefits observed with mTOR inhibitors in terms of preservation of renal function translate into improved long-term graft survival and protection from chronic allograft nephropathy. There is also a need to determine the extent to which any such benefits outweigh the long-term side effects of mTOR inhibitors, particularly their adverse effect on the lipid profile. Confirmation of the anticancer properties of mTOR inhibitors in renal transplant recipients also is awaited, but initial data are encouraging.

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Chapter 20

Antibodies and Fusion Proteins

Allan D. Kirk

Renal transplantation is the preferred treatment for most end-stage renal diseases. The success of transplantation has been counterbalanced, however, by its dependence on immunosuppressive drugs with their related infectious, metabolic, and malignant complications. Consequently, a common goal throughout the history of clinical transplantation has been the minimization and individualization of immunosuppressive therapy. Typically, drugs with highly specific mechanisms of action have been preferred over drugs with broad effects, and the search for increasingly specific drugs has provided a major impetus for the development of immunosuppressive therapies in general, and of antibodies and fusion proteins in particular.

Antibodies and other glycoprotein cell surface receptors are defined by their ability to bind to a particular ligand with unambiguous specificity. Although they may mediate diverse effects through associated downstream signaling pathways, their function is characterized by fidelity to distinct binding motifs. This trait has been long recognized as having great potential for targeted therapeutic use with minimal unintended effects, and organ transplantation historically has been a preferred testing ground for receptor-based therapeutics, such as monoclonal antibodies (MAbs), polyclonal antibody preparations, and engineered glycoprotein receptor-antibody hybrids known as fusion proteins, collectively known as biologics. The initial success of biologics in transplantation has more recently led to an explosion in the number developed for clinical use.[232] In addition to transplant-related indications, biologics have been developed for the treatment of many oncologic and autoimmune conditions, and there are now at least 200 preparations in some level of clinical or preclinical development.[231] Importantly, although renal allograft rejection was the original indication for MAb therapy,[63] most modern development has been spurred by indications serving larger population bases. In addition to using agents developed for transplantation, clinicians are increasingly adopting therapies from other immunologically relevant indications. This so-called off-label use is now increasingly common and is becoming a primary means of biologics development for transplantation.

This chapter provides an overview of antibody-based and receptor-based therapies for kidney transplantation. Drugs developed and approved for use in transplantation are described; drugs with relevant actions that have been developed for other indications but evaluated in transplantation also are described. Investigational agents that have been tested clinically are reviewed.

HISTORICAL PERSPECTIVE

The early experiences in renal transplantation were marked by very high rates of rejection and complications related to the effects of the two available immunosuppressants of the day, glucocorticosteroids and azathioprine; this, combined with the recognition that lymphocytes were the predominant effectors in rejection, stimulated interest in alternative lymphocyte-directed strategies. By the mid-1960s, several investigators had shown that animals injected with lymphocytes would produce sera containing lymphocyte-specific antibodies, which could be used to reduce the lymphocyte counts when injected into other experimental animals. This technology gave rise to the initial lymphocyte depletion trials using antilymphocyte antibody preparations—antilymphocyte serum, antilymphocyte globulin, and antithymocyte globulin.[26,62,72,269] These agents were collectively called polyclonal preparations because they were composed of antibodies with many, largely undefined, specificities. Their ability to prevent and reverse rejection, particularly in patients refractory to the drugs of the day, led to their increasing use over the ensuing decade.[64]

The increased use of polyclonals made many of their limitations apparent. The imprecise in vivo methods for producing polyclonal antibodies resulted in preparations with promiscuous binding to many nonlymphocyte cell types. Although each antibody in the preparation bound to a single target, collectively, the preparation bound to a broad array of cell surface molecules. Cross-reactivity with many hematopoietic cells made anemia, neutropenia, and thrombocytopenia dose limiting. The method of production also led to wide batch-to-batch variability. The clinical effect of the agent varied considerably, making it difficult to establish prospectively proper dosages and estimate the magnitude of anticipatable side effects. In addition, because the preparations were made in animals, usually rabbits or horses, they contained proteins that were antigenic to humans.[201,281] They had the potential to induce a neutralizing antibody response and evoke adverse effects, such as serum sickness or anaphylaxis.[222] Finally, some lymphocyte cell surface receptors, when bound by antibody, would induce cell activation, leading to a release of anaphylatoxins and cytokines, producing a syndrome of flu-like and, in extreme cases, septic-like symptoms subsequently termed cytokine release syndrome.

In the 1970s, Kohler and Milstein[159] presented a landmark development in the field of protein therapeutics—a means of producing antibody preparations with a single, genetically defined monoclonal specificity. The development of MAbs addressed many of the shortcomings associated with polyclonal preparations, particularly specificity and variability. The first such preparation approved for clinical use was muromonab (OKT3), a MAb of mouse origin specific for human cluster of differentiation (CD) 3 (described later).[63] OKT3 rapidly and specifically cleared T cells from the peripheral circulation and was shown to be a very effective treatment for allograft rejection.[63,87,209,217,235] Although many of the problems associated with the diffuse nature of polyclonal antibodies were addressed, some were not. The immune response against heterologous animal proteins and the cytokine release syndrome remained. OKT3's heightened specificity for the T cell receptor (TCR) not only produced more reliable T cell clearance but also more reliable T cell activation and cytokine release. The antimouse antibody response also limited prolonged dosing in a subset of patients.[131]

With the genetic engineering advances of the 1980s, the production of MAbs became much more efficient, theoretically allowing any surface molecules to be targeted. Effort was redirected from pan–T cell depletion toward fine targeting of relevant T cell subsets and blockade of functions unique to effector T cell activation. An example was the high-affinity interleukin (IL)-2 receptor, CD25 (described later), expressed predominantly on activated T cells. Additionally, methods of genetic engineering were developed to allow DNA encoding for binding sites from heterologous proteins to be grafted onto genetic sequences encoding the monomorphic scaffold of human antibodies to create chimeric or humanized MAbs.[31,132,191] These techniques also allowed for unique fusion proteins to be created combining the Fc portions of antibodies with nonantibody receptors and ligands, and allowing for cell surface molecules to be created in a soluble form with prolonged half-lives.

The humanization of antibodies and the use of human-derived receptors has practically eliminated the problem of antibody clearance and opened the possibility for prolonged treatment regimens. More recently, the production of fully human antihuman antibodies has become a practical reality.[313] Techniques including phage display mutagenesis and the transgenic production of mice containing human immunoglobulin genes that respond to immunization with human antibody now offer the promise of highly specific, nonimmunogenic, well-tolerated protein reagents. Human and humanized biologics are now making possible prolonged therapy with highly specific therapeutic agents.

Multiple surface molecules have been targeted by biologics investigationally, and several are now accepted as clinical therapies in transplantation and other indications. Biologic therapy is being increasingly adopted into standard practice, with 70% of kidney transplants performed in the United States now using prophylactic antibody therapy of some sort.[1,250] Despite this general trend, however, it has not been established whether this strategy is necessary in all cases. Although antibody induction reduces acute rejection rates in the first year after transplantation, the lasting effects of induction remain incompletely defined.[278,279] The modern era is now characterized by the availability of many promising agents and the challenge of understanding their most appropriate clinical use.

ANTIBODY STRUCTURE AND FUNCTION

The clinical effects of MAbs in transplantation relate closely to the physiological effects and structural characteristics of antibodies in general. Antibodies are one of two common glycoprotein antigen receptors that result from somatic gene rearrangements in specialized lymphocytes, the other being TCRs.[98,127] Five different heavy chain loci (μ, γ, α, ε, and δ) and two light chain loci (κ and λ), each with variable, diversity, or junctional (V, D, or J) and constant (C) regions, are brought together randomly by the recombination associated gene (RAG)-1 and RAG-2 apparatus to form a functional antigen receptor with highly variable binding ability. Antibodies have a basic structure of two identical heavy chains and two identical light chains (Fig. 20-1). The heavy chain usage defines the immunoglobulin type as being IgM, IgG, IgA, IgE, or IgD. This structure forms two identical antigen-binding sites brought together on a common region known as the Fc portion of the antibody. Although all of these subtypes have therapeutic potential, IgG antibodies have been the most commonly used clinically. IgG molecules are the most common result of peripheral immunization and are structurally easier to produce and manipulate.

Physiologically, antibodies exist as surface molecules on B cells, facilitating their antigen-specific activation and, importantly, are secreted into the serum to bind to and neutralize circulating antigens. Heterologous nonhuman antibodies are sufficiently similar to their human counterparts to facilitate most physiological effector functions when used in humans. Antibodies produced by mice, rabbits, and horses can be used in humans and still evoke biologically important effects. There is no animal that is a priori superior, however, and all heterologous antibodies have the potential to induce a neutralizing antibody response.

Antibodies can have a broad range of effects when they bind (Fig. 20-2). They can mimic the native ligand of a molecule and lead to signal transduction, or they can bind to the molecule in such a way as to prevent it from binding to its intended ligand.[289,315] Antibodies can be either activating or

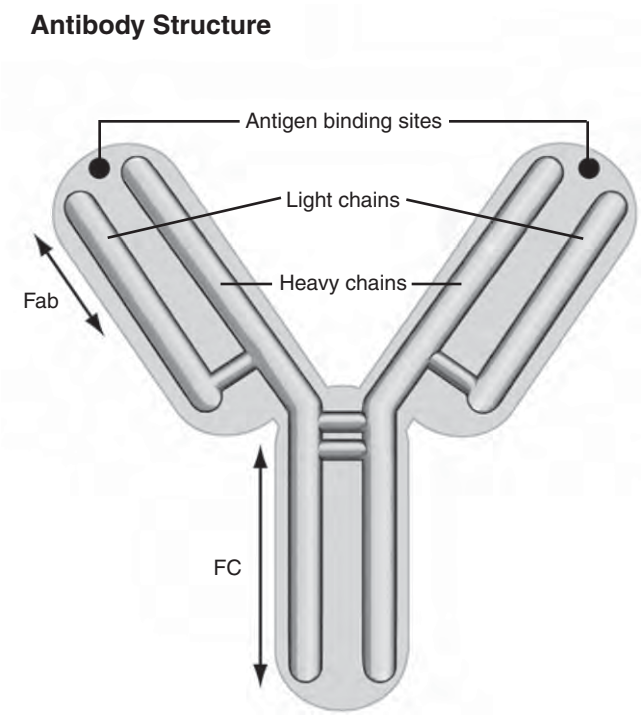

Figure 20–1 General antibody structure. The prototypic structure of an IgG molecule is shown.

inhibiting, and the predominant effect can be determined only through empirical in vivo analysis. Antibodies can bind to cells in such a way as to have no appreciable effect.[133] Antibody binding cannot be equated with functional significance. In some cases, a combined effect occurs whereby the antibody activates the targeted molecule but induces surface molecule internalization, effectively clearing the molecule from the cell surface and inhibiting its subsequent function.[144] This transient activation effect can lead to a burst of target cell activity (e.g., cytokine release), resulting in undesirable side effects, or can simply lead to surface modulation of the targeted molecule. Antibodies cannot target molecules that are not present on the cell surface. Although they can influence intracellular pathways, they cannot bind intracellular molecules directly.

Antibodies also activate the classical complement cascade and in doing so can induce complement-mediated lysis of a targeted cell. In addition, many phagocytic cells have receptors for the constant Fc region of antibodies and preferentially engulf cells coated with antibody through a process known as antibody-dependent cellular cytotoxicity (ADCC). Both of these activities facilitate the most noticeable effect of antibody therapies—target cell depletion. Depletion is only the most obvious effect of antibody therapy, however, and should not be assumed to be the most relevant or desired. Additionally, these effects depend on their antigen binding region and their nonvariable Fc region for effectiveness.[89] The importance of Fc segment effects is shown by nonspecific antibody infusion, which can mediate important effects presumably by neutralizing complement or saturating Fc receptors.[43,226]

It has become apparent that the maturation state of the targeted cells also can influence the response to antibody treatments. Specifically, cells that have matured into a memory phenotype have some degree of resistance to antibody-mediated depletion.[214] The mechanisms involved in depletion resistance remain to be defined, but memory cells differ from naive cells in many potentially relevant ways, including enhanced antiapoptotic and complement regulatory gene expression. The ultimate effect of antibody therapy may vary not only with the antibody preparation but also with the phenotype of the targeted cell and even the immune history of the recipient.

All of these effects can alter the function of molecules and cells, giving antibodies broad therapeutic potential. This array of effects makes antibody development difficult, however. Minor changes in antibody structure can radically alter their effects, and at present it is impossible to predict an antibody's properties on a structural basis alone. Certain IgG isotypes support complement and ADCC functions better than others, but generally an antibody must be tested in vivo to determine which of its many potential effects would be dominant.[103]

GENERAL CLINICAL CONSIDERATIONS FOR THE USE OF ANTIBODY PREPARATIONS

Immunosuppressive regimens used for organ transplantation can be generally characterized as induction, maintenance, or rescue therapies. Induction immunosuppression is intense treatment designed to inhibit immune responsiveness at the time of transplantation. It is usually potent to the point that its prolonged use is prohibitively toxic. Maintenance immunosuppression is of lesser potency, but is tolerable for long-term use and forms the basis of most immunosuppressive regimens. Rescue therapy is similar to induction in that it is intense, effective, and chronically intolerable, but differs in that it is used to reverse established rejection. Immunosuppressive medications can conceivably fall into any or all of these categorizations based on the dose and route used. Biologics currently are primarily indicated as rescue agents and are used in approximately 20% of all acute rejection episodes.[308] Their use as induction agents is growing; 50% to 70% of patients undergoing kidney transplantation now receive biologic induction.[1,250]

Antibody preparations also have been generally classified as depleting or nondepleting based on whether or not they deplete cells expressing the targeted antigen. Generally, T cell–depleting antibody preparations are primarily indicated for the treatment of refractory (e.g., steroid resistant) acute cellular rejections, acute rejections occurring in high-risk settings (e.g., marginal kidneys), and particularly aggressive vascular (e.g., Banff grade 2 or 3) rejections. Depleting antibodies also are being increasingly used as induction agents, although this is often an off-label use. Nondepleting antibody preparations and fusion proteins have been most commonly studied as induction agents and typically have less efficacy in rescue indications. Maintenance applications of biologics remain investigational.

Many depleting and nondepleting antibody preparations have been studied in randomized trials and have been proven efficacious in reducing the rate of acute rejection when used as an induction agent combined with standard maintenance regimens and compared with bolus methylprednisolone induction. Few prospective studies compare the prominent agents, however, and no agent has distinguished itself as clearly superior in all clinical circumstances. Most trials

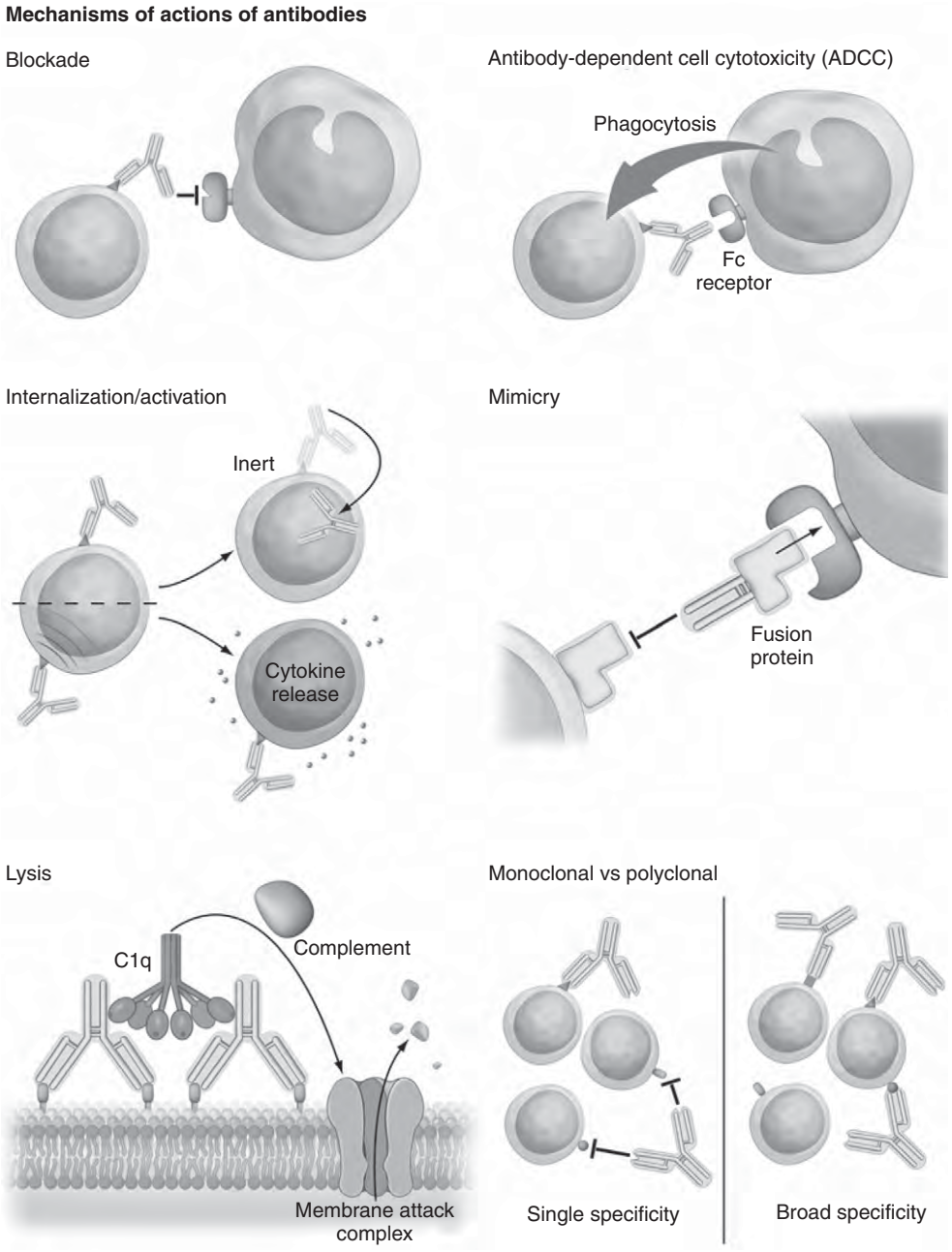

Figure 20–2 Mechanisms of action for antibody and fusion protein function. Antibodies can work via many mechanisms as depicted here and described in more detail in the text.

have used the surrogate end point of acute rejection, rather than more definitive outcome measures, such as patient or graft survival.

When considered as a whole, biologics have been convincingly shown to be more effective than steroids in reversing acute rejection.[308] When used as induction agents, they reduce the incidence of acute rejection in the first 6 months of transplantation in kidney recipients, particularly recipients who are sensitized, compared with the historical standard of bolus methylprednisolone induction and maintenance with cyclosporine, azathioprine, and prednisone.[278,279] Despite these benefits, there is no evidence that biologics alter long-term patient or graft survival in the era of modern immunosuppression.[278,279,308] Long-term analysis suggests that a measurable effect in kidney transplantation disappears after 5 years. This analysis may indicate that the side effects of maintenance therapy or comorbidities supersede early graft outcome and are the dominant determinants of outcome over time.

Antibody preparation use does not generally influence the rate of technical complications[128] but seems to reduce the risk of graft thrombosis in children.[259] Several induction strategies, in particular polyclonal antibodies and OKT3, have been shown, however, to increase measurably the risk of post-transplantation lymphoproliferative disease (PTLD) and death from malignancy when combined with conventional maintenance immunosuppression.[53,178,186,215,216] PTLD is a product of the intensity of the overall immunosuppressive therapy in combination with the recipient's preexisting immunity to the causative agent, Epstein-Barr virus.

Specifically, the expected PTLD rate is 0.5% in patients who do not receive antibody induction or who receive CD25-specific therapy. OKT3 induction carries a significantly higher rate of 0.85%, as does polyclonal depletion at 0.81%, particularly in recipients newly exposed to Epstein-Barr virus at transplantation.[53]

Other early complications, including cardiovascular and infectious deaths, correlate with antibody use, but the interpretation of this relationship is confounded by the preferential use of antibodies in high-risk patients.[37,186] Viral infection is a substantial concern, however, when using potent antibody therapy, particularly agents associated with T cell depletion. When used for induction or rescue, antibody preparations should be accompanied by broad prophylaxis against opportunistic infection. Antiviral therapy, such as ganciclovir or acyclovir,[15,118,291] should be initiated and continued for at least 3 months. The choice of agent is based on the pretransplant status of the donor and recipient. Oral candidiasis prophylaxis with nystatin or clotrimazole and *Pneumocystis* therapy with trimethoprim/sulfamethoxazole also should be maintained for several months. Individual clinical risks often dictate substantially longer periods of prophylaxis. Each antibody preparation has a unique side-effect profile and indication, which are discussed subsequently.

The use of antibody preparations for maintenance therapy had been limited until more recently by the immune response formed against the antibody itself. Recombinant humanized or chimeric antibodies and fusion proteins have essentially eliminated this as a concern, however. It is likely that future development of these molecules will investigate the role of antibodies in sustained preventive therapy.

POLYCLONAL ANTIBODY PREPARATIONS

Heterologous antibody preparations can be derived from many animals immunized with human tissues or cells (e.g., human lymphocytes). When reinfused into humans, these antibodies bind to antigens expressed on the original immunogen, where they mediate the effects discussed earlier. Given that these preparations are produced through whole-cell immunization, the resulting preparations contain a vast array of antibodies binding many epitopes expressed on the immunogen cells—some intended, and some not. Because each animal produces a unique immune response to an antigen, clinical-grade preparations are generally the result of pooled responses from many animals. For practical reasons, most polyclonal preparations are derived from rabbit or horse immunizations.

Ideally, a single renewable cell type equivalent to the effector cell in rejection could be used as a reproducible immunogen free from elements such as stromal tissue and neutrophils. No such cell has been developed, however. Commercially available polyclonal preparations continue to be made using heterogeneous cell populations or tissues such as thymus obtained from cadaver donors or surgical specimens. After immunization, the immunized animals are bled to obtain hyperimmune serum. The serum is typically absorbed against platelets, erythrocytes, and selected proteins to remove antibodies that could result in undesirable effects such as thrombocytopenia. Historically, hyperimmune serum was administered without additional purification, but now all commercially available products are purified to obtain only IgG isotypes. Even so, polyclonal antibody preparations are not fractionated to separate relevant from irrelevant antibodies preexistent from the environmental immune responses of the immunized animals. Greater than 90% of antibodies found in polyclonal preparations are likely not involved in therapeutically relevant antigen binding.[29,30,229,258]

Many groups have prepared polyclonal antibody preparations for their own institutional use, and this practice gave rise to a highly variable literature with little standardization or objective comparisons between products.[123,271,273,286] More recently, three dominant commercial polyclonal preparations have emerged: two rabbit-derived antibody preparations, antithymocyte globulin–rabbit (ATG-R, Thymoglobulin, or ATG-Meriux) and antithymocyte globulin–Fresenius (ATG-F), and one horse-derived product (ATGAM). Of these, Thymoglobulin is used most commonly in North America, with both rabbit preparations used in Europe.[1,250]

As discussed earlier, antibodies can mediate many effects when they bind to their target antigen, and a significant factor determining their effect is the antigenic specificity of the preparation. By their very nature, polyclonal preparations are composed of a wide variety of antibodies, and complete characterization has remained elusive.[29,30,229] Detected specificities include many T cell molecules involved in antigen recognition (CD3, CD4, CD8, and TCR), adhesion (CD2, lymphocyte function antigen [LFA]-1, and intracellular adhesion molecule [ICAM]-1), and costimulation (CD28, CD40, CD80, CD86, and CD154), and non–T cell molecules (CD16 and CD20) and class I and class II major histocompatibility complex (MHC) molecules (Fig. 20-3). Although all of these targets hypothetically can influence an immune response, and when studied individually, they do, it is unclear which of these specificities are crucial to the ultimate therapeutic effect. This broad reactivity with adhesion molecules and other receptors upregulated on activated endothelium has led many authors to advocate the preferential use of polyclonal antibody preparations in situations, such as prolonged ischemic times, where endothelial activation and ischemia-reperfusion injury is anticipated.[19,47]

Most polyclonal antibodies have prolonged serum half-lives of several weeks.[36,230] Nondepleted cells have been shown to be coated with heterologous antibody for months, suggesting that these preparations could influence the function of lymphocytes long after treatment has stopped. Lymphocyte subsets are abnormal for years after therapy, with particularly low $CD4^+$ T cell counts.[195] It also is reasonable to assume that antibodies targeting differing specificities would have variable effective half-lives based on the rates of surface molecule recycling, the affinity of the binding interaction, and the mechanism of action. Stimulating antibodies may have effects whenever they are bound, whereas inhibitory compounds could mediate an effect only when the natural ligand being antagonized is present. Polyclonal preparations likely have mechanisms of action that vary by batch, circumstance of use, and degradation state. It is unlikely that any single generalized mechanism exists. For the purposes of following the clinical effect, bulk T cell depletion is used as a general estimate of antibody potency, and polyclonal antibody preparations are considered depletional agents.

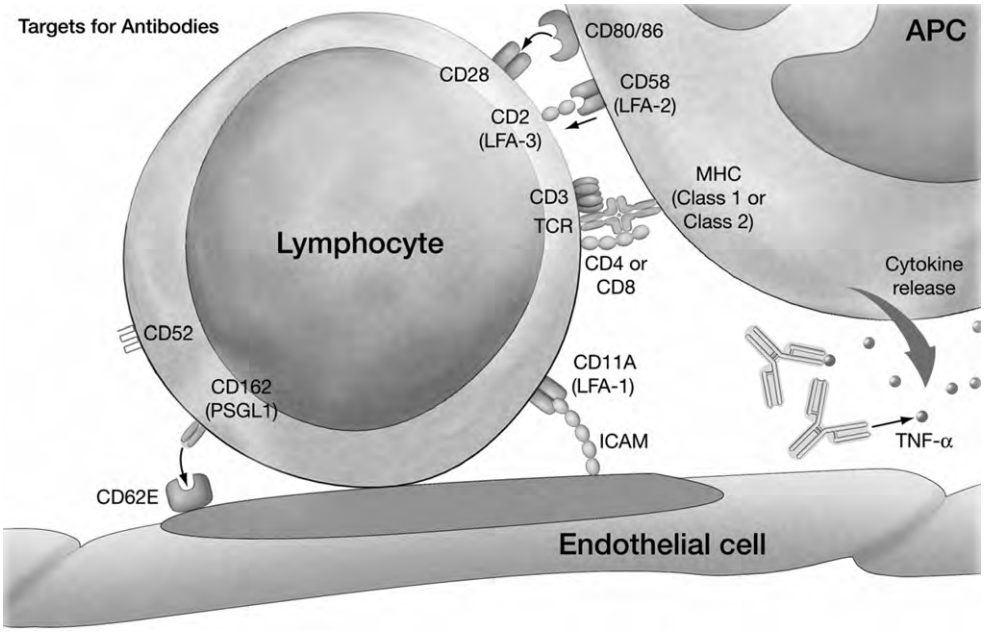

Figure 20–3 Sites of action for antibody and fusion proteins in clinical use. Shown are the surface molecules that have been targeted in clinical transplant trials and their respective ligands when known. APC, antigen-presenting cell.

SPECIFIC CLINICAL APPLICATIONS OF POLYCLONAL ANTIBODY PREPARATIONS

Polyclonal antibody preparations have been used in transplantation to achieve immunosuppression since the 1960s.[269] They are used as induction and rescue therapies, but the immune response to the proteins has precluded attempts to use them as maintenance drugs. As discussed previously, no single mechanism of action has been established, and they likely mediate their antirejection properties through depletion and other effects, including costimulation blockade, adhesion molecule modulation, and B cell depletion.[29,30,221,229]

Induction

Historically, polyclonal antibody preparations were used to bolster the effect of steroids and azathioprine in an attempt to reduce the unacceptably high rejection rates typical of the 1960s and 1970s. Generally, a 2- to 3-week course of a polyclonal antibody delayed the onset of acute rejection and reduced the requirement for high-dose steroids in the early postoperative period without significantly altering long-term survival.[62,63,126,273,309] After the introduction of cyclosporine, the use of polyclonal antibody induction fell from favor with the realization that this potent combination was associated with increased infectious and malignant morbidity.[185,211] With improved viral prophylaxis, a better understanding of the infectious etiology of PTLD, and more standardized commercial polyclonal products, there has been a marked resurgence of interest in polyclonal antibody induction.[1,250]

Most modern trials have evaluated polyclonal antibodies added to an otherwise rigorous maintenance regimen (typically triple immunosuppressive therapy). This intense regimen has statistically reduced acute rejection rates, but has reciprocated with increased infectious morbidity without changing long-term outcome.[49,193] This increased infectious risk may be acceptable in selected higher risk patient populations, such as recipients of donation after cardiac death donors, recipients of extended criteria donation, and patients with a high risk of rejection such as retransplant recipients and recipients with delayed graft function,[19,44,45,47,99,255,271] particularly when avoidance of prolonged calcineurin inhibitors is desired.[78,248,262]

More recent trials have attempted to address the increased infectious risk by pairing aggressive polyclonal induction with substantially reduced maintenance therapy. Two pilot studies have shown that ATG-R induction facilitates reduced maintenance immunosuppression in highly selected, closely followed patients, leading to graft and patient survivals comparable to the current standard.[270,277] These studies have emphasized administration before reperfusion, theoretically to take maximal advantage of anti–adhesion molecule effects, and relatively high-dose therapy, to limit the proinflammatory effects of reperfusion and to achieve rapid and lasting T cell depletion. Although these studies indicate that such an approach is possible, it remains to be seen if it can be generalized to noninvestigational settings.

Rescue

Although polyclonal antibodies remain controversial for induction, their use for the treatment of steroid-refractory rejection is an established indication. Many polyclonal preparations have shown their utility in this setting, spanning several decades of associated maintenance regimens. The first randomized trial showing that antilymphocyte serum was superior to high-dose steroids for the treatment of established rejection was reported in 1979.[253] In the context of azathioprine and prednisone maintenance

immunosuppression, antilymphocyte serum reversed rejection faster than bolus glucocorticosteroids, reduced the rate of recurrent rejection, and led to improved survival at 1 year.[199] Most rejection episodes occurring in the cyclosporine era and beyond respond to bolus steroids. Polyclonal agents have been indicated as a second-line therapy for steroid-resistant acute cellular rejection.[21,95,183,234] Recurrent rejection can be treated with repeated courses of polyclonal antibodies in situations where antirabbit (or antihorse) antibodies have not been formed.[27,179]

Of the currently available polyclonal preparations, ATG-R is used most commonly for rescue. It has been shown to be superior to ATGAM in terms of reversal of steroid-resistant rejection and persistence of a rejection-free state.[95] This difference has not been shown, however, to influence patient or graft survival.

Non–T cell–specific polyclonal antibody preparations also reverse established cellular acute rejection. Although not typically considered alongside T cell–depleting polyclonal antibody preparations, high-dose human IgG fractions (intravenous immunoglobulin) are polyclonal antibodies of random specificity pooled from human donors. Because they are not derived from animals, are not the products of heterologous immunization, and do not target a specific cell type, most of the adverse effects associated with polyclonal antibodies are not applicable. Nevertheless, high-dose human IgG fractions have been shown to reverse rejection despite the absence of any T cell–depleting abilities. Although a course of polyclonal anti–T cell antibody typically consists of 5 to 20 mg/kg given over several days, intravenous immunoglobulin is infused at much higher doses, 500 to 1000 mg/kg over 1 to 3 days, and at this dose has been shown to reverse established rejection with the same overall reversal rate as OKT3.[43] At least at high dose, nonspecific antibody infusion can modulate immune responses, perhaps through Fc receptor binding and resultant downregulatory effects of Fc receptor–expressing antigen-presenting cells (APCs).[143]

Administration and Adverse Effects

The polyclonal preparations used in modern clinical practice are generally given through a large-caliber central vein to avoid thrombophlebitis. In experienced hands, a dialysis fistula can be accessed for this purpose. More recent reports have suggested that polyclonal antibodies can be administered peripherally when diluted and formulated with heparin, hydrocortisone, or bicarbonate solutions.[227,314] An in-line filter is recommended to prevent infusion of precipitates that may develop during storage. The protein content should not exceed 4 mg/mL, and dextrose-containing solutions should be avoided because they induce protein precipitation.

Given the weeks-long half-lives of polyclonal antibodies, divided doses are not required for steady-state levels. The tolerability of these compounds is markedly improved, however, by spaced dosing. The rate of infusion is associated with the severity of side effects, and the course of therapy is generally over several days, with individual doses given over 4 to 6 hours. This time course depends on the dose used and is most applicable to the standard doses of ATG-R and ATG-F (1.5 mg/kg/dose for a total of 7.5 to 10 mg/kg) or ATGAM (15 mg/kg/dose for a total of 75 to 100 mg/kg). More recent investigational induction studies have employed substantially higher doses given over 12 to 24 hours or, alternatively, while the patient is anesthetized.[99,270,277] With a growing emphasis being placed on reduced length of stay after transplantation, larger infusions over fewer days are being employed.

Generally, rabbit-derived polyclonal preparations seem to be significantly better tolerated and more efficacious than ATGAM when used in a quadruple regimen for renal transplantation.[32,110] The most common acute symptoms associated with polyclonal antibody use are the result of transient cytokine release. Chills and fevers occur in at least 20% of patients and are generally treatable by premedication with methylprednisolone, antipyretics, and antihistamines. The use of polyclonal antibodies, particularly in the treatment of rejection, has been associated with an increase in the reactivation and development of primary viral disease caused by cytomegalovirus, herpes simplex virus, Epstein-Barr virus, and varicella.[2,101] It is likely, however, that this is not a class-specific association, but rather an indication of more intensive immunosuppression in general.

Dosage adjustment is warranted to counter leukopenia and thrombocytopenia. Peripheral cell counts drawn immediately after infusion tend to exaggerate cytopenic effects, and most side effects are promptly remedied by time. T cell counts or, more easily, absolute lymphocyte counts can be monitored to ensure that the preparation is achieving its desired effect. Absolute lymphocyte counts less than 100 cells/μL are typical. Attempts to tailor therapy to a specific peripheral cell count have been made to limit the use of these costly preparations. Rejection can occur and persist with very low T cell counts, however, and there is little evidence that dose variation by cell count alters efficacy.

As discussed earlier, polyclonal antibody preparations evoke a humoral immune response to themselves.[201,222,281] This response can be detected by enzyme-linked immunosorbent assay–based assays for antirabbit or antihorse antibody, but these tests typically are unavailable in most clinical settings. Failure to achieve significant T cell depletion suggests the presence of these antibodies. Serum sickness and anaphylaxis also can occur.[222] Preemptive skin testing is not practiced often because these tests have not correlated well with clinical outcome.[25,33] Rather, slow infusion rates should be employed during the initial exposure. Antianimal antibodies are most likely to occur in individuals with prior exposure to the preparation involved, but also can exist in individuals with significant prior exposure to the animals themselves.

The most common adverse symptoms related to polyclonal antibodies are fever, urticaria, rash, and headache. These are most likely related to the release of pyrogenic cytokines, such as tumor necrosis factor (TNF)-α, IL-1, and IL-6, which results from activating antibody binding to targeted cell surface receptors and subsequent cell lysis.[51,75,295] Infrequently, pulmonary edema and severe hypertension or hypotension can result in death. As the number of target cells decreases with repeated dosing, this response typically abates. The most concerning response is within the first 24 hours of the first dose, and patients should be monitored closely during this period. The response is limited considerably by methylprednisolone premedication. The rash associated with polyclonal antibody administration conversely tends to occur late in the treatment or at times after the last dose. It is generally self-limiting and requires only symptomatic treatment for urticaria. Antiendothelial antibodies in

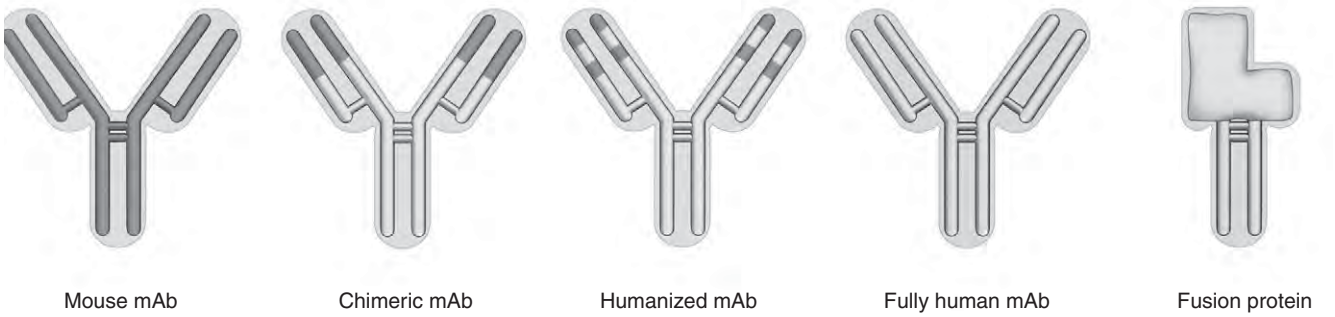

Figure 20–4 Types of monoclonal antibodies and fusion proteins. Dark areas represent portions of the molecule of nonhuman origin, and light areas represent human proteins.

polyclonal antibodies have been suggested to bind to donor endothelia and activate complement, inducing humoral rejection in some patients.[58]

MONOCLONAL ANTIBODY PREPARATIONS

MAb preparations differ from polyclonal preparations in that all antibody molecules are derived from a single genetic template and are identical. Batch-to-batch variation is eliminated, allowing the mechanism of action and half-life to be extrapolated based on a single ligand receptor interaction (although this still can be influenced by many individualized circumstances). This preparation narrows the scope of effect, however, making the use of these drugs more dependent on precise knowledge of the pathology involved.

Historically, MAbs are the product of clonally immortalized B cell hybridomas. More recently, genetically engineered mammalian cells have been the source. Use of other production methods, including viral and prokaryotic, or even plant cells, is being investigated.[9] As the production cell becomes increasingly distant from human, the resultant antibodies have increasingly aberrant glycosylation, which can radically alter their efficacy.[89] Regardless of the production cell, the resultant antibody can be purified of any extraneous proteins or other antibodies and used as an infused drug.

The most common method for deriving a MAb typically has been to immunize a mouse with a cell or cell fraction containing the antigen desired. Splenocytes are isolated from the immunized animal, and fused with an immortalized cell, producing many diverse antibody-producing cells. These cells are cloned (grown from single cell suspensions), and the supernatant from each clone is tested for reactivity against the desired antigen. A single robust clone with the desired antibody production characteristics is chosen and grown either in vitro or in a carrier animal. The supernatant from the clone is purified for therapeutic use. Because many MAbs are made by mouse B cells, they are mouse antibodies. Similar to animal-derived polyclonal antibodies, they can be cleared from the circulation by an antibody-directed immune response.[50] This immune response can cause anaphylaxis and neutralize the effect of the MAb in subsequent administrations.[246]

To improve the efficiency of antibody production and eliminate animal-derived protein epitopes, the gene fragment encoding the binding site of murine antibodies can be isolated and engineered onto the gene that encodes for nonpolymorphic regions of a human antibody, such as IgG1.[31,113,191] The resultant hybrid antibody gene can be transfected into a high expressing eukaryotic cell line and grown in vitro to produce antibodies that are predominantly human antibody, yet still bind to a specific human epitope (Fig. 20-4). These hybrid antibodies can be considered chimeric, if the entirety of the murine antibody binding site is used in the construct, or humanized, if the only murine portion is the specific complementary determining regions of the parent antibody.[132] Generally, chimeric antibodies preserve the specificity of the original antibody better, whereas humanized antibodies have less chance of evoking a neutralizing response.[83] Practically speaking, both are effective strategies that avoid the problem of antibody clearance.

The entire IgG gene has been transgenically expressed in a mouse.[313] This animal, when immunized, makes human, not mouse, antibody, which can be prepared for monoclonal production. This method is likely to be more efficient for producing truly human antihuman antibodies without the need to engineer each antibody individually.

When approved for clinical use, MAbs must be named based on their structural characteristics (Table 20-1). The generic name of a MAb gives the practitioner a reasonable understanding of the origins and specificity of the MAb.

MONOCLONAL ANTIBODIES IN CURRENT CLINICAL TRANSPLANTATION PRACTICE

Because each MAb has a singular specificity, each agent available for general clinical use is considered individually (see Fig. 20-3). Most MAbs are defined based on their targeted cell surface protein, and these generally are classified based on the CD nomenclature. A numerical CD designation does not define an antigen, but rather defines a molecule or group of molecules. MAbs that bind to the same CD molecule can bind to the same or different epitopes and have similar or different effects.

Table 20–1 **Nomenclature for Monoclonal Antibodies**

Prefix	Target		Source		Suffix
Varies based on preference of developer	-vi(r)-	Viral	-u-	Human	-mab
	-ba(c)-	Bacterial	-o-	Mouse	
	-li(m)-	Immune	-a-	Rat	
	-le(s)-	Infectious lesions	-e-	Hamster	
	-ci(r)-	Cardiovascular	-i-	Primate	
	-co(l)-	Colonic tumor	-xi-	Chimeric	
	-me(l)-	Melanoma	-zu-	Humanized	
	-ma(r)-	Mammary tumor			
	-go(t)-	Testicular tumor			
	-go(v)-	Ovarian tumor			
	-pr(o)-	Prostate tumor			
	-tu(m)-	Miscellaneous tumor			

Muromonab (OKT3; Murine Anti-CD3)

The TCR is linked to a transmembrane complex of proteins collectively known as CD3. The CD3 complex conveys an activating signal to the nucleus via a calcineurin-dependent pathway and serves as the fundamental signal in antigen-specific T cell activation. CD3 is present on essentially all T cells, defining the cell type. The TCR signal is generally known as signal 1 because it is primarily required for T cell activation and defines the antigen specificity of the T cell. Given that T cells are a crucial mediator of acute cellular rejection, CD3 was one of the first molecules to be targeted with MAbs, and OKT3 (muromonab) was the first MAb to gain clinical approval for therapeutic use in humans.[209]

Although the molecular target of OKT3 is singular and precise, its effects are many. The mechanism by which OKT3 mediates its immunosuppressive effect remains ill-defined. OKT3 is an IgG2a mouse antibody that binds to the ε component of human CD3. On binding, the antibody mediates complement-dependent cell lysis and ADCC and in doing so rapidly clears T cells from the peripheral circulation.[295] This binding event also leads to pan–T cell activation before their elimination, resulting in systemic cytokine release. The result is a marked cytokine release syndrome that is responsible for most of the adverse effects associated with the drug (see later).

When antigen binds to the TCR, TCR-CD3 internalization occurs; physiologically, this ensures that antigen binding is reflective of antigen burden and avoids activation mediated by continuous binding of a low-prevalence antigen. Similarly, OKT3 binding to CD3 leads to TCR-CD3 internalization.[51] T cells that are not cleared are often rendered void of surface TCR. These T cells that fail to express the TCR are incapable of receiving a primary antigen signal and are immunologically inert.

Bulk T cell clearance likely is not the primary mechanism of action of OKT3. Clinical rejection can occur with exceptionally low T cell counts achieved by other means, and stable graft function can occur with large T cell infiltrates within the graft itself.[124,151] Although the peripheral circulation is rapidly cleared by OKT3, many T cells can be found in the periphery and in the allograft itself.[144] A substantial amount of the rapid T cell clearance from the circulation is likely related to lymphocyte marginalization perhaps induced by the cytokines released and by the methylprednisolone that is given with OKT3. The overall effect of OKT3 is likely an aggregate effect of interrupted TCR binding, TCR internalization, cytokine-mediated regulatory changes, disrupted trafficking, and cell depletion. OKT3 has proven efficacy as an induction and a rescue agent. Its immunogenicity has prevented its use as a maintenance agent, and the drug is effective only in combination with other immunosuppressive compounds.[297]

Induction

Initial trials with OKT3 have shown that this MAb is an efficacious induction agent in kidney transplantation,[76,188,205] but only when combined with otherwise effective maintenance immunosuppression.[297] OKT3 cannot prevent rejection beyond the period of its actual infusion without additional maintenance therapy. Its usefulness as an induction agent is most pronounced in sensitized patients[208] and patients with delayed graft function, in whom it facilitates the delay of calcineurin inhibitor administration and the resultant nephrotoxicity.[22,136] It reduces the number of acute rejection episodes and the time to first rejection episode. In more recent literature, OKT3 has been shown to reduce acute rejection episodes compared with cyclosporine, azathioprine, or mycophenolate mofetil and steroids without changing patient or graft survival,[3,115,206] but to be equivalent to intravenous cyclosporine induction in children.[20] Despite its early prominence, use of OKT3 as an induction agent has dramatically declined in recent years, primarily as a result of its side-effect profile.

Because OKT3 is an entirely mouse-derived antibody, its use leads to the development of an antibody response directed against OKT3 in a significant percentage of patients. The development of antimouse antibodies varies based on the concomitant immunosuppression given, but is seen in at least 30% of patients.

Rescue

The primary modern indication for OKT3 is for the treatment of biopsy-proven, steroid-refractory, acute cellular rejection. In this indication, the side-effect profile is justifiable, and the efficacy of OKT3 is undeniable.[63,80,209,283,284] OKT3 is successful in providing sustained reversal of approximately 80% of

these vigorous rejections. It is effective even in the presence of prior aggressive lymphocyte depletion, suggesting that its mechanism of action is not primarily a result of bulk T cell depletion.[69,151,217] The incidence of steroid-refractory rejection, defined as failure to respond to 3 consecutive days of bolus methylprednisolone (e.g., 500 mg daily), has declined considerably with improved maintenance immunosuppressive agents, as has the incidence of rejection in general. The need for OKT3 has been reduced to only a few transplant recipients.

It is appropriate to consider OKT3 in patients with biopsy-proven acute rejection who have failed 3 days of therapy with high-dose methylprednisolone or some other vigorous rescue agent. Excessive delay beyond this time increases the complications of rescue therapy.[282] Additional indications for OKT3 include rejection associated with vasculitis (Banff grade 2 or 3 rejection) and rejections in clinical situations in which the organ is unlikely to tolerate prolonged immune attack.[137] Because misdiagnosis also can be responsible for apparent steroid resistance, a renal biopsy is indicated before the administration of OKT3 to confirm that acute cellular rejection is the cause of the renal dysfunction.

Administration and Adverse Effects

OKT3 targets T cells. It does not induce the pancytopenia typical of polyclonal antibody preparations. Its propensity to activate T cells induces a sometimes serious cytokine release syndrome, however, which is dependent on the number of T cells affected.[51,96] During the first dose, most T cells in the body are involved, many of which are in a highly activated state when rejection is ongoing. Cytokine release is worse at the first infusion, particularly for rescue indications. The effect abates with T cell clearance and after 3 days is usually negligible. Although many cytokines are likely involved in this syndrome, TNF-α is a dominant player because its sequestration can markedly attenuate the symptoms.[48,86]

Cytokine release can result in fever, nausea, vomiting, rigors, and general malaise reminiscent of severe flu-like symptoms.[285] It increases vascular permeability and can precipitate severe pulmonary edema. Patients with severe fluid overload owing to renal dysfunction should undergo dialysis before the first infusion. Occasionally, OKT3 can induce aseptic meningitis,[182] which in its most severe form can induce transtentorial herniation and death. Allograft thrombosis also has been reported.[3] Use of OKT3 increases the risk of PTLD, particularly in Epstein-Barr virus–naive recipients of kidneys from Epstein-Barr virus immune individuals.[53,285]

OKT3 generally is given as a peripheral infusion of 5 to 10 mg/dose. A central line is not required. Patients should be premedicated with methylprednisolone, acetaminophen, and diphenhydramine 1 to 2 hours before the initial infusion.[52,254] It is advisable to infuse the first dose over 1 to 2 hours to minimize the initial cytokine release. As the side effects abate with subsequent doses, the drug can be given over 5 minutes without adverse events. Patients should be monitored closely at the time of the initial infusion in an inpatient setting equipped to deal with cardiopulmonary arrest. Daily dosing is continued for 10 to 14 days, targeting a total dose of 70 mg. As OKT3 eliminates the TCR signal transduction pathway, calcineurin inhibitors can be safely discontinued or reduced substantially during therapy; this eliminates concomitant calcineurin inhibitor toxicity and facilitates more rapid return to normal renal function.[79,284] As the treatment course reaches an end, calcineurin inhibitor levels can be optimized for subsequent maintenance therapy to avoid rebound.

Human antimouse antibodies are formed in response to OKT3 administration in approximately one third of patients, depending partly on the concomitant immunosuppression used during therapy.[59,246] These antibodies should be documented in the event that subsequent OKT3 administration is contemplated, and reuse of the drug should be preceded by a test for antimouse antibody immunity.[169] Antibodies can be directed against the mouse IgG in general, or specifically against the OKT3 idiotype.[50,82,169] Measurement of CD3-expressing cells by flow cytometry during therapy ensures that the drug is effectively clearing T cells. Clinical presence of a pronounced cytokine release is substantial evidence, however, of a clinical effect. OKT3 is not used as a maintenance agent because of its side-effect profile and its immune clearance with time.

Interleukin-2 Receptor (CD25)-Specific Monoclonal Antibodies

The receptor for IL-2 is composed of three chains (α, β, and γ), of which the α and γ chains are constitutively expressed, and the β chain is induced with activation. The presence of the β chain, now designated as CD25, indicates prior T cell activation and identifies cells that have undergone some degree of effector maturation. CD25 has been targeted to suppress activated cells, while sparing resting cells.

There are two commercially available anti-CD25 antibodies, both of which have been engineered to avoid antimurine antibody responses. Daclizumab is a humanized anti-CD25 IgG1, and basiliximab is a chimeric mouse-human anti-CD25 IgG1. Both agents avoid immune clearance and can be used for prolonged periods without inducing a neutralizing antibody.[8,161,298] CD25 was the first molecule to be targeted successfully with a humanized MAb in transplantation.[155] These agents also avoid the serum sickness associated with mouse-derived, rabbit-derived, or horse-derived proteins.

Anti-CD25 antibodies are thought to work primarily through steric hindrance of IL-2 binding to CD25 and deprive T cells of this cytokine during early activation. There is little evidence for a depletional effect, or if there is one, it is limited to a few cells. More recently, it has become clear that CD25 induction is involved not only in the activation of cytotoxic T cells but also in the activation of cells with potentially salutary effects on the allograft, such as T regulatory cells.[275] T cells that have been previously activated and are responding in an anamnestic response are less dependent on IL-2 for proliferation. Heterologous responses (cross-reactive responses between a previously encountered pathogen and an alloantigen) or memory alloimmune responses seem not to be affected significantly by CD25 interruption. Given this biology primarily focused on naive T cell early activation, CD25-directed antibodies have found a role in induction, but have no role in the treatment of established rejection. Although there has been anecdotal experience using these antibodies for maintenance immunosuppression in the setting of calcineurin inhibitor toxicity with recurrent rejection, no study has formally evaluated this approach.

Induction

Many anti-CD25 antibodies, including anti-Tac,[155] 33B3.1,[264] LO-Tact-1,[119] and BT563,[296] have been tested in humans and been shown to delay modestly or reduce the onset of acute rejection when used with conventional maintenance immunosuppression. The experimental rodent antibodies have been generally abandoned in favor of the humanized/chimerized antibodies.

Daclizumab and basiliximab have been shown to reduce modestly the incidence of acute cellular rejection compared with methylprednisolone induction when used in triple or double immunosuppressive regimens, with exceptional patient tolerability in kidney and extrarenal transplantation.[23,117,135,197,198,200,249,299] Studies comparing basiliximab with polyclonal antibodies in regimens using cyclosporine, mycophenolate mofetil, and steroids have shown comparable outcomes.[168,194,261] The magnitude of the antirejection effect seen with anti-CD25 therapy depends to some extent on the intensity of the maintenance regimen, with earlier trials using cyclosporine-based and azathioprine-based regimens showing a 25% reduction and later trials in the tacrolimus/mycophenolate mofetil era showing a more modest 10% improvement. Anti-CD25 induction also has been used successfully in steroid-free regimens in kidney transplantation.[28,237] The use of anti-CD25 has not been shown, however, to facilitate more aggressive maintenance reduction regimens, such as monotherapy or calcineurin avoidance.[212,300]

Administration and Adverse Effects

Although the efficacy of anti-CD25 therapies is modest, the safety profile is highly favorable.[23,117,135,197,198,200,249,261,299] Binding of anti-CD25 antibodies does not mediate T cell activation, and no perceptible cytokine release occurs. Clinical trials generally have shown no increase in infectious complications or delayed wound healing. The risk of PTLD with anti-CD25 induction is similar to that when no induction agent is employed.[53]

Alemtuzumab (Humanized Anti-CD52)

Given the reduction in rejection achieved with prolonged polyclonal antibody–mediated T cell depletion, the ease of administration and consistency of MAbs, and the benefits of humanization, clinicians have sought agents with a combination of these traits. The CD52-specific humanized MAb alemtuzumab has emerged as a promising candidate.[177,306]

Alemtuzumab (Campath-1H) is a humanized IgG1 derivative of a rat antihuman CD52.[306] CD52 is a nonmodulating, glycosylphosphatidylinositol-anchored membrane protein of unknown function found in high density on most T cells, B cells, and monocytes.[108] CD52 is not found on hematopoietic precursor cells and does not seem to be an adhesion molecule; it is not necessary for T cell activation. Several versions of the nonhumanized anti-CD52 predecessors of alemtuzumab have been studied and been shown to be effective in mediating rapid T cell depletion and reversing steroid-resistant rejection. The humanized form has been studied in several indications and is currently approved for the treatment of lymphogenous malignancies.

Although not approved for use in solid organ transplantation, alemtuzumab has been used off-label as an induction agent.[250,251] Its mechanism of action seems to be predominantly related to bulk T cell depletion, with lesser depletion of B cells and monocytes. It rapidly depletes CD52-expressing lymphocytes centrally and peripherally in renal transplant recipients.[151] The use of alemtuzumab as a rescue drug is burgeoning, and there has been anecdotal investigation in this drug as a maintenance therapy.

Induction

In preliminary, uncontrolled studies, alemtuzumab has been shown to facilitate reduced maintenance immunosuppressive requirements without an apparent increase in infectious or malignant complications in kidney and extrarenal transplantation compared with historical controls.* Specifically, alemtuzumab has been used to achieve perioperative depletion in combination with triple immunosuppression and early steroid weaning; steroid-free regimens with calcineurin inhibitors and mycophenolate mofetil maintenance; and with monotherapy regimens of cyclosporine, tacrolimus, or sirolimus. Graft and patient survivals have been comparable to contemporaneously reported registry data, although the incidence of reversible rejection has predictably increased with decreases in concomitant maintenance therapy. Although the efficacy of alemtuzumab as an induction agent has been encouraging to date, prospective comparison with other regimens is just beginning.

More recent studies investigating alemtuzumab induction have shown that although it depletes all T cell subsets, it has a modest selectivity for naive cell types.[214] Nondepleted T cells exhibit a memory phenotype and seem to be most susceptible to calcineurin inhibitors. Maintenance regimens including calcineurin inhibitors seem to do best in alemtuzumab-based maintenance reduction strategies. The rapid and profound depletion has allowed for a delay in the initiation of therapeutic calcineurin inhibitor levels, however, and has made this an attractive option for patients with delayed graft function.[158]

Although alemtuzumab depletes B cells, its effect on T cells is more profound and lasting. It does not clear plasma cells. Some investigators have associated alemtuzumab administration with an increase in antibody-mediated rejection or at least post-transplant development of donor-specific alloantibody.[38] Whether this association is related to the effects of the antibody or the reductionist maintenance regimens used with alemtuzumab remains to be determined.

Rescue

The rodent antihuman CD52 predecessors of alemtuzumab, Campath-1M and Campath-1G, were originally tested as rescue agents.[90-92,107] In the original studies using anti-CD52 for steroid-resistant rejection, the antibodies were used with triple immunosuppression and steroid bolus therapy, leading to a prohibitively immunosuppressive regimen with excess infectious morbidity and mortality. With the success of alemtuzumab as an induction agent, there has been a resurgence of interest in its use as a rescue agent. Several anecdotal reports have recently emerged.[14,68] Additional study is required to define its role in this setting, although its predilection for naive cells may limit its efficacy after sensitization.

*References 13, 39, 40, 106, 139, 140, 151, 152, 157, 158, 184, 251, 280, 294.

Administration and Adverse Effects

Alemtuzumab can be administered through a peripheral intravenous catheter and can be dosed as a 30-mg flat dose or at 0.3 mg/kg dose over 3 hours. Almost total elimination of peripheral $CD3^+$ T cells can be expected within 1 hour of the first infusion, although secondary lymphoid depletion requires 48 hours and at least two doses.[151,214] Higher doses have not been shown to be of additional benefit in transplantation.

The rapid depletion characteristic of alemtuzumab is associated with a cytokine release phenomenon similar to, but less severe than, that seen with polyclonal antibodies or OKT3. Administration should be preceded by a bolus of methylprednisolone, diphenhydramine, and acetaminophen. The first dose should be given in a setting capable of dealing with hypotension, anaphylaxis, and other sequelae of cytokine release. Neutralizing antibodies have not been described for alemtuzumab.

Early trials investigating alemtuzumab as a therapy for multiple sclerosis suggested an association between its use and the development of autoimmune thyroiditis.[57] Specifically, patients with multiple sclerosis receiving high-dose investigational therapy with alemtuzumab had a significantly increased risk of hyperthyroidism developing 1 to 3 years after therapy. It has been hypothesized that T cell depletion, particularly depletion that selectively spares activated cells, could disrupt T cell regulation and unmask autoreactive clones. This effect could be most evident in individuals with low-level adjuvant maintenance immunosuppression, as was the case in the multiple sclerosis trials. There has been a case report of autoimmune thyroiditis in an alemtuzumab-treated renal transplant patient, leaving the potential for autoimmune disease as an unresolved matter of concern.[153]

Rituximab (Humanized Anti-CD20)

Rituximab is a chimeric MAb specific for CD20. CD20 is a cell surface glycoprotein involved in B cell activation and maturation whose natural ligand is unknown.[73] Similar to alemtuzumab, it has been developed and approved for use in lymphogenous malignancies, particularly $CD20^+$ B cell lymphomas and PTLD.[105] Given its specificity for B cells (and despite its lack of specificity for antibody-producing plasma cells), rituximab has been suggested to be a therapy for antibody-mediated rejection and rejections involving vasculitis.[17,18] Rituximab also has been used in regimens designed to facilitate transplantation in sensitized individuals, such as ABO-incompatible donor recipient pairs or transplants across a positive crossmatch following antibody removal.[263,293] At present, the role of rituximab in transplantation is largely investigational; however, similar to alemtuzumab, its off-label use is increasing considerably.

The mechanism of action of rituximab is presumed to be depletional, primarily through induced apoptosis.[74] Treatment with this antibody rapidly and specifically clears $CD20^+$ cells from the circulation. The role of $CD20^+$ cells in alloimmune responses is currently incompletely defined. Although these cells are precursors to antibody-producing plasma cells, they do not produce antibody without further maturation. Their role in acute antibody production is not well established, and it is unlikely that they have a direct effector cell role in rejection. Several authors have documented $CD20^+$ infiltrates as a marker for particularly recalcitrant acute rejection.[121,243] These cells are known to have APC function and it has been postulated that they serve to facilitate intragraft antigen presentation. Currently, rituximab is being used in induction and rescue indications.

Induction

The use of rituximab as an induction agent has been limited to patients with known donor-specific sensitization. In particular, rituximab has been suggested to be a surrogate for recipient splenectomy in patients undergoing donor desensitization with plasmapheresis or intravenous immunoglobulin infusion, or both.[263,293] It has not been prospectively studied, but rituximab seems to have some effect in reducing the rebound of alloantibody in these complex patients.

Rescue

Several reports have emerged suggesting that rituximab has a role in the treatment of vascular rejection (Banff classification 2 and 3) and in reversing emerging alloantibody formation.[17,18] This would be presumed to be relevant for allograft infiltrates shown to contain $CD20^+$ cells, although specific guidelines for the use of rituximab remain forthcoming. As with its use as an induction therapy, use of rituximab as a rescue agent remains investigational.

Rituximab's most important indication in organ transplantation is not as a rescue agent for rejection, but rather as a primary treatment for PTLD.[276] Although immunosuppression reduction is the primary therapeutic maneuver in PTLD, rituximab has emerged as an effective and well-tolerated maneuver to be interjected between immunosuppressive withdrawal and more aggressive chemotherapy.

Administration and Adverse Effects

Rituximab can be administered through a peripheral vein and is associated with few overt side effects. As with all proteins, anaphylaxis can occur, and initial doses should be given in a monitored environment. When used as a treatment for PTLD, it is typically given at a dose of 375 mg/M^2. Dosing as an immunosuppressant has empirically followed this regimen. Rituximab persists in the circulation for weeks to months, and a single dose effectively eliminates $CD20^+$ cells for a similarly prolonged period. The presence of rituximab in the serum artificially produces a pan-positive B cell crossmatch by complement dependent cytotoxicity and flow techniques. Characterization of alloantibody after the use of rituximab requires alloantigen-specific methods, such as solid-phase bead array assays.

Humax-CD20 is a new, fully human form of CD20-specific antibody.[16] It is currently in phase II clinical trials for rheumatoid arthritis and chronic lymphocytic leukemia. Although it is not used in transplantation to date, its application is anticipated.

FUSION PROTEINS

Fusion proteins are molecules that have been engineered from a single receptor targeting a ligand of interest fused to another protein that provides another salutary property. In transplantation, this secondary molecule is typically the Fc portion of an IgG molecule that gives the receptor an antibody-like half-life.[138,165,174] Fusion proteins also can involve the fusion of a specific toxin to a MAb to facilitate

epitope-directed drug delivery.[156] Fusion proteins are similar to MAbs because they have a single homogeneous specificity and can be composed of human or humanized components, limiting their immune clearance and opening their use for prolonged administration. There are no fusion proteins approved for use in transplantation at present. There are notable examples, however, of transplant-relevant fusion proteins in late stage development that are discussed subsequently.

Monoclonal Antibodies and Fusion Proteins in Clinical Transplantation Investigation

The promise of MAb therapy has led to the development of a rapidly expanding number of antibodies and fusion proteins targeting a wide variety of surface molecules. Several of these agents have shown efficacy in large animal transplant models and in early clinical transplant trials. Even more have been developed for autoimmune indications, such as psoriasis and rheumatoid arthritis, but their immunomodulating effects have clear potential in transplant indications. The following agents have been studied in early phase clinical transplant trials or have received approval for clinical use in nontransplant indications and have preclinical trials suggesting efficacy in transplantation. These agents are discussed based on their targeted ligand. All new antibodies under clinical development are now humanized or fully human.[232]

CD2-Specific Approaches

CD2, also known as LFA-2, is an adhesion molecule expressed on T cells and natural killer cells that binds to CD58 (LFA-3) on APCs and facilitates TCR binding and signal transduction. It has been targeted by the rat IgG2b anti-CD2 MAb, BTI-322, and more recently by siplizumab (also known as MEDI-507), a humanized IgG1 version of BTI-322. BTI-322 was investigated initially as an induction and rescue agent for cadaver donor renal and hepatic allografts and for graft-versus-host disease, and was shown to have biological activity and to give results consistent with the standard therapies available at the time.[173,192,224,267]

Clinical trials in psoriasis using siplizumab began in 1999 and were met with an unexpected propensity toward agent immunogenicity.[161] This agent has been used in nonhuman primate transplant tolerance trials with success in mixed chimerism–directed approaches[141] and has been used clinically as part of a nonmyeloablative conditioning regimen to achieve mixed hematopoietic chimerism.[266] Siplizumab currently is being investigated in phase I trials for T lymphocytic malignancies.[56]

Alefacept is a human fusion protein of the CD2 ligand (CD58, LFA-3) with IgG1 that has been shown to inhibit T cell proliferation. Its administration also has been shown to have a relative selective depleting effect on effector memory T cells, the same cells that have been relatively spared by other depleting MAbs and polyclonal preparations.[100,244] It has gained increased attention more recently in experimental transplantation. Alefacept is currently approved for the treatment of plaque-like psoriasis. Preclinical trials in nonhuman primate transplantation have shown that alefacept has minimal effect on graft survival when used alone, but that it does extend graft survival when used with adjuvant therapies.[84] Its use as a combination therapy remains to be completely explored.

CD3-Specific Antibodies

Targeting CD3 is a proven strategy as shown by the success of OKT3. Significant effort has been directed toward modernizing the anti-CD3 approach to avoid the many side effects associated with CD3 activation. Several CD3-specific antibodies, including huOKT3γ1, aglycosyl CD3, and visilizumab (HuM291), have been humanized and otherwise engineered to eliminate their undesirable activating properties and immunogenicity.[93,207,317] Phase I studies have indicated that modified versions of a CD3-specific antibody can achieve T cell depletion without the confounding problems of cytokine release or an antibody neutralization. Phase II trials using visilizumab in marrow transplantation have shown initial efficacy against graft-versus-host disease,[42] and huOKT3γ1 has shown promise as a prophylactic agent in new-onset diabetes mellitus.[116] These studies have shown that the side effects related to OKT3 use are not inherent in CD3-directed therapies, opening the door for more refined targeting of this receptor complex. Currently, huOKT3γ1 is in a single clinical study in islet transplantation. Visilizumab is in phase III trials for ulcerative colitis.[56]

CD4-Specific Antibodies

CD4 is a cell surface glycoprotein that binds to a monomorphic region of MHC class II molecules and in doing so stabilizes the interaction between the TCR and MHC class II. It is expressed on approximately two thirds of peripheral T cells and has partially defined several functional T cell subsets, including helper T cells and T regulatory cells. CD4 also is expressed by peripheral monocytes and other APCs, where its function is poorly characterized. It likely plays a crucial role in facilitating cell-to-cell communication among lymphoid cells, and it has lesser effects on physiological effector functions. Given its central role in cellular immune responses, CD4 has long been a target for immune manipulation, and several antibodies have been tested in transplantation. Generally, the efficacy has been exceptional in defined rodent models and modest in more clinically relevant settings; this may relate to the growing recognition that $CD4^+$ T cells have a potential role in tempering immune responses.[6,275,305]

Many studies have shown that anti-CD4 antibody induction dramatically inhibits the development of acute rejection in rodents, particularly when combined with supplementary donor antigen, such as donor-specific transfusion.[176,239,256,316] Given that the distribution of MHC class II molecules differs substantially between rodents and humans, however, these studies have not been predictive of the anticipated effect in humans. Depleting[81,218,240] and nondepleting[10,65,71,170,203,311] antibodies have shown an effect in experimental models suggesting that cell elimination, disruption of cell-cell communication, or signal transduction through CD4 may be mechanistically relevant. Two humanized anti-CD4 preparations have shown significant prolongation of nonhuman primate renal allograft survival.[65,218]

Initial clinical transplantation trials using anti-CD4 MAbs employed murine-derived antibodies, including OKT4A, BL4, MT151, and B-F5.[70,81,166] Predictably, these agents were subject to immune clearance, but nevertheless were shown to lead to $CD4^+$ T cell clearance. Regardless, patients experienced

rejection rates of 50%, and the agents were not sufficiently efficacious to warrant further development. Subsequent trials investigating the humanized OKT4A[61] and the chimeric cM-T412[187] have been evaluated in conjunction with cyclosporine-based maintenance therapy in kidney and heart transplant recipients.[235] In both cases, the antibody was well tolerated, and treated patients had low rates of rejection, suggesting that this approach is promising. Antibody responses toward the remnant murine portions of the MAb were surprisingly frequent, however. $CD4^+$ T cell depletion was not achieved using OKT4A, but was common with cM-T412.

The mouse antihuman CD4 MAb, Max.16H5, has been tested in pilot fashion as a clinical rescue agent.[233] Max.16H5 depleted $CD4^+$ T cells and was associated with reversal of rejection in most treated patients. Neutralizing antibodies were not detected. No trials investigating humanized anti-CD4 MAbs have been reported for rescue therapy.

Many human or humanized CD4-specific MAbs, including HuMax-CD4, TNX355, and 4162W94,[54,164,260] have been evaluated in phase I, II, and III trials for nontransplant indications, such as psoriasis and rheumatoid arthritis. These studies have shown that CD4-specific antibodies can influence immune responses and that their use is relatively safe in humans. Currently, there are no active anti-CD4 MAb trials registered in transplantation.[56]

Costimulation-based Therapies

Interest in the costimulation pathways as targets for immune manipulation has exploded in recent years.[111] Generally, these agents interfere with pathways that act to influence the outcome of antigen binding to the TCR. Costimulatory molecules can exert positive or negative influences on the efficiency of antigen presentation and recognition and alter the threshold for activation of naive T lymphocytes without having a primary activating or inhibitory function. Costimulatory molecule manipulation influences only cells with ongoing TCR activation and should have effects only on cells actively undergoing antigen recognition; this has been thought to allow for antigen-specific immune manipulation.

The most studied costimulatory receptor on T cells is CD28. It has two known ligands, CD80 (B7-1) and CD86 (B7-2), both of which are expressed on APCs. CD28 is constitutively expressed on most T cells and on ligation reduces the threshold for TCR activation.[134] CD152 (cytotoxic T lymphocyte–associated antigen 4 [CTLA4]) is an induced molecule expressed on T cell activation that is structurally similar to CD28 and competitively binds CD80 and CD86, transmitting an inhibitory signal that acts to terminate the immune response.[307] CD28 and CD152 serve reciprocal roles, both stimulated by the B7 molecules and facilitating (CD28) or quelling (CD152) a T cell response.

An additional receptor ligand pair that has gained considerable attention involves CD40 and CD154. CD154, also known as CD40-ligand, is expressed on activated T cells and other cells, including platelets.[11,102,114,150] CD40 is expressed on APCs. Although the specific effect of CD154 on T cells is incompletely defined, CD40 has a major influence on APC activation. CD40 ligation leads to marked APC activation, including increased expression of the B7 molecules and MHC, and stimulatory cytokine production greatly facilitating antigen presentation.[46] CD154 expressed by activated platelets greatly augments alloimmune responses and can serve as the sole source of CD154 responsible for rejection.[319] CD154 exists as a large inducible reservoir that can be triggered by platelet activation and augment antigen presentation at the time of a traumatic injury, including a transplant procedure. Many other costimulatory molecules have been investigated, but none has yet been exploited as a target for clinical manipulation.[55]

Costimulatory molecules can be targeted with blocking MAbs to inhibit their stimulatory effects. Because it is difficult to determine prospectively whether a MAb is stimulatory or inhibitory in vivo, and because costimulatory molecules have stimulatory and inhibitory effects, it has been challenging to find therapeutically reliable agents. Because CD152 and CD154 are upregulated on activated T cells, these costimulatory molecules also may serve as targets for selective elimination of activated effector cells.[189]

Although most experimental use of MAbs directed against costimulatory molecules has focused on tolerance induction (elimination of a need for any maintenance therapy), the clinical focus has been on pairing costimulation-directed biologics with maintenance minimization strategies, particularly calcineurin-sparing approaches. Agents interfering with the CD28/B7 and the CD40/CD154 pathways have reached clinical trials, with the B7-specific agents being developed the most (discussed in depth subsequently). Two humanized MAbs specific for CD154, hu5c8 and IDEC-131, have been shown in nonhuman primates to prevent acute rejection for months to years without additional immunosuppression and have been paired with sirolimus monotherapy and donor-specific transfusion to lead to operational tolerance in some cases.[146,147,220,318] Early human trials with hu5c8 were hindered by unimpressive efficacy and concerns for thromboembolic risk.[142,148]

CD154-specific therapies have not been studied clinically in recent years, and most preclinical attention has turned toward intervention with CD40 as opposed to CD154.[5] Nevertheless, investigational interest in CD154 manipulation remains intense.

A cocktail of two humanized MAbs specific for the B7 molecules CD80 and CD86 has been shown to facilitate prolonged renal allograft survival in nonhuman primates.[149] These antibodies reached clinical trials in organ transplantation and were shown to have initial safety in humans. Their development has not been pursued. A similar approach has been exploited with the fusion protein belatacept (see later).[165]

Although there are many costimulation molecules that have been targeted in rodents with dramatic results, no MAbs have been successfully transitioned to the clinic. This situation likely relates to the fundamental role that costimulation molecules have in general immunity and immune homeostasis. In addition to the thromboembolic concerns, marked adverse reactions have been associated with costimulation-directed MAbs. Severe autoimmune enteritis and vasculitis have been triggered by a humanized antibody directed against CTLA4, showing that CTLA4 signaling and its resultant negative T cell regulation is vital to preserving a balance with the activating effects of CD28 signaling.[122] Similarly, severe septic-like responses have been reported after the administration of TGN1412, a CD28-specific MAb tested in phase I trials.[274] There seems to be a fundamental balance between the two B7-specific T cell molecules CD28 and CD152 that is required to avoid dysregulated autoimmunity.

Greater success has been achieved with agents that target the B7 molecules CD80 and CD86, providing inhibition of potential CD28 and CD152 signals; this has been achieved through the use of B7-specific fusion proteins.

B7-Directed Fusion Proteins

Two costimulation-based therapies that are immediately relevant to renal transplantation are fusion proteins combining the extracellular domain of CD152 (CTLA4), a costimulatory receptor that binds to the costimulatory molecules CD80 and CD86 (collectively known as the B7 molecules), and the Fc portion of IgG1.[165,174] It is crucial to recognize that CTLA4 fusion proteins do not bind to CTLA4, but rather bind with high affinity to the B7 molecules CD80 and CD86. In doing so, they inhibit CD28 and CD152 signaling, rather than having the unopposed CD28 or CD152 signaling that has been associated with adverse events. Although, theoretically, inhibiting both could lead to immunosuppression through inhibition of CD28/B7 interactions or immunostimulation through prevention of CD152/B7 interactions, in practice the effect seems to be immunosuppressive.

Abatacept is a direct fusion of the extracellular domain of CTLA4 to the Fc portion of IgG1 and has been studied extensively preclinically under the name CTLA4-Ig. Rodent studies have shown that administration of CTLA4-Ig can prevent allograft rejection.[172,292] Abatacept has been shown to delay modestly the onset of acute rejection up to 30 days when used in nonhuman primate models of renal transplantation.[146] Although not dramatic, this is similar to the efficacy of clinically used polyclonal antibodies and anti-CD25 therapies in primate models. Currently, abatacept is approved for use in the treatment of rheumatoid arthritis, but has not been developed further in transplantation.[97,190]

Belatacept is a second-generation form of abatacept that has been investigated preclinically under the name LEA29Y. It has been mutated to contain two amino acid substitutions (L104E and A29Y) to give slower dissociation rates for its binding to CD86 and CD80. It has been shown to prolong the onset of acute rejection in nonhuman primates and to synergize with basiliximab and other clinically available immunosuppressants. Given its superior performance in preclinical models relative to abatacept, belatacept has been developed in clinical renal transplantation. In a phase II study, belatacept was used in lieu of cyclosporine in combination with mycophenolate mofetil and prednisone and was shown to give similar acute rejection rates with improved renal function at 1 year and a lower incidence of effects typically attributed to cyclosporine.[302] Based on these promising results, phase III trials in renal transplantation have begun. This agent is likely to become the first costimulation blockade agent developed for transplantation and to make possible many mechanistically novel therapeutic approaches toward tolerance induction.

Belatacept is currently being developed with a specific intention to be used to facilitate calcineurin inhibitor avoidance in renal transplantation.[165,302] It is being specifically envisioned not only as an induction agent but also as the first biologic to be intended for use as maintenance immunosuppression. Given its tolerability and apparent efficacy in phase II trials, belatacept represents an amalgam of antibody-relevant technologies that have been combined to create a nondepletional, nonactivating, human protein construct that can be administered without inducing a neutralizing response.

Tumor Necrosis Factor-α–based Approaches

Sequestration of cytokines using MAbs has long been contemplated as a therapeutic strategy in many inflammatory diseases. Although many cytokine-specific agents have been developed, only TNF-α–specific agents have gained widespread clinical use. TNF-α is a cytokine produced by many immune cells that is ubiquitously present in most inflammatory responses and has numerous general proinflammatory effects, including increased chemotaxis, vascular permeability, and fever. It has been considered as an attractive target for many inflammatory aspects of transplantation, including depletion-associated cytokine release syndrome, ischemia-reperfusion injury, and rejection. Three TNF-α–specific agents are currently approved for nontransplant conditions, and their use in transplantation is emerging.

Infliximab is a chimeric IgG1 MAb that binds to cell-bound and circulating TNF-α, sequestering it from the TNF receptor and inhibiting TNF-dependent proinflammatory effects. It has been developed for the treatment of numerous autoimmune disorders, including rheumatoid arthritis (its primary approved clinical use), psoriasis, Crohn's disease, and ulcerative colitis.[257,312] It has been used in pilot studies of many transplant indications, including renal, bone marrow, intestinal, and islet transplantation with suggestive success. Its predominant therapeutic effect in transplantation seems to be to limit paracrine cytokine-mediated activation within the graft and to mute the clinical sequelae of rejection without altering the overall infiltrate of inciting allosensitization.[67,94,213]

Etanercept is a soluble recombinant TNF receptor–IgG fusion protein that acts to absorb soluble TNF-α and limit its availability in the circulation. It is approved for the treatment of rheumatoid arthritis and has been increasingly evaluated for a role in the treatment of graft-versus-host disease.[130] Use of etanercept in solid organ transplantation has not been reported. Similarly, adalimumab is a TNF-α–specific MAb that has been approved for the treatment of psoriatic arthritis.[312] Golimumab is a fully human TNF-α–specific MAb that is in phase II trials for rheumatoid arthritis. No reports have been made of use of these agents in transplantation, although there are more than 20 trials in autoimmune indications.[56]

PSGL1 (CD162)

PSGL1-Ig is a fusion protein combining the extracellular domains of P-selectin glycoprotein ligand-1 (CD162) with the Fc portion of IgG1. CD162 is a ligand for P-selectin, E-selectin, and L-selectin, all of which have been shown to facilitate leukocyte and platelet adhesion. Because cell adhesion has been implicated as a primary event in reperfusion injury and in allorecognition, this drug has been contemplated as a therapy to limit the impact of events occurring during initial implantation. Treatment with PSGL1-Ig has been shown to attenuate ischemia-reperfusion injury, most prominently in rodent models of hepatic warm ischemia.[41,85,88] This agent is currently in a phase I/II evaluation in kidney transplantation to determine its efficacy in preventing reperfusion injury.[56]

Other Experimental Antibodies and Fusion Proteins

Almost all surface molecules expressed by leukocytes have been considered for therapeutic targeting. Many have been formally investigated in early clinical trials without sufficient promise to warrant additional clinical development. Others have significant promise in advanced preclinical settings but have yet to be tested in humans. Knowledge of these agents is useful for a complete understanding of the field.

Targeting CD5

CD5 is an adhesion molecule that is constitutively expressed on T cells and a subset of B cells.[228] It binds to CD72 and is thought to regulate the intensity of antigen receptor signal transduction. Its primary function may be costimulatory or inhibitory, but mounting evidence suggests that it has a role in self-tolerance. XomaZyme-CD5 Plus (XomaZyme H65) is a ricin-conjugated CD5-specific MAb that has been evaluated in clinical trials to prevent graft-versus-host disease after bone marrow transplantation, without apparent efficacy.[181] As the biology of this molecule is better understood, its re-evaluation as a therapeutic target may be warranted.

Targeting CD6

The human CD6 is a cell surface glycoprotein expressed by T cells and a subset of B cells. It has been shown to act as a costimulatory molecule and can stimulate T cells when cross-linked with CD28.[210] Anti-CD6 MAbs inhibit the interaction of CD6 with its ligand, activated leukocyte cell adhesion molecule.[268] Anti-T12, an anti-CD6 MAb, has been evaluated clinically, but has not shown consistent efficacy.[154] More recently, an anti-CD6 has been used ex vivo to T cell deplete bone marrow before its use in marrow transplantation.[242]

Targeting CD7

CD7 is a cell surface costimulatory molecule expressed on human T and natural killer cells and on cells in the early stages of T, B, and myeloid cell differentiation.[247,272] Its expression is augmented on activated alloimmune-responsive T cells. CD7 has been thought to be an attractive target for MAbs, offering the possibility of alloimmune-activated T cell–specific depletion.

SDZCHH380 is a chimeric mouse antihuman CD7 IgG1 that has been studied in initial clinical renal transplant trials.[167] SDZCHH380 induction was prospectively compared with OKT3 induction with comparable results. At 4 years, SDZCHH380-treated patients had good allograft function and did not develop neutralizing antibodies.[252] Additional development has not been reported.

Targeting CD8

CD8 is a glycoprotein present on approximately one third of T cells in lieu of CD4. Similar to CD4, it binds to a monomorphic region MHC, although it binds to class I rather than class II antigens. CD8 defines cytotoxic effector cells and perhaps a subset of regulatory cells. It facilitates binding between the TCR and class I molecules and is important in protective immune lysis of virally infected parenchymal cells. $CD8^+$ T cells are known to infiltrate allografts and to participate in allograft rejection.[236] Despite this demonstrated role in rejection, CD8 has not been successfully targeted in transplantation, perhaps because $CD8^+$ T cells are recruited late in an alloimmune response and have less regulatory control over immune responses than $CD4^+$ T cells. The CD8-specific MAb anti-Leu2a has been shown to deplete peripheral blood $CD8^+$ cells in humans; however, when tested as a rescue agent, it had limited effects in reversing renal allograft rejection.[310] More recently, 76-2-11, a mouse anti-swine CD8-specific MAb, has been shown to delay the onset of cardiac allograft vasculopathy in a miniature swine model of cardiac transplantation, suggesting that there may be a limited role for this approach.[7] Additionally, ex vivo depletion of $CD8^+$ T cells with anti-Leu2a has been investigated as a means of reducing graft-versus-host disease with promising preliminary results.[204] Anti-CD8 induction has not been investigated clinically in solid organ transplantation.

Targeting CD45

CD45 is a transmembrane protein tyrosine phosphatase expressed on T cells. It is physically associated with the TCR and facilitates the signal transduction function of CD3 through interactions with the zeta and zeta-associated protein-70 components of CD3.[175] CD45 exists in several isoforms (CD45RA, CD45RB, and CD45RO) that result from RNA spliced variants, and these are differentially expressed on T cells with varying degrees of maturity and activation. Of these, CD45RB has been most aggressively targeted as T cells expressing high amounts of this isoform skewing toward an aggressive T helper type 1 phenotype. CD45RB-specific MAbs have been shown to induce transplant tolerance in some rodent models and to prolong the survival of nonhuman primate renal allografts significantly.[175] Several antibodies have entered phase I trials for lymphocytic leukemia,[56] and at least one humanized anti-CD45RB is anticipated to enter early-phase clinical trials in renal transplantation.[301] ChA6, a chimeric MAb binding CD45RB and CD45RO (an isotype found on memory T cells), has been shown to prevent islet allograft rejection in mice by deleting memory T cells and being permissive for the persistence of protolerant regulatory T cells.[104]

Targeting Cell Adhesion

Given the fundamental requirement for adhesion molecules in most inflammatory responses, there has been long-standing interest in blocking adhesion interactions to prevent lymphocyte infiltration. As discussed previously, polyclonal antibodies are thought to bind to and inhibit some adhesion molecules. Several MAbs have been developed to target adhesion pathways. Among the most prominent is the LFA-1/ICAM-1 pathway. LFA-1 (CD11a/CD18) is expressed on mature T cells and binds to ICAM-1 (CD54) expressed on APCs and endothelial cells.[180,238,265] The pathway greatly facilitates initial lymphocyte recruitment at sites of injury and inflammation.[196] Adhesion pathways have been studied in several preclinical settings, including rodents[129,225] and nonhuman primates,[24,66,138] with survival being markedly prolonged in rodents and prolonged 30 days in primates.

Enlimomab, a murine anti-CD54 MAb, was successfully tested in a phase I trial involving high-risk deceased donor kidneys[112] and subsequently evaluated in a placebo-controlled phase II study combined with conventional triple-drug maintenance therapy.[241] No significant difference was detected between the treated and the placebo groups, and further development was not pursued. Similarly, odulimomab,

a murine anti-LFA-1 MAb, was studied as an induction agent compared with R-ATG in renal transplantation, with no significant difference being found between the groups.[125] A single rescue trial using the anti-LFA-1 murine MAb 25-3 failed to show efficacy.[171]

Efalizumab (Raptiva) is a recombinant humanized MAb that binds to human CD11a and inhibits the LFA-1/ICAM-1 interaction.[77,202] Efalizumab has been tested in phase I studies for renal transplantation and is currently in one clinical trial for islet transplantation to improve initial engraftment and function.[56] It is now approved by the Food and Drug Administration for treatment of mild-to-moderate psoriasis.

Targeting the T Cell Receptor

T cells bind their cognate antigen through their heterodimeric glycoprotein TCR. There are two general forms, an α/β form, expressed on 95% of peripheral T cells and responsible for specifying most alloimmune responses, and a γ/δ form, which is involved in innate immune responses and appears late in allograft rejection.[145] The TCR is a result of somatic gene rearrangement similar to that seen in antibody formation, and the specificity of each T cell can be defined by its individual TCR. Rejections based on specific TCR/MHC interactions select for specific TCR types showing that each MHC mismatch is recognized by a few clones, rather than by the entire T cell repertoire.[109] Although this finding fostered initial enthusiasm for targeting antigen-specific T cells through custom MAbs specific for a given TCR, this approach has been deemed impractical given the vast number of TCRs generated during T cell maturation and their variable cross-reactivity with variable MHC polymorphisms. Nevertheless, the success of targeting TCR-associated proteins such as CD3 has generated some interest in targeting monomorphic portions of the TCR directly. More recently, the realization that TCR signaling is required for T cell apoptosis and regulation has made preservation of the TCR a competing strategy.

T10B9, also known as Medi-500, is a murine IgM specific for a monomorphic determinant on α/β and γ/δ TCRs. It is effective in mediating T cell depletion in vitro and in vivo[34] and has been studied as a rescue and induction agent in renal and cardiac transplantation.[303,304] In both trials, the antibody-mediated T cell depletion was well tolerated. Its efficacy as a rescue agent seemed to be similar to that of OKT3, and the cardiac trial suggested efficacy as an induction agent. Nevertheless, the agent has not been developed further in organ transplantation, likely as a result of comparably effective humanized MAbs. T10B9 has been studied as a conditioning agent of bone marrow transplantation,[288] and a phase III trial using T10B9 as an ex vivo depletional agent for bone marrow transplantation has been completed.[56]

Targeting Complement

Proteins of the complement cascade have long been known to be crucial in mediating antibody-associated cytotoxicity.[60] Many approaches have been contemplated to achieve complement elimination in the setting of antibody presensitization, including plasmapheresis and intravenous immunoglobulin administration. More recently, it has been shown that complement, specifically that produced locally within the kidney itself, is a contributing factor facilitating peripheral T cell maturation and rejection.[219] Polymorphisms in complement expression have been shown to influence the incidence of rejection and renal allograft survival in ways not previously recognized.[35]

Two complement-specific agents have been used clinically and have been shown to be biologically active with promise for application in transplantation. Eculizumab is a humanized MAb specific for C5a, a key initiation factor in complement membrane attack complex formation. It has been shown to be a potentially effective therapy for paroxysmal nocturnal hemoglobinuria and is currently in phase III trials for this indication.[56,120] TP-10 (soluble complement receptor type 3) is a recombinant soluble protein that binds and inactivates the central activating component of the complement cascade, C3. It has been used in numerous preclinical settings and shown to be effective in preventing humoral xenograft rejection in a pig-to-nonhuman primate model.[223] It is currently being investigated in a clinical trial for its role in preventing cardiopulmonary bypass–related complications.[56]

Immunotoxins

Antibodies that have been joined either chemically or genetically with a specific cytotoxic agent (e.g., ricin or diphtheria toxin) have been termed immunotoxins.[163] These compounds have the specificity of MAbs but can exert a cytotoxic effect beyond that related to complement or ADCC. Many immunotoxins are now being investigated as tumor-specific cytotoxic agents for malignancies and have been shown to have potent antitumor effects. Two CD25-specific immunotoxins currently in clinical trials for lymphoblastic leukemia, LMB-2 and RFT5.dgA, have shown the ability to clear $CD25^+$ cells effectively from the circulation.[12,56,162] These agents could be envisioned to perform in a means analogous to the CD25-specific MAbs currently available, with a more potent depletional effect rather than acting predominantly through steric inhibition of CD25. Similarly, a CD22-specific immunotoxin is in trials for $CD22^+$ lymphoblastic leukemia and might be envisioned as an agent similar to other B cell–specific MAbs such as rituximab.[56,245]

Although immunotoxins have not been clinically tested in transplantation, ample preclinical data suggest that they have great therapeutic potential. Specifically, a macaque CD3-specific diphtheria immunotoxin, FN18-CRM9, has been used in nonhuman primate renal transplantation with remarkable success.[156,287] Treatment with FN18-CRM9 induces a rapid 3-log–fold depletion of T cells in the peripheral circulation and in the secondary lymphoid organs. Rhesus monkeys so treated before transplantation experience markedly prolonged allograft survival with no other maintenance immunosuppression, and a significant proportion survive for years after T cell repopulation. Although most of these animals eventually develop chronic allograft nephropathy,[290] the induction effect is impressive, and it has served as the conceptual inspiration for many clinical trials using T cell depletion.[39,151,157] Because most adults have antibodies against diphtheria toxin, this approach has not been successfully transferred to a human-specific MAb. Nevertheless, this is a promising approach for future development.

CONCLUSION

Antibodies are now established as valuable agents for the treatment and prevention of allograft rejection.

Currently, several polyclonal and monoclonal anti–T cell antibodies have proven roles in the treatment of steroid-resistant acute rejection. The last decade has seen increasing justification for the use of antibodies as induction agents. Antibody induction has been shown to be an effective means of achieving very low rates of acute rejection in renal transplantation. The trials performed to date have shown, however, that antibodies produce a modest benefit over regimens with calcineurin inhibitors, antiproliferative agents, and steroids, or that they are associated with increased morbidity. Nevertheless, it is appropriate to consider antibody induction as emerging from an adolescence of sorts, and less morbid target strategies and reduced maintenance regimens are expected to improve the side-effect profile of antibody induction schemes.

The optimal use of antibody induction is still being determined, but it is increasingly clear that the benefits derived from antibodies will be determined by their appropriate application. Modern immunosuppressive regimens should be individualized, specifically pairing induction agents based on their mechanism of action to a specific clinical need, and combining them with complementary maintenance therapies.

The future of transplantation continues to be cloaked by a need for more specific therapies with broader therapeutic indices. Antibodies are highly specific and have proved to be safe and effective drugs whose side effects are generally confined to the specific effects of the target antigen bound. Although the early hopes of clinicians have been slow to materialize, the technology associated with antibody design, construction, and production have consistently improved to yield a diverse array of agents to be tested and added to the transplant armamentarium. The future is likely to see almost exclusive use of humanized or human antibodies and fusion proteins as opposed to xenogeneic protein constructs. Past problems of antigenicity and severe cytokine release effects are surmountable, and as the targeted antigens become more rationally selected based on growing understanding of biology, antibodies and fusion proteins are expected to continue to establish themselves as crucial agents not only for induction and rescue but also, importantly, for maintenance therapy. Trials are beginning to explore this facet of antibody and fusion protein administration. Additionally, the use of antibody combinations may become an attractive way of manipulating the immune response. Transplant clinicians will need to become increasingly aware of immune therapies developed for autoimmune and malignant indications.

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Chapter 21

Other Forms of Immunosuppression

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SMALL MOLECULES

Inhibitors of Pyrimidine Biosynthesis

Brequinar sodium and leflunomide, initially developed as an antitumor drug (brequinar sodium) and an agriculture herbicide (leflunomide), were explored as immunosuppressants because of their ability to inhibit the enzyme dihydroorotate dehydrogenase, a key enzyme in pyrimidine biosynthesis. In addition, they have now been shown to exert immunosuppressive activity through the suppression of several tyrosine kinases.

Leflunomide and Malononitrilamides

The immunosuppressive effects of leflunomide were first shown in models of adjuvant arthritis and graft-versus-host disease,[16] and clinically it is known to be effective and safe for the treatment of rheumatoid arthritis.[237] The potential of leflunomide as an immunosuppressant in transplantation was extensively shown in various experimental studies, but its long half-life (several days) may pose the problem of potential overimmunosuppression in transplant patients. Analogues of the active metabolite of leflunomide (A771726 or 2-cyano-3-hydroxy-but-2-enoic acid-[trifluoromethylphenyl-amide]) have been developed and are called malononitrilamides (MNAs). FK778 (also known as MNA 715, HMR1715 or 2-cyano-3-hydroxy-*N*-[4-(trifluoromethyl)-phenyl]-2-hepten-6-enoic acid) is the best-studied synthetic MNA, and because it has a much shorter half-life than leflunomide (6 to 45 hours versus 15 to 18 days), it is an attractive alternative to leflunomide for application in organ transplantation.[112]

CHEMICAL STRUCTURE AND PHARMACOLOGY

Leflunomide (N-(4)) trifluoro-methylphenyl-5-methylisoxazol-4-carboximide) is a prodrug and is easily converted to its open ring metabolite A771726, which, in almost all in vitro and in vivo assays described, exhibits the activities described for leflunomide. The MNAs are designed to be structurally similar to A771726.

Leflunomide is insoluble in water and is suspended in 1% carboxymethylcellulose for oral administration. The half-life of leflunomide in humans is long (>10 days), and the drug is metabolized predominantly by the liver. Oral bioavailability of FK778 is not substantially affected by food, and no gender effect on pharmacokinetics was observed in phase I studies.[46]

MECHANISM OF ACTION

Leflunomide and its analogues have strong antiproliferative effects on T lymphocytes and especially on B lymphocytes. The production of IL-2 is not, or is only partially, inhibited by leflunomide.[50]

Kinetic studies on activated lymphocytes have shown that addition of exogenous uridine reversed the antiproliferative effects of leflunomide,[234] and that leflunomide retained its inhibitory activity when uridine was added 24 hours after initiation of stimulation. Inhibition of pyrimidine synthesis was proposed to be an important mechanism of action and was molecularly confirmed by showing a direct leflunomide-mediated inhibition of the enzyme dihydroorotate dehydrogenase.[306] Lymphocytes rely entirely on the de novo pathway of pyrimidine biosynthesis and cannot use another, so-called pyrimidine salvage, pathway. Dihydroorotate dehydrogenase inhibition leads to depletion of the nucleotide precursors uridine triphosphate and cytidine triphosphate, which are necessary for the synthesis of RNA and DNA, and hence strongly suppresses DNA and RNA synthesis.

Although in some reports it was mentioned that the immunosuppressive effect of A771726 in vivo was overcome by administering uridine,[246] this was not confirmed in other models.[270] The in vivo mechanism of action of leflunomide may depend on factors such as drug levels, disposable uridine pools, and immune activation pathways involved, but in particular, studies have indicated that in addition to inhibition of dihydroorotate dehydrogenase, leflunomide and the MNAs may act through inhibition of tyrosine kinases. Phosphorylation of the epidermal growth factor receptor of human fibroblasts has been shown to be inhibited

by leflunomide.[168] It also was shown that leflunomide directly inhibited the interleukin (IL)-2–stimulated protein tyrosine kinase activity of p56lck[168] and of p59fyn, which is associated with activation through the T cell receptor/CD3 complex. At higher concentrations, A771726 also inhibited IL-2–induced tyrosine phosphorylation of Janus kinase 1 (JAK1) and JAK3 protein tyrosine kinases, which initiate signaling by the IL-2 receptor.[70] In studies attempting to design inhibitors of the antiapoptotic tyrosine kinase Bruton's tyrosine kinase (BTK), leflunomide analogues were shown to exhibit strong inhibitory activities.[154] Because BTK is a key factor for T cell–independent antibody formation, this effect of leflunomide may explain its high potency in the suppression of T cell–independent IgM xenoantibody formation (see later).

The hypothesis that leflunomide may exhibit more than one mechanism of action in vivo was illustrated further in mice in which uridine restored proliferation and IgM production by lipopolysaccharide-stimulated B cells, whereas suppression of IgG production was not reversed. This phenomenon correlated in a dose-dependent manner with tyrosine phosphorylation of JAK3 and STAT6 proteins, known to be involved in IL-4–induced signal transduction pathways.[233] This double in vivo mechanism of action was confirmed in rats, in which xenogeneic reactivity was counteracted by the administration of uridine, whereas alloreactivity was not.[51] Other effects of leflunomide and MNAs have been described, such as inhibition of various macrophage functions, in particular the production of oxygen radicals,[120,160,161] the inhibition of IgE-mediated hypersensitivity responses,[110] the expression of IL-8 receptor type A,[169] and tumor necrosis factor (TNF)–mediated nuclear factor κB (NFκB) activation.[160]

FK778 has equivalent or stronger immunosuppressive activity than leflunomide in vitro and in vivo.[112,227] The immunosuppressive effect is synergistic with that of calcineurin inhibitors and mycophenolate mofetil.[23,66,148,206]

FK778 and leflunomide have been shown to possess antiviral effects. Both inhibit viral replication of members of the herpesvirus family by preventing tegument acquisition by viral nucleocapsids during the late stage of virion assembly.[71,128,299,300] Leflunomide is effective against multidrug-resistant cytomegalovirus in vitro,[299] although this in vitro activity is modest, and the selectivity index is low.[72] In a rat model of heterotopic heart transplantation, this anticytomegalovirus effect of leflunomide and FK778 was confirmed and was unaffected by uridine administration.[52,322] The successful treatment with leflunomide of polyomavirus type BK nephropathy[116,304] and cytomegalovirus in renal transplant patients has been reported.[113]

Leflunomide and FK778 have vasculoprotective effects, independent of the inhibition of dihydroorotate dehydrogenase.[224] FK778 also inhibits maturation of dendritic cells in vitro, by preventing upregulation of activation markers and IL-12 production. This phenomenon was not reversible by exogenous uridine.[323,324]

EXPERIMENTAL EXPERIENCE

In various transplantation experiments in rats, leflunomide was shown to be at least equal in potency as cyclosporine[16] and able to synergize with cyclosporine to induce tolerance.[149] Specific characteristics of leflunomide-mediated immunosuppression in rats were its ability to interrupt ongoing acute rejections[305] and its efficacy in preventing and treating chronic vascular rejection.[310]

One of the most attractive characteristics of leflunomide and the MNAs is their strong capacity to delay xenograft rejection[150] and to induce partial xenograft tolerance.[146] This capacity may be related to the strong suppressive effects of leflunomide on T cell–independent xenoantibody formation and to its ability to induce natural killer cell nonresponsiveness[146] and modulate xenoantigen expression.[147] Monotherapy with FK778 in rats,[191] and its combination with microemulsified cyclosporine in dogs[133] or tacrolimus in nonhuman primates,[205] reduced chronic allograft nephropathy[191] and significantly prolonged renal allograft survival.[133,191,205]

CLINICAL EXPERIENCE

Leflunomide has not been used in studies involving transplant patients yet because of its suboptimal pharmacokinetic profile. In a double-blind, randomized multicenter trial in rheumatoid arthritis patients,[237] the efficacy of leflunomide was found to be superior to placebo and similar to sulfasalazine. Overall, it was well tolerated.

A phase II multicenter study was performed with FK778 involving 149 renal transplant patients,[294] in which FK778 was combined with tacrolimus and corticosteroids. The patients receiving FK778 experienced fewer acute rejections, but there was no effect on graft survival at week 16. The reduction of acute rejection episodes was most pronounced in the subgroup in which target levels were obtained in the second week. Mean total and low-density lipoprotein cholesterol levels were 20% lower in the FK778 group than in the placebo group.

TOXICITY

Although rats tolerate leflunomide well after long-term administration, dogs develop anemia and gastrointestinal ulcerations. The most frequent side effects in arthritis patients receiving long-term leflunomide treatment were reported to be diarrhea (17%), nausea (10%), alopecia (8%), and rash (10%),[237] leading to a dropout rate of ± 5% in arthritis trials. In the previously mentioned phase II study involving FK778, there was a dose-dependent increase in side effects, including anemia, hypokalemia, symptomatic myocardial ischemia, and esophagitis.[294]

CONCLUSION

Leflunomide, and the newer designed analogues, MNAs, warrant careful investigation in transplant patients, especially their effect on antibody formation and on chronic vascular lesions. Their synergism with cyclosporine or tacrolimus may be valuable.

Brequinar Sodium

Brequinar sodium originally was developed as an antitumor drug. With the extensive data on safety issues regarding the use of brequinar as an antineoplastic agent, interest in the drug as an immunosuppressant to control graft rejection was stimulated.`

CHEMICAL STRUCTURE AND PHARMACOLOGY

Brequinar is a substituted 4-quinoline carboxylic acid (6 fluoro-2-(2-fluoro-1,1-biphenyl-4-yl)-3 methyl-4-quinoline-carboxylic acid, sodium salt). It is a water-soluble compound

that is readily absorbed after oral administration.[67] Peak concentrations are obtained approximately 2 hours after oral administration, with the half-life in humans reported to be about 8 hours. Two thirds of the breakdown products are excreted in feces, and one third are excreted in urine.

Brequinar inhibits the mixed lymphocyte reaction in a dose-dependent manner. The concentration required to produce a 50% inhibition is species dependent and varies from 0.025 μg/mL in humans to 40 μg/mL in monkeys. In humans, there is substantial interindividual variation in 50% inhibition values.[155]

MECHANISM OF ACTION

As previously mentioned, a first mechanism of action of brequinar is inhibition of the enzyme dihydroorotate dehydrogenase,[45] as evidenced by the fact that in vitro and some in vivo effects of brequinar can be reversed by the administration of uridine.[315] This mode of action explains the antiproliferative effect of brequinar and its ability to reduce mRNA levels of interferon (IFN)-γ, IL-2 and IL-10.[273] T lymphocytes and B lymphocytes are affected, explaining the effects of brequinar on cell-mediated and humoral immunity. Some immunosuppressive effects of brequinar are unaffected by uridine supplementation, however, suggesting that another mechanism of action may be involved. In this respect, it has been shown that brequinar can inhibit tyrosine phosphorylation in anti-CD3–stimulated murine T lymphocytes.[315] It was shown that brequinar-mediated control of lymphadenopathy and autoantibody production in MRL-lpr/lpr mice depended only partially on inhibition of pyrimidine nucleotide synthesis and that it was rather associated with in vivo inhibition of protein tyrosine phosphorylation.[314]

EXPERIMENTAL EXPERIENCE

In rats, brequinar treatment, three times weekly for 30 days, was in most recipients associated with permanent kidney and liver allograft survival. Prolongation of heart allograft survival was more difficult to achieve and required longer periods of treatment.[59] Survival times of small bowel allografts and hamster xenografts in rat recipients have been shown to be prolonged equally by brequinar treatment.[60]

The difference in mechanism of action of brequinar and cyclosporine led to the expectation that potential synergistic action would allow significant dose reductions in brequinar and fewer side effects. Brequinar was shown to be very active on B lymphocytes, whereas the principal target cells of cyclosporine are T cells. Although a synergistic effect of brequinar with cyclosporine was documented in various experimental models,[143] this combination was complicated by enhanced toxicity of the two compounds as a result of drug accumulation.[189]

In xenograft rejection, the humoral immune response is crucial and was shown to be successfully inhibited by combined treatment with brequinar and cyclosporine.[60] Similarly, brequinar treatment before the transplantation of allogeneic hearts to previously sensitized recipients significantly delayed graft rejection and was associated with suppression of antibody responses to donor tissues.[319]

CLINICAL EXPERIENCE

Following its approval for phase I studies in 1991, brequinar was tested in 32 patients receiving kidney transplants. Patients received standard cyclosporine and steroid therapy; in addition, brequinar was initiated within 48 hours after the transplant and given on alternate days, aiming at plasma levels of less than 2 mg/mL. In this first series of patients, evidence indicated that the number of rejection episodes was significantly reduced.[58] These initial positive results were not confirmed in other studies, however, and enthusiasm for the drug was tempered because of its narrow range of therapeutic effectiveness and the risk of thrombocytopenia at high doses.[117]

TOXICITY

In rats, the combination of brequinar and cyclosporine was shown to lead to enhanced toxicity of both compounds as a result of drug accumulation.[189] In humans, the most common side effects at high doses were thrombocytopenia and mucositis.[58,117]

CONCLUSION

Although the characteristics of brequinar suggest that it would be an attractive immunosuppressant, the suboptimal pharmacologic profile jeopardizes its use in transplant patients. The future use of this drug in transplantation would require the development of analogues exhibiting a shorter half-life and less toxicity.

15-Deoxyspergualin

In 1981, spergualin (a water-soluble peptide) was isolated from the culture filtrate of *Bacillus latersporus* and explored as a new anticancer or antibiotic substance.[266] Its analogue 15-deoxyspergualin subsequently became widely known as a promising new immunosuppressant.

Chemical Structure and Pharmacology

Spergualin (1-amino-19-guanitido-11,15-dihydroxy-4,9, 12-triazathioprinenonadecane-10,13-dione) was synthetically dehydroxylated to produce 15-deoxyspergualin. Because of its poor oral bioavailability, 15-deoxyspergualin must be delivered parenterally.[272] The drug is rapidly eliminated, primarily through the kidney.[280]

Mechanisms of Action

The precise mode of action of 15-deoxyspergualin is unknown. It specifically binds to Hsp 70, a heat-shock protein[177] and is believed to have its principal effect by inhibiting activation of transcription factor NFκB in antigen-presenting cells and monocytes.[99] This premise may explain why 15-deoxyspergualin inhibits monocyte and macrophage functions such as antigen presentation, major histocompatibility class II upregulation, IL-1 release, or superoxide production.[68,296] T cell–specific functions, such as concanavalin A blastogenesis, mixed lymphocyte reaction responsiveness, and IL-2 production, are only poorly affected or not affected at all.[261] In contrast, B lymphocyte maturation and antibody production are sensitive to 15-deoxyspergualin.[244] On the basis of these characteristics, 15-deoxyspergualin is considered to be a particular immunomodulatory agent with a unique mechanism of action.

Experimental Experience

In most animal experiments, 15-deoxyspergualin did not seem to be effective when used to prevent rejection.

When treatment was initiated several days after transplantation, however, the drug was found to be much more effective.[228] This observation suggested that 15-deoxyspergualin may be useful for the treatment of rejection crises. This suggestion was confirmed in dogs,[8] and treatment of rejection subsequently became the major indication for clinical use (see later). Because of its effects on monocytes, macrophages, and B lymphocytes, 15-deoxyspergualin seems promising for xenotransplantation; this is illustrated by the fact that it is effective in stringent xenogeneic transplant models, such as primary nonfunction of islet xenografts[271] and the induction of xenogeneic chimerism in the pig-to-baboon combination.[217]

Clinical Experience

In clinical transplantation, experience with 15-deoxyspergualin was obtained mostly in patients with rejection. Between 1988 and 1991, several clinical trials evaluated the effects of 15-deoxyspergualin in the treatment of kidney allograft rejection. Overall, results indicated that a 7- to 10-day course of 15-deoxyspergualin monotherapy reversed 70% of the acute rejections and 40% of the rejections that were already in a more chronic phase. When a 3-day course of high-dose methylprednisolone was added, the results improved to 90% and 60%, respectively.[7] Overall, treatment of recurrent rejection was as effective as treatment of first episodes of rejection.

Because of its effects on antibody formation, 15-deoxyspergualin also was explored in conjunction with cyclosporine, prednisolone, and antilymphocyte globulin for its capacity to inhibit secondary antibody production in ABO-incompatible or HLA-presensitized kidney transplant recipients and in pig islet xenograft recipients.[94,262] 15-Deoxyspergualin was safe and effective in ABO-incompatible and preformed antibody–positive kidney transplantation in a prophylactic and a therapeutic regimen for acute rejection.[262] In two of three 15-deoxyspergualin–treated patients, small amounts of urinary porcine C-peptide were detectable for several weeks, indicating some survival of xenogeneic fetal porcine islets.[94] More recently, Kirk and colleagues[124] found that the combination of alemtuzumab and 15-deoxyspergualin failed to induce tolerance in a small series of living donor kidney transplant recipients, but experience is too limited to draw firm conclusions.

Toxicity

In the clinical studies involving 15-deoxyspergualin, the most common side effects were subjective complaints of facial numbness and gastric discomfort. These symptoms disappeared as soon as the infusion was interrupted. Bone marrow suppression was the most common serious side effect, but it responded effectively to treatment with recombinant granulocyte colony-stimulating factor.[7,262]

Conclusion

Until analogues are developed that allow for oral administration,[137] the major clinical indication of 15-deoxyspergualin is limited to the treatment of rejection crises. 15-Deoxyspergualin may be an alternative to steroids or antilymphocyte agents. The fact that it remains effective after recurrent administration is promising. In the future, if xenotransplantation becomes a reality, 15-deoxyspergualin may become important, especially for islet xenotransplantation. Because of its effects on macrophages and B lymphocytes, it may be essential to tackle the difficult problem of primary graft nonfunction.

FTY720

Origin and Chemical Structure

FTY720 is a synthetic structural analogue of myriocin, a metabolite of the ascomycete *Isaria sinclairii*, a fungus that vegetates on wasps.[83,84,223] FTY720 has a molecular weight of 344 daltons and is a 2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride. This chemical structure is different from cyclosporine, FK506, and other current immunosuppressants.

Antirejection Properties in Small and Large Animals

FTY720 given daily by oral gavage has marked antirejection properties in mice, rats, dogs, and monkeys. FTY720 (0.1 to 10 mg/kg) prolongs survival of skin allografts in highly allogeneic rodent models.[47] In a DA-to-Lew rat combination, a short course of peritransplant oral FTY720 (5 mg/kg; day −1 and 0) prolongs cardiac allograft survival and is as efficient as a 10-day post-transplant treatment with FK506 at 1 mg/kg.[312] Cardiac and liver allograft survivals are prolonged in the ACI-to-Lew rat model by either induction or maintenance treatment with FTY720.[257] Even delayed administration of FTY720 interrupts an ongoing allograft rejection suggesting a role for FTY720 as a rescue agent.[257,313] FTY720 blocks not only rejection but also graft-versus-host disease after rat intestinal transplantation.[170] Peritransplant and post-transplant FTY720 (0.1 to 1 mg/kg/day) also has profound immunosuppressive properties in kidney transplantation in monkeys and dogs and in liver transplantation in dogs.[123,259,279,318]

Synergy with Other Immunosuppressants

Small and large animal models provide evidence that FTY720 acts in synergy with calcineurin inhibitors, cyclosporine, and FK506 and that this benefit does not result from pharmacokinetic interactions.[258] An induction course with FTY720 acts in synergy with post-transplant FK506 in prolonging cardiac allograft survival in rats.[312] A similar phenomenon has been observed when FTY720 is used after transplantation in combination with cyclosporine in rat skin and heart allografts.[47,104,123,258] FTY720 shows synergistic effect with FK506 and cyclosporine in heart and liver transplants in the ACI-to-Lew rat model.[318] FTY720 shows synergy with cyclosporine in kidney transplantation in dogs (0.1 to 5 mg/kg/day) and monkeys (0.1 to 1 mg/kg/day).[279] Finally, FTY720 (0.1 mg/kg) synergizes with cyclosporine and FK506 in dog liver transplantation.[260] Synergy between FTY720 and rapamycin also was observed in cardiac transplantation in rats.[302]

Mechanisms of Action

In contrast to cyclosporine and FK506, FTY720 is a poor inhibitor of T cell function in vitro.[279] In particular, FTY720 does not influence antigen-induced IL-2 production. This lack of in vitro immunosuppressive activity contrasts with the marked antirejection properties of FTY720 seen in vivo.

Rats receiving one oral dose of 10 mg/kg of FTY720 show a rapid and profound decrease in peripheral

lymphocyte counts. These counts remain significantly depressed, but return to pretreatment levels within 14 days.[257] Fluorescence-activated cell sorter analysis indicates a specific reduction in CD3 cells, with unchanged CD4-to-CD8 cell ratio.[313]

It was first suggested that FTY720-induced lymphocytopenia results from apoptotic lymphocyte death. In vitro exposure to high FTY720 concentrations (4×10^{-6} M) induces chromatin condensation, typical DNA fragmentation, and formation of apoptotic bodies.[258] Apoptosis after administration of FTY720 also has been documented in vivo.[47,145,163,258] FTY720 causes intragraft apoptotic lymphocytic death in animals with ongoing liver allograft rejection.

A second mechanism of action of FTY720 is through alteration of lymphocyte trafficking.[48,98,159,167] After FTY720 administration (4 mg/kg or 8 mg/kg) in mice, labeled B cells and T cells immediately leave the peripheral blood and migrate to the peripheral lymph nodes, mesenteric lymph nodes, and Peyer's patches. The labeled cells return to the peripheral blood after withdrawal of the drug and do not undergo apoptotic death. Migration is equivalent for T cells, CD4 cells, CD8 cells, and B cells.[321] This altered cell trafficking is accompanied by a reduction of lymphocyte infiltration into grafted organs,[321] a phenomenon that would contribute to the antirejection property of the drug.

Lymphocytes treated ex vivo with FTY720 and reintroduced in vivo similarly migrate to the peripheral lymphoid tissues, indicating that FTY720 acts directly on lymphocytes. The effect of FTY720 is abolished by previous exposure to pertussis toxin, suggesting that FTY720 modulates G protein–coupled chemokine receptors on the cell surface of the lymphocytes.[33] In addition, the process of accelerated homing was completely blocked in vivo by coadministration of anti-CD62L, anti-CD49d, and anti-CD11a monoclonal antibody, suggesting that FTY720 directly affects the homing receptors.[48] It has been suggested that $CD4^+CD25^+$ T regulatory cells are differently affected by FTY720 compared with T effector cells.[225] $CD4^+CD25^+$ T regulatory cells express lower levels of sphingosine 1-phosphate 1 ($S1P_1$) and $S1P_4$ receptors and show reduced response to S1P. In vitro FTY720-treated $CD4^+CD25^+$ T regulatory cells possess an increased suppressive activity in an antigen-specific proliferation assay.[225]

FTY720, in the presence of TNF-α, increases the expression of certain intercellular adhesion molecules on human umbilical vein endothelial cells in vitro.[144] Alteration of cell trafficking by FTY720 may result not only from its direct action on lymphocytes but also from an effect on endothelial cells. S1P receptors also are present on murine dendritic cells. On administration of FTY720, dendritic cells in lymph nodes and spleen are reduced; the expression of CD11b, CD31/PECAM-1, CD54/ICAM-1, and CCR-7 is downregulated; and transendothelial migration to CCL19 is diminished.[136]

In a murine model of cardiac transplantation, alloantigen-specific effector-memory T cells were sequestrated in regional lymphoid tissue, and a decreased T cell infiltration in the allograft was observed after FTY720 treatment.[97,325] Delayed administration of FTY720 attenuated the progression of vasculopathy and interstitial fibrosis, suggesting that FTY720 interrupts the trafficking of activated effector-memory T cells.[97]

Toxicity

Pulmonary, cardiac, and neurologic toxicities have been reported, but only in animals exposed to very high doses of FTY720. The parent compound of FTY720 (myriocin) induces severe digestive toxicity, but FTY720 itself does not.[46,84] At therapeutic doses, FTY720 seems to be well tolerated. Doses of 5 mg/kg cause no clinical toxicity in rats. Studies in dogs indicate that doses of 5 mg/kg are equally well tolerated for 90 days.[47,123] At 10 mg/kg, no toxicity was observed in cardiac transplantation rats receiving post-transplant FTY720.[47,104,258] A single dose of FTY720 at 10 mg/kg was lethal, however, when given before transplantation to rat liver recipients. Monkeys treated with FTY720 (0.1 to 1 mg/kg) showed no specific side effects.[279] Typical side effects of calcineurin inhibitors—nephrotoxicity, neurotoxicity, and diabetogenicity—have not been observed with FTY720.

FTY720 in Humans

Stable renal transplant patients maintained on cyclosporine tolerate well one oral dose of FTY720 (0.25 to 3.5 mg).[25,35,36,235] In particular, no pulmonary toxicity was noted. Although clinically asymptomatic, a few episodes of bradycardia were observed. One episode of headache led to drug withdrawal.

Similar to its effect in animals, single doses of FTY720 cause a lymphocytopenia that is dose dependent in intensity and duration and that affects CD4 cells, CD8 cells, memory T cells, naive T cells, and B cells equally. Monocyte and granulocyte counts remain unchanged. Doses of 1 mg caused a rapidly reversible decrease in lymphocyte count with a nadir at about 6 to 12 hours. Higher doses of FTY720 result in more sustained and more profound lymphocytopenia.

Maximal concentration and area under the curve are proportional to the dose, indicating that the pharmacokinetic profile of FTY720 is linear. The volume of distribution is larger than the blood volume, indicating a widespread tissue penetration. FTY720 undergoes hepatic metabolism and has a long half-life (about 100 hours), indicating extended pharmacological action. Bioavailability is adequate, and intersubject variability is low.

In a phase II study in de novo renal transplantation, FTY720 at 2.5 mg was found to be as effective as MMF in combination with cyclosporine for the prevention of acute rejection after renal transplantation. FTY720 was well tolerated and not associated with the side effects commonly observed with immunosuppressant therapies.[269]

Conclusion and Future Prospects

FTY720 is a promising new type of immunosuppressive agent (immunomodulator) with unique structure and mechanism of action (S1P receptor modulator) and marked antirejection effect. FTY720 modifies lymphocyte trafficking through alteration of the expression or function of adhesion molecules. This provokes a migration of lymphocytes from the peripheral blood to the secondary lymphoid tissues, a reduction in allograft lymphocyte infiltration, and a peripheral lymphocytopenia. The effect is dose dependent and reversible on discontinuation of the drug. FTY720 also may cause lymphocyte apoptosis, but probably only at higher doses. FTY720 can ameliorate or prevent rejection when used as an induction or maintenance therapy.

Ongoing acute rejection can be interrupted by post-transplant FTY720, which acts in synergy with calcineurin inhibitors cyclosporine and FK506 and with rapamycin. Ongoing experimental work suggests that FTY720 also may protect from ischemia-reperfusion injury.[13,158,253,278] In addition to its role in clinical organ transplantation, FTY720 may prove useful in the treatment of inflammatory/autoimmune conditions.[121]

The first studies in rats involving KRP-203 (2-amino-2-(2-[4-3(-benzyloxyphenylthio)-2-cholorophenyl]ethyl)-1, 3-propanediol hydrochloride), which has some similarity of molecular structure to FTY720, have been published. KRP-203 alone or in combination with low-dose cyclosporine or mycophenolic acid prolonged skin and heart allograft survival with attenuated bradycardia.[230,256,263]

1,25-Dihydroxyvitamin D_3 and Its Analogues

Mechanism of Action

1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and some of its new synthetic structural analogues are promising immunomodulators, with effects in autoimmunity and transplantation immunology. The detection of the receptor for $1,25(OH)_2D_3$ (vitamin D receptor) in almost all cells of the immune system, especially in antigen-presenting cells (macrophages and dendritic cells) and in activated T lymphocytes, led to the investigation of a potential role for $1,25(OH)_2D_3$ as an immunomodulator.[164,291] In addition, activated macrophages and dendritic cells are able to synthesize and secrete $1,25(OH)_2D_3$ in a regulated fashion.[102,245] After macrophage activation by IFN-γ, the secretion of classic macrophage products, such as IL-1, TNF-α, and IL-12, precedes the transcription of the vitamin D 1α-hydroxylase enzyme (responsible for the final and rate-limiting step in the synthesis of $1,25(OH)_2D_3$) and consequently the production of $1,25(OH)_2D_3$ itself.[185] The timing of its synthesis and secretion is compatible with that of a suppressive negative feedback signal.

$1,25(OH)_2D_3$ stimulates the differentiation of monocytes toward good phagocytosis and killing of bacteria, while suppressing their antigen-presenting capacity.[138,236] Essential for the latter is the suppression of surface expression of HLA class II molecules and of classic adhesion molecules necessary for full T cell stimulation, such as CD86.[55] This inhibition of HLA class II and costimulatory molecule (CD86, CD80, CD40, CD54) expression also is observed on the surface of dendritic cells after in vitro or in vivo treatment with $1,25(OH)_2D_3$ or its analogues.[20,93,197,203,292,293] Dendritic cells, being the antigen-presenting cells par excellence, are deviated toward a more immature or tolerogenic phenotype having in vitro and in vivo capacity to induce the development of regulatory T cells.[91,165,166,197,292,293]

The crucial cytokines secreted by antigen-presenting cells (monocytes and dendritic cells) for recruitment and activation of T cells are directly influenced by $1,25(OH)_2D_3$. IL-12, being the key cytokine determining the direction in which the immune system is to be activated, is inhibited by $1,25(OH)_2D_3$ and its analogues.[61,140,293] Thereby, $1,25(OH)_2D_3$ directly interferes with the heart of the immune cascade, shifting the immune reaction toward a T helper type 2 (Th2) profile. In addition, expression by dendritic cells of the immunosuppressive IL-10, opposing the effects of IL-12, is increased by treatment with $1,25(OH)_2D_3$ or its analogues.[197,293]

Although the major immunomodulatory effects of $1,25(OH)_2D_3$ are mediated through its action on antigen-presenting cells, T cells also are direct targets of $1,25(OH)_2D_3$. The Th1 cytokines IL-2 and IFN-γ are directly inhibited by $1,25(OH)_2D_3$,[6,54,264] whereas the Th2 cytokine IL-4 is stimulated.[27,37,186] The molecular pathways by which $1,25(OH)_2D_3$ modulates the expression of these and other genes in the immune system varies widely.[290] Next to the classic interaction with vitamin D receptor–specific binding sites in the promoter region of target genes (vitamin D–responsive elements) as in the inhibition of IFN-γ,[54] $1,25(OH)_2D_3$ also interferes with other pathways of transcription regulation. $1,25(OH)_2D_3$-mediated inhibition of IL-2 is due to impairment of NFAT/AP-1 complex formation and subsequent association with its binding site within the IL-2 promoter.[6,264] During the inhibition of IL-12 in monocytes and dendritic cells, $1,25(OH)_2D_3$ targets the NFκB pathway. Activation and binding of NFκB to its binding site within the promoter of the p40 subunit of IL-12 are repressed by $1,25(OH)_2D_3$.[61]

Preclinical Models

The fact that $1,25(OH)_2D_3$ and its analogues influence the immune system by immunomodulation through the induction of immune shifts and regulator cells makes these products appealing for clinical use, especially in the treatment and prevention of autoimmune diseases. In the animal model of autoimmune diabetes in the NOD mouse, upregulation of regulator cells and a shift away from Th1 toward Th2 could be observed in $1,25(OH)_2D_3$-treated mice locally in the pancreas and in the peripheral immune system.[186] A restoration of the defective sensitivity to apoptosis characteristic for NOD T lymphocytes was observed, resulting in a better elimination of autoreactive effector cells.[39,41,64,65] This increased sensitivity to apoptosis has been described for different apoptosis-inducing signals. This mechanism may explain why an early and short-term $1,25(OH)_2D_3$ treatment before the clinical onset of autoimmunity can lead to long-term protection and restoration of self-tolerance.[42] This arrest in the progression of autoimmune diabetes in NOD mice treated with an analogue of $1,25(OH)_2D_3$ was shown to be associated with an enhanced frequency of regulatory T cells in the pancreatic lymph nodes.[92] A clear additive and even synergistic effect was observed between $1,25(OH)_2D_3$ or its analogues and other, more classic immunosuppressants, such as cyclosporine, sirolimus, or mycophenolate mofetil, in vitro and in different in vivo autoimmune disease models, such as autoimmune diabetes[40,42,95] and experimental autoimmune encephalomyelitis.[31,32,288]

$1,25(OH)_2D_3$ and its analogues were investigated in various transplantation models, such as pancreatic islet allotransplantation and xenotransplantation in mice[91,96]; allogeneic heart[115] and skin[22,295] transplantation in mice; and allogeneic aorta,[207] bone marrow,[187] heart,[107,139] kidney,[208] and liver[209] transplantation in rats. The overall conclusion that can be drawn from these studies is that as monotherapy, $1,25(OH)_2D_3$ and its analogues provoke only a modest prolongation of graft function. This is not surprising in view of the weak intrinsic effects of $1,25(OH)_2D_3$ and its analogues on T cells. In conjunction with other immunosuppressants, strong synergistic effects often can be observed, however.[91,96,114,118,187,207-209,295] In addition, in view of its effect

on antigen presentation and on directing the immune system in the Th2 direction, $1,25(OH)_2D_3$ may help to induce tolerance.[91] A major concern remains, however, the side effects of $1,25(OH)_2D_3$ on calcium and bone metabolism. The use of $1,25(OH)_2D_3$ analogues, which have maintained or amplified immunomodulatory effects in combination with reduced effects on calcium and bone, already partially conquer this problem.[30,289] The additional use of calcium-lowering methods, such as limited nutrient calcium intake, and bone resorption inhibitors, such as bisphosphonates, aid in further bypassing the negative side effects of hypercalcemia and excessive bone resorption,[287] facilitating the step toward the clinical applicability of $1,25(OH)_2D_3$ and its analogues for their potent immunomodulatory properties.

Cyclophosphamide

Cyclophosphamide (2-[bis(2-chloroethyl)amino]-2H-1,3,2-oxazaphosphorinane 2-oxide) is an oxazaphosphorine that was first synthesized in 1958 by Arnold and colleagues.[10] On cellular uptake, it is extensively metabolized.[24,63] The drug is first transformed to hydroxylated intermediates by the cytochrome P-450 system.[195] The hydroxylated intermediates undergo breakdown to form the active compounds phosphoramide mustard and acrolein, and reaction of the phosphoramide mustard with DNA results in cell death.[63]

At high doses, cyclophosphamide is an effective immunosuppressive agent in experimental allograft models,[307] with perhaps some specificity for B lymphocytes.[281] On the basis of a short-term follow-up of a small series of patients, Starzl and coworkers[239] suggested that cyclophosphamide might be substituted for azathioprine because very good results with few complications were achieved using triple therapy with antilymphocyte globulin, cyclophosphamide, and prednisolone. Previous experience with cyclophosphamide in small series had not been good, probably because high doses were being administered.[194]

Cyclophosphamide has been used in combination with azathioprine and prednisolone[21] in the treatment of chronic steroid-resistant rejection, and although some benefit was achieved,[285] serious complications were noted. Two small controlled trials have shown that cyclophosphamide, in intermittent boluses in the first few weeks after transplantation, was not beneficial.[111,303]

The complications of cyclophosphamide can be severe, such as leukopenia, thrombocytopenia, hemorrhagic cystitis, nausea, and vomiting. These complications were found to be rare, however, in a study of a few patients given low-dose cyclophosphamide as a replacement for azathioprine for liver dysfunction, and there was no evidence of inadequate immunosuppression. It is possible that the immunosuppressive effect of cyclophosphamide has never been adequately tested at dosages sufficiently low to avoid complications. This possibility is suggested further by the report of Yadav and colleagues,[316] who showed that in living related transplant recipients who were given cyclophosphamide instead of azathioprine because of hepatic dysfunction or because of the high cost and unavailability of azathioprine, complications attributed directly to cyclophosphamide were minimal. The authors concluded that cyclophosphamide was a safe and effective alternative to azathioprine.

The only standard indication for cyclophosphamide in transplantation today is the desensitization of highly sensitized recipients before renal transplantation. Most of these protocols involve repeated plasmapheresis, in combination with cyclophosphamide, either with or without continuation of steroids, until a kidney transplant can be performed.[1]

Bredinin (Mizoribine)

Bredinin, 4-carbamoyl-1-β-D-ribofuranosylimidazolium-5-olate, is a nucleoside analogue that is structurally similar to ribavirin. It was isolated from the culture media of the soil fungus *Eupenicillium brefeldianum* as an antibiotic agent with activity against *Candida albicans*. Bredinin exerts its immunosuppressive function through selective inhibition of the enzymes inosine monophosphate dehydrogenase and guanosine monophosphate synthetase, both of which are required for the generation of guanosine monophosphate from inosine monophosphate in the de novo pathway.

Previously, bredinin has been used mainly in Japan and is infrequently used elsewhere. In a canine model of renal transplantation, bredinin prolonged graft survival.[9] In humans, compared with azathioprine, bredinin showed equally potent immunosuppressive activity and fewer adverse effects.[12,129,173,265,267] Because of its similarity in structure to ribavirin, bredinin also exhibits in vitro antiviral activity against cytomegalovirus, respiratory syncytial virus, measles, hepatitis C virus, coronavirus, parainfluenza, and influenza virus.[105,179,219,229,231]

In conclusion, experience with bredinin today is limited, but results show that it is a safe and effective immunosuppressant in human kidney transplantation. Phase III trials are under way in France, Germany, and the United Kingdom in renal transplant patients.

Janus Kinase 3 Inhibitors

JAK3 is a tyrosine kinase essential for the signal transduction from the common γ chain of the cytokine receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 to the nucleus. Its expression is restricted to immune cells, and this feature makes it an attractive target for new immunosuppressants. Deficiency in JAK3 results in severe combined immunodeficiency syndrome.[152,212,214,215] Because bone marrow transplantation is curative for severe combined immunodeficiency syndrome patients, it can be concluded that JAK3 has no other essential functions in other systems or organs.[182]

Several JAK3 inhibitors have been developed—tyrphostin AG-490, PNU156804, dimethoxyquinazoline compounds (WHI-P131), CP-690 550, and Mannich base NC1153. From studies on acute lymphoblastic leukemia cells, it was concluded that tyrphostin AG-490 was a selective JAK2 inhibitor, with only bystander inhibitory activity against JAK3. In other T cell lines, AG-490 showed specific inhibitory activity against JAK3.[301] In rats, the combination of tyrphostin AG-490 and cyclosporine resulted in a prolongation of heart allografts.[19,125,126]

PNU156804 is an antibiotic of the undecylprodigioisin family and is an inhibitor of JAK3.[172] In a rat model of heart transplantation, it prolonged allograft survival and showed synergism with cyclosporine.[70,233] WHI-P131 was originally designed as an antileukemic drug.[252] WHI-P131 prevented acute graft-versus-host disease, while preserving graft-versus-leukemia effect[284] and prevented the onset of diabetes in NOD mice.[43] Platelet function is disturbed by WHI-P131,

and this effect is independent of JAK3 inhibition, raising issues of selectivity of this drug.[274]

CP-690 550 is the most potent (inhibitory potency of 1 nM) and selective JAK3 inhibitor to date. In rodents and nonhuman primates, CP-690 550 exerted strong suppression of immune reactions and prolongation of heart and kidney allograft survivals. In monotherapy, it significantly delayed the onset of rejection in kidney allografts.[28,29,44,130] In nonhuman primates, CP-690 550 significantly reduced T cell IL-2–enhanced IFN-γ production and CD25 and CD71 expression, and it inhibited cellular alloimmune responses in vitro.[44,192] Administration in vivo resulted in a reduction of natural killer cell and T cell numbers, whereas $CD8^+$ effector memory T cells were unaffected.[56,192] The most common side effect of CP-690 550 is anemia, and this is due to inhibition of JAK2-mediated signaling through the erythropoietin receptor. Another possible detrimental result of interference with IL-2 signaling relates to the fact that tolerance induction essentially depends on the IL-2 pathway.[132,156,157] Mannich base NC1153 preferentially inhibited JAK3, prolonged kidney allograft survival, and induced transplantation tolerance in rats without toxic effects.[243]

In conclusion, specific JAK3 inhibitors show great promise as new effective immunosuppressants, with few side effects. Clinical studies in autoimmune disease and organ transplantation are in progress.

Others

Cladribine is an adenosine deaminase–resistant analogue of deoxyadenosine and is used in the treatment of leukemia and lymphoma. Many studies have explored the immunosuppressive capacity of cladribine. In vitro, cladribine inhibits B cell and T cell proliferation.[88] In vivo, cladribine monotherapy was shown to prolong skin allograft survival in mice[89]; in combination with cyclosporine, it prolonged liver and heart allograft survival in rats[226]; and it was more effective than cyclosporine monotherapy in small bowel allografts.[183] No clinical trials are published to date.

The farnesyltransferase inhibitor A 228839 was developed as an anticancer compound that inhibits Ras guanosine triphosphatases. A 228839 inhibited lectin-induced proliferation and antigen-presenting cell–induced T cell proliferation. The compound also inhibited lymphocyte Th1 cytokine production and promoted apoptosis in lectin-activated lymphocytes.[232]

FR 252921, an immunosuppressive agent isolated from the culture of *Pseudomonas fluorescens*, inhibits activating protein-1 transcription activity and acts predominantly against antigen-presenting cells. FR 252921 showed synergy with tacrolimus in vitro and in vivo. In murine models of skin transplantation, compared with the optimal dose of tacrolimus alone, the combination of FR 252921 and tacrolimus prolonged graft survival.[80-82]

TOTAL LYMPHOID IRRADIATION

For several decades, total lymphoid irradiation (TLI) has been used to treat Hodgkin's disease.[119] The possibility of applying TLI as an immunosuppressive regimen rather than as an anticancer treatment was discovered by investigators at Stanford University.[85] In a study involving patients with Hodgkin's disease, they showed that cellular immune functions were severely impaired, whereas secondary hematological tumors were rare, and the only infections commonly observed after TLI were localized herpes zoster infections.[87]

Procedure of Total Lymphoid Irradiation

TLI is delivered through two ports. A first, so-called mantle, port includes the lymph nodes of the neck, axillae, and mediastinum. The other port is called the "inverted Y" and encompasses aortic, iliac, and pelvic lymph nodes and spleen. Usually, a total dose of 40 to 50 Gy (1 Gy = 100 rad) is administered in daily fractions of 1.5 to 2.5 Gy.

Mechanisms of Action

Much of the currently available experimental evidence on the immunological mechanisms underlying TLI-induced tolerance points to the importance of suppressor cells.[247] Strober's group identified post-TLI suppressor cells as host-type natural killer T cells because the protective effect of TLI against graft-versus-host disease was abrogated in mice with a CD1d inactivated gene.[134] These host-type natural killer T cells produced IL-4 and stimulated donor-type cells also to produce IL-4.[134,135] Definitive evidence of the functional importance and activity of these suppressor cells was provided by the demonstration that they could prevent graft-versus-host disease in vivo.[101]

Post-TLI attenuation of effector T lymphocyte reactivity was proposed to be equally responsible for the observed immunosuppressed state after TLI.[18,73,74] This intrinsic T cell defect depended on the irradiation of thymus and extrathymic tissues.[188] After TLI, anergized T cells were shown to be incapable of proliferating even in the presence of exogenous IL-2.[76]

In other studies, TLI was shown to lead to thymic clonal deletion of donor-reactive or host-reactive lymphocytes.[220] TLI-treated mice also exhibited decreased antidonor cytotoxic T cell precursor frequencies.[78] Finally, Strober's group showed that Th2 lymphocytes recover soon after TLI, whereas Th1 lymphocytes remain deficient for several months,[17] and they showed that this defect also can be prevented by thymic shielding during irradiation.[18] This Th2 dominance after TLI has been confirmed by other groups in rodents[75] and in large animals.[238]

Experimental Experience

TLI-treated BALB/c mice receiving a fully allogeneic C57BL6 bone marrow and skin graft on the first day after TLI became stable hematopoietic chimeras without signs of graft-versus-host disease, and they developed permanent donor-specific tolerance with preserved anti–third-party reactivity.[250] Tolerance induction was critically dependent on the width of the irradiation field, the time of transplantation after TLI, the total dose of TLI, and the absence of presensitization.[250,297,298]

Following these promising results in rodents, transplantation experiments using TLI were performed in dogs. Although bone marrow chimerism could be easily induced, tolerance to either heart[90] or kidney[106] allografts was not obtained, suggesting that TLI-induced bone marrow chimerism does not create tolerance toward organ-specific antigens.

The combination of TLI and low-dose cyclosporine was found to be effective and clinically safe in rats,[216] and TLI with postoperative antithymocyte globulin induced permanent and specific transplantation tolerance toward heart allografts in about 40% of transplanted dogs.[249] These encouraging results led to a similar trial in clinical kidney transplantation (discussed later). Myburgh and associates[176] applied a modified TLI regimen in baboons, with low dosage and wide field exposure, and showed that tolerance can be achieved in larger animals without concomitant bone marrow transplantation.

The principal disadvantage for the clinical application of TLI is that the complete regimen of fractionated daily irradiation needs to be administered and completed before, but sufficiently close to, the moment of transplantation, and finding a suitable donor organ within such a restricted time frame is problematic. Investigators have explored the possibility of using TLI after transplantation. In mouse and rat heart allograft models, post-transplantation TLI significantly prolonged graft survival when combined with monoclonal anti-CD4 antibodies[277] or infusion of donor-type dendritic cell precursors.[100] Pretransplantation TLI combined with cyclosporine,[242] cyclosporine and pretransplant splenectomy,[317] cyclosporine and anti-CD4 monoclonal antibody,[241] or deoxyspergualin[162] resulted in significantly longer graft survival rates than any other combination previously used.

Also, in heart or heart-lung transplantation experiments between xenogeneic nonhuman primate species, preoperative TLI, when administered in combination with cyclosporine and antithymocyte globulin,[218] cyclosporine and splenectomy,[26] or cyclosporine and methylprednisolone,[193] was more efficient than any other treatment regimen. Pretransplantation TLI, combined with cyclosporine and methotrexate in a pig heart-into-baboon model resulted in a graft survival time of more than 2 weeks. This regimen inhibited xenoreactive natural antibody production, but not the xenoreactivity of macrophages.[311] In a pig islet-into-rat xenograft model, TLI in combination with deoxyspergualin was extremely effective,[271] and even in a discordant lamb-into-pig model, TLI synergized with cyclosporine and azathioprine to provoke a 30-fold increase of the mean xenograft survival time.[275]

Clinical Experience

The first clinical kidney transplants using TLI were performed at the University of Minnesota in 20 patients who had previously rejected a renal allograft.[178] Because similar results (an increase of about 30% 1-year graft survival compared with historical control data) were achieved in this patient population using cyclosporine, and because of the ease of administration, the investigators concluded that cyclosporine was preferred over TLI.

In the 1980s, a controlled trial was performed at the University of Leuven, Belgium, in patients with end-stage diabetic nephropathy receiving cadaver kidney allografts, investigating the effect of pretransplantation TLI (20 daily fractions of 1 Gy, followed by once-weekly TLI doses until a suitable donor was found), followed by low-dose post-transplantation prednisone maintenance treatment. Long-term (8-year) follow-up revealed that rejection episodes were more frequent and patient and graft survivals were significantly inferior in the TLI-treated group. The excess mortality in the TLI-treated patients was due to sepsis, resulting from high-dose steroid therapy needed to treat rejection crises. This clinical experience confirmed the animal data, which also showed that TLI alone is insufficient to provoke long-term graft survival or tolerance and that extra manipulations are needed.

In a study at Stanford University, 24 patients received a first, and 1 patient a second, cadaver renal allograft using TLI and antithymocyte globulin.[142] The actuarial graft survival was 76% and 68% at 1 and 2 years. Ten of the 25 patients never had a rejection crisis despite an overall poor HLA matching between donor and recipient. As in the Leuven study, phenotyping of the suppressor/cytotoxic lymphocytes revealed that only 10% of the post-TLI suppressor/cytotoxic cells were cytotoxic (compared with ± 50% in control subjects). The expansion within the suppressor/cytotoxic subpopulation observed after TLI was entirely due to an increase of suppressor cells.

In follow-up studies, a specific antidonor mixed lymphocyte culture hyporesponsiveness or nonresponsiveness was shown,[53] and in some patients, all immunosuppressive drugs could be withdrawn.[248] An evaluation in a larger group of 52 patients treated with the same protocol at the same center showed a 3-year graft survival of about 50%, which is less than in cyclosporine-treated patients (about 75%).[142]

Synergism between TLI and cyclosporine was studied in comparison with the conventional immunosuppressive regimen (ALG, prednisolone, azathioprine) in 20 patients at Rome University.[57,171] Only 1 of the patients treated with conventional immunosuppression retained a functioning graft, whereas 7 of the TLI-treated patients had a functioning graft, among whom 4 never had a rejection crisis.

The use of a wide-field TLI regimen, shown to be effective in baboons,[176] was studied in humans at the University of Johannesburg.[174,175] The 1-year and 5-year actuarial graft survivals were 86% and 60% and were significantly better for unsensitized patients (80% at 5 years). Seven patients (9.6%) died from transplant-related causes, five with functioning grafts. The facts that in two patients all immunosuppressive drugs could be stopped for several years, and that, in most of the others, only low-dose maintenance immunosuppression (cyclosporine, 3 mg/kg, and prednisolone, <10 mg/day orally) was used without any rejection crisis, seem to confirm the results obtained in the baboon model, in which more than 50% of the animals became specifically tolerant.[176]

Post-transplant TLI combined with anti-CD3 monoclonal antibodies or with antithymocyte globulin and donor-specific blood transfusions seemed effective in a rat heart allograft model.[309] On the basis of these results, the efficacy of TLI was evaluated in heart transplant patients with therapy-resistant or early vascular rejection.[108,141,222] TLI resulted in a significant reduction of rejection recurrences, an effect that was maintained for at least 2 years. These favorable results have been confirmed by several other groups.[11,49,153,276,286] Also, TLI-treated patients develop less coronary atherosclerosis than matched controls despite multiple rejection episodes.[196]

TLI in the treatment of progressive bronchiolitis obliterans syndrome after lung transplantation was retrospectively evaluated in 37 patients in a more recent study. TLI significantly reduced the rate of decline in forced expiratory volume in 1 second, was well tolerated, and was associated with few severe complications.[77]

Conclusion

Although TLI has been shown to be a safe immunosuppressive regimen, it also has become evident that it is inefficient at inducing tolerance in large animal models and humans and is cumbersome to administer. Consequently, TLI has been abandoned in clinical practice except for the treatment of therapy-resistant rejection of heart or heart-lung transplant. In view of the increasing interest in xenotransplantation, the potential of TLI to interfere with xenogeneic reactivity must be explored further. The fact that TLI may concomitantly influence T cell–dependent and T cell–independent immunity may be important because both immune arms are now known to be equally important for the rejection of xenografts.

PHOTOPHERESIS

Extracorporeal photopheresis is a technique in which leukocytes, removed from patients by leukapheresis, are exposed to 8-methoxypsoralen and ultraviolet A light. It was developed as an immunoregulatory treatment for erythrodermic cutaneous T cell lymphoma.[69] Subsequently, the procedure was shown to be safe as an alternative treatment for various human immune and autoimmune diseases,[201] and in rats[199] and monkeys,[198] the regimen was shown to result in extended skin allograft and cardiac allograft and xenograft survivals. Different mechanisms have been shown to contribute to the immunomodulatory effect of photopheresis, including selective inhibition of effector cells,[199,200] induction of a high rate of apoptosis,[320] increased capacity to phagocytose apoptotic T cells resulting in the induction of anticlonotypic immune responses,[213] and a shift toward Th2 immune activation.[14]

In clinical transplantation, photopheresis has been applied as a therapeutic and prophylactic option. It has been applied in the treatment of recurrent or resistant acute rejection in renal transplant patients,[14,62,86,103,131,254,308] but the number of patients included in these studies is limited, and prospective, randomized trials are needed. The safety and efficacy of photopheresis in the prevention of acute rejection of cardiac allografts have been evaluated in primary cardiac allograft recipients randomly assigned to standard triple-drug immunosuppressive therapy (cyclosporine, azathioprine, and prednisone) alone or in conjunction with 24 photopheresis sessions performed during the first 6 months after transplantation. After 6 months of follow-up, photopheresis-treated patients developed significantly fewer rejections, and there were no significant differences in the rates or types of infection. Although there was no significant effect on graft survival rates at 6 or 12 months, this study indicated that photopheresis may be an effective new immunosuppressive regimen in transplant recipients.[15] In patients with refractory bronchiolitis obliterans after lung transplantation, photopheresis resulted in a stabilization of graft function, and in some of these patients it resulted in histological reversal of rejection.[181,221]

SPLENECTOMY

Splenectomy in the recipient before transplantation was first proposed by Starzl and colleagues[240] in 1963 as a means to improve graft survival. Although splenectomy is a standard procedure for patients who develop hypersplenism or azathioprine-associated leukopenia, evidence on the role of splenectomy in enhancing graft survival is controversial.[122,184,204,211,240,251] A large prospective randomized trial in Minneapolis showed splenectomy to improve graft survival significantly,[79] but longer term follow-up showed loss of beneficial effects because of an increased infection-related mortality.[255] Several other single-center studies have shown an alarming risk of sepsis and death, nullifying any early benefits of splenectomy on graft survival,[2,202] and a multicenter analysis from the South Eastern Organ Procurement Foundation confirmed a modest improvement in graft survival after splenectomy but a relentless increase in patient mortality.[151]

Splenectomy may have a place in the preparation of a recipient who is to receive an ABO-incompatible graft, a practice that is likely to become more widely used in living related donor transplantation, in which an ABO-incompatible but otherwise suitable donor is the only available donor. Alexandre and associates[3,4] reported a series of 38 such ABO-incompatible living donor transplants in which the recipient was prepared by plasmapheresis, donor-specific platelet transfusion, and splenectomy. Although the authors believe that the need for plasmapheresis and donor-specific platelet transfusion should be re-evaluated, splenectomy was thought to be important because 3 recipients who did not have a splenectomy lost their grafts from acute vascular rejection, in contrast to only 5 of 33 who did undergo splenectomy.[3,4,210] Ishikawa and colleagues[109] in Japan reported a small-scale but successful experience with postsplenectomy, ABO-incompatible, living donor kidney transplantation. Antigen-specific immunoadsorption and rituximab treatment have been developed more recently, however, as alternatives to plasmapheresis and splenectomy in the setting of ABO-incompatible kidney transplantation.[282,283]

PLASMAPHERESIS

Plasmapheresis has been applied in three settings. The first is in the treatment of steroid-resistant acute rejection that is morphologically predominantly vascular and considered to be antibody-mediated rather than cell-mediated. Although some initial reports suggested a beneficial effect,[38] controlled trials were unconvincing.[5,127] Nojima and colleagues[180] reported the successful treatment of antibody-mediated acute renal allograft rejection by combining plasmapheresis with 15-deoxyspergualin. The second setting is in the preparation of recipients of ABO-incompatible living donor kidneys, referred to earlier,[3,210] although Brynger and coworkers[34] have reported some successful ABO-incompatible grafts without prior plasmapheresis of the recipient. In the third setting, plasmapheresis is used in an attempt to reduce the titer and the broad reactivity of HLA antibodies in highly sensitized candidate transplant dialysis patients; it is combined with cyclophosphamide therapy to prevent reappearance of the antibodies. Encouraging early results of this approach have been reported, although they were associated with considerable morbidity.[268] Immunoadsorption has been applied as an alternative to plasmapheresis and was found to be an equally efficient method.[190,282,283] Studies of this approach in highly sensitized candidate transplant recipients are continuing, in particular, the search for drugs that selectively prevent synthesis of antibodies but perhaps may be less toxic than cyclophosphamide.

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Chapter 22

Transplantation in the Sensitized Recipient and Across ABO Blood Groups

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Protocols have been developed to allow patients with antibody against either donor human leukocyte antigens (HLAs), termed positive crossmatch, or donor blood group, termed ABO incompatible, to undergo successful kidney transplantation. This chapter discusses the rationale for these transplants, the clinical protocols employed, and the role of immunological risk in the observed outcomes. In addition, what is known regarding the mechanism of antibody production and its impact on renal allografts is outlined, highlighting important gaps in current knowledge in this emerging field.

SENSITIZED PATIENTS

The presence of alloantibody in a potential renal allograft recipient severely limits the options for successful kidney transplantation. Historically, because of the risk of hyperacute rejection or early post-transplant humoral rejection, donor-specific alloantibodies (DSA) have been considered an absolute contraindication to kidney transplantation. New technologies and new protocols have greatly increased the chances of transplantation in these candidates, however.

Alloantibody Detection (see Chapter 10)

To understand the therapeutic options for sensitized patients, one first must understand the various assays used to determine the presence of alloantibody (Table 22-1). A more detailed description of these assays is presented elsewhere in this book; here a brief description of these assays and the historical context of their development is provided to shed light on the current understanding of kidney transplantation in sensitized patients.

Some of the first evidence for alloantibody was the retrospective study of Patel and Terasaki in 1969.[48] This study showed that the ability of a recipient's serum to lyse donor cells in vitro was associated with allograft loss within hours of transplantation in a high percentage of cases. Similar cell-based methodology to detect alloantibody is still in use today.

The panel-reactive antibody (PRA) assay is a screening test that seeks to measure the breadth of sensitization.[16] In the PRA, recipient serum is tested for its ability to lyse a panel of T lymphocytes that is a surrogate for a group of potential donors. Historically, the PRA was a cytotoxicity assay of very low sensitivity that was enhanced by adding antihuman globulin (AHG). The PRA assay also may be performed using a more sensitive flow cytometric technique that detects very low levels of cytotoxic and noncytotoxic alloantibody. The PRA has several limitations, however, including the following: It detects only anti–class I antibody, the panels do not reflect all donors, and the data provide only limited information regarding the anti-HLA specificities of the antibodies.

To detect the presence of antibody against an individual donor kidney, a crossmatch assay is performed.[16] The first crossmatches were cell-based cytotoxicity assays in which recipient serum was mixed with donor lymphocytes—either T cells or B cells. The T cell cytotoxicity crossmatch assay is now routinely performed with AHG enhancement and is termed the T cell AHG crossmatch. This assay was the most commonly performed assay for DSA detection for many years. Because the primary goal of the crossmatch assays at that time was to avoid hyperacute rejection, a positive T cell AHG crossmatch was (and usually still is) considered an absolute contraindication to kidney transplantation.

Subsequently, the use of flow cytometric crossmatch (FXM) techniques allowed for the detection of very low levels of alloantibody and noncytotoxic alloantibody. The ability to detect low levels of DSA stimulated a new discussion. Were these alloantibody levels too low to cause hyperacute rejection? Before the development of FXM techniques, some patients had been transplanted unknowingly with low levels of DSA and had done well. The significance of a positive FXM remained unclear for many years, with some experts considering it an absolute contraindication to kidney transplantation and others considering it an unimportant finding that merely represented yet another barrier for sensitized patients. Most experiences, including our own, have suggested that patients with a negative T cell AHG crossmatch and a positive T cell FXM are at very low risk for

Table 22–1 Alloantibody Detection Assays

Screening Assays	
Panel-reactive antibody (T cell only)	
Multi–HLA antigen solid phase assay (class I and II)	
Donor-specific Alloantibody Detection Assays	
Anti–Class I	
T cell cytotoxicity (NIH-CDC) assay	Very low sensitivity
T cell AHG-CDC assay	Low sensitivity
T cell FXM assay	High sensitivity
Solid phase bead or ELISA assay	Highest sensitivity
Anti–Class I or Anti–Class II (or Both)	
B cell cytotoxicity (NIH-CDC) assay	Low sensitivity
B cell FXM assay	High sensitivity
Solid phase bead or ELISA assay	Highest sensitivity

AHG-CDC, antihuman globulin–Centers for Disease Control and Prevention; ELISA, enzyme-linked immunosorbent assay; FXM, flow cytometric crossmatch; NIH-CDC, National Institutes of Health–Centers for Disease Control and Prevention.

hyperacute rejection, but are at increased risk early after transplantation for humoral or cellular rejection, or both.

Another historically controversial area surrounds the significance of a positive B cell crossmatch.[17,32,38] Because B cells express class I and class II, a positive B cell crossmatch may be due to the presence of anti–class I antibody or anti–class II antibody, or a combination of both. In addition, some B cell crossmatches may be positive secondary to non-HLA antibodies or innocuous autoantibodies. Finally, because most sensitized patients have a combination of anti–class I and anti–class II antibodies, a positive B cell crossmatch in the absence of a positive T cell crossmatch is rare, limiting further the ability to study the importance of alloantibody against class II. Our own data, described in detail subsequently, suggests that a B cell crossmatch secondary to anti–donor class II alloantibody is associated with a high rate of humoral rejection and can lead to hyperacute rejection. The immunological risk of a positive B cell crossmatch in patients without evidence of alloantibody to either class I or class II is unclear, but is likely low.

A major source of confusion regarding the significance of the various cell-based assays is a general lack of standardization in the manner in which crossmatches are done in different laboratories. Registry data of sensitized patients contain heterogeneous information, and most published reports are based on small numbers of patients from single centers.

The introduction of so-called solid phase assays has brought significant changes to alloantibody characterization.[51] These assays are based on novel technology in which purified HLAs (class I and class II) attached to flow cytometry beads or to enzyme-linked immunosorbent assay plates are used as targets for alloantibody rather than intact human cells. Solid phase assays with multiple HLA types attached to synthetic substrates now provide a sensitive screen for the presence of anti-HLA antibodies against a wide range of (but not all) HLA types. With their ability identify anti–class I and anti–class II alloantibody and their inclusion of a wide variety of antigens, solid phase screening assays are rapidly replacing the traditional PRA. This technology also seems to be much more reproducible than cell-based assays.

Using single-antigen solid phase assays, the HLA specificity of the alloantibody usually can be determined. These assays have the ability to determine if the candidate has alloantibody against specific donor HLAs. Currently, these sensitive assays are not quantitative and have the same limitations of the FXM in that they identify antibodies that may not represent an increased risk of antibody-mediated graft damage. A combination of solid phase assays and cell-based crossmatch assays is still needed. One study suggests that when antibody against donor HLA is identified using single-antigen assays, the FXM is almost always positive.[15] Showing the lack of anti–donor HLA antibody using single-antigen assays predicts a negative crossmatch less successfully.

Immunological Risk

Clinicians now have the ability to estimate DSA levels across a spectrum ranging from very high to very low. In clinical practice today, DSA detected as a positive crossmatch or in solid phase assays is no longer considered an absolute contraindication to kidney transplantation, but rather it represents the immunological risk of antibody-mediated injury.[16] This concept of immunological risk has emerged as one of the core principles in the transplantation of sensitized patients. The increased immunological risk ranges from an increased risk of hyperacute rejection, such as that seen in sensitized patients with high levels of DSA, to an increased risk of early humoral rejection, such as that seen in sensitized patients with low levels of DSA. Very low levels may represent no increased risk at all. Quantifying this risk is an important aspect to designing protocols to enable successful kidney transplantation in sensitized patients. As described later, a combination of the various previously described assays allows clinicians to better determine the risk of antibody-mediated graft damage in sensitized patients. Current assays cannot completely determine the entire immunological risk of all patients. In addition, sensitized patients are at increased risk for T cell–mediated rejection, and patients may possess antibodies against antigens not detected by current assays.

Current best practice for antibody determination involves initial screening with multiantigen solid phase assays. These multiantigen assays are inexpensive and can be performed simultaneously on several patients. If positive, the specificity is determined using single-antigen assays. If the sensitized candidate has a potential living donor, the "level" of DSA is estimated semiquantitatively using the various crossmatch assays (serial dilutions in the cytotoxicity assays quantified or by channel shift in the FXM, or both).[67]

Clinical Approaches to Sensitized Patients

Cadaver Donors

If a sensitized patient has no prospective living donors, the only option is to be placed on the cadaver donor waiting list. The current system provides only a limited ability to provide a cadaver donor kidney transplant to sensitized patients. In the United States, approximately 15,000 patients on the Organ Procurement and Transplantation Network (OPTN)/United Network for Organ Sharing (UNOS) cadaver donor kidney waiting list are "sensitized" (i.e., have alloantibody to at least one or more HLA types).[72] Approximately 8000 are

sensitized broadly with a PRA greater than 80%. Despite being awarded additional "points" for this level of sensitization, fewer than 500 of these patients are transplanted each year.[66] Most patients never receive a transplant. In addition, the graft survival of patients who do receive a transplant is decreased, with the risk of graft loss at 1 year 1.8 times that of unsensitized patients. Another 7000 or so waitlisted candidates have a PRA of 20% to 80%. Currently, these patients receive no points for being sensitized and have approximately half the transplantation rate of nonsensitized patients.

Protocols to decrease alloantibody to levels below that associated with immediate allograft injury have been termed desensitization protocols. Some of these protocols have been used successfully in sensitized patients waiting for a cadaver donor kidney. In a multicenter, double-blinded study, 101 sensitized renal allograft candidates received high-dose intravenous immunoglobulin (IVIG) (2 g/kg monthly × 4) or equivalent volume placebo.[28] Baseline PRA levels as determined by a T cell cytotoxicity assay were similar in both groups (80% in both). IVIG treatment decreased the PRA by approximately 10% by 4 months, but the PRA returned to baseline at 6 months (2 months after the last IVIG infusion) and was equal to that of placebo-treated patients at that time point. Among dose-adherent patients, 35% (n = 16) IVIG and 17% (n = 8) placebo patients were able to be transplanted. Nine of 17 patients transplanted after IVIG infusion had a rejection episode, however, compared with only 1 of 10 placebo-treated patients.

Treatment of nine sensitized patients with the anti-CD20 antibody rituximab met with only limited success.[70] Two of nine subjects had no change in PRA; one had a decrease in PRA from 87% to 51%; five had changes in histogram architecture suggesting loss of antibody specificity; and one patient had a fourfold decrease in PRA titer from 1:64 to 1:16 at 6 months after treatment. Only one of the seven patients converted a donor-specific crossmatch to negative and underwent successful living donor kidney transplantation.

The fact that desensitization protocols involving multiple plasmapheresis treatments require coordination of the timing of transplantation[67] severely limits their applicability to cadaver donor kidney transplantation. A new proposal for the allocation of cadaver donor kidneys to sensitized candidates that would incorporate solid phase and crossmatch assays is under consideration by the OPTN/UNOS. In the new schema, all candidates are screened using multiantigen solid phase assays and, if positive, their alloantibody specificities are determined using single-antigen assays.

An analysis by the Scientific Registry of Transplant Recipients has updated the HLA frequency of the U.S. donor pool for individual HLA types and linked haplotypes. Combining these national data with the HLA specificities as determined by single-antigen solid phase assays, the probability that the candidate would have a positive crossmatch against the entire donor pool can be calculated. This probability of a positive crossmatch would replace the PRA as the metric for the breadth of sensitization. An 80% positive probability would mean that the candidate would have antibodies against 80% of the donor pool. Kidneys, likely from a relatively large donor pool (i.e., larger than the current local area), would be allocated to sensitized candidates only when they have been shown to lack antibodies to donor HLA. Final T cell and B cell crossmatches would be required to verify the "virtual crossmatch." Using a similar approach in one local area, one group reported an increase in the transplantation rate of sensitized patients with outcomes similar to those of nonsensitized patients.[15]

Paired Donation

If a sensitized candidate has potential living donors, all should be tested to find a crossmatch-negative donor. If no such donor can be found, sensitized candidates may opt to enter into one of the growing number of paired living donor programs. These "exchange" schemas have been shown to increase the transplantation rate of ABO-incompatible and sensitized patients.[42,57] Paired schemas employ the same "unacceptable antigen" schema described earlier to find a crossmatch-negative donor for sensitized patients. Although these programs increase the number of potential donors for sensitized patients, patients with antibodies against a wide variety of HLA types are still unlikely to find a crossmatch-negative donor, unless the prospective donor pool is very large. A variation of the schema might be used to identify a donor against whom a sensitized candidate has low levels of DSA. In this situation, desensitization protocols can be employed to achieve a successful transplant.

Even if the logistical and ethical hurdles associated with paired donation can be overcome, clinical judgment is needed to assess the best treatment course for a particular sensitized candidate. Broadly sensitized patients with a living donor against whom they have low levels of DSA might be served best by desensitization and positive-crossmatch kidney transplantation. Patients who have a positive crossmatch against all of their potential living donors, but are not broadly sensitized patients (i.e., patients with a low probability of a positive crossmatch) might be served best by paired donation.

Positive-Crossmatch Living Donor Kidney Transplantation

An increasingly viable option for sensitized candidates with an otherwise suitable living donor is to perform the transplant despite the presence of a positive crossmatch.[19,23,30,41,43,56,67] The treatment protocol depends primarily on the immunological risk of the recipient (i.e., the level of DSA as measured by the crossmatch at baseline). In patients with high levels of DSA, such as patients with a positive cytotoxicity crossmatch at baseline, protocols that reduce antibody levels or "desensitize" patients are required to prevent hyperacute rejection or early antibody-mediated damage. Patients with lower levels of DSA may not require pretransplant desensitization, but do benefit from close post-transplant monitoring.

HIGH-LEVEL DONOR-SPECIFIC ALLOANTIBODY RECIPIENTS

Some patients are at extremely high risk for antibody-mediated injury and may not be best served by transplantation. One such group are patients who have such high levels of DSA that they cannot achieve a negative T cell AHG crossmatch despite intensive desensitization.

Early in our experience, we transplanted 10 patients who, despite multiple plasmapheresis treatments (mean 10 treatments), were unable to achieve a negative T cell AHG crossmatch.[67] Given that these highly sensitized patients had almost no other option, we performed the transplant despite the persistence of low titers in the T cell AHG crossmatch (undiluted to 1:8) on the day of transplantation. Of these 10 patients, 70% developed humoral rejection, two of which were hyperacute. The 1-year graft survival was only 50%.

Table 22–2 **Success of Desensitization with Various Protocols Stratified by Baseline T cell Antihuman Globulin Crossmatch Titer***

Antihuman Globulin Crossmatch Titer	Intravenous Immunoglobulin	Plasmapheresis	Plasmapheresis / Monitoring
Undilute			X
1:2	X	XXXXXXXXXX	XXXXX
1:4	XX	XXXXXXXXXX	X
1:8	XOO	XX	X
1:16	XOO	XXXXXOO	XXX
1:32	OO		X
1:64			
1:128	O	OOO	
1:256	O		O†O†

*X = achieved a negative crossmatch; O = crossmatch remained positive despite desensitization.
†Nonresponsive to desensitization protocol, not transplanted.
From Stegall MD, Gloor JM, Waters J, et al: A comparsion of plasmapheresis vs high dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. Am J Transplant 6:348, 2006.

The inability to achieve a negative T cell AHG crossmatch generally correlated with a baseline crossmatch titer of 1:32 or greater. Based on this experience, patients with very high levels of DSA at baseline and patients who fail to achieve a negative T cell AHG crossmatch despite desensitization represent an extremely high-risk group for immunological graft loss.

The current goal of the published desensitization studies has been to achieve a negative cytotoxicity crossmatch at the time of transplantation. The two major approaches involve either high-dose IVIG or multiple plasmapheresis treatments.

High-dose IVIG (typically in the range of 2 g/kg body weight) has been shown to be successful in ameliorating a positive complement-dependent cytotoxicity crossmatch and permitting successful transplantation.[42] Patients who fail to respond to one dose of IVIG may respond subsequently to repeated doses. The likelihood of an individual responding to high-dose IVIG therapy may be predicted by performing an in vitro National Institutes of Health–Centers for Disease Control and Prevention crossmatch after adding IVIG to the sera to be studied. A decrease or blockade of the crossmatch suggests that the patient would respond "in vivo" after administration of IVIG.

Using this method, Jordan and colleagues[30] have reported that 75% of patients with DSA detected using a complement-dependent cytotoxicity crossmatch are found to be "in vitro responders." In this responder group, 90% are successfully converted to a negative crossmatch. Approximately 70% of all patients with high-level DSA are able to achieve a negative crossmatch using high-dose IVIG. In a series of 47 patients who underwent transplantation, the 1-year allograft survival was 80%, with a humoral rejection rate of 40%. Similarly, Glotz and coworkers[23] reported successful desensitization and transplantation of 4 patients with cytotoxic DSA levels.

The other major approach to desensitization in patients with high levels of DSA involves multiple plasmapheresis treatments.[19,41,43,56,67] In this approach, the goal of plasmapheresis is to remove DSA physically before transplantation and obtain a negative crossmatch at the time of transplantation. IVIG usually is given in conjunction with plasmapheresis at a lower dose of IVIG (typically 5 to 10 g based on body weight) than that used when IVIG is given alone for desensitization. IVIG in this approach is given to prevent hypogammaglobulinemia associated with multiple plasmaphereses, although it is possible that it provides some of the immunomodulatory effect.

Although high-dose IVIG and plasmapheresis-based regimens successfully decrease antibody, comparative studies are few. In one report,[67] a single dose of high-dose IVIG was compared with two plasmapheresis-based protocols in a series of 37 patients who at baseline had positive T cell AHG crossmatch against their living donor (Table 22-2). High-dose IVIG and plasmapheresis protocols were effective in producing a negative crossmatch in patients with lower levels of DSA (AHG-CDC T cell crossmatch titer ≤ 1:4). Neither high-dose IVIG nor plasmapheresis was effective in producing a negative crossmatch in patients with crossmatch titers exceeding 1:16. In patients with titers of 1:8 to 1:16, however, high-dose IVIG rarely produced a negative crossmatch, whereas plasmapheresis-based protocols predictably did so. This study also showed that although high-dose IVIG caused antibody reduction in most patients, its effect was not as reproducible as multiple plasmapheresis treatments.

LOW-LEVEL DONOR-SPECIFIC ALLOANTIBODY RECIPIENTS

Some patients have such low levels of DSA that risk of hyperacute rejection is very low. Generally, these patients have a negative cytotoxicity crossmatch but a positive FXM. In our experience, these patients do not require the intensive preconditioning used in the preparation of patients with high levels of DSA to prevent hyperacute rejection.[21] Nevertheless, patients with low levels of DSA are at increased risk for humoral rejection during the first days to weeks after transplantation compared with nonsensitized patients.[1]

Humoral rejection in these patients usually occurs when DSA levels increase significantly above pretransplant levels. One such case is presented in Figure 22-1. At baseline, the patient had negative T cell and B cell cytotoxic crossmatch assays and only mildly positive T cell and B cell FXM. The patient received conventional immunosuppressive therapy without antibody induction. After living donor kidney transplantation, the creatinine level increased on postoperative day 4, and the biopsy specimen showed clear-cut humoral rejection. At this time, DSA levels were

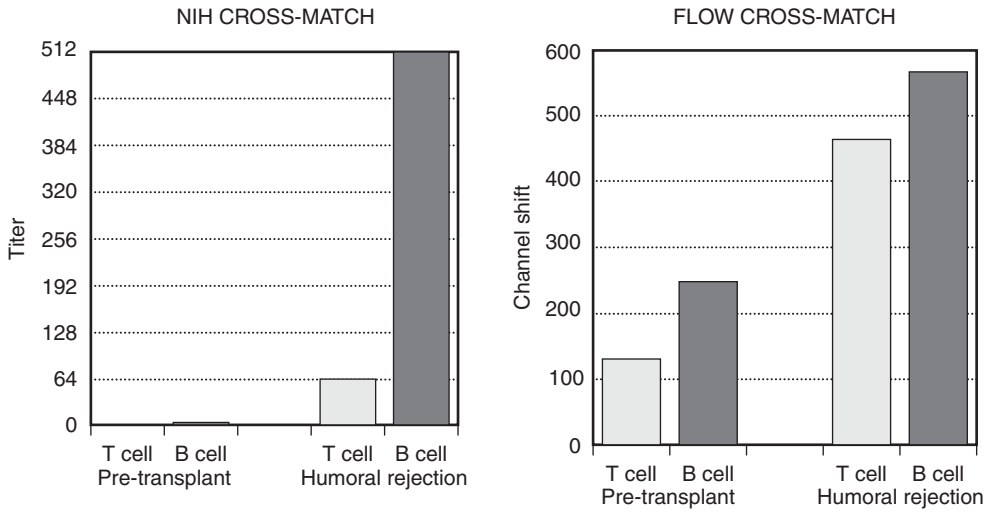

Figure 22–1 Evidence for an increase in donor-specific alloantibody from baseline (as measured by crossmatch levels) in a patient who developed humoral rejection. At baseline, T cell and B cell cytotoxicity assays were negative, and T cell and B cell crossmatches were mildly positive (*left bars* in both figures). By postoperative day 4, the cytotoxicity crossmatch assays were positive at very high titers, and the channel shift on T cell and B cell crossmatches had increased markedly. A biopsy confirmed the presence of humoral rejection. Despite aggressive treatment with plasmapheresis, the graft was lost by day 8. NIH, National Institutes of Health.

markedly increased. All crossmatches were positive, including cytotoxic T (titer 1:64) and B (titer 1:512), and there was a marked increase in T cell and B cell FXM channel shifts. Despite multiple plasmapheresis treatments, the patient lost the graft as a result of uncontrolled humoral rejection by day 8. This increase in DSA likely occurred as a result of the anamnestic memory response produced by re-exposure of the recipient memory B lymphocytes to circulating donor antigen. A possible approach to preventing humoral rejection in patients with low levels of DSA would be to prevent the memory B cell response.

Akalin and colleagues[1] reported a series of eight patients with positive FXM treated with pretransplant high-dose IVIG and antithymocyte antibody induction.[1] In that group, no humoral rejection occurred. Similarly, Gloor and associates[21] reported a series of 18 patients in whom FXM was positive and cytotoxicity crossmatch was negative who received high-dose IVIG and antithymocyte induction. Humoral rejection was diagnosed in 11% of patients.[21] Our protocols also have used a single pretransplant dose of rituximab, with similar rates of humoral rejection. The optimal management of low-level DSA is unclear, and it is possible that close observation might lead to similar results.

ANTI–CLASS II DONOR-SPECIFIC ALLOANTIBODY

The importance of anti–class II DSA is less clear than that of anti–class I DSA. Many sensitized patients have a combination of anti–class I and anti–class II antibodies. Because the B cell crossmatch is affected by both groups of antibodies (B cells express class I and class II), assessing the level of anti–class II antibodies using the B cell crossmatch assay is unreliable. In addition, we and others have found a poor correlation between the B cell crossmatch assays and clinical outcomes.

More recent data show that anti–class II DSA can cause hyperacute and early humoral rejection. We identified 12 sensitized patients in our series who had a positive B cell FXM and a negative T cell FXM. Six of 12 patients (50%) had class II DSA detectable by single-antigen flow beads, whereas the other 6 patients had no demonstrable DSA by single-antigen flow beads. In the first 2 weeks after transplantation, all 6 of the patients with detectable class II DSA had C4d present on protocol biopsy specimens, whereas only 33% (2 of 6 patients) without evidence of class II DSA had C4d present ($P = .021$). Four of 6 patients (67%) with anti–class II DSA developed humoral rejection within 4 weeks after transplantation, whereas no patient who was positive for B cell FXM and negative for anti–class II DSA developed humoral rejection. These data show that sensitized patients with clearly defined anti–class II DSA have a high incidence of humoral rejection. The significance of positive B cell crossmatch in the absence of class II DSA by single-antigen flow beads seems to carry a low risk for antibody-mediated injury.

Assessing Immunological Risk Clinically

How does one quantify immunological risk clinically? All of the antibody detection assays are semiquantitative only, and comparisons between cytotoxicity and FXM are uncommon. In our laboratory, most, but not all, patients with a channel shift greater than 300 on the T cell FXM also have a positive T cell AHG crossmatch (Fig. 22-2). Early in our experience, we considered only patients with a positive T cell AHG crossmatch to be "high risk" and candidates for desensitization therapy. Now we consider any patient with a T cell FXM channel shift greater than 300 to be high risk, however, regardless of their T cell AHG, and patients with levels above this undergo pretransplantation desensitization.

Figure 22-3 shows that this channel shift provides a useful division of risk of post-transplant humoral rejection. Despite receiving pretransplant desensitization with multiple plasmapheresis treatments, the humoral rejection rate was 60% (9 of 15) in a cohort of patients whose baseline T cell FXM channel shift was greater than 300 at baseline. Conversely, in a cohort of patients with baseline T FXM channel shifts less than 300 treated with anti-CD20 antibody before transplantation, the rejection rate was 22% (2 of 11; $P < .001$ compared with the high-level DSA group).

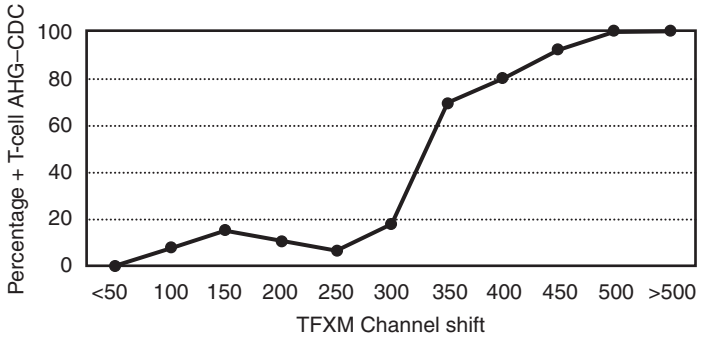

Figure 22–2 Relationship between T cell antihuman globulin (AHG)–Centers for Disease Control and Prevention (CDC) crossmatch positivity and T cell flow cytometric crossmatch (TFXM) channel shift in positive-crossmatch kidney transplant recipients. Recipient serum was tested in parallel for reactivity against donor T cells in the T cell AHG-CDC and the TFXM assays (n = 50 tests). In patients with a TFXM channel shift of 300 or less, the incidence of a positive T cell AHG-CDC crossmatch was always less than 20%. Conversely, in patients with a TFXM channel shift greater than 300, the incidence of a positive T cell AHG-CDC crossmatch was greater than 70% and approached 100% with increasing channel shifts.

What about patients who have a combination of anti–class I and anti–class II or anti–class II DSA alone? We have taken a more conservative approach over time in this situation. Our current protocols require that at the time of transplantation, the T cell and the B cell FXM have channel shifts less than 300. This approach has avoided hyperacute rejection in all but 1 of the last 54 sensitized recipients.

Treatment of Humoral Rejection

Significant advances have been made in the diagnosis of humoral rejection, primarily as a result of the recognition that the histological appearance of humoral rejection differs significantly from that of acute cellular rejection.[39,69] The identification of the complement degradation product C4d as a marker for the interaction of antibody, antigen, and complement system has permitted more timely and accurate diagnosis of humoral rejection.[13,26,46] The Banff 97 classification for allograft histology has been modified to take these factors into account.[52] Currently, the approach to the treatment of humoral rejection is based on removal or inactivation of circulating DSA and efforts to decrease antibody production.

Similar to the preconditioning regimens used to prepare for transplantation, plasmapheresis and high-dose IVIG have been used to treat humoral rejection.[29,50] Early reports on the efficacy of plasmapheresis in treating humoral rejection gave contradictory results.[4,7] Nevertheless, in these older reports, the criteria used to define humoral rejection were not standardized. Additionally, in many reports, therapy was delayed after the diagnosis of rejection, and plasmapheresis was implemented after the rejection episode had been treated unsuccessfully using other modalities. More recent studies report successful reversal of humoral rejection in most patients treated with plasmapheresis-based protocols, although chronic allograft nephropathy may follow.[27] Pascual and colleagues[50] reported successful reversal of humoral rejection using a combination of plasmapheresis and increased maintenance immunosuppression. Similarly, high-dose IVIG has been shown to be effective in reversing humoral rejection in a few patients.[27] Doses are similar to those used in pretransplant conditioning regimens.

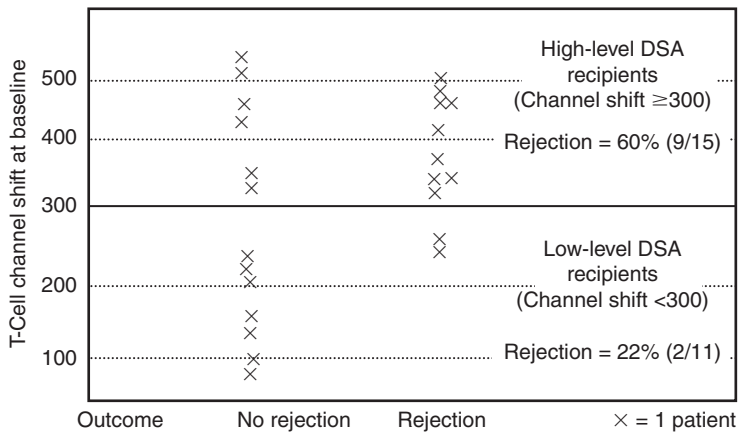

Figure 22–3 Correlation between the baseline T cell flow cytometric crossmatch channel shift and subsequent humoral rejection. Patients with channel shifts of 300 or greater at baseline received pretransplant plasmapheresis, anti-CD20, rabbit antithymocyte globulin (Thymoglobulin), and post-transplant antibody monitoring. Patients with channel shifts less than 300 received anti-CD20 antibody. All patients received Thymoglobulin induction, tacrolimus, mycophenolate mofetil (CellCept), and prednisone. The humoral rejection rate was 60% (9 of 15) in patients with channel shifts of 300 or greater versus 22% (2 of 11) in patients with baseline channel shifts less than 300. DSA, donor-specific alloantibody.

Finally, a difficult group comprises patients who have persistent humoral rejection despite intensive treatment. In this setting, graft loss is common; we have employed splenectomy empirically in an effort to reduce antibody production.

Post-Transplant Monitoring

Early after transplantation, especially in the first 2 weeks, the development of acute humoral rejection correlates with high levels of antidonor antibody. Post-transplant monitoring of antibody levels with the goal of keeping these sufficiently low might avoid allograft injury. Although this seems to be the case in ABO-incompatible renal allografts, the success of low antibody levels in avoiding allograft injury in positive-crossmatch transplants is still unclear. Currently, our protocol is to maintain the T cell and the B cell channel shift at less than 300 in the first 2 weeks after transplantation.

Late Outcomes

Acute humoral rejection is rare beyond 2 months after transplantation in ABO-incompatible and positive-crossmatch kidney transplant recipients. Generally, antidonor antibody is lower at this point after transplantation and is well tolerated by the allograft. In three instances in which the T cell AHG crossmatch has remained positive beyond 1 month after transplantation, all three patients developed accelerated transplant glomerulopathy and lost their grafts in the first year.

Late after transplantation (≥ 3 months), antidonor antibody levels tend to remain at relatively low levels or even disappear (especially common in sensitized patients).[18,73] Is the persistence of low level of DSA deleterious to the allograft in positive-crossmatch renal allograft recipients? Our data suggest that at 1 year it is not.

We compared histological findings at 1 year on protocol surveillance biopsy specimens in 37 positive-crossmatch living donor kidney transplant recipients with 198 conventional transplants and 18 ABO-incompatible recipients.[22] Table 22-3 shows that at 12 months after transplantation, the histology of most positive-crossmatch and ABO-incompatible renal allografts is similar to that of conventional kidney transplants. The mild fibrosis and tubular atrophy seen in these grafts (positive-crossmatch and conventional grafts) generally have been associated with good long-term graft survival in previous studies.[9] Positive-crossmatch recipients show an increase, however, in the incidence and severity of chronic glomerulopathy. The primary event associated with chronic glomerulopathy was a previous humoral rejection episode. Neither C4d staining of the peritubular capillary endothelium nor persistence of antidonor antibody seemed to correlate with glomerulopathy. Although the presence of glomerulopathy had little impact on renal function 1 year after transplantation, longer follow-up is needed to determine the true impact of persistent DSA on the allograft. Nevertheless, given these findings, our current approach is not to treat persistent DSA levels even if histological changes are present.

In contrast to our results, several lines of evidence suggest that antibody can cause chronic renal injury in native and transplanted kidneys. The most common site of injury is the vascular endothelium, such as the glomeruli. Several studies have suggested that chronic rejection of renal allografts is preceded by prolonged exposure to alloantigen.[33,37] In these studies, most renal allograft recipients with detectable alloantibody had good functioning grafts, however, suggesting that anti-donor antibody does not always produce damage. At least four different explanations have been suggested for this lack of damage.

Graft survival rates of positive-crossmatch kidney transplants have been good. Reported 1-year graft survival rates have been approximately 80% in patients with a positive cytotoxicity crossmatch against their living donor at baseline who were successfully desensitized with either high-dose IVIG or multiple plasmapheresis treatments. In our experience, 1-year graft survival in patients with low-level DSA is approximately 90%. Graft survival data beyond 5 years are still lacking in positive-crossmatch recipients.

ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION

The presence of antibody against donor blood group also has been considered a contraindication to kidney transplantation, with high levels associated with hyperacute and early

Table 22–3 **Percentage of Recipients Who at Time 0 and at 12 Months Had Histologic Scores >0 in Conventional, ABO-Incompatible, and Positive-Crossmatch Living Donor Kidney Transplants**

Histology	Conventional	ABO-Incompatible	Positive-Crossmatch	*P**
Glomerulopathy				
Time 0 (*N* = 201)	0	0	0	0.281
12 months (*N* = 260)	8	13	22	0.03†
Interstitial Fibrosis				
Time 0	9	7	0	0.654
12 months	59	71	68	NS
Vasculopathy				
Time 0	33	33	28	0.386
12 months	44	42	49	NS

*χ^2.

†Higher incidence in positive-crossmatch than in conventional group.

Adapted from Gloor M, Stegall MD, Cosio FC, et al: Histologic findings one year after positive crossmatch or ABO-incompatible kidney transplantation. Am J Transplant 6: 1841, 2006.

humoral rejection. Although more recent studies have suggested that blood subgroup A2 donors may be transplanted into B recipients with low anti–A blood group antibody titers, success in other combinations is problematic—even in A2 to O combinations.[3,6,20,45,64] The options for nonsensitized patients whose only donor is ABO incompatible are similar to the options of positive-crossmatch patients[65] and include (1) being placed on the cadaver donor waiting list, (2) entering into a paired living donor program, or (3) undergoing a desensitization protocol and receiving the ABO-incompatible living donor kidney despite the presence of antidonor antibody.[2,54,61,68]

Being placed on the cadaver donor waiting list is the most commonly used option. Because most ABO-incompatible candidates are blood group O (78% in our series), their current mean waiting time for a cadaver donor kidney is approximately 5 years in the United States. This long waiting time translates into increased morbidity and mortality pre- and post-transplantation, especially in older patients and diabetics.

The level of anti–blood group antibody that causes hyperacute rejection has not been determined exactly and may vary. We have shown that an anti–blood group isohemagglutination titer of less than 1:8[20] seems to be "safe" at the time of transplantation in that evidence of antibody deposition is not seen on 30-minute postreperfusion surveillance biopsy specimens. Achieving these "safe" levels of antibody can be difficult in some patients, however. Patients who at baseline (before any therapy) have high levels of anti–blood group antibody (e.g., ≥ 1:512) rarely can be "desensitized" (have antidonor antibody reduced to safe levels) using our current protocols. Performing splenectomy either before or at the time of transplantation might allow successful ABO-incompatible transplantation even in patients with very high anti–blood group antibody levels.[60] In addition, because our early experience showed a high incidence of humoral rejection in O recipients of A2 donor kidneys, we have not used different criteria for A2 versus non-A2 donors.

Our protocols have evolved to include preemptive plasmapheresis treatments[71] and antibody monitoring aimed at maintaining low levels of antidonor antibody in the first 2 weeks after transplantation. Our goal is to keep the isoagglutination anti–blood group antibody titer less than 1:16 for 2 weeks. Using this approach, our group and several groups in Japan have shown that ABO-incompatible living donor kidney transplantation can achieve graft survival rates approaching those of other living donors. Long-term graft survival also has been good, suggesting that anti–blood group antibody rarely causes chronic graft injury.

A major barrier to the widespread application of ABO-incompatible and positive-crossmatch kidney transplantation is the increased cost compared with conventional transplants. We performed a retrospective study comparing 40 ABO-incompatible with 77 matching ABO-compatible living donor renal allografts with respect to complications, resource use, and cost from day −14 to 90 days post-transplantation.[55] Overall, surgery-related complications and resource use were increased in the ABO-incompatible group, primarily because of the desensitization protocol and antibody-mediated rejection. In the absence of rejection, the mean number of complications was similar for both groups. ABO-incompatible kidney transplantation was approximately $38,000 more expensive than ABO-compatible transplants, but was cost-effective compared with maintaining the patient on dialysis while waiting for a blood group–compatible cadaver donor kidney.

Although similar data are lacking in positive-crossmatch kidney transplantation, we expect that it too is cost-effective. The fact that ABO-incompatible and positive-crossmatch kidney transplantation increase the number of living donors and are cost-effective compared with maintenance dialysis should encourage third-party and governmental payers to underwrite the increased costs of these procedures.

MECHANISTIC VIEW OF ANTIBODY PRODUCTION AND ANTIBODY-MEDIATED INJURY

Although emerging experience shows acceptable graft survival in ABO-incompatible and positive-crossmatch kidney transplants, antibody-mediated graft losses still occur early and late after transplantation. Increased understanding of antibody production and its impact on the graft would lead to improvements in therapy for these patients.

Over the past decade, the pathway to antibody production has been clearly delineated in numerous animal and human studies.[3,5,6,12,25,31,40,62,63] The phenotypes of the various B cell subsets are shown in Table 22-4. The bone marrow continuously generates a large variety of naive B cells expressing cell surface immunoglobulin. Although each naive B cell's immunoglobulin is unique, as a population these naive B cells are capable of interacting with an enormous variety of antigens, including all types of class I and class II HLA molecules. These mature, but naive, B cells remain in a quiescent state until they encounter antigen in secondary lymphoid tissue, such as the spleen. Activation of B cells, which requires T cell help, may lead to the development of plasma cells (either short-lived or long-lived) and to the development of memory B cells. Naive B cells express cell surface immunoglobulin, yet only plasma cells are capable of antibody secretion. Memory B cells also express cell surface immunoglobulin and are capable of rapid conversion to plasma cells within hours of re-exposure to antigen. Memory B cells do not secrete immunoglobulin, however.

Long-lived plasma cells can persist for years in special microenvironments of the marrow and spleen, continuously producing antibody even in the absence of antigenic stimulation. They are terminally differentiated and are resistant to most pharmacologic agents. Most of the anti-HLA antibody detected in sensitized recipients is likely produced by long-lived plasma cells.

Plasma cells seem to be resistant to most immunomodulatory agents commonly in use in clinical transplantation. They do not use interleukin-2 for their function and are not

Table 22–4 Cell Surface Phenotypes of B Cell Subsets

Naive B Cell	Memory B Cell	Plasma Cell
$CD20^+/CD27^-$ $CD38^-/CD138^-$ Intracytoplasmic immunoglobulin negative	$CD27^+/CD20^{+/-}$ $CD38^-/CD138^-$ Intracytoplasmic immunoglobulin negative	$CD27^-/CD20^-$ $CD38^+/CD138^+$ Intracytoplasmic immunoglobulin positive

significantly inhibited by either calcineurin inhibitors or antibodies against the interleukin-2 receptors. They do not express CD52, the target for alemtuzumab (Campath). They also do not express CD20 and would seem to be resistant to treatment with the anti-CD20 antibody, rituximab. Indeed recent studies by our group have demonstrated that plasma cells are resistant to desensitization with IVIG, rituximab, and thymoglobulin.[51a,52a]

The presence of DSA is only the first step in the development of humoral rejection. The next step is the binding of antibody to allograft. Using immunohistological techniques, donor-specific IgG and IgM are not detectable on renal allograft vascular endothelium even in the setting of clear-cut antibody-mediated rejection.[14,52] Indirect evidence of antibody binding to an allograft has been the demonstration of C4d in the peritubular capillaries, but C4d binding alone does not seem to be damaging to renal allografts.[13,26,46] More distal terminal complement activation is associated with kidney damage, however.[47] The presence of membrane attack complex of C5b-9 has been shown to mediate neutrophil influx and synthesis of proinflammatory cytokines and may cause direct cell injury, apoptosis, and necrosis.[34,35,58] Similarly, the anaphylatoxin, C5a, is a chemoattractant for neutrophils and macrophages. The C5a receptor on endothelial cells, neutrophils, and macrophages activates these and other cells to produce cytokines, chemokines, and adhesion molecules[10,11] and may regulate apoptosis.[24,59] Antibody also has been shown to cause endothelial cell damage by complement-independent mechanisms.

It has been suggested in clinical and experimental studies that organ allografts seem to develop resistance to antigraft antibody. This was first described and is well established in ABO-incompatible allografts.[8] Other investigators have shown evidence for this process in allosensitized recipients of renal allografts. The mechanisms of accommodation are unknown; however, data suggest that the stimulation of anti-apoptotic molecules, such as hemoxygenase 1, Bcl-xl, and Bcl-2, may be important early.[44,53] Our group showed that normal functioning ABO-incompatible renal allografts 1 year after transplantation develop a unique intragraft gene expression profile different from ABO-compatible grafts.[49] There is molecular evidence of accommodation in human ABO-incompatible grafts. In our opinion, the evidence for accommodation is much stronger for ABO-incompatible grafts than for positive-crossmatch renal allograft recipients.

Combining what is known about antibody production with existing clinical studies, a mechanistic model of alloantibody production and antibody-mediated damage in sensitized renal allograft recipients can be constructed. In this model, baseline DSA is the product of long-lived plasma cells that generally are resistant to current therapy. Desensitization therapy primarily removes or blocks DSA without significantly affecting ongoing antibody production. After transplantation, alloantibody is the product of persistent production by preexisting plasma cells and the recruitment of memory B cells to become plasma cells. This conversion of memory B cells to plasma cells is the major mechanism by which patients with low levels of DSA at baseline develop humoral rejection after transplant.

Humoral rejection in patients with high levels of DSA at baseline involves a memory B cell response and ongoing DSA production by preexisting plasma cells. We hypothesize that the recruitment of naive B cells is not a mechanism of antibody production either at baseline or during a humoral rejection episode. The basis for this assumption is our clinical observation that humoral rejection occurs despite treatment with either rabbit antithymocyte globulin (Thymoglobulin) (which would decrease T cell help) or rituximab (which removes naive T cells). Studies of complement in humoral rejection have been limited primarily to histological studies of C4d binding to the allograft. The role of complement is likely much more complex, however, and merits much more detailed study. Late after successful transplantation, the levels of DSA measurable in the peripheral blood decrease in most patients. The cause of this decrease is unclear, but it may be the result of a gradual decrease in antibody production or other processes, such as accommodation or absorption of antibody by the graft.

CONCLUSION

Positive-crossmatch or ABO-incompatible kidney transplant may be the best treatment option for some patients with end-stage renal disease. Although desensitization protocols have shown remarkable success, humoral rejection with its associated increase in immediate and late graft loss remains a major barrier to success. From the few patients transplanted to date, important lessons have been learned. The immunological risk of patients varies from very low to prohibitively high, and protocols can be tailored to the risk of antibody-mediated damage. Future research efforts focusing on the mechanisms of antibody production and its impact on the graft should provide for continued progress in this new and challenging field.

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Chapter 23

Approaches to the Induction of Tolerance

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HISTORICAL PERSPECTIVE

In 1951, Billingham and Medawar[18] published a landmark article entitled "The Technique of Free Skin Grafting in Mammals" in the *Journal of Experimental Biology*. In it, Billingham and Medawar provided the foundation for what would become the field of transplant immunology. Classic experimental observations, which included a noticeable acceleration in rejection responses after transplanting a second full-thickness allogeneic skin graft harvested from the same donor as the initial graft, set the standard for what eventually would become the groundwork for immunological memory.[18,186] Further work that was based on earlier writings of Owen[187] involved skin grafting dizygotic mammalian twin calves. The observations that these grafts are accepted by both hosts led to the hypothesis that a phenomenon of tolerance to the grafts was achieved secondary to "foreign" blood cells persistent in each twin owing to placental fusion.[18]

These breakthroughs in research translated to the clinic in 1954, when Murray and colleagues performed the first successful kidney transplant between monozygotic twins at the Peter Bent Brigham Hospital in Boston, Massachusetts. The success of this operation was partly due to the lack of immunosuppression needed in the transplant of monozygotic twins. Allografts that were subsequently attempted failed initially because of uncontrolled acute rejection responses. The quest to identify methods of immunosuppression and tolerance induction in transplantation began.[290] The impact of all of this work is still felt today, as many of the experimental models and methods are reproduced in transplant immunology laboratories around the world.

DEFINITION OF TOLERANCE

Generally, the concept of tolerance (operational) refers to the persistent survival of a transplanted allograft in the absence of continuing immunosuppressive therapy and an ongoing destructive immune response targeting the graft. The functional and nonspecific nature of this definition may be appropriate in that multiple immunological mechanisms and donor-recipient conditions are required to induce and maintain tolerance to a defined set of donor antigens in vivo. Achieving functional tolerance in transplant recipients mandates that specific allograft-destructive responses are "switched off," while the global immune response to pathogens and carcinogens remains intact. The most robust form of transplantation tolerance has to be donor-specific, as opposed to mere immunoincompetence, a requirement that can be tested experimentally by grafting third-party transplants and by challenging tolerant recipients to respond to virus infections and tumor loads. The concept of graft-specific tolerance is essential to maintain long-term survival of the graft and host and to eliminate the adverse events associated with lifelong nonspecific immunosuppression.

NEED FOR TOLERANCE IN CLINICAL TRANSPLANTATION

The human immune system broadly comprises a balance between the innate and adaptive responses.[96,182] First, these responses recognize antigens from pathogens or foreign material, and then they mount a response against invading tissue or cells to destroy it and clear the body from potential harm. The key difference between the two pathways relies on antigen specificity, that is, the innate response neither is specific nor is altered with multiple antigenic challenges; however, the adaptive response is specific for a particular antigen and "remembers" the infectious agent on each successive insult. The adaptive response improves with each encounter of a particular foreign agent. When the immune system encounters an antigen, it has to decide which type of response to make. Multiple factors are taken into account in making this decision,

Table 23–1 Immunosuppressive Agents Used in Solid Organ Transplantation

Class of Agent	Agent
Corticosteroid	Prednisone
	Methylprednisolone
Antiproliferative	Azathioprine
	Mycophenolate mofetil
	Mycophenolate sodium
Calcineurin inhibitor	Cyclosporine
	Tacrolimus
mTOR inhibitor	Sirolimus
	Everolimus
Polyclonal antilymphocyte antibodies	ALG
	ATG
	ALS
Monoclonal antibodies (with target)	Muromonab (CD3)
	Basiliximab (IL-2a receptor–CD25)
	Daclizumab
Costimulation blockade	LEA 29Y (CTLA4Ig)

Adapted from Taylor AL, Watson CJ, Bradley JA: Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol 56:23-46, 2005.

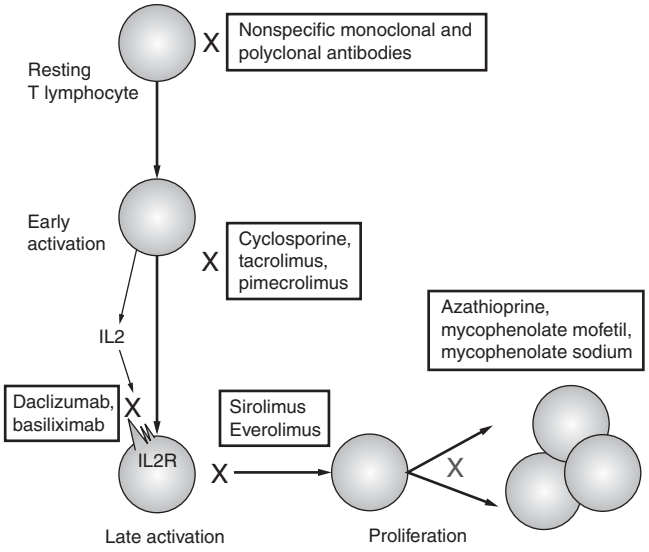

Figure 23–1 Schematic sites of action of common immunosuppressants. Each immunosuppressive agent targets a specific step in the activation and proliferation of T lymphocytes. (Adapted from Taylor AL, Watson CJ, Bradley JA: Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol 56:23-46, 2005.)

including where the antigen is "seen" and the conditions at the time of presentation, in particular, the presence or absence of inflammation. Components of the innate and adaptive arms of the immune system participate in this decision-making process.[57]

The ability to manipulate the outcome—either activation or unresponsiveness—of these immunological responses to foreign antigens on a molecular level may provide insight into therapeutics that mediate acceptance of a graft after transplantation. Currently, a variety of immunosuppressive agents are available that are used to control unwanted immune responses against an allograft. The improvements in short-term (1 year) graft survival seen since the 1970s in large part are due to the use of immunosuppressive pharmacotherapies in transplant recipients, and 1-year graft survival is now greater than 90% after kidney transplantation at most centers worldwide.[169,246,248] In the context of solid organ transplantation, the drugs that currently are available for clinical use, including azathioprine, cyclosporine, tacrolimus, mycophenolate mofetil, rapamycin, antithymocyte globulin, anti-CD25 monoclonal antibodies, and steroids (Table 23-1), are effective at suppressing the processes that lead to early activation of the immune system. Each immunosuppressive agent acts, however, on a specific area of the immune response to an allograft, and all are globally nonspecific. Each agent has its own deleterious side effects.

These drugs can be used successfully to prevent or control acute allograft rejection; however, they are less effective at controlling the long-term response to injury and activation of the immune system, or chronic rejection. They also seem to be unable to promote the development of unresponsiveness or tolerance to the donor antigens consistently in the way they are used clinically at present. Experimental studies suggest that some of these agents may block the development of unresponsiveness under certain circumstances.[135,276] For nearly all transplant recipients, the continued survival of the allograft depends on lifelong administration of several immunosuppressive drugs.

The inability of current immunosuppressive drug regimens to induce tolerance to donor antigens may be partly due to the nonspecific nature of the immunosuppression achieved by using drug therapy. Drugs, including those mentioned previously, are unable to distinguish between the potentially harmful immune response mounted against the organ graft and responses that could be beneficial, protecting the recipient from infectious pathogens and providing mechanisms to control the development of malignant cells. Generally, the drugs act by interfering with lymphocyte activation or proliferation regardless of the antigen specificity of the lymphocyte targeted (Fig. 23-1). This lack of immunological specificity means that the immune systems of patients treated with these medications are compromised not only in their ability to respond to the transplant but also in their ability to respond to any other antigenic stimuli that may be encountered after transplantation. Patients are more susceptible to infections and are at a higher risk for developing cancer.[90,195]

It has been suggested that some of the drugs used to treat transplant patients, in particular cyclosporine and tacrolimus, may have additional properties that play a role in enhancing tumor growth in a manner that is unrelated to the drugs' effects on the immune system.[50,89,155] On the contrary, pharmacotherapies such as rapamycin and its analogues which inhibit the mammalian target of rapamycin (mTOR) that is necessary for cellular growth and proliferation, have shown antineoplastic properties.[212] The promotion of $CD25^{+}CD4^{+}$ regulatory T cells by rapamycin along with interleukin (IL)-10, bolstering the suppression of allograft-mediated rejection, also has been shown.[12]

The full potential of organ transplantation may not be realized until alternative approaches to nonspecific immunosuppression are identified. Novel strategies that lead to the targeting of only the immune response directed against the transplant in the short-term or the long-term are needed. If tolerance to donor antigens of the graft could be achieved

reliably, it would ensure that only lymphocytes in the patient's immune repertoire responding to donor antigens were suppressed, leaving most lymphocytes immunocompetent and able to perform their normal function of protecting the body from infection and cancer after transplantation. The development of specific unresponsiveness to donor alloantigens in the short-term or the long-term after transplantation seems to offer the best possibility of achieving effectiveness and specificity in the control of the immune system after transplantation in either the absence or at least reduced loads of nonspecific immunosuppressive agents. This chapter discusses the mechanisms underlying tolerance induction and strategies used to induce unresponsiveness in transplanted allografts.

UNDERSTANDING THE IMMUNOLOGICAL MECHANISMS BEHIND TOLERANCE INDUCTION (see Chapter 2)

Overview of T Cell Activation

Understanding the mechanisms of activation and regulation of the immune system is important in the development of novel approaches for tolerance induction in the context of transplantation. The constant wealth of data on immunological activation appearing in the literature may be overwhelming at times; however, these findings are crucial if strategies for targeting the immune system are to be developed in the future. This section sets the scene for discussing the different approaches to tolerance induction being explored most actively at present.

Developing thymocytes containing mature T cell receptors (TCRs) with low affinity for self-antigen are "neglected" in the thymus and do not proliferate. TCRs with a high affinity for self-antigen undergo programmed cell death and are "deleted," leaving the T cells with receptors that have an intermediate affinity to enter the bloodstream and recirculate between blood and peripheral lymphoid tissue. Naive T cells continue to circulate, receiving survival signals along the way via IL-7 receptors and in the form of self-peptide/self–major histocompatibilty complex (MHC) complexes; however, when these naive cells encounter a specific antigen, they can differentiate and proliferate into an effector population. Naive T cells encounter antigen in the form of a peptide/MHC complex on the surface of antigen-presenting cells (APCs), of which there are many forms. Antigen presentation to T cells occurs via macrophages, B cells, and dendritic cells (DCs). DCs are the most professional of the APCs and are highly specialized in ingesting and presenting antigen.

During the immediate postoperative phase of transplantation, innate immunological responses induce inflammatory reactions and the increased maturation of tissue-specific DCs, hastening antigen uptake and migration to lymphoid tissue for subsequent presentation to naive T cells.[96] When activated, $CD4^+$ T cells differentiate early on into T helper type 1 (Th1) or Th2 cells, each with its own portfolio of cytokines. Th1 cells secrete macrophage-activating cytokines, including interferon (IFN)-γ, and are responsible for eliciting cell-mediated immune responses. In contrast, Th2 cells stimulate antibody production by B cells and secrete a variety of cytokines distinct from Th1 cells, including IL-4 and IL-10.[96]

There has been strong evidence to suggest a paradoxical influence of IFN-γ on cell and organ transplantation.[247,288] On the one hand, IFN-γ is a key mediator in the dysregulated Th1 response that results in a variety of autoimmune diseases, including type 1 diabetes and multiple sclerosis.[288] On the other hand, IFN-γ has been identified to play a role in the induction and maintenance of immunological tolerance to alloantigens. Experiments using costimulation blockade in wild-type versus IFN-$\gamma^{-/-}$ murine allograft models revealed the inability to prolong allograft survival in the absence of IFN-γ.[85,127] Evidence from our laboratory and others corroborates data linking the suppressive effects of IFN-γ to immunoregulatory T cells.[113,218,288] Specifically, our laboratory has shown that the rapid and transient nature of IFN-γ secreted early by alloantigen-induced regulatory T cells may inhibit the proliferation of effector lymphocytes and delay the effects of the adaptive immune response.[288]

APCs and T lymphocytes are pivotal to the adaptive arm of the immune response. They can act as helper and effector cells and play a role in the destructive immune response that occurs after transplantation of a mismatched graft.[161] T lymphocytes also may have immunoregulatory suppressive actions to induce tolerance in peripheral lymphoid tissues, controlling ongoing immune responses and suppressing unwanted actions.[1,214,287]

After transplantation, donor-derived passenger leukocytes are triggered to migrate out of the graft, partly by the proinflammatory environment created as a result of the transplantation procedure itself.[133] The release of chemokines and cytokines and complement and endothelial cell activation influence the events leading to the initiation of the immune response. In particular, secondary lymphoid tissue chemokine has been reported to play an important role in the migration of DCs in vivo to T cell compartments of the spleen and lymph node.[43] As DCs home from the graft to host lymphoid tissue under conditions of inflammation (i.e., after transplantation), they undergo a maturation process that results in the upregulation of costimulatory and adhesion molecules and MHC/peptide complexes, which are essential to trigger the response of naive T cells. In this way, immunostimulatory APCs expressing donor-type MHC/peptide complexes are brought into close proximity to naive T cells that may have TCRs capable of recognizing the donor antigens via the direct pathway of allorecognition.

The interaction of the MHC/peptide complex and TCR forms an immunological synapse, which depends on the successful dynamic rearrangement and polarization of the filamentous actin in the DC cytoskeletal membrane to bring the MHC/peptide complex in close relation to the TCR, initiating an activation response.[48,132,156] Specific T cell membrane compartments termed lipid rafts serve as recruitment centers for costimulatory molecules to concentrate on the cytoskeleton, allowing for closer interactions with molecules on the APCs.[92,156] T cell activation has been shown to be inhibited when this cytoskeletal arrangement does not occur (Fig. 23-2).[3]

Damage to the graft as a result of removal from the donor and implantation into the recipient causes the release of donor antigen from the graft. The proinflammatory environment within the graft attracts recipient-derived APCs to the graft site. In this situation, donor alloantigens are taken up by recipient APCs. Immature forms of the cells are well designed to capture antigen because they are phagocytic and

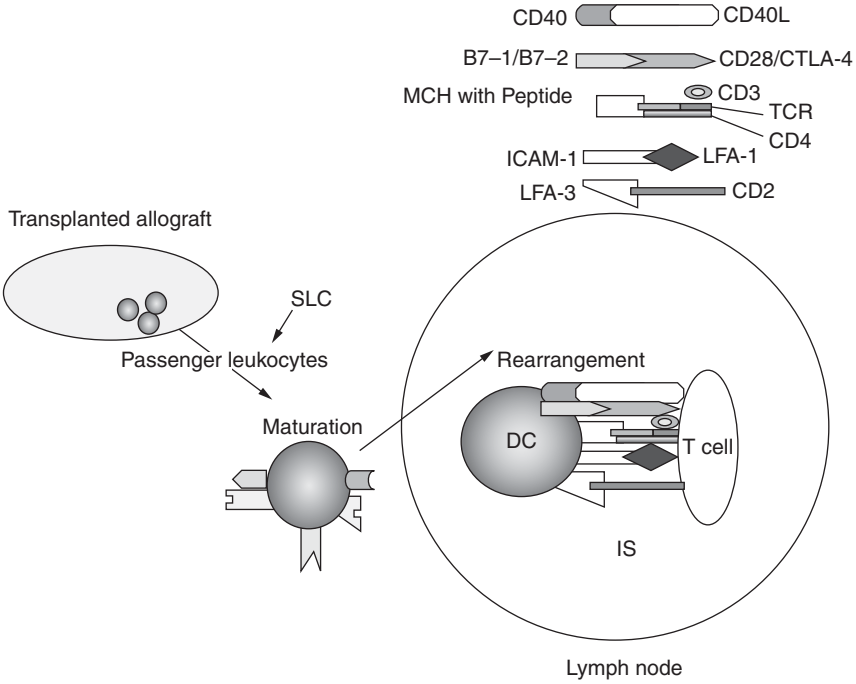

Figure 23–2 Formation of the immunological synapse. Passenger leukocytes from a transplanted allograft emigrate from the organ and under the influence of secondary lymphoid tissue chemokine (SLC) migrate to the lymph nodes and spleen. En route, these dendritic cells (DC) undergo maturation and upregulation/rearrangement of their cell surface markers using mechanisms linked to lipid rafting. When in the lymph node, T cell activation ensues on the formation of the immunological synapse (IS). T cell activation requires at least two signals. Signal 1 is delivered to the T cell when MHC class II peptide complexes on the antigen-presenting cells (APC) are recognized specifically by the T cell receptor/CD3 complex expressed by the T cell. CD4 (T cell) interacts with the MHC class II molecule, fulfilling an adhesion and a signaling function. Signal 2 or costimulation is provided by additional cell surface interactions. CD28 (T cell) can bind to B7.2 (CD86) and B7.1 (CD80) expressed by the APC. This interaction delivers a signal to the T cell that lowers the threshold for T cell activation. CD40 on the APC can bind to its ligand, CD40L (CD154) (T cell). This interaction provides additional signals to the T cell but, in contrast to the CD28 pathway, also delivers signals to the APC, resulting in an increase in expression of B7.1 and B7.2. To ensure that the T cell engages the APC for sufficient time for the signaling events to occur, adhesion molecules, including intercellular adhesion molecule (ICAM)-1 and lymphocyte function antigen (LFA)-1, also engage each other.

have the ability to take up material by micropinocytosis.[209] Antigens taken up by one of these routes enter the endocytic pathway and are processed into peptides that can be expressed at the cell surface bound to recipient MHC class II molecules. In addition, recipient DCs can take up apoptotic cells that may be generated as a result of ischemia-reperfusion injury after transplantation, and this can lead to antigen presentation in the context of MHC class I molecules. More recent evidence suggests that another pathway may exist wherein antigen processed by apoptotic cells may be cross-presented by DCs to generate a MHC class I/peptide complex.[4,19] Presentation of donor-derived allopeptides by recipient APCs triggers recipient T cells to respond to donor alloantigen through the indirect pathway of allorecognition.[227] T cells responding through the direct and indirect pathway of allorecognition contribute to allograft rejection.[72]

For a T cell to become activated fully, a threshold number of TCRs needs to be engaged.[261] TCR recognition of a donor MHC/peptide complex present on an APC results in signal transduction through the CD3 proteins that associate with the TCR at the cell surface. This signal transduction initiates a cascade of biochemical signaling pathways that are contributed to by interactions between accessory, costimulatory, and adhesion molecules and culminate ultimately in cytokine production and proliferation of the triggered T cell and its differentiation into an effector cell (Fig. 23-3).

Accessory and costimulatory molecules that have been shown to be important in triggering T cell activation on the T cell side include CD4, CD11b/CD18 (leukocyte function associated antigen [LFA]-1), CD28, and CD154 (CD40 ligand). These molecules must engage their ligands on APCs, MHC class II, intercellular adhesion molecule (ICAM), CD86/80 (B7-1/B7-2), and CD40 to ensure that the threshold for activation of a naive T cell is overcome when antigen recognition has occurred.

It is well established that T cell activation occurs in the two-signal pathway described previously, wherein the MHC/peptide complex interacts with TCR constituting the first signal, and then various costimulatory molecules interact with each other to complete the induction of activation. The process is much more complex, however. When CD28 molecules on the T cell surface interact with B7 molecules on the APC, lipid rafts become rapidly polarized even in the absence of TCR/MHC complex formation. Some downstream effects of TCR triggering, such as increases in intracellular calcium levels and translocation of nuclear factor κB to the nucleus, may occur with B7-CD28 interaction alone, questioning the actual sequence of the traditional signaling hypothesis.[128]

The cytokine and chemokine milieu present at the time these molecular engagements occur affects the differentiation pathway a T cell takes and the course of the response.[180] Cytokines and chemokines can modulate the expression of

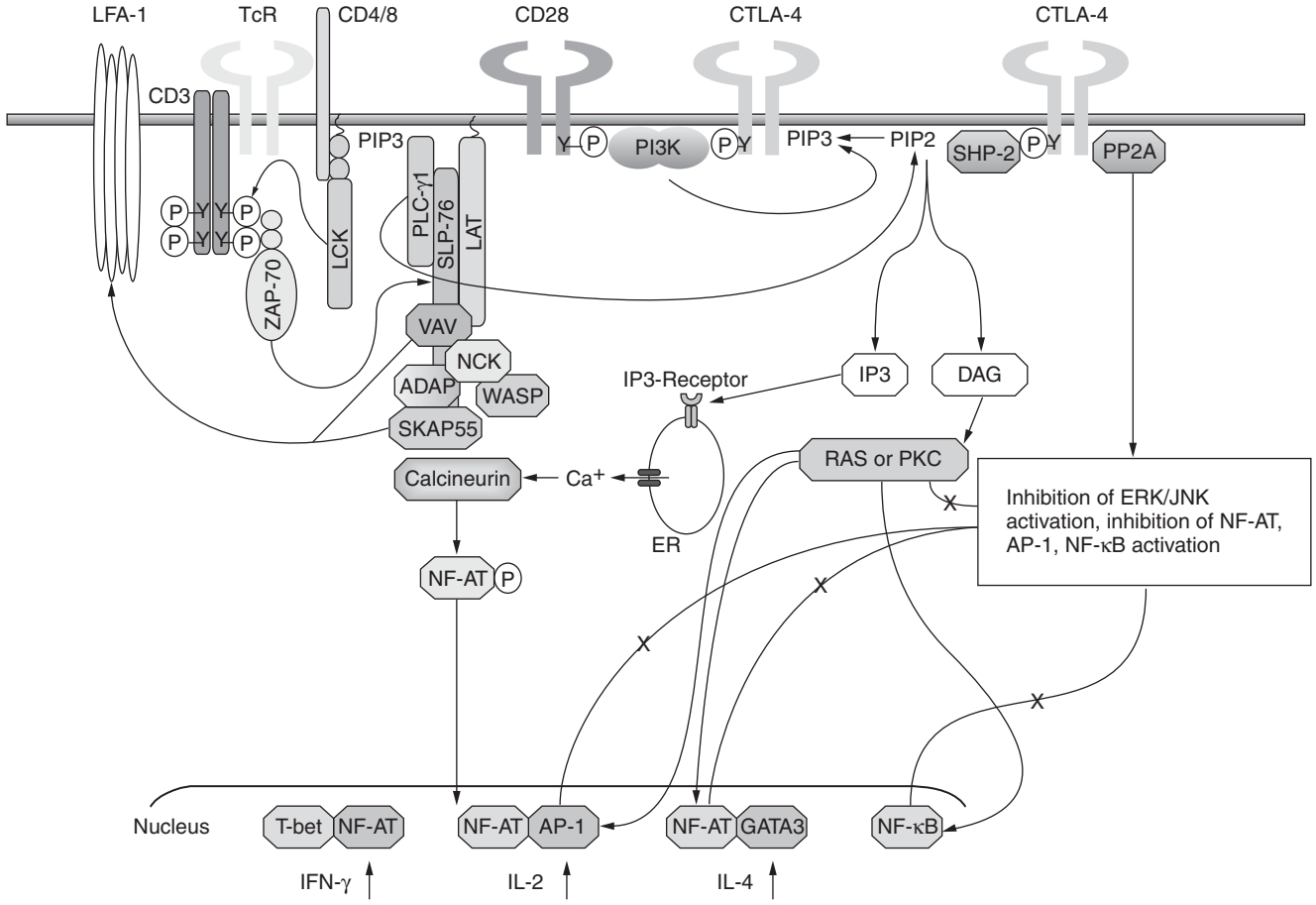

Figure 23–3 Model for T cell receptor (TCR) and costimulator signaling during T cell activation. Engagement of the TCR leads to rapid activation of Src kinase Lck, which phosphorylates ITAM motifs in the CD3 and TCR chain, followed by the activation of ZAP-70, which contributes to the downstream phosphorylation of adapters, such as LAT, SLP-76, and ADAP. These adapters form a signaling complex that includes NCK, VAV, PLCγ1, and other molecules. The phosphorylated PLCγ is important for regulating calcium flux and activating PKC and MAPK/ERK, leading to activation of transcription factors (i.e., NF-AT, AP1, and NFκB) and cytokine production. NF-AT proteins cooperate with T-bet in Th1 cells and GATA3 in Th2 cells to maintain and commit to T helper cell differentiation through the induction of IFN-γ or IL-4. Also, CD28 associates with PI3K and VAV to upregulate cytokine production through RAS/PKC or PI3K effector-involved signal pathways. CTLA4 might interact with PP2A, PI3K, or SHP-2. Cross-linking of CTLA4 has been found to reduce TCR-dependent activation of MAPK, ERK, and JNK and of transcription factors (i.e., NF-AT, AP1, and NFκB). VAV-NCK-WASP contributes to actin polymerization, and SLP76-ADAP-SKAP55 regulates integrin-mediated T cell adhesion.

the cell surface molecules mentioned previously and the expression of cytokine and chemokine receptors themselves. This modulation can result in differential signaling in the T cell and APC, tipping the balance of the response from full to partial activation or, in some circumstances, inactivation of the cells involved, dramatically modifying the downstream events (i.e., cell migration patterns and the generation of effector cells). Activation signals in the form of cytokines propagate the responses initiated by signals 1 and 2 and are often referred to as the third signal in T cell activation.[138]

Mechanisms of Tolerance to Donor Antigens

The human immune system has evolved naturally to respond to challenges in a precise and controlled way. A constant balance exists to ensure an effective, but not excessive, response to any unwanted stimuli. It may be possible to take advantage of these mechanisms to induce or maintain tolerance to donor antigens. Many mechanisms of tolerance are continuously used by the body to prevent reactions against self-antigens, which ultimately would lead to autoimmune pathologies.[181] The self-tolerance of the immune system comprises a conglomeration of mechanistic pathways all working together to discriminate between self and nonself. Many of these mechanisms may be applied to alloantigens. The mechanisms identified as responsible for inducing or maintaining tolerance to donor antigens include the following[59]:

- Deletion of donor reactive cells centrally in the thymus and in the periphery
- T cell ignorance or a state of effector unresponsiveness that is relevant to grafts placed at "immunologically privileged" sites, such as the cornea or brain
- Exhaustion, in which the ability of donor reactive cells is eliminated as a result of overstimulation
- Anergy, defined as a state of unresponsiveness that is refractory to further stimulation

More recently, a state of antiallograft/antibody persistence, termed accommodation, has appeared in the literature. This term must not be confused with that of tolerance, in that allograft accommodation is a series of physiological

changes that allow a transplanted organ to function in the face of responses directed against the graft.[126] The induction and maintenance of tolerance is a dynamic process and operates as multiple mechanisms in concert with one another, similar to that of self-tolerance and prevention of autoimmune diseases. Each facet varies in its degree of function as the process develops.

Methodology of Tolerance Induction and Maintenance

Persistence of Donor Antigen

An overriding feature in all of the above-mentioned mechanisms of tolerance is the persistent presence of donor antigen throughout the period of tolerance in vivo. Many experimental models have established that donor antigen must be present continuously to maintain a tolerant state, before or after transplantation, regardless of the precise nature of the mechanism that is operating.[27,81,116,223] The source of the antigen can be donor-derived cells introduced before transplantation, as is the case in models of mixed chimerism,[116] or the graft itself after transplantation.[81,217,234] In the absence of antigen, tolerance is lost gradually because the mechanisms responsible for maintaining tolerance are no longer stimulated. During the induction phase and the maintenance phase of tolerance, the presence of alloantigen is the key factor driving the outcome. As is often the case with the immune system, the same element can influence the response positively and negatively. In the case of donor antigen, presentation in the wrong context, as in a proinflammatory environment, as outlined earlier, could lead to activation with the potential of destroying the tolerant state and triggering graft rejection.

Deletion of Donor Reactive Leukocytes

Tolerance to peripheral self-antigens is achieved routinely by processes that begin with selective propagation or deletion in the thymus. These developing thymocytes undergo successive levels of TCR and cell surface molecule expression in their central development. The stochastic mechanism of TCR development renders many formed TCRs useless. Through thymic selection, a mature T cell repertoire is developed that not only is diverse but also can react to foreign antigen, while remaining tolerant to self-antigens. The newly formed TCRs that the thymocytes express are challenged by self-MHC and are selected based on response. Thymocytes that are positively selected express TCRs that relay a signal on activation; cells that have no response to self-MHC/peptide die through neglect. Cells containing TCRs that transmit a robust signal in response to self-MHC/peptide complexes are deleted via programmed cell death.[112] Thymocytes expressing a functional $\alpha\beta$ TCR develop into mature T cells in the thymus only if the constraints for positive and negative selection are met.

Central tolerance by clonal deletion of T cells in the thymus is the major mechanism by which tolerance to self-antigens is induced.[64] This process is essential to ensure that a diverse T cell repertoire is produced and maintained. Thymocyte selection is so meticulous that only 1% to 3% of thymocytes actually succeed in survival and export.[244] Despite the stringency of selection, however, the process of deletion of T cells in the thymus may be incomplete. Although residual T cells have a TCR with only a lower affinity and avidity for the selecting ligand, they still are present and have the potential to react with the selecting antigen or by cross-reactivity with another antigen at a later stage.[107]

Central deletion of T cells in the thymus can be exploited as a mechanism for inducing tolerance to donor antigens. This mechanism has been particularly successful in the context of therapeutic strategies using donor bone marrow in combination with nonmyeloablative therapy, such as T cell depletion or costimulation blockade, for the induction of tolerance.[275] The clinical applicability of this strategy can be shown by kidney transplant recipients who have previously undergone bone marrow transplantation from the same donor because of hematologic indications. Macrochimerism in these patients leads to long-term graft acceptance without immunosuppression.[275] In mixed allogeneic chimeras in the mouse, donor-derived DCs have been shown to reside and persist in the recipient thymus.[157,255] As a result, there is continuous deletion of donor reactive thymocytes, leading to the absence of donor reactive T cells in the periphery and tolerance. The challenge of these approaches is to achieve a sufficient level of chimerism reliably without using a treatment regimen that is excessively toxic. More recent shifts in paradigm have allowed the use of costimulation blockade as conditioning regimens in maintenance therapy rather than tolerance induction, eliminating long-term calcineurin inhibition and its harmful side effects.[138]

Intrathymic injection of donor antigen or allopeptides directly into the thymus results in the deletion of donor reactive cells.[98,159,184,197] If this injection of antigen is combined with leukocyte or T cell depletion in the periphery, it can lead to the successful induction of operational donor-specific tolerance in rodents.[97] In contrast to the situation that occurs in stable mixed chimeras, after intrathymic delivery of donor antigen, the antigen persists in the thymus for only a defined period after injection. Intrathymic delivery of donor antigen provides a window of opportunity in which to transplant a solid organ graft, rather than producing persistent deletion of thymocytes in the long-term.[98]

Antigen-reactive T cells also may be deleted in the periphery.[272] The introduction of high doses of defined antigens intravenously or orally has been shown to result in deletion of mature T cells in the peripheral lymphoid organs.[14,111] $CD4^+$ and $CD8^+$ T cells can be eliminated by peripheral deletion, but in many cases deletion is incomplete even when high doses of antigen are used. When analyzed, these residual antigen-reactive cells remaining in the periphery were shown to be hyperresponsive to further stimulation by the same antigen, showing that additional mechanisms of tolerance were in operation.[190]

The mechanisms by which T cells are deleted in the thymus and the periphery have been an area of active investigation. To maintain the longevity of self-antigen and to protect against foreign invasion, autoreactive thymocytes are believed to undergo programmed cell death centrally. These T cells continue to be pruned by apoptosis in the periphery as well.

Two distinct modes of apoptosis have been implicated as the mechanism essential for T cell death. Activation-induced cell death (AICD) is a receptor-driven, caspase-8–dependent pathway wherein high doses of antigen or repetitive stimulation is necessary for cellular demise. Activated T cell autonomous death, formerly known as passive cell death,

is a caspase-8–independent and death receptor–independent pathway wherein a downregulation of the T cell–protective, Bcl-2–related protein, Bcl-2 interacting mediator of cell death (Bim), causes signals that lead to apoptosis.[87]

AICD was a term originally coined to describe death of thymocytes after activation via their CD3 molecules,[224] but AICD also can occur in the periphery.[276] Subsequent reports proved, however, that in vitro thymocyte death occurs through pathways initiated by TCR and possibly tumor necrosis factor (TNF)-α receptor engagement.[141] These receptors subsequently go on to propagate signals through the Fas pathway, which has been shown to play an essential role in the homeostasis of the peripheral lymphocyte compartment and in effector mechanisms used by cytotoxic T lymphocytes and natural killer cells to destroy target cells.[172] The Fas receptor (CD95, APO-1) is a type 1 membrane protein of the TNF receptor superfamily. When it finds its natural ligand (CD95L, Fas-ligand), a complex signaling cascade is initiated, leading to caspase activation, which can result in the death of the Fas-expressing cell by apoptosis.[87,172] High levels of FLIP, an inert homologue of caspase-8, are expressed in primary T cells and render these cells resistant to AICD. It has been shown, however, that during the S phase of the cell cycle, IL-2 sensitizes T cells to AICD by downregulating levels of FLIP.[5] Although there are conflicting data about the role of the Fas pathway in the thymus, the overall impression from many analyses suggests that the Fas pathway can play a role in antigen-specific deletion of thymocytes, but only at high concentrations or repetitive stimulation of antigen. It is possible in these scenarios that increased antigenic exposure leads to upregulation of IL-2 expression, attenuating the levels of FLIP, creating a proapoptotic milieu.[87]

More relevant to negative selection in the thymus may be the role of activated T cell autonomous death. During the first checkpoint of thymocyte development, or TCR-β selection, $CD4^-CD8^-CD3^-$ thymocytes transition to double-positive cells and pass through a second checkpoint of positive selection where single-positive $CD8^+$ or $CD4^+$ T cells are chosen to develop in the thymic cortex based on signal delivery via MHC class I ($CD8^+$) or MHC class II ($CD4^+$).[260] As mentioned previously, thymocytes with TCRs that express exceedingly intense signals to self-MHC/peptide complex are seen as autoreactive and destroyed. Thymic deletion of these autoreactive cells is thought to be less dependent on the Fas pathways described earlier and more dependent on the dynamic process of activated T cell autonomous death. During activated T cell autonomous death, Bim, a member of the Bcl-2 family of proteins, is thought to be essential for initiation of cytokine withdrawal, calcium flux, and ultimately Bcl-2–regulated apoptotic signaling.[235]

It has been well established by previous studies that autoreactive thymocytes harbor an increased level of intracellular calcium. More recent evidence shows that signals of negative selection induce calcium-dependent Bim transcription via protein kinase C signaling pathways.[31] This pathway differs from that of AICD in that it is triggered by growth factor (IL-2) withdrawal or various cytotoxic drugs and induces the mitochondrial release of cytochrome *c*, which forms an apoptosome with the adapter protein APAF-1, ultimately activating the proapoptotic aspartic acid–specific cysteine protease, caspase-9.[236]

In contrast to central mechanisms, the Fas pathway may play a greater role, in combination with other mechanisms, in deletion of T cells at particular sites in the periphery, so-called immune privileged sites.[13] At these sites, transplantation of allogeneic tissues results in the prolonged survival of the transplanted tissue relative to the survival obtained after transplantation of the same tissue at other sites. These sites include the anterior chamber of the eye and the testis.[60,174] Fas ligand expression has been shown to be important for these sites to maintain their immune privileged status. More recent studies have shown that islet allograft transplantation in the testis not only generated fewer $CD8^+$ memory cells but also generated an increase in $CD4^+CD25^+$ regulatory T cells compared with islets that were transplanted conventionally under the kidney capsule. When costimulatory pathways were blocked, there was an induction of tolerance in the testicular islet allografts, but not in those transplanted under the kidney capsule.[174]

Fas ligand–mediated apoptosis has been shown to be the mechanism by which inflammatory cells entering these sites are eliminated. The Fas pathway also has been implicated in deletional tolerance after administration of allogeneic bone marrow.[69] In the periphery, the Fas pathway may be more important in deletion of antigen-reactive cells when antigen is present at high concentration or at particular sites of the body where Fas ligand is expressed endogenously. Many other attempts have been made to harness the immunological potential of these immune privileged sites and have had varying degrees of success.[75,258]

In the periphery, AICD maintains homeostasis in the lymphocyte compartment. In addition to the Fas pathway, many other peripheral mechanisms have been implicated in clonal downsizing after the elimination of antigen, including upregulation of expression of CD152 (CTLA4) on T cells, a molecule that prevents further costimulation by competing for and binding to CD80 and CD86 (B7-1 and B7-2) on the APC and by delivering negative signals to the responding cell, shutting down further clonal expansion.[36,262] Similar to CTLA4 are the CD28-related programmed cell death 1 receptors (PD-1), which share a 23% homology with CTLA4. In contrast to CTLA4, however, PD-1 is not restricted to T cells alone but can be found on myeloid cells and B cells as well, suggesting a broader role in immunological regulation. The binding of PD-1 to its ligands PD-L1 and PD-L2, which are upregulated on the surface of T cells, B cells, macrophages, and DCs on activation, leads to the inhibition of lymphocyte activation.[185]

Loss of antigen-reactive cells through AICD rapidly eliminates reactivity toward the stimulating antigen. In normal circumstances (i.e., during responses to nominal antigens), this process is used to balance the response. Antigen-reactive T cells no longer are activated when the antigen has been eliminated. After transplantation, antigen stimulation potentially continues as long as the organ continues to function. Expansion of donor reactive T cells could occur indefinitely, unless the response was actively controlled. AICD may be one of the mechanisms that is used to ensure that the size of the population of leukocytes responding to donor antigen is kept at a manageable level. Certain immunosuppressive drugs, such as rapamycin, may be able to facilitate this process.[145,276]

The reappearance of donor reactive cells at a functional level can be controlled or prevented by the continuing

presence of donor antigen in the form of the organ graft or active immunoregulation. This process results in the long-term survival of the graft provided that the rate of deletion is maintained or that additional mechanisms that can promote tolerance to the graft are induced. In some situations, this process is described as exhaustion because the response to a particular antigen can be effectively exhausted by chronic stimulation of the responding populations. Such a situation occurs most commonly in chronic viral infection.[297]

Suppression and Regulation of Immune Responses

Although the concept of antigen-specific suppression is not new, over the past decade there has been a resurgence of interest in the characterization and functional dissection of T cell–mediated suppression, now more often called immunoregulation.[281] Suppression was described first in the 1970s after the demonstration that antigen-specific unresponsiveness could be transferred from one recipient to another.[70] Antigen-reactive T cell balance, in this case, is controlled by suppression of homeostatic proliferation, rather than the mechanisms of deletion that occur during T cell development. When transferred between recipients, populations of cells present among those transferred adoptively must be capable of regulating the response of naive cells to the same antigen.

The human immune system has developed to protect self-tissues from external pathogens and autoreactive cells. Although many autoreactive cells are deleted centrally in the thymus, some manage to escape and are stymied further in the periphery via the previously discussed mechanisms of ignorance, peripheral deletion, and anergy. Even so, although rare, autoimmune diseases occur when mature effector T cells are unable to distinguish between self and nonself, abandoning basic self-tolerance. Peripheral prevention of autoimmunity has been described to be regulated with "active" mechanisms of tolerance by using a unique subset of T cells with regulatory function.[150,281] Maintenance of tolerance and active regulation of self-reactive leukocytes is essential in the prevention of autoimmune diseases.[200] Regulatory T cells also have been implicated as being a key factor in the active induction and maintenance of unresponsiveness to donor alloantigen in vivo, a characteristic that may prove to be crucial in the realm of transplant tolerance.[287]

Phenotypic Characterization of Regulatory T Cells

Although many varieties of regulatory T cells have been reported (e.g., natural killer cells, γδ T cells, regulatory DCs, $CD8^+$ regulatory cells), an enriched subset of $CD4^+$ T cells have enjoyed much attention in the literature.[9] These $CD4^+$ regulatory T cells may be subdivided further into cells that are induced and secrete IL-10 and transforming growth factor (TGF)-β or T regulatory-1 cells and the so-called naturally occurring regulatory T cells. In recent years, many laboratories have attempted to find markers that are exclusive to this natural regulatory population to isolate and manipulate immunoregulatory cells in vitro and in vivo for potential therapeutics.

Originally, $CD4^+$ effector cells causing colitis were shown to be controlled by a population of naive $CD4^+$ $CD45RB^{hi}$ cells in an adoptive transfer model.[198] Later, these $CD4^+CD45RB^{hi}$ cells were found to express constitutively the alpha chain of the IL-2 receptor, CD25.[154] Although CD25 is not solely expressed on regulatory cells and is upregulated on activation of T cells, it seems to be the most useful marker to sort this population. Naturally occurring regulatory T cells have been reported to represent 5% to 10% of the human T lymphocyte population.[181,287] Further studies on cord blood found that $CD25^+CD4^+$ regulatory T cells that were able to suppress proliferation by anti-CD3Ab expressed twofold higher levels of the gene *FOXP3*, which encodes the forkhead/winged-helix transcription factor scurfin.[280] A deficiency in the *FOXP3* gene has been shown to cause autoimmune and inflammatory disease in rodents and humans. Humans lacking *FOXP3* develop the X-linked recessive disease IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) or XLAAD (X-linked autoimmunity-allergic dysregulation syndrome).[279] More recent evidence has revealed that *FOXP3* is expressed predominantly in regulatory T cells and is essential for their development and function.[62] Ectopic expression of *FOXP3* has been shown to influence suppressive activity on peripheral effector populations.[62]

Expression of other markers to identify and isolate regulatory T cells also has been reported. A host of surface markers on $CD25^{high}$ cells have been identified in murine models and span a wide spectrum of variability. Examples include CTLA4 and CD122 and members of the TNF receptor superfamily, such as glucocorticoid-induced TNF receptor–related protein. Many other markers, such as chemokine receptors, Toll-like receptors, and homing receptors, also have been described; however, many of these markers have not been confirmed in humans and are upregulated on nonregulatory $CD25^-$ cells as well, making their utility as isolation molecules difficult to discern.[281]

Mechanism of Regulation

To exploit suppression and regulation of the immune response to an organ graft for therapeutic purposes, a clearer understanding of the mechanisms by which this phenomenon operates is required. Although regulation could be operating exclusively through deletional mechanisms, at present there is little evidence to support this as the dominant mechanism for active immunoregulation or suppression. The demonstration that immunoregulatory cells can be used to transfer unresponsiveness adoptively from a transplant recipient with a long-term surviving graft to a fresh naive recipient through many generations of cells, the process known as infectious tolerance, suggests that this population of regulatory or suppressor cells can generate further cohorts by influencing the differentiation patterns of naive cells in vivo.[178,270] These cells seem to function not by eliminating donor reactive aggressive leukocytes but by silencing their functional activity in vivo.

Multiple mechanisms are employed by regulatory T cells to suppress effector populations. The methods of suppression used by naturally occurring regulatory T cells and Tr1 type cells vary and include the induction of effector cell anergy,[54,67] suppression of an effector phenotype by T cells,[160] and conversion of potential effector cell populations into regulatory subsets.[63,100,105] Finally, the suppressive abilities of regulatory T cells may extend beyond acting on T effector cells alone; there is evidence to suggest that $CD25^+CD4^+$ regulatory T cells may control the ability of APCs to trigger T cell activation.[35]

Naturally occurring $CD25^+CD4^+$ regulatory T cells undergo positive selection in the thymus and enter the periphery as committed cells.[41] These thymically derived regulatory T cells exhibit a cell contact–dependent, cytokine-independent mechanism of action, in contrast to the Tr1 cells, which function via cytokine-dependent and contact independent–mechanisms.[205] Cell contact–dependent mechanisms seem to be essential for induction of anergy, yet a shift to cytokine dependence and contact independence may occur when these T cells are anergized.

Although controversial, the cytokines IL-10 and TGF-β have been suggested as having significant roles in rendering T effector populations anergic. Experiments done mainly in the murine inflammatory bowel disease model exhibit classic inflammatory bowel disease lesions when $CD25^-CD4^+$ T cells are transferred to immunodeficient mice. These lesions are prevented when the effector cells are cotransferred with $CD25^+CD4^+$ T cells. When an anti–IL-10 receptor–blocking monoclonal antibody is administered to the pretreated mice, the prevention of inflammatory bowel disease is neutralized.[215] Roles for TGF-β and IL-10 alone or in combination have been proposed in many different models of immunoregulation and anergy, including the anterior chamber of the eye, after oral or nasal delivery of antigen and in models of tolerance to self-antigen or alloantigen.[7,101,199,278] TGF-β has been reported to modulate the function of the APC promoting Th2 responses.[25,118] TGF-β has been shown to influence naive T cells into a regulatory phenotype, expressing Foxp3, with suppressive activity ex vivo.[93] The ability to convert naive T cells to cells with a regulatory phenotype and ability may prove to be beneficial in diseases of autoimmunity and transplantation tolerance.[63]

The relationship between TGF-β and IL-10 in the development of tolerance still is being characterized as different models show differential requirements for one or both of these mediators at particular stages in the response.[163] From these data, it seems reasonable to propose that there are certain soluble mediators that can promote the development of unresponsiveness when present in the correct microenvironment, TGF-β and IL-10 being two examples. Similar to many immunological mediators, the presence of TGF-β and IL-10 in the right place at a certain concentration is integral to the way in which they function. When present in the wrong place at the wrong time with respect to tolerance induction, TGF-β can cause fibrosis, and IL-10 can trigger acute graft rejection.[20,168,170,204,206,267] These and other soluble mediators not yet identified likely act in combination with cell surface structures to promote the development of tolerance.

Inhibition of allograft rejection also may be mediated by a process whereby potential effector cell populations may be converted into regulatory T cells themselves. Additionally, it is now known that T cells capable of regulatory function are not dependent on the thymic emigrant population of naturally occurring regulators. Models of thymectomized mice, which have undergone donor-specific transfusion pretreatments under the cover of anti-CD4 antibody, are able to accept skin allografts long-term. To dispel the concern that the pretreatment protocol may expand preexisting populations of regulatory T cells, thymectomized mice rendered immunodeficient by $CD8^+$ and $CD4^+$ T cell–depleting antibodies were reconstituted with $CD25^-CD4^+$ effector cells and administered peripheral donor-specific transfusions along with anti-CD4 antibody. These mice were found to generate cells capable of regulating allogeneic skin grafts long-term, suggesting that regulatory T cells may develop from $CD25^-CD4^+$ precursors in the periphery independent of any centralized thymic influence.[105]

Finally, an alternative but complementary hypothesis to explain the action of regulatory T cells suggests that regulatory T cells may manipulate the ability of an APC to activate T cells.[144,243] APCs have been shown to become licensed to trigger effector cell activation when they have encountered an activated T helper (CD4) cell.[17,131,210] This hypothesis eliminates the need for clusters of helper and cytotoxic T cells to be brought together in the vicinity of the APC at the same time to ensure that only effector cells with the appropriate antigen specificity are activated. Rather, the hypothesis suggests that when an APC has presented an antigen and activated a T helper cell, the T helper cell changes the functional activity of the APC to enable activation of an effector T cell to be triggered in its absence.

A similar scenario has been envisaged for regulatory T cells. When regulatory cells are mixed into cultures of APCs and helper T cells, they can inhibit proliferation of the responding T cells. It has been shown that regulatory T cells can inhibit the upregulation of costimulatory molecules on APCs when they are present in these cultures.[145,215,243] These and other data suggest that regulatory cells can change the function of APCs, preventing them from triggering T cell activation.

Evidence, from our laboratory and others, tracking the movement and proliferation of effector T cell populations in the presence of regulatory T cells shows that regulatory T cells delay T cell priming at the level of the lymph nodes.[22,33] These regulatory T cells intensify their response and home to the localized site of the affected tissue in the event of inflammation.[22,33]

Linked Unresponsiveness

The phenomenon of antigen-induced tolerance was originally thought to be specific to a sole antigen, which served as the initial tolerogen.[9] A potential powerful effect of regulatory and suppressor cells is a process known as linked unresponsiveness, wherein the immune response is manipulated to accept a variety of different antigens by initially targeting just one.[49,153,282] If a recipient's immune system is exposed to a defined alloantigen before transplantation, alone or in combination with a T cell modulating agent, the response to that antigen can be blunted in vivo.[153,213,283] This unresponsive state may spread beyond the scope of this sole antigen and may be linked to other molecules present on a graft provided that the initiating antigen is present (Fig. 23-4).[287] One hypothesis as to how regulatory T cells suppress the rejection response is via linked unresponsiveness.

Regulatory T cells that recognize MHC molecules via the indirect pathway develop when donor alloantigens interact with a recipient either before or after transplantation.[287] These regulatory cells have been shown to use the mechanism of linked unresponsiveness as their mode of suppression. In our studies using a mouse model of transplantation, we have shown that when recipients are pretreated with cells expressing a single donor class I molecule, such as $H2K^b$ alone[153] or in combination with anti-CD4 monoclonal antibody,[213,282,283] specific unresponsiveness to $H2K^b$ is induced before transplantation. After transplantation, this state of unresponsiveness to $H2K^b$ can be linked to MHC

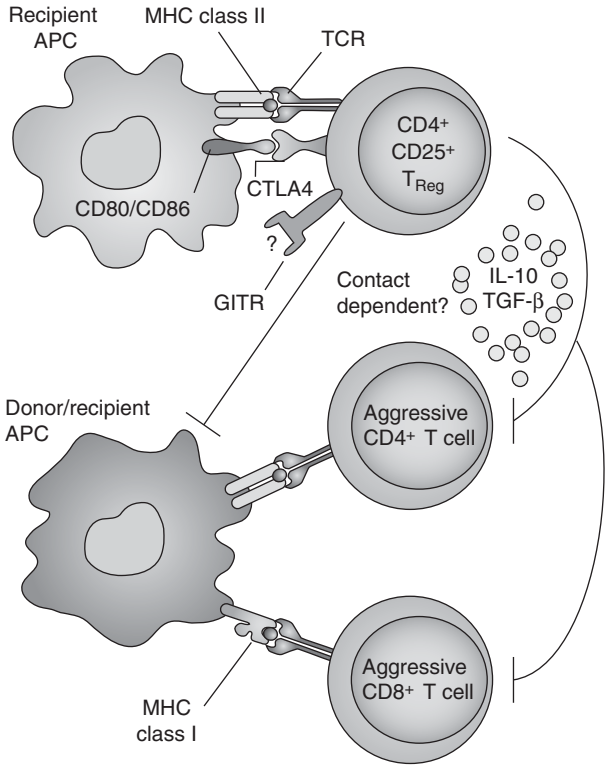

Figure 23–4 Linked unresponsiveness as a mechanism of immunoregulation in transplantation. When donor alloantigens are encountered under certain conditions, either before or after transplantation, regulatory T (Treg) cells that recognize the donor antigen through the indirect pathway develop. When the graft is completely mismatched with the recipient, such Treg cells can recognize the donor alloantigen as an allopeptide bound to a recipient MHC class II molecule after processing by recipient antigen-presenting cells (APCs). The Treg cells are triggered to manifest their regulatory potential, which can affect other donor-specific alloantigen T cells responding through either the direct or the indirect pathways of allorecognition. The functional activity of Treg cells in this in vivo setting has been shown to depend on cell-associated molecules, including CTLA4 and GITR, and soluble mediators such as interleukin (IL)-10 and transforming growth factor (TGF)-β. (From Wood KJ, Sakaguchi S: Regulatory T cells in transplantation tolerance. Nat Rev Immunol 3:199-210, 2003. Copyright 2003 Macmillan Magazines Ltd.)

and minor histocompatibility complex antigens expressed by the graft (Table 23-2). If one transplants an organ graft expressing the initial antigen and other alloantigens, unresponsiveness to the triggering antigen and the alloantigens expressed by the transplant develops in the long-term after transplantation.

The mechanisms underlying linked unresponsiveness are under active investigation. Data from the analysis of anergized T cell clones in vitro and regulatory cells in vivo show that the process is active and requires cell-to-cell contact.[83,149] In many systems, the initiating antigen is seen indirectly by the recipient's immune system,[73,83] after processing of the donor molecule by recipient APCs. The cells have been described as possessing the phenotype of regulatory cells because they can function in adoptive transfer systems.

This phenomenon has important clinical implications, particularly when alloantigen is administered before transplantation in the form of blood transfusions. The mechanism implies that tolerance established to one set of antigens can spread to others if they are presented on the same graft or the same APCs. It might be possible to expose a recipient to one or more defined human leukocyte antigens (HLAs) that they themselves do not express. When an organ donor is available, the graft might express at least one of the antigens to which unresponsiveness has been induced before transplantation. In this way, the presentation of this same donor molecule on an allograft would allow linked unresponsiveness to develop to the mismatched antigens expressed by the organ donor. Evidence from Ochando and colleagues[183] suggests that alloantigens introduced into the host intravenously are acquired and processed by plasmacytoid DCs. These plasmacytoid DCs have been shown to play a distinct role in inducing and maintaining tolerance to vascularized allografts by phagocytizing alloantigen and homing to peripheral lymph nodes ultimately to aid in the induction of $CD4^+CD25^+Foxp3^+$ regulatory cells.[183]

The characterization and expansion of these regulatory T cells may be the way forward in the induction of tolerance in clinical transplantation. Studies already have begun to try to isolate cells that suppress the rejection response in vivo and in vitro.[66,189,216] Additionally, current immunosuppression protocols may be tailored to the individual based on the tracking of expansion or deletion of regulatory T cells that is specific to each transplant recipient.[2]

INFORMATION FROM ANALYZING TOLERANT RECIPIENTS

Operational tolerance, whereby an allograft remains functional and rejection-free for more than 1 year without the influence of immunosuppression, is the "holy grail" of transplantation and an extremely rare event in the clinical setting.[211] Clinical reports of patients with spontaneously tolerant allografts not only are infrequent but also are usually limited

Table 23–2 **Experiments Showing Linked Unresponsiveness in a Cardiac Allograft Mouse Model***

Source of Antigens Used to Pretreat CBA ($H2^k$) Recipients in Combination with Anti-CD4	Strain and MHC Haplotype of Heart Donor	Initiating Antigens	Graft Survival (Median Survival Time) (days)
B10-$H2^b$	B10-$H2^b$	B10-$H2^b$	100
B10-$H2^b$	BALB-$H2^d$	None	25
CBK-$H2K^b$ + $H2^d$	B10-$H2^b$	$H2K^b$	100
CBK-$H2K^b$ + $H2^k$	(CBK x BALB)F1 $H2K^b$ + $H2^d$	$H2K^b$	100
CBK-$H2K^b$ + $H2^k$	(CBK x BALB)F1 $H2^k$ + $H2^d$	None	25

*The recipient is pretreated with antigen in the form of blood under an umbrella of anti-CD4 monoclonal antibody.

to liver transplants[51,245]; however, there have been reports of graft acceptance in kidney transplant recipients without the administration of immunosuppressive agents.[188,211,298] Specific reports of the spontaneous development of transplantation tolerance in rodent models exist for liver grafts across a full MHC mismatch and of kidney and heart grafts that are mismatched for one or more major or minor antigens in some donor-recipient combinations.[65,103,196,203] In some large animal models, such as the pig, liver and kidney allografts are accepted after administration of only a short course of immunosuppression.[30,71] The results of these large animal models have been reported in clinical settings as well in the form of prope or minimal immunosuppression tolerance. Prope tolerance, a term coined by Calne and colleagues in 1998,[29] refers to maintenance of a tolerant graft with low, nontoxic doses of immunosuppression.[167] Prope tolerance is believed by some investigators to be the more pragmatic approach to tolerance induction.

The mechanisms of operational tolerance are unclear and under active investigation. Patients who exhibit tolerance to their grafted organs after immunosuppressive withdrawal, usually owing to noncompliance, may provide key insights into the process of tolerance development. As discussed previously, the key mechanisms of tolerance in experimental models include clonal deletion, anergy, and immunological regulation/suppression.[59] Donor-specific hyporesponsiveness does not seem to rely solely on clonal deletion, however, as a means to achieve operational tolerance.[84] Reports suggest that operational tolerance may be achieved even in the presence of anti–donor reactive antibodies.[59,238]

Graft-specific tolerance has been shown to correlate with mechanisms of regulation either with or without anergy. Immune regulation, via regulatory cell activity, as a means to achieve donor-specific hyporesponsiveness has been described in kidney allograft recipients using a human-to-mouse trans vivo delayed-type hypersensitivity assay.[264] Further evidence of the role that regulatory T cells have in donor-specific tolerance has been described using $CD25^{+}CD4^{+}$ T cells isolated from the peripheral blood of living related liver transplant recipients who have achieved graft acceptance without immunosuppression.[294] In most cases, the suppression displayed by these regulatory cells were donor-specific alloantigen.[294] $CD25^{hi}CD4^{+}$ cells occurred with a higher frequency in phenotyped peripheral blood mononuclear cells of operationally tolerant liver transplant recipients compared with age-matched nontransplanted volunteers.[146] Additionally, reports of gene transcription analyses suggest that Foxp3 transcripts are significantly greater in patients exhibiting operational tolerance compared with patients with chronic rejection.[151]

Ultimately, multiple mechanisms play a role in graft-specific tolerance. Donor hyporesponsiveness also has been described as a result of antigen load in the form of multiorgan transplantation. It generally is accepted that there is a hierarchy with respect to the ease of inhibition of immune response directed against different organs, with liver allografts and skin grafts being at the two opposite ends of the spectrum. In clinical transplantation, it is often noted that the liver seems to protect other organs that are transplanted alongside it from the full force of the rejection response—the liver effect. Liver allografts seem to promote the development of unresponsiveness. The initial post-transplant phase after liver grafting is associated with the activation of donor-specific helper and cytotoxic T cells and infiltration of the graft by T cells and macrophages.[103] The level of infiltration subsides after a few months, however, and the graft survives long term. When the characteristics of the cellular infiltrates and the cytokines that the infiltrating leukocytes produce have been examined in the early post-transplant period in tolerant and rejecting liver allografts, they have been found to be essentially the same, with some changes in the B cell compartment.[55,239] An early downregulation of IL-4 expression also has been reported, but the relationship between this and the tolerant state has not been clarified.[56]

Analyses of rejecting and nonrejecting kidney allografts in the early post-transplant period have failed to identify one key parameter that distinguishes rejecting from nonrejecting grafts.[45,285] The ability of cells infiltrating the accepted allografts to respond to IL-2 was compromised owing to lack of expression of the high-affinity IL-2 receptor.[46] We also have shown more recently that leukocytes infiltrate accepted allografts with accelerated kinetics, and a proportion express high levels of FoxP3 (Carvalho-Gaspar M, Wood KJ, unpublished data, 2005). Evidence suggests that the blood T cells of drug-free tolerant renal transplant recipients show an altered repertoire of TCR Vβ usage and cytokine profile suggestive of hyporesponsiveness. The specific cytokine transcript profile lacked the key molecules of rejection, including IFN-γ.[26] The search for new genes that may hold the key to tolerance expressed in tolerant T cells continues.

It has been proposed that the ability of a liver allograft to protect itself from acute rejection and in the long term reverse the rejection response to itself and to a second organ graft may be due to the large antigen load delivered by the liver itself.[240] This hypothesis is supported, in some sense, by the finding that simultaneous transplantation of multiple hearts or kidneys into the same host also can promote acceptance of all of the grafts in the absence of immunosuppression, whereas in the same situation transplantation of a single graft would result in rapid rejection. Transplantation of multiple heart or kidney grafts does not by itself induce transplantation tolerance even though graft survival is prolonged. If donor leukocytes also are infused, however, tolerance is induced. This observation may offer more clues as to why the liver is particularly potent in inducing unresponsiveness on its own.[240]

The liver contains numerous passenger leukocytes.[286] It has been suggested that these leukocytes hold the key to the liver effect.[233,286] The migration of these cells from the graft in the early post-transplant phase might contribute to the inactivation of donor reactive cells and provide a long-term source of donor antigen in the recipient—microchimerism. It has been shown that elimination of these cells before transplantation prevents tolerance induction and, as mentioned earlier, tolerance can be restored by infusing extra donor leukocytes.[231,240]

It has been postulated that the persistence of donor-derived passenger cells from the liver allograft is key to the development of the unresponsive state in the long term. Data suggest, however, that the presence of the donor leukocytes is required only in the short term after transplantation, and that thereafter the passenger leukocytes play no significant role.[125,129] These data imply that in the long term, other mechanisms are responsible for maintaining the survival and integrity of the liver graft. Other mechanisms that have been proposed to explain the spontaneous acceptance of

liver grafts include the production of large quantities of soluble donor class I molecules that may block the functional activity or induce apoptosis of $CD8^+$ T cells and setting up regulatory populations of T cells that can control the downstream response made by the host against the graft[202,295] and the production of immunoregulatory molecules such as IL-10 by the liver after transplantation.[140]

CURRENT STRATEGIES USED TO INDUCE IMMUNOLOGICAL TOLERANCE TO AN ALLOGRAFT

The strategies for tolerance induction being explored most actively at present invoke one or more of the mechanisms of tolerance described previously. These mechanisms include the continuous deletion of donor reactive leukocytes by establishing the presence of high levels of donor cells in the recipient (mixed chimerism); short-term depletion or deletion, or both, of donor reactive leukocytes combined with the establishment of immunoregulation and suppression of responses to donor alloantigens in the longer term after transplantation; and costimulation blockade leading to the induction of T cell unresponsiveness in the presence of an organ graft.

Most of the approaches being explored, with the exception of mixed chimerism, do not aim to induce tolerance to donor antigens before transplantation. Instead, they attempt to use novel strategies that are nonspecific in their mode of action at the time of transplantation to create an environment that promotes the development of operational tolerance to the graft in the long term. Although in an ideal world it would be preferable to switch off the response to donor antigens before the graft is transplanted, in the short term this may be unrealistic with the tools currently available. The development of tolerance to the graft in the long term would have major benefits for patients because it would enable the total amount of immunosuppressive drug therapy to be reduced over the transplant course, and it might enable drug therapy to be eliminated from the treatment regimen at some point. Many of the approaches being developed rely on the use of biological molecules, monoclonal antibodies, or soluble recombinant ligands in the form of fusion proteins, alone or in combination with donor antigen to enable targeting of specific components of the immune system.

Mixed Chimerism

Stable mixtures of donor and recipient cell types that may coexist within a species and confer an alloantigen-specific tolerant state is an idea that initially was restricted to bone marrow transplantation. The limited presence of donor leukocytes found in long-term surviving organ transplant recipients changed the preexisting dogma that successful organ engraftment operated on a different mechanism than that of bone marrow transplantation.[233,234] Although few donor-specific immunological cell types are detected in the phenomenon of microchimerism, lymphoablative therapy is not a requirement, and hematopoietic stem cell engraftment does not occur.[166] Further studies attempting to correlate microchimeras to states of tolerance showed no relationship between the presence or absence of microchimerism and allograft rejection.[88]

The induction of macrochimerism with the use of cytoreductive techniques generates hematopoietic stem cell engraftment and is often associated with transplantation tolerance.[166] To attain a macrochimeric state, donor reactive leukocytes must be deleted from the recipient's immunological system. Deletion of donor reactive cells is an effective way of eliminating recipient-derived donor reactivity if deletion can be maintained throughout the post-transplant course.

It has been shown elegantly that the development of macrochimerism as a result of bone marrow infusion under the appropriate conditions can be used to achieve this goal.[242] A few bone marrow transplant recipients who subsequently required a renal transplant were transplanted with a kidney from their bone marrow donor.[95,219,229] In these cases, long-term immunosuppression was unnecessary because the recipient already was unresponsive to the donor alloantigens as a result of the allogeneic chimerism that developed after the successful bone marrow transplant. Bone marrow transplantation is an inappropriate approach to consider for most recipients on transplant waiting lists. Fully allogeneic chimerism has the drawback of reducing the immunocompetence of the recipient's immune system in some situations. Nevertheless, these cases provided a foundation for in vivo tolerogenic studies wherein donor bone marrow cells are introduced into recipients under conditions allowing for the development and maintenance of macrochimerism and long-term allograft survival.[162,284]

Many different approaches have been used to achieve macrochimerism. Total lymphoid irradiation alone or in combination with bone marrow infusion has been shown to be effective at inducing tolerance in some recipients in rodents, primates, and humans.[171,228,237] The requirement for irradiation in these systems has inhibited their development and clinical application to the fullest extent, however.

Because of the limitations of myeloablative therapy, alternative approaches in the mouse model were developed and refined, wherein high-dose bone marrow infusions combined with nonmyeloablative conditioning regimens promote deletion of donor reactive cells in the thymus.[157,242,255,275] Use of costimulation blockade has been shown to eliminate the need for cytoreduction and provide experimental long-term graft survival across multiple organ systems.[77,273,274] In a large animal model, T cell depletion also has been shown to be effective in producing stable mixed chimerism.[94]

Transient macrochimerism, via nonmyeloablative conditioning, has been used as a tool to achieve tolerance to renal allografts that are concomitantly transplanted with donor bone marrow in nonhuman primate models.[108,109,117,165] These chimeric protocols translated successfully to the clinic when Spitzer and coworkers[230] reported mixed chimerism used to treat a patient with multiple myeloma who required a renal transplant. In the animal and human experiences, macrochimerism disappeared after several months, but tolerance persisted. In primates in which fully mismatched allografts were transplanted, it has proved difficult to eliminate some of the more toxic elements of the pretransplant protocol.[117]

The pretransplant workup includes thymic irradiation, whole-body irradiation, splenectomy, and donor marrow infusion and then relies on the administration of a short course of cyclosporine after transplantation. To reduce or eliminate the toxicity of the protocol, alternative approaches for achieving reliable, stable mixed chimerism in large

animals are required. The finding that T cell depletion or costimulation blockade is effective in small animals is encouraging, and both of these strategies require careful evaluation in large animal models. Although in the short term that state of chimerism established by bone marrow infusion is important in inducing tolerance to the graft, in large animals it may not be the only mechanism that operates in the long term after transplantation, when immunoregulation may become an important contributor to the unresponsive state that persists. The maintenance of the macrochimeric state up to the time of transplantation may be sufficient to enable the graft to be transplanted without long-term immunosuppressive drug therapy.

Costimulation Blockade

As discussed previously, the activation of a T cell depends on multiple signals. The interaction of a TCR with an MHC/peptide complex triggers signal 1. Cell surface costimulatory molecules activate signal 2, which proceeds to induce naive T cell activation.[24,130] When signal 1 is forced to act on its own, T cells have been shown to undergo anergy or apoptosis.[221] Monoclonal antibodies and recombinant fusion proteins targeting costimulatory molecules are capable of inducing tolerance to donor antigens in vivo. The utility of these costimulation pathways as targets for pharmacotherapeutics in the induction of transplantation tolerance has proved to be a new and exciting aspect of transplant immunology in recent years. Members of the immunoglobulin and TNF/TNF receptor superfamilies have been elucidated and found to make up many of the costimulatory molecules that are integral to positive costimulation in the pathway of T cell activation. Two pairs of ligand-receptor interactions that seem to play key roles in positive costimulation are CD40/CD40 ligand (CD154), which are members of the TNF/TNF receptor superfamily, and CD80/CD86 and CD28, which belong to the immunoglobulin superfamily.[138]

Although the precise mechanisms of these costimulatory pathways have yet to be deciphered, the complete abrogation or attenuation of these pathways has been a target of extensive research in the laboratory. Development of CD28 blockade by CTLA4 immunoglobulin (CTLA4Ig) (abatacept) for rheumatoid arthritis and clinical trials with modified CTLA4Ig (belatacept) for transplantation shows promise for the use of costimulation blockade in suppressing effector responses.[23]

CD40/CD154 Pathway

The CD40/CD154 pathway has been targeted using monoclonal antibody therapy to inhibit graft rejection.[134,135] CD154, or CD40 ligand, is a type 2 membrane protein of the TNF family and is expressed predominantly by activated $CD4^+$ T cells and by a small proportion of $CD8^+$ T cells, natural killer cells, and eosinophils,[36] and more recently CD154 has been found on platelets.[6,138] Structural models predict that CD154 forms a homotrimer that binds to CD40 on the surface of APCs. CD40 also may be found on B cells, macrophages, DCs, and thymic epithelium and is inducible on the surface of endothelial cells and fibroblasts.[136]

The CD40/CD154 pathway interaction is pivotal for the induction of humoral and cellular responses. The importance of CD154 for B cell activation was first shown by in vitro studies. A CD40-immunoglobulin fusion protein and a blocking monoclonal antibody to CD154 were shown to inhibit B cell cycling, proliferation, and differentiation into plasma cells in response to T cell–dependent antigens.[179] In vivo studies using the anti-CD154 monoclonal antibody, CD40 knockout mice, or CD154 knockout mice[106,292] all showed a crucial role for this interaction in the generation of primary and secondary humoral responses to T cell–dependent antigens, class switching to antigen switching RGG1 responses, and development of germinal centers. The lack of humoral response in the absence of CD40/CD40 ligand interaction is not only due to a lack of signaling through CD40 on the B cell surface but also to the inhibition of priming of $CD4^+$ T cells through CD40 ligand.[76]

The CD40/CD154 pathway is bidirectional. CD154 engagement on T cells augments not only T cell activation but also CD40 triggering on the APC that primes the APC for stimulation. Signals through CD40 have been shown to upregulate expression of CD80 and CD86 and induce IL-12.[78] Activation of DCs through CD40 promotes their ability to present antigen to T cells; this may explain why targeting CD154 and blocking its ability to interact with CD40 has a profound effect on T cell–dependent immune responses in vivo. If modification of APC function is a route to tolerance, this pathway also may be involved when the behavior of APCs is modified after the interaction with immunoregulatory and suppressor T cells.[148]

USING THE CD40/CD154 PATHWAY FOR THERAPEUTICS

The idea of targeting the CD40/CD40 ligand to induce tolerance to transplanted allografts initially enjoyed much success in rodent and nonhuman primate models, but complications were encountered when the translation to the clinic was attempted. Long-term acceptance of cardiac, renal, and islet allografts in several murine and nonhuman primate models was achieved with CD40 blockade using anti-CD154 monoclonal antibody as monotherapy or in conjunction with anti-CD28.[134,135,158,176,191] So-called tolerant states generated by anti-CD154 therapy alone have been shown to disappear when therapy is withdrawn, however, leading to rejection. Even with CD28 blockade, anti-CD154 therapy must be sustained to promote permanent engraftment of cardiac or islet grafts.[82,191,226] Induced tolerant states in rodents tend to be more robust when anti-CD154 therapy has been combined with donor antigens before transplantation tolerance has been induced.[158,176,191] Although promising results were reported in experimental models, anti-CD154 therapy was found to have the unexpected complication of thrombogenesis.

CD154 was found to play key roles in coagulation. Some reports suggest that CD154 acts to stabilize thrombi, whereas others implicate CD154 in platelet activation.[6] Whatever the role that CD154 may play in transplantation tolerance, it is clear that this molecule acts via independent pathways in a variety of cascades unrelated to tolerance induction.[138]

Interest in this approach also was reflected in reports that a humanized monoclonal antibody specific for CD154 (hu5c8) was capable of prolonging the survival of renal and islet allografts in rhesus monkeys.[114,119,120] The initial data from these primate studies looked encouraging with rejection-free survival of the kidney grafts obtained provided that antibody therapy at a high dose (25 mg/kg) was continued in the first 6 months after transplantation. When anti-CD154

therapy was discontinued after the first month post-transplantation, rejection episodes did occur. Analysis of the status of recipients with long-term surviving grafts showed that peripheral lymphocytes from the monkeys do not respond in vitro to donor antigen. The recipients do develop antidonor antibody, however, and when biopsy samples were taken from some of the long-term surviving grafts, a T cell infiltrate was present.

Together, these observations were sufficiently encouraging to initiate a pilot clinical study using hu5c8 in renal transplantation. In this study, hu5c8 was administered to seven patients with low-dose steroid alone, and five patients went on to experience episodes of rejection.[121] Other variants of costimulatory blockade that target different epitopes of CD154 have been developed with hopes of improved tolerance induction and thrombogenic suppression. Experimental results in cardiac allografts of cynomolgus monkeys treated with an inhibitor of CD154, IDEC-131, either alone or in combination with leukocyte depletion in the form of antithymocyte globulin, prolonged allograft survival; however, tolerance was not induced because alloantibody production and transplant vasculopathy, although delayed, still occurred.[8] The use of triple therapy with an abbreviated course of mTOR inhibition (sirolimus), a donor-specific transfusion, and IDEC-131 prevented allograft rejection and induced operational tolerance in rhesus monkeys undergoing MHC-mismatched renal transplantation.[201] Still other antibodies, such as ABI793, have been developed, but they have been plagued with continued thromboembolic complications.[104]

Anti-CD154 monoclonal antibody therapy alone, although capable of promoting graft prolongation in some situations, may be unable to prevent transplant vasculopathy. This observation was first reported by Larsen and colleagues,[134,135] and analysis of data from our own studies have confirmed this observation.[53] When we investigated this observation in more detail in a vascular allograft model, we found that the blockade of CD154 alone does not have a significant effect on the development of transplant arteriosclerosis, a finding that is corroborated by primate models.[8] The disease state was inhibited only when $CD8^+$ T cells were removed from the recipient. Long-term follow-up of these recipients has revealed that the disease process in the absence of $CD8^+$ T cells is delayed, rather than inhibited completely. These observations have important implications for the use of monoclonal antibodies in the clinical setting.

Exploration of the efficacy of CD154 blockade in different donor recipient combinations in mouse models has revealed that only when rejection depends on $CD4^+$ T cells is CD154 blockade on its own effective. It is unclear why therapeutics centered around CD154 blockade were so successful in primate models, if the $CD8^+$ compartment remained intact and functional. In donor-recipient combinations in which $CD8^+$ T cells also play a role in rejection, many studies have shown that the $CD8^+$ T cell subset is unaffected by CD154 monoclonal antibody therapy.[53,91,99,256] In some cases, this situation can lead to the rejection of grafts despite CD154 blockade. $CD8^+$ T cells become activated, proliferate, and home to the graft in the presence of high-dose continued anti-CD154 monoclonal antibody therapy in vivo.[99] These data together with other data from transplant models and virus infection studies[277] raise questions as to the potential ability of anti-CD154 monotherapy to control rejection in every situation.

Further studies have been undertaken to evaluate antibodies to CD40 to bypass the potential ramifications of CD154 blockade. Initial animal knockout models reveal a propensity of CD154 knockout mice to develop unstable thrombi, a phenomenon not seen in CD40 knockout mice.[42] It seems a logical next step to assess the potential of antibodies targeted to CD40, circumventing CD154 mechanisms and consequences. Preliminary studies in the rhesus monkey renal allograft model reveal promising results with a short course of low-dose calcineurin therapy administered concomitantly with anti-CD40/anti-CD86 costimulation blockade. In this study, two of four animals developed 3-year drug-free graft survival, and none of the animals developed alloantibodies to the donor, suggestive of tolerance induction.[80] Continued studies are necessary to evaluate thoroughly the efficacy of CD40-targeted therapeutics.

B7:CD28/CTLA4 Pathway

CD80 (B7-1) and CD86 (B7-2) are expressed as cell surface molecules by APCs and are responsible for delivering additional signals to T cells when they interact with CD28.[74,222] CD86 seems to interact preferentially with CD28 and may be the most important ligand for T cell activation. CD86 and CD80 can interact with a second molecule, CD152 (CTLA4), which is expressed by T cells later in the activation process. CD80 may bind preferentially to CD152.[253] In contrast to CD28, CTLA4 negatively regulates T cell activation when it engages its ligand on the APC and, as described previously, is implicated in the control of clone size to maintain normal homeostasis in the immune system.[21,254] In contrast to CD28, CTLA4 is expressed only after T cells are activated and constitutively expressed on regulatory T cells.[232] The role of each of these pathways in alloimmune responses is being investigated with a battery of reagents, including monoclonal antibodies, fusion proteins, and knockout mice.[102]

USING THE B7:CD28/CTLA-4 PATHWAY FOR THERAPEUTICS

When CTLA4Ig, an immunoglobulin fusion protein of CTLA4, was produced, it was shown to inhibit graft rejection in xenogeneic and allogeneic systems.[135,142] In rodent models, CTLA4Ig therapy alone has been shown to induce tolerance to the graft,[142,194] an effect that was enhanced when donor antigen was included in the treatment protocol.[147,194,220] This effect has not been found in every experimental model examined, however. The use of CTLA4Ig monotherapy in primates has not been reported to be capable of inducing long-term graft survival.[119]

The mechanism by which CTLA4Ig promotes long-term graft survival has been investigated in a mouse model. Blockade of CD80 and CD86 at the time of alloantigen recognition triggers deletion of antigen-reactive cells in the early phase after transplantation.[145,276] When an antiapoptotic gene, *bcl-x*, was expressed in the responding lymphocytes, deletion did not occur, and graft prolongation was prevented. This finding suggests that an early reduction in clone size facilitates the development of long-term graft function promoted by treatment with CTLA4Ig by reducing the number of donor reactive cells that have to be controlled downstream in the post-transplantation course.

Although primate models using CTLA4Ig to induce tolerance proved to be largely unsuccessful, the theoretical foundation of blocking this pathway to promote graft survival continued to intrigue researchers. Additionally, it was known that the binding properties of CTLA4 could be manipulated to optimize the ligation of CD80 and CD86, a crucial component to experimental efforts of tolerance induction.[138]

Belatacept

Experiments using CTLA4Ig laid the groundwork for further pharmacotherapeutic developments targeted at the B7:CD28/CTLA4 pathway. The most promising of these developments is the introduction of belatacept. Belatacept, LEA29Y, originally was derived from the fusion protein CTLA4Ig, or abatacept.[23,137] It differs from CTLA4Ig by two amino acid sequences, which confers an approximately twofold greater ligation capacity to CD80 and CD86. This increase in avidity allows for a 10-fold increase in the in vitro suppression of T cell activation compared with CTLA4Ig.[137] Originally in nonhuman primate studies, belatacept was found to prolong renal allograft survival and inhibit donor-specific alloantibody production alone and in combination with other traditionally used immunosuppressive regimens.[137] These and other findings allowed for the translation of LEA29Y to renal transplant patients in the clinics.

To date, results of phase II trials comparing belatacept with cyclosporine in partially randomized studies of more than 200 patients across 22 centers in North America and Europe suggest that belatacept is not inferior to cyclosporine. Results of this trial revealed that patients with belatacept-based therapy had improved renal function, reduction in chronic allograft nephropathy, decreased calcineurin-related toxicity, and no thromboembolic complications secondary to the exclusion of the CD154 pathway.[266] Additionally, more recent experiments in nonhuman primates using neonatal porcine islet grafts have revealed long-term xenograft survival under the cover of CD28-CD154 blockade with maintenance immunosuppression of sirolimus and belatacept.[32] Although promising, further trials and vigilant follow-ups are necessary to assess accurately the efficacy of these new therapeutic regimens.

Targeting CD3 and Accessory Molecules

Initially, administration of depleting anti-CD4 and anti-CD8 monoclonal antibodies was shown to result in prolonged graft survival.[37,38,152,225] That this treatment strategy resulted in antigen-specific tolerance was shown first most clearly when a protein antigen was administered in conjunction with a depleting anti-CD4 monoclonal antibody.[15,16,79] Refinements of these types of protocols have resulted in the ability to achieve long-term T cell unresponsiveness to protein and alloantigens in the absence of T cell depletion in experimental models.[16,39,47,207] Many other accessory molecules, other than anti-CD4 and anti-CD8, have been targeted in an attempt to induce tolerance in models of bone marrow,[34,61] islet,[177,293] renal,[139,177] and cardiac allografts,[11,34,173] among others.[175]

OKT3, a murine anti–human CD3 monoclonal antibody, received approval for human use in 1986 in kidney transplant patients experiencing rejection and eventually liver and cardiac transplant recipients as well.[143] Although widely used, OKT3 brings with it the undesirable complications of the human antimouse antibody response and a first-dose reaction characterized by fevers, chills, and gastrointestinal, respiratory, and cardiac complications.[68,249] These ramifications are thought to be the result of T cell activation and subsequent cytokine release.[143] Many investigators have devoted time to the construction of pharmacotherapeutics that mimic the efficacy of OKT3 with less immunogenicity. A few of these OKT3-derived molecules in preliminary studies, such as hu12F6, hOKT3γ1(Ala-Ala), and ChAglyCD3, have proved to be more effective in T cell suppression and less immunogenic compared with OKT3.[86,115,143]

Along with anti-CD3, antibodies to CD11a (LFA-1) and its ligands, ICAM-1, ICAM-2, and ICAM-3, have been investigated and have suggested prolonged graft survival in many of the aforementioned models. Although anti–LFA-1 therapy either alone or in combination with anti-ICAM therapy has been suggested for long-term allograft survival, the mechanism of action of these monoclonal antibodies is still quite contentious. LFA-1 has been implicated as an essential molecule for cellular trafficking and motility and T cell activation.[10,52] Reports also suggest that the interaction of LFA-1 and the ICAM molecules serves as a costimulatory pairing for T cell activation.[263]

Operational tolerance induced by these strategies has been shown to develop over several weeks after the initial antigen encounter.[193,223] When a combination of donor antigen and monoclonal antibody therapy targeting accessory molecules is used, the precise mechanism of tolerance induction depends partly on the amount of antigen infused.[192] With high doses of donor bone marrow, deletion also may be used as one of the mechanisms of tolerance initially.[14,28] With lower doses of antigen, immunoregulation is the mechanism in operation. When antibodies targeting accessory molecules are used as therapeutic agents at the time of transplantation, immunoregulation is the dominant mechanism that comes into play to maintain tolerance in the longer term.[269]

In these systems, tolerance to donor antigens is either induced or maintained, or both, as a result of the development of a population of regulatory and suppressor T cells that can mediate unresponsiveness to the initiating donor antigen and other antigens present on the graft—the phenomenon of linked unresponsiveness.[282] In mice and rats, this type of tolerance has been shown to be infectious[208]; it can be transferred from one generation of cells to another provided that there is a sufficient period of contact between the two populations.

The maintenance of tolerance in these systems requires the persistent presence of antigen in the form of the organ when the thymus is still functional.[81] In the absence of donor antigen, tolerance is eventually lost, presumably as a result of the export of naive cells T cells from the thymus into the periphery. Quantitatively, if these cells fail to encounter antigen, they eventually outnumber the unresponsive T cells induced by the monoclonal antibody therapy.

LEUKOCYTE DEPLETION AT THE TIME OF TRANSPLANTATION

Many tolerance induction strategies that have been investigated in small and large animal studies result in the depletion of leukocytes (antithymocyte globulin, anti-CD52) or T cells (anti-CD3 with or without immunotoxin, -CD2, -CD4,

and -CD8).[268] In small animals, the short-term depletion of T cells seems to be sufficient in some situations for tolerance to develop and be maintained in the long term. The success rate can be enhanced by removing the thymus before transplantation to prevent repopulation of the periphery with T cells after transplantation.[164] Initial data from primates using anti-CD3 immunotoxin conjugated alone before transplantation or in combination with deoxyspergualin, a drug that inhibits nuclear factor κB (NFκB) and monocytes and macrophages, at the time of transplantation suggested that T cell depletion can be used to induce tolerance to donor alloantigens.[58,124,252]

Follow-up trials in humans undergoing renal transplantation and T cell depletion with the anti-CD52 monoclonal antibody alemtuzumab with or without deoxyspergualin revealed that profound T cell depletion either alone or in combination with deoxyspergualin failed to induce tolerance in humans.[122,123] Clinical results across organ systems reveal, however, that steroid-free regimens with reduced maintenance doses of immunosuppression may be used after alemtuzumab therapy.[259,265,271]

Depletion of leukocytes at the time of transplantation creates a transient immunodeficiency in the recipient, compromising the recipient's ability to reject the transplant. The degree and duration of leukocyte depletion achieved determine how effective and for how long the graft is protected from immune attack. The downstream events that occur when leukocytes begin to reappear in the recipient's circulation are not clearly understood. Using TCR transgenic recipients, we have shown that when leukocytes are depleted, the maintenance of tolerance depends on transplantation of the graft within a window of depletion of donor reactive cells in the thymus and periphery.[98] If the organ graft is transplanted at the appropriate time, donor reactive cells fail to repopulate from the thymus in an antigen-selective manner. Although donor reactive cells do not reappear in the periphery, cells with reactivity to other antigens are present in the periphery of recipients with long-term surviving organ grafts.

These data can be used to suggest a mechanism for the long-term survival observed in nonhuman primates treated with anti-CD3 immunotoxin complex. In this case, one can argue that the $CD3^+$ T cells are depleted by the immunotoxin before transplantation. A window of opportunity is created such that when a renal allograft is transplanted, no donor reactive cells are present in the periphery. As cells repopulate the periphery with time after the transplantation, donor reactive cells are deleted or eliminated as a result of the presence of the surviving graft. We have shown in clinically based trials that the number of residual donor reactive cells can be controlled using reduced immunosuppression after leukocyte depletion using alemtuzumab (Campath-1H).[257] Although clinical trials using anti-CD52 and antithymocyte globulin as T cell depletion strategies have yet to show proper immunological tolerance, they have allowed for further larger scale trials, and have fueled ideas to combine this strategy with administration of donor antigen in an attempt to achieve some level of mixed chimerism.[38,110,241,250,251,289]

EFFECT OF IMMUNOSUPPRESSION ON TOLERANCE INDUCTION

The introduction of any novel strategy for tolerance induction into clinical practice at present necessitates combining the approach with one or more immunosuppressive drugs at the time of transplantation. How successful this approach would be is unclear. Data from many experimental studies in which a biological agent has been combined with one of the calcineurin inhibitors given simultaneously at the time of transplantation suggest that calcineurin inhibitors might block or inhibit the development of unresponsiveness.[120,135] Additionally, more recent evidence suggests that regulatory T cells cultured with sirolimus have a much stronger suppressive capability compared with regulatory cells in the presence of cyclosporine.[40]

The inhibition of unresponsiveness could be linked to the inhibition of IL-2 gene transcription in the presence of calcineurin inhibitors. Studies in IL-2 knockout mice have shown that operational tolerance to alloantigens is not induced in this setting.[44] Apoptosis triggered by costimulation blockade is blocked in the presence of calcineurin inhibitors.[276,291] Calcineurin inhibitors have been shown to inhibit the suppressive function of $CD4^+CD25^+$ regulatory cells, leading to an increase in severity of graft-versus-host disease and diminished survival in experimental animals.[296] If deletion of alloantigen reactive cells is an essential part of the mechanism that operates during the induction of unresponsiveness as a consequence of costimulation blockade at the time of antigen recognition, it may not be surprising that inclusion of calcineurin inhibitors in the treatment protocol blocks graft prolongation.

Examination of data from clinical studies in which biological agents have been used as part of the therapeutic strategy supports the experimental findings outlined previously. When OKT3 was given simultaneously with cyclosporine at the time of transplantation, the long-term graft survival rate was poorer than when cyclosporine was introduced in a delayed fashion. The protocol adopted for the Campath 1H study has been designed to take these observations into account.[29]

Whether all immunosuppressive drugs have a similar effect on the development of unresponsiveness when used in combination with biological agents requires further careful evaluation. Preliminary data from primate studies using anti-CD154 suggest that there are differential effects.[120] Further work is essential to enable an acceptable treatment regimen for use in combination with novel agents to be identified if the translation to the clinic of strategies designed to promote the development of tolerance is going to be successful.

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Chapter 24

Pathology of Kidney Transplantation

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RENAL ALLOGRAFT BIOPSY

Renal biopsy remains the "gold standard" for the diagnosis of episodes of graft dysfunction that occur commonly in patients after transplantation. The results of a renal allograft biopsy changed the clinical diagnosis in 30% to 42% of patients and therapy in 38% to 83%, even after the first year.[163,165,267] Most importantly, unnecessary immunosuppression was avoided in 19% of patients.[267] The biopsy also is a gold mine of information on pathogenetic mechanisms—a generator of hypotheses that can be tested in experimental animal studies and in clinical trials. Finally, the biopsy serves to validate the hypothesis tested in such trials. Interpretation of the renal biopsy specimen currently relies primarily on histopathology complemented by immunological molecular probes and, perhaps in the future, quantitative gene expression.

This chapter describes the relevant light, immunofluorescence, and electron microscopy findings of the most common lesions that affect the renal allograft and their differential diagnoses; references cited are largely limited to human pathological studies after 1990. The discussion is broadly divided into allograft rejection and nonrejection pathology, with an emphasis on differential diagnosis of acute and chronic allograft dysfunction. Grading systems of acute and chronic rejection are discussed further in the appropriate sections. Additional references and details are available in a comprehensive review.[57]

Optimal Tissue

At least seven nonsclerotic glomeruli and two arteries (bigger than arterioles) must be present in a renal allograft biopsy specimen for adequate evaluation.[53,335] Using these criteria, the sensitivity of a single core is approximately 90%, and the predicted sensitivity of two cores is about 99%.[53] Adequacy depends entirely on the lesions seen in the biopsy specimen, however. One artery with endarteritis is sufficient for the diagnosis of acute cellular rejection, even if no glomerulus is present; similarly, immunofluorescence or electron microscopy of one glomerulus is adequate to diagnose membranous glomerulonephritis. In contrast, a large portion of cortex with a minimal infiltrate does not exclude rejection. Subcapsular cortex often shows inflammation and fibrosis and is not representative. Diagnosis of certain diseases is possible with only medulla (e.g., acute humoral rejection, polyomavirus nephritis). A normal medulla does not rule out rejection, however.[370] Frozen sections for light microscopy are of limited value because freeze artifacts preclude accurate evaluation. The diagnostic accuracy of frozen sections was 89% compared with paraffin sections.[44] Rapid (2-hour) formalin/paraffin processing is used at Massachusetts General Hospital for urgent and weekend biopsies.

Microscopy

The biopsy sample is examined for glomerular, tubular, vascular, and interstitial pathology, including (1) transplant glomerulitis, glomerulopathy, and de novo or recurrent glomerulonephritis; (2) tubular injury, isometric vacuolization,

tubulitis, atrophy, and intranuclear viral inclusions; (3) endarteritis, fibrinoid necrosis, thrombi, myocyte necrosis, nodular medial hyalinosis, and chronic allograft arteriopathy; and (4) interstitial infiltrates of activated mononuclear cells, edema, neutrophils, fibrosis, and scarring. The diagnostic lesion often is located in arteries and arterioles, so they are particularly scrutinized.

Our standard immunofluorescence panel detects IgG, IgA, IgM, C3, C4d, albumin, and fibrin in cryostat sections. C4d, a complement fragment, is used to identify antibody-mediated rejection; the other stains are primarily for recurrent or de novo glomerulonephritis.[47] Immunohistochemistry in paraffin sections is indicated in the differential diagnosis of lymphoproliferative or viral diseases and may be used for C4d. Electron microscopy is valuable when de novo or recurrent glomerular disease is suspected and to evaluate peritubular capillary (PTC) basement membranes.[146]

Classification of Pathological Diagnoses in the Renal Allograft

The ideal diagnostic classification of renal allograft pathology should be based on pathogenesis, have therapeutic relevance, and be reproducible. The current classification based on Banff and other systems (Table 24-1) meets these criteria.[57]

DONOR KIDNEY BIOPSY

Biopsy of a cadaver donor kidney is sometimes used to determine the suitability of the kidney for transplantation. Objective pathological criteria based on outcome that could be applied to the renal biopsy specimen as a screening test have not been established because donor biopsies are not routinely performed, and controlled trials have not been done. A major problem in assessing the donor kidney is that this is usually carried out with cryostat sections, often by local pathologists in the middle of the night. Arbitrary criteria risk that kidneys would be discarded needlessly. In two large studies, the outcome at 1 to 5 years was not measurably correlated with pathological lesions.[38,257] As rejection and patient death from complications diminish as a cause of graft loss, the influence of the quality of the graft is likely to increase.

Glomerulosclerosis is one feature that is readily assessed in frozen section by the most casual observation. Glomerulosclerosis greater than 20% correlates with poor graft outcome in several studies.[86,104,283] Donor serum creatinine did not distinguish the different degrees of glomerulosclerosis found on biopsy specimens. The odds ratio remained significant after adjustment for donor age, rejection episodes, or panel-reactive antibody.[283] Five-year graft survival was strikingly diminished in recipients of grafts with greater than 20% glomerulosclerosis compared with grafts with 0% sclerosis (35% versus 80%).[86] Other large studies have failed to detect a major effect of glomerulosclerosis greater than 20%, however, if adjusted for the age of the donor[274] or renal function.[81]

At least 25 glomeruli are needed to correlate with outcome.[371] A wedge biopsy sample may not be representative because it includes mostly outer cortex, the zone where glomerulosclerosis and fibrosis secondary to vascular disease is most severe; a needle biopsy is recommended. Even though many other studies try to correlate fibrosis or vascular disease, reproducibility of scoring these lesions, even on permanent sections in broad daylight, is notoriously poor.[100] At this time, histological evaluation is recommended in donors with any evidence of renal dysfunction, with a family history of renal disease, or whose age is greater than 60 years. Histological selection of kidneys from donors older than 60 years can result in a graft survival rate similar to that of grafts from younger patients.[287]

Other lesions may cause the transplant surgeon or pathologist to argue against use of the graft. Arterial intimal

Table 24–1 Pathological Classification of Renal Allograft Disease

I. Immunological rejection
- A. Hyperacute rejection
- B. Acute rejection
  1. Acute T cell–mediated rejection (acute cellular rejection, $C4d^-$)
     - a. Tubulointerstitial (Banff type I)
     - b. Endarteritis (Banff type II)
     - c. Arterial fibrinoid necrosis/transmural inflammation (Banff type III)
     - d. Glomerular (transplant glomerulitis; no Banff type)
  2. Acute antibody-mediated rejection (acute humoral rejection, $C4d^+$)
     - a. Tubular injury
     - b. Capillaritis/thrombotic microangiopathy
     - c. Arterial fibrinoid necrosis
- C. Chronic rejection
  1. Chronic T cell–mediated rejection (with T cell activity)
  2. Chronic antibody-mediated rejection (with antibody activity, $C4d^+$)

II. Alloantibody/autoantibody–mediated diseases of allografts
- A. Anti-GBM disease in Alport's syndrome
- B. Nephrotic syndrome in nephrin-deficient recipients
- C. Anti-TBM disease in TBM antigen–deficient recipients
- D. De novo membranous glomerulonephritis
- E. Anti–angiotensin II receptor autoantibody syndrome

III. Nonrejection injury
- A. Acute ischemic injury (acute tubular necrosis)
- B. Drug toxicity
  1. Calcineurin inhibitor (cyclosporine, tacrolimus)
  2. mTOR inhibitors (sirolimus, everolimus, rapamycin)
- C. Acute tubulointerstitial nephritis (drug allergy)
- D. Infection (viral, bacterial, fungal)
- E. Major artery/vein thrombosis
- F. Mechanical
  1. Obstruction
  2. Urine leak
- G. Renal artery stenosis
- H. Arteriosclerosis
- I. De novo glomerular disease
- J. Post-transplant lymphoproliferative disease
- K. Chronic allograft nephropathy, not otherwise classified (interstitial fibrosis and tubular atrophy)

IV. Recurrent primary disease
- A. Immunological (e.g., IgA nephropathy, lupus nephritis, anti-GBM disease)
- B. Metabolic (e.g., amyloidosis, diabetes, oxalosis)
- C. Unknown (e.g., dense deposit disease, focal segmental glomerulosclerosis)

mTOR, mammalian Target of Rapamycin; GBM, glomerular basement membrane; TBM, tubular basement membrane.

From Colvin RB, Nickeleit V: Renal transplant pathology. In Jennette JC, Olson JL, Schwartz MM, et al (eds): Heptinstall's Pathology of the Kidney. Philadelphia, Lippincott-Raven, 2006, p.1347.

fibrosis increases the risk of delayed graft function[154] and has a slight effect on 2-year graft survival (6% decrease).[350] Thrombotic microangiopathy with widespread, but less than 50%, glomerular thrombi increases the likelihood of delayed graft function and primary nonfunction,[274] but is compatible with unaltered 2-year graft survival.[198] Reversal of diabetic glomerulosclerosis,[1] IgA nephropathy,[151] membranous glomerulonephritis,[233] lupus nephritis,[180] membranoproliferative glomerulonephritis,[34] and endotheliosis secondary to preeclampsia (personal observation) have been reported.

HYPERACUTE REJECTION

Hyperacute rejection refers to immediate rejection (typically within 10 minutes to 1 hour) of the kidney on perfusion with recipient blood, where the recipient is presensitized to alloantigens on the surface of the graft endothelium. During surgery, the graft kidney becomes soft and flabby and livid, mottled, purple, or cyanotic; urine output ceases. The kidney subsequently swells, and widespread hemorrhagic cortical necrosis and medullary congestion appears. The large vessels are sometimes thrombosed.

Early lesions show marked accumulation of platelets in glomerular capillary lumens that appear as amorphous, pale pink, finely granular masses in hematoxylin and eosin–stained slides (negative on periodic acid–Schiff stains). Neutrophil and platelet margination occurs over the next hour or so along damaged endothelium of small arteries, arterioles, glomeruli, and PTCs, and the capillaries fill with sludged (compacted) red blood cells and fibrin.[376] The larger arteries usually are spared. The neutrophils do not infiltrate initially, but form "chain-like" figures in the PTCs without obvious thrombi.[376] The endothelium is stripped off the underlying basal lamina, and the interstitium becomes edematous and hemorrhagic. Intravascular coagulation occurs and cortical necrosis ensues over 12 to 24 hours. The medulla is relatively spared but is ultimately affected as the whole kidney becomes necrotic.[164]

Widespread microthrombi usually are found in the arterioles and glomeruli and can be detected even in totally necrotic samples. The small arteries may show fibrinoid necrosis. Mononuclear infiltrates are typically sparse. One case showed $CD3^+$ cells in the adventitia of small arteries and in the surrounding interstitium.[103] By electron microscopy, neutrophils attach to injured glomerular endothelial cells.[376] The endothelium is swollen and separated from the glomerular basement membrane (GBM) by a lucent space. Capillary loops and PTCs are often bare of endothelium. Platelets, fibrin thrombi, and trapped erythrocytes occlude capillaries.[57]

The site of antibody and complement deposition is determined by the site of the target endothelial alloantigens. Hyperacute rejection as a result of preexisting anti–HLA class I antibodies may show C3, C4d, and fibrin throughout the microvasculature.[122] ABO antibodies (primarily IgM) also deposit in all vascular endothelium. Cases with anti–class II antibodies may have IgG/IgM primarily in glomeruli and PTCs, where class II is normally conspicuous.[3] In anti–endothelial-monocyte antigen cases, IgG is primarily in PTCs rather than glomeruli or arteries.[269] Often, antibodies cannot be detected in the vessels,[325] even though they can be eluted from the kidney.[185,214] In these cases, C4d should be positive in PTCs[47] and more useful than immunoglobulin stains. In occasional cases, intraoperative biopsy specimens may be negative for C4d (Cohen AH: personal communication), perhaps related to focally decreased perfusion or insufficient time to generate substantial amounts of C4d.[57]

The differential diagnosis of hyperacute rejection includes ischemia and major vascular thrombosis.[57] The major diagnostic feature of hyperacute rejection is the deposition of C4d in PTCs and the prominence of neutrophils in capillaries. Although the finding of antibody and C4d deposition in PTCs is diagnostic when present, negative immunofluorescence stains do not exclude hyperacute rejection. Exogenous antibody (rabbit or horse antilymphocyte serum) can cause severe endothelial injury, sometimes with C4d deposition mimicking hyperacute rejection.[48] Hyperacute rejection typically has more hemorrhage, necrosis, and neutrophil accumulation in glomeruli and PTCs than acute tubular necrosis, although glomerular neutrophils alone are associated with ischemia.[102] Major arterial thrombosis has predominant necrosis with little hemorrhage or microthrombi, and PTC neutrophils are not that prominent. Renal vein thrombosis shows marked congestion and relatively little neutrophil response.

ACUTE RENAL ALLOGRAFT REJECTION

Acute rejection typically develops in the first 2 to 6 weeks after transplantation, but it can arise in a normally functioning kidney 3 days to 10 years or more later or in a graft affected by other conditions, such as acute tubular necrosis, calcineurin inhibitor toxicity, or chronic rejection. Acute rejection may be cell mediated or humoral, or both (see Table 24-1). Acute cellular rejection is mediated primarily by T cells reacting to donor histocompatibility antigens in the kidney and is much more common than acute humoral rejection, due to donor-specific antibodies, although the latter is now recognized with greater frequency and has a worse prognosis. The distinction between the two has been made clearly in the literature only since 1999.

Acute Cellular Rejection

T cells react to donor histocompatibility antigens expressed in the tubules, interstitium, vessels, and glomeruli, separately or in combination (Table 24-2). The approximate frequencies of histological involvement are 45% to 70% tubulointerstitial, 30% to 55% vascular, and 2% to 4% glomerular, with considerable center variation.[57] The donor ureter also is affected but is rarely sampled.[101]

Tubulointerstitial Rejection (Type I)

The prominent microscopic feature of acute cellular rejection is a pleiomorphic interstitial infiltrate of mononuclear cells, accompanied by interstitial edema and sometimes hemorrhage (Fig. 24-1). The infiltrate is typically patchy in the cortex and the medulla. The infiltrating cells are primarily T cells and macrophages. Activated T cells (lymphoblasts) with increased basophilic cytoplasm, nucleoli, and occasional mitotic figures indicate increased synthetic and proliferative activity.[162] Granulocytes are commonly present but rarely prominent. When neutrophils are conspicuous, the possibility of antibody-mediated rejection or pyelonephritis should be considered.

Table 24–2 Banff/Types of Acute T Cell–Mediated Rejection*

Suspicious/ borderline	Any tubulitis + infiltrate of 10-25%, *or* Any infiltrate of ≥10% + tubulitis of 1-4 cells/tubule
Type I	Tubulitis >4 cells/tubule + infiltrate >25%
A	With 5-10 cells/tubule (t2)
B	With >10 cells/tubule (t3)
Type II	Mononuclear cells under arterial endothelium
A	<25% luminal area
B	≥25% luminal area
Type III	Transmural arterial inflammation, *or* fibrinoid arterial necrosis with accompanying lymphocytic inflammation†

*All cases should be analyzed for C4d deposition. If C4d is present, an additional diagnosis of concurrent antibody-mediated rejection is made.

†Cases with these features are often due to alloantibody. To use as a category of T cell–mediated rejection requires C4d in peritubular capillaries to be negative.

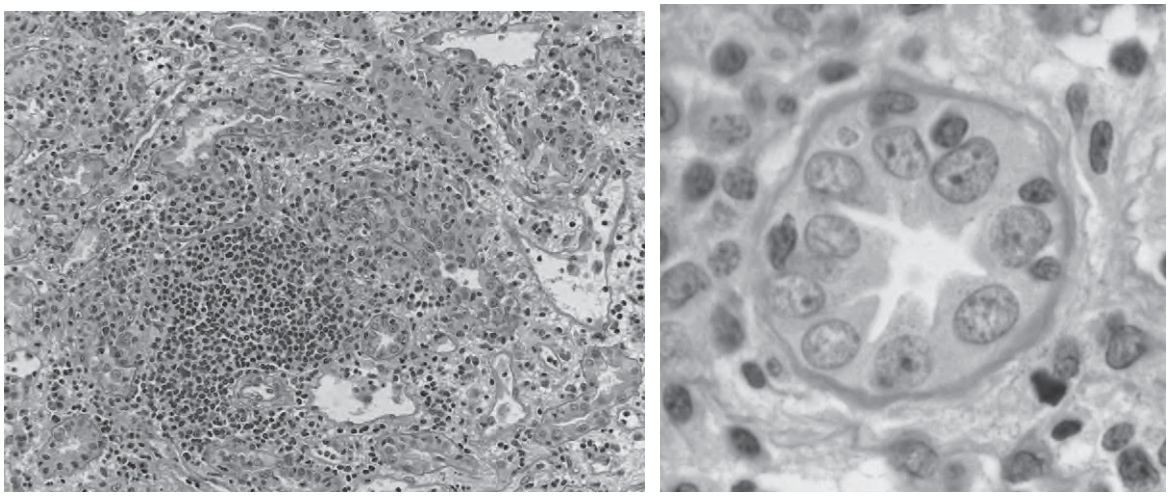
From Colvin RB, Nickeleit V: Renal transplant pathology. In Jennette JC, Olson JL, Schwartz MM, et al (eds): Heptinstall's Pathology of the Kidney. Philadelphia, Lippincott-Raven, 2006, p 1347.

Eosinophils are present in about 30% of biopsy specimens with rejection and can be abundant but are rarely more than 2% to 3% of the infiltrate.[7,248] Abundant eosinophils (10% of infiltrate) are associated with endarteritis (Banff type II).[207] Mast cells increase, as judged by tryptase content, and correlate with edema.[68] Acute rejection with abundant plasma cells has been described in the first month after transplantation and is associated with poor graft survival.[4,41,203] Some cases of acute rejection have $CD20^+$ B cells, a finding sometimes correlated with poorer prognosis.[309] Infiltrating T cells express cytotoxic molecules, including perforin,[158,266] Fas ligand,[5,266] granzyme A and B,[170,210,266,297] TIA-1/GMP-17,[202,210] and tumor necrosis factor (TNF)-β (lymphotoxin).[254]

Mononuclear cells invade tubules and insinuate between tubular epithelial cells, a process termed tubulitis (see Fig. 24-1B), which is best appreciated in sections stained with periodic acid–Schiff reagent to delineate the tubular basement membrane (TBM). All cortical tubules (proximal and distal), the medullary tubules, and the collecting ducts may be affected. Disruption of the TBM and leakage of Tamm-Horsfall protein into the interstitium has been described in biopsy specimens, especially evident on periodic acid–Schiff stains,[43] sometimes forming a granuloma.[57] Tubular cell apoptosis occurs,[15,142,202,255] which correlates with the number of cytotoxic cells and macrophages in the infiltrate.[202,255] Tubular epithelial cells express HLA-DR, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1 in increased amounts in acute cellular rejection* and express the costimulatory molecules CD80 and CD86.[250] Tubules also synthesize TNF-α,[225] transforming growth factor-β1, interleukin (IL)-15, osteopontin, and vascular endothelial growth factor.[6,263,377] Increased expression of S1004A may signal the process of epithelial-to-mesenchymal transition.[292] Some tubular cell–derived molecules have the potential to inhibit acute rejection, such as protease inhibitor-9 (PI-9), the only known inhibitor of granzyme B,[297] and IL-15, which inhibits expression of perforin.[377]

$CD8^+$ and $CD4^+$ cells invade tubules.[359] Intratubular T cells with cytotoxic granules[202] and $CD4^+FOXP3^+$ cells[365] accumulate selectively in the tubules compared with the interstitial infiltrate. T cells proliferate when inside the

*References 18, 23, 30, 31, 88, 96-98, 243, 262, and 369.



A B

Figure 24–1 Acute cellular rejection, type I. **A**, Mononuclear cells, composed of activated lymphocytes and macrophages, infiltrate the edematous interstitium and invade tubules. Tubulitis affects proximal and other tubules, where mononuclear cells are interposed between the tubular epithelial cells. **B**, The invading mononuclear cells appear dark with scant cytoplasm, which distinguishes them from tubular epithelial cells. The tubular basement membranes are stained red by the periodic acid–Schiff stain, which is useful to delineate the boundary between the tubule and the interstitium. (See color plate.)

tubule, as judged by the marker Ki67 (MIB-1), which contributes to their concentration within tubules, in addition to selective invasion.[202,293] Increased tubular HLA-DR,[23,97] TNF-α,[225] interferon-γ receptor,[254] IL-2 receptor,[167] and IL-8 are detectable by immunoperoxidase study in acute cellular rejection. Several adhesion molecules are increased on tubular cells during rejection, including ICAM-1 (CD54) and VCAM-1, which correlate with the degree of T cell infiltration.[30]

Signs of tubular cell injury can be detected by TdT-uridine-nick end label (TUNEL) for apoptosis. Increased numbers of TUNEL-positive tubular cells are present in acute rejection compared with normal kidneys.[142,202] The frequency was significantly lower in cyclosporine toxicity or acute tubular necrosis.[202] The degree of apoptosis correlates with the cytotoxic cells in the infiltrate, consistent with a pathogenetic relationship.[202] Prominent apoptosis of the infiltrating T cells also has been detected at a frequency comparable to that in the normal thymus (1.8% of cells).[202] Other investigators have described occasional TUNEL-positive lymphocytes.[142] Apoptosis probably occurs in infiltrating T cells as a result of activation-induced cell death and would serve to limit the immune reaction.[202]

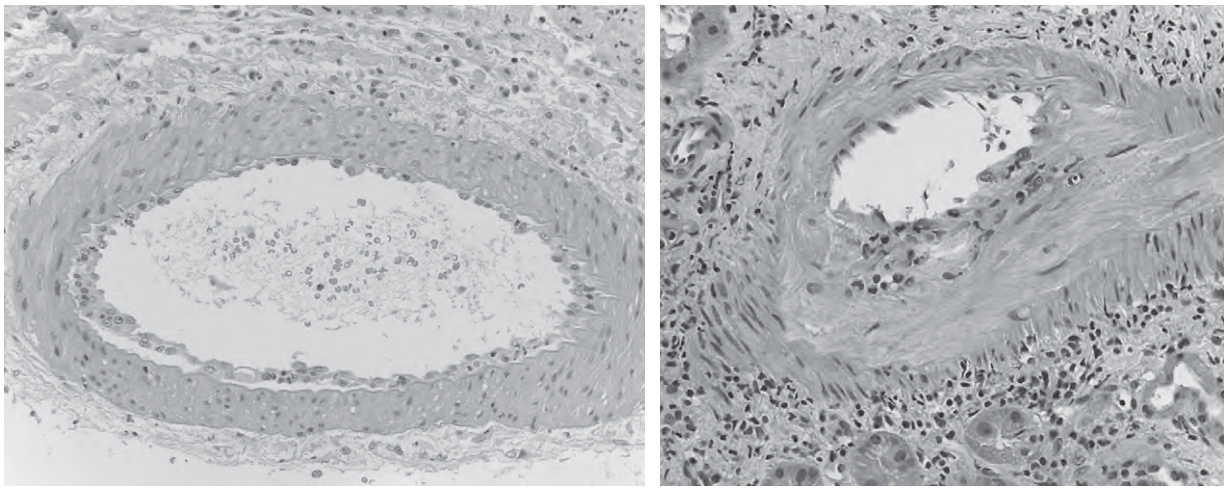
Little, if any, immunoglobulin deposition is found by immunofluorescence in acute cellular rejection, which is characterized primarily by accumulation of extravascular fibrin in the interstitium and commonly increased C3 and C5b-9 along the TBM.[85] The C3 is largely derived from tubular cells.[11] C3 may have a role in the pathogenesis of acute rejection because mouse kidneys deficient in C3 have prolonged survival.[278] C4d deposition in PTCs indicates an antibody-mediated component.

Electron microscopy is not required for the diagnosis of acute rejection, but it does reveal significant pathogenetic features, notably endothelial injury in PTCs (which also can be appreciated by light microscopy in 1-μ Epon embedded sections). The PTCs show mononuclear cells in the lumen, mostly lymphocytes, which are sometimes flattened in contact with the endothelium or when emigrating through the wall.[145] Loss of endothelium, disruption of the basement membrane, and balloon degeneration occur focally in association with the mononuclear leukocytes.[145,320] The endothelium becomes activated, as judged by cytoplasmic and nuclear enlargement, increased ribosomes, endoplasmic reticulum, mitochondria, and Golgi profiles; the fenestrations may disappear completely.[145] The endothelial hypertrophy has been compared with normal postcapillary venules, which are otherwise not anatomically recognized in the kidney.[145]

Gene expression studies of graft tissue have revealed that transcripts for proteins of cytotoxic T lymphocytes (CTLs), such as granzyme B, perforin, and Fas ligand,[72,132,181,319,343,344,348] and the master transcription factor, are characteristic of acute cellular rejection.[132] Graft CTL-associated transcripts precede tubulitis in mouse kidney grafts.[83] Treatment of rejection is followed by a measurable decrease of CTL-associated transcripts.[343] Knockout of either granzyme or perforin does not prevent acute rejection, however, suggesting they are not essential.[82] Interferon-γ mRNA is detectable in fine-needle aspirates 1 week before the clinical onset of rejection.[238] Other genes associated with acute rejection are TNF-β, TNF-α, CCL5, and macrophage inflammatory protein-1α.[132] No elevation of transforming growth factor-β or IL-10 has been detected.

Endarteritis (Type II Rejection)

Infiltration of mononuclear cells under arterial and arteriolar endothelium is the pathognomonic lesion of acute cellular rejection (Fig. 24-2). Many terms have been used for this process, including endotheliitis, endovasculitis, intimal arteritis, and endarteritis. We prefer the last term, which emphasizes the type of vessel (artery versus vein) involved and the site of inflammation. Mononuclear cells that are



A B

Figure 24–2 Acute cellular rejection, type II. **A**, Endarteritis in a medium–sized artery. The endothelium is lifted by undermining mononuclear cells, without involvement of the media. **B**, Subendothelial infiltration in a small artery with underlying arteriosclerosis (donor disease). This acute process should be distinguished from chronic transplant arteriopathy. (See color plate.)

sometimes attached to the endothelial surface are insufficient for the diagnosis of endarteritis; however, they probably represent the early phase of this lesion. Endarteritis in acute cellular rejection must not be confused with fibrinoid necrosis of arteries. The latter is characteristic of acute humoral rejection and can be seen in thrombotic vasculopathy. Some clinicians still do not separate these lesions, regarding all "vascular rejection" as predominantly humoral.

Endarteritis has been reported in 35% to 56% of renal biopsy specimens with acute cellular rejection.[20,53,168,248,312] Many pathologists do not find the lesion as often, which possibly may be ascribed to inadequate sampling, overdiagnosis of rejection (increasing the denominator), or the timing of the biopsy with respect to antirejection therapy. Endarteritis lesions affect arteries of all sizes, including the arteriole, although the lesions affect larger vessels preferentially. In a detailed analysis, 27% of the artery cross sections were affected versus 13% of the arterioles.[248] A sample may not be considered adequate to rule out endarteritis unless several arteries are included. A sample of four arteries would have an estimated sensitivity of about 75% in the detection of type II rejection.[248] Arteriolitis has the same significance as endarteritis.[21] Endarteritis can occur in cases with little or no interstitial infiltrate or tubulitis, arguing that it has a distinct pathogenetic mechanism.[57] In severe cases, a transmural mononuclear infiltrate affects the media, with focal necrosis of the myocytes, features that constitute type III rejection (transmural inflammation or fibrinoid necrosis). Although this focal necrosis occasionally occurs in the absence of demonstrable antibodies, it is more typical of antibody-mediated rejection.

Endothelial cells often are reactive with increased cytoplasmic volume and basophilia. The endothelium shows disruption and lifting from supporting stroma by infiltrating inflammatory cells.[9] Occasionally, endothelial cells are necrotic or absent; however, thrombosis is rare. Endothelial apoptosis occurs,[142,202] and increased numbers of endothelial cells appear in the circulation.[379] The media usually shows little change. In severe cases, a transmural mononuclear infiltrate may be seen (termed type III rejection). The cells infiltrating the endothelium and intima are T cells and monocytes, but not B cells.[9] $CD8^+$ and $CD4^+$ cells invade the intima in early grafts, but later $CD8^+$ cells predominate,[359] suggesting that class I antigens are the primary target.[202]

Normal arterial endothelial cells express class I antigens, weak ICAM-1, and little or no class II antigens or VCAM-1. During acute rejection, the endothelium of arteries expresses increased HLA-DR,[96,359] ICAM-1, and VCAM-1.[31,88] The upregulation of the adhesion molecules occurs in association with $CD3^{+}$[30] and $CD25^{+}$[98] infiltrating mononuclear cells. Endothelial cells also have decreased endothelin expression in rejection with endarteritis, but not in tubulointerstitial rejection.[372]

Glomerular Lesions

In most cases of acute cellular rejection, the glomeruli are spared or show minor changes, typically a few scattered mononuclear cells (T cells and monocytes) and occasionally segmental endothelial damage (Fig. 24-3).[357] A severe form of this glomerular injury, termed transplant glomerulitis or acute allograft glomerulopathy, develops in a few cases (< 5%), manifested by hypercellularity, injury and enlargement of endothelial cells, infiltration of glomeruli by mononuclear cells, and webs of periodic acid–Schiff–positive material.[290] Crescents and thrombi are rare. Endarteritis often accompanies the transplant glomerulitis.[213] The glomeruli contain numerous $CD3^+$ and $CD8^+$ T cells and monocytes.[129,359] Fibrin and scant immunoglobulin and complement deposits are found in glomeruli. This variant of cellular rejection has been associated with certain viral infections, such as cytomegalovirus and hepatitis C virus,[61] although viral antigens are not in the glomerular lesions.

Electron microscopy reveals enlarged, reactive endothelial cells, with a marked increase in cytoplasmic organelles (ribosomes, mitochondria, endoplasmic reticulum), an enlarged nucleus with open chromatin, prominent nucleoli, and loss of fenestrae. The endothelial swelling may obliterate the lumen. Some GBM may be bare of endothelium or wrinkled and collapsed. The glomerular capillary lumens contain monocytes and activated lymphocytes, with occasional neutrophils, platelets, and fibrin. The mesangium has loose matrix and sometimes monocytes. Amorphous electron dense deposits are sparse and limited to subendothelial spaces and the mesangium.[290]

Differential Diagnosis

Acute cellular rejection typically has a diffuse, interstitial mononuclear cell infiltrate, whereas patients with calcineurin inhibitor toxicity and patients with stable function have only focal mononuclear cell infiltrates (Table 24-3). Endarteritis or $C4d^+$ is found extremely rarely, if ever, in calcineurin inhibitor toxicity, and if either is present, it is the most discriminating feature for acute rejection.[239,326,351] Prominent tubulitis favors acute rejection because it is less prominent in acute tubular necrosis, particularly in the proximal tubules.[190] Tubulitis has been documented, however, in renal transplants with dysfunction resulting from lymphoceles (obstruction) or urine leaks, possibilities that need to be considered and excluded by other techniques.[66] Acute obstruction typically has some dilation of the collecting tubules, especially in the outer cortex. Edema and a mild mononuclear infiltrate also are common.

Interstitial mononuclear inflammation and tubulitis occur in a variety of diseases other than acute rejection, such as drug-induced (allergic) or infectious tubulointerstitial nephritis. When eosinophils are more abundant than usual for rejection, and eosinophils invading tubules are identified, drug allergy may be favored over rejection. The presence of endarteritis permits a definitive diagnosis of active rejection.[248] Lymphocytes commonly surround vessels (without medial involvement), a nonspecific feature, and must not be confused with endarteritis. Tubulitis is often present in atrophic tubules and does not indicate acute rejection. The diagnosis of acute pyelonephritis should be considered when active inflammation and abundant intratubular neutrophils are present. The pathologist should be cautious, however, because in acute humoral rejection, neutrophilic tubulitis with neutrophil casts can be seen; a C4d stain helps in distinguishing between these conditions. Positive urine and blood cultures also separate infection from rejection.

The usual diagnostic features of polyomavirus interstitial nephritis (BK virus) are the enlarged, hyperchromatic tubular nuclei with lavender viral nuclear inclusions, often in collecting ducts. These nuclei may be inconspicuous, however, and diligent study of multiple sections may be required. Other clues are prominent apoptosis of tubular cells, and abundant

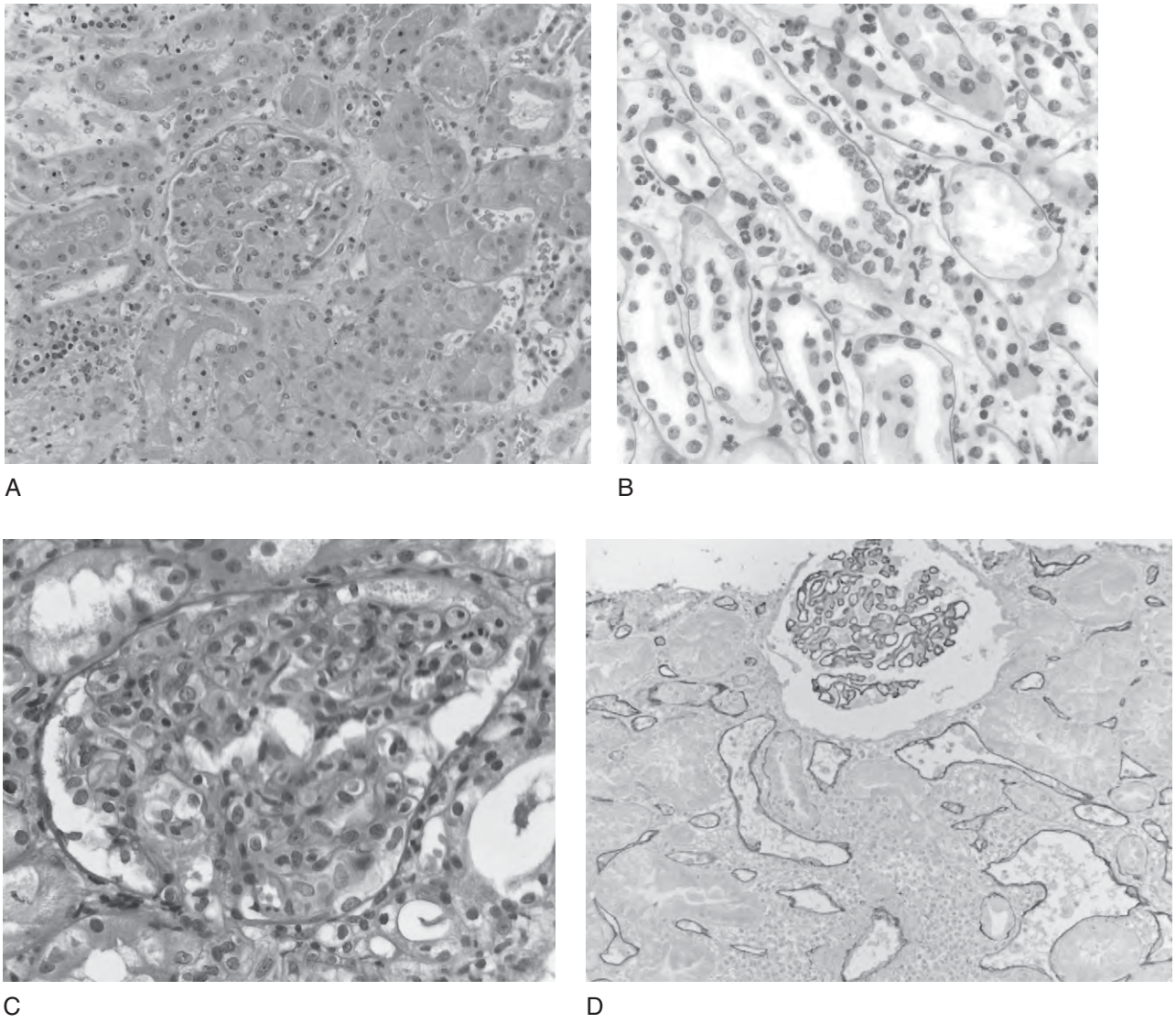


Figure 24–3 Acute humoral rejection. **A**, At low power, mild interstitial inflammation, focal hemorrhage, neutrophils, and thrombi are seen in glomerular capillaries and dilated peritubular capillaries with leukocytes (hematoxylin and eosin stain). **B**, At high power, neutrophils can be seen in the peritubular capillaries with little tubulitis (periodic acid–Schiff stain). **C**, Acute transplant glomerulitis is prominent in this case of acute humoral rejection. Glomerular endothelial cells are swollen, and the capillaries are filled with mononuclear cells, mostly macrophages (periodic acid–Schiff stain). **D**, C4d stain of a case of acute humoral rejection shows prominent, diffuse staining of dilated peritubular capillaries, sometimes containing inflammatory cells, and linear staining along the glomerular basement membrane (immunohistochemistry with a polyclonal anti–C4d rabbit antibody). (See color plate.)

plasma cells that invade tubules (a pathognomonic finding in our experience). Immunohistochemistry for polyoma large T antigen and electron microscopy (even of paraffin) confirm the diagnosis. Sometimes BK virus infection, with its exuberant plasmacytic infiltration and activated immunoblasts, may be confused with the plasmacytic hyperplasia form of post-transplant lymphoproliferative disease (PTLD).

Acute Antibody-Mediated Rejection

Acute antibody-mediated rejection (also known as acute humoral rejection) is a form of renal allograft rejection caused by the post-transplant production of circulating antibodies to donor alloantigens on endothelium, including HLA class I and class II antigens,[47,121,317] ABO blood group antigens,[91] and other non–major histocompatibility complex (MHC) antigens.[46,168,383] The antibodies arise after transplantation, in contrast to hyperacute rejection, although the patient may be presensitized with low titers before transplantation, insufficient to trigger an immediate rejection. Other terms used historically for acute humoral rejection are accelerated acute rejection, necrotizing arteritis, and fibrinoid necrosis.

Circulating cytotoxic antidonor class I antibodies were present in 25% of patients with acute rejection[121,182] associated with an increased risk of graft loss.[121,182] Identification of acute humoral rejection in biopsy specimens is difficult because none of the histological features is diagnostic, and immunoglobulin deposition usually is not detectable in the graft.[194,244,296] Techniques for showing C4d in PTCs, pioneered by Feucht and colleagues,[90] have substantially improved detection of this condition.[47,64,195,279,296] Acute humoral rejection may occur in the absence of evidence for

Table 24–3 **Differentiation between Acute Rejection and Acute Calcineurin Inhibitor Toxicity**

	Acute Rejection	Calcineurin Inhibitor Toxicity
Interstitium		
Infiltrate	Moderate-marked	Absent-mild
Edema	Usual	Can be present
Tubules		
Tubular injury	Usual	Usual
Vacuoles	Occasional	Common
Tubulitis	Prominent	Minimal-absent
Arterioles		
Endotheliitis	Can be present	Absent
Smooth muscle degeneration	Absent	Sometimes present
Mucoid intimal thickening with red blood cells	Absent	Sometimes present (TMA)
Arteries		
Endotheliitis	Common	Absent (rare mononuclear TMA)
Peritubular Capillaries		
C4d	May be positive	Negative
Glomeruli		
Mononuclear cells	Often	Rare
Thrombi	Occasional	Occasionally prominent (TMA)

TMA, thrombotic microangiopathy.

T cell–mediated injury, although commonly both are present.[47] Acute humoral rejection typically manifests with clinically severe acute rejection[121] 1 to 3 weeks after transplantation, but it also can arise months to years later, often in association with decreased immunosuppression or noncompliance.[349] With current therapy, about 5% to 7% of recipients develop an episode of acute humoral rejection, and about 25% of biopsy specimens taken for acute rejection have pathological evidence of an acute humoral rejection component.[57] The main risk factor is presensitization by blood transfusion, pregnancy, or prior transplant[184]; however, most have a negative crossmatch at the time of transplantation.

Diagnostic Criteria

The three diagnostic criteria for acute humoral rejection are (1) histological evidence of acute injury (neutrophils in capillaries, acute tubular injury, fibrinoid necrosis), (2) evidence of antibody interaction with tissue (typically C4d in PTCs), and (3) serological evidence of circulating antibodies to antigens expressed by donor endothelium (typically HLA).[195,279] If only two of the three major criteria are established (e.g., when antibody or C4d is negative or not done), the diagnosis is considered suspicious for acute humoral rejection. Biopsy specimens that meet the criteria for acute humoral rejection and acute cellular rejection are considered to have both forms of rejection. Biopsy specimens with C4d and no pathology are likely a manifestation of accommodation (see later).

Pathological Features

Histological findings are typically a scant to moderate mononuclear interstitial infiltrate, sometimes with prominent neutrophils[127,195,286,357] and increased numbers of macrophages (see Fig. 24-3).[186] The extent of mononuclear infiltration often does not meet the criteria for acute cellular rejection.[286] PTCs have neutrophils in about 50% of cases and are classically dilated (see Fig. 24-5A). Interstitial edema and hemorrhage can be prominent. Glomeruli have accumulations of neutrophils (approximately 25% of cases) and macrophages (approximately 50% of cases) (see Fig. 24-3)[195,249,286,357] and occasionally fibrin thrombi or segmental necrosis.[121,195,357] Acute tubular injury, sometimes severe, can be identified in many cases and may be the only initial manifestation of acute humoral rejection. Focal necrosis of whole tubular cross sections, similar to cortical necrosis, has been reported; 38% to 70% of acute humoral rejection cases may have patchy infarction.[182,357] Little mononuclear cell tubulitis is found, although a neutrophilic tubulitis with or without neutrophil casts may be prominent,[357] resembling acute pyelonephritis. Plasma cells can be abundant in acute humoral rejection, either early[4] or late[71,273] after transplantation, sometimes associated with severe edema and increased interferon-γ production in the graft.[71] B cells also can be present, but have no apparent diagnostic value.

In about 15% of cases, small arteries show fibrinoid necrosis, with little mononuclear infiltrate in the intima or adventitia, but with neutrophils and karyorrhectic debris (Fig. 24-4).[182,357] Arterial thrombosis can be found in 10%, and a pattern resembling thrombotic microangiopathy has been reported.[182] Of 26 published cases with fibrinoid necrosis, 73% (19 of 26) were $C4d^+$.[127,195,249,357] Presumably, the $C4d^-$ cases had T cell–mediated rejection or thrombotic microangiopathy. Antibodies to the angiotensin II type 1 receptor have been detected in a few cases with arterial fibrinoid necrosis, in the absence of C4d deposition in capillaries.[80] The presence of mononuclear endarteritis in cases of

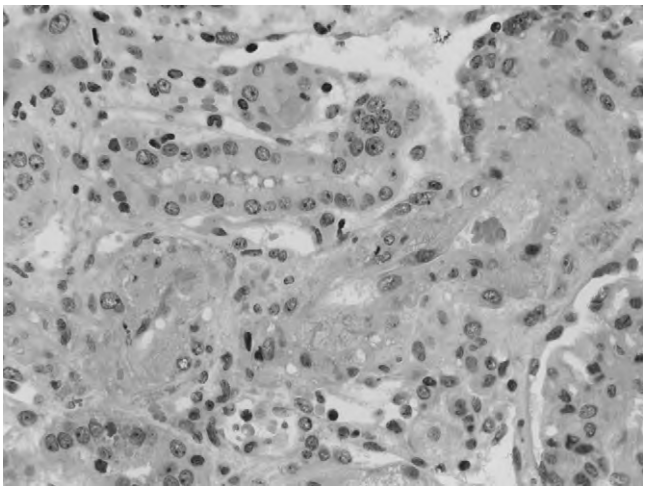


Figure 24–4 Fibrinoid arterial necrosis. An arteriole with destruction of the medial wall smooth muscle cells by fibrinoid necrosis. Some neutrophils are present underneath the reactive and swollen endothelium. This vascular change distinctly differs from endarteritis (compare with Figure 24–2) and can be seen in acute humoral rejection and type III acute cellular rejection. This case had positive C4d. (See color plate.)

acute humoral rejection strongly suggests a component of T cell–mediated rejection. Normal arteries in a biopsy specimen do not exclude acute humoral rejection. In biopsy specimens from patients with circulating anti–class I antibody, 25% had no arterial lesions at all.[357]

By electron microscopy, the PTCs are dilated and contain neutrophils. The endothelium is reactive and shows loss of fenestrations. The glomerular endothelium is separated from the GBM by a widened lucent space with swelling of endothelial cells[357] and loss of endothelial fenestrations, indicative of injury. Platelets, fibrin, and neutrophils are found in glomerular cells and PTCs. The small arteries with fibrinoid necrosis show marked endothelial injury and loss, smooth muscle necrosis, and deposition of fibrin.

C4d Interpretation

Feucht and colleagues[90] first drew attention to C4d as a possible marker of an antibody-mediated component of severe rejection. C4d, a fragment of complement component C4, is released during activation of the classical complement pathway by antigen-antibody interaction. C4d forms a thioester bond that binds covalently to tissues at the local site of activation. The covalent linkage explains why C4d remains for several days after alloantibody disappears because antibody binds to cell surface antigens that can be lost by modulation, shedding, or cell death.

Although immunoglobulin deposition is found in only a few cases, C4d is characteristically detected in a widespread, uniform ring-like distribution in the PTCs by immunofluorescence in cryostat sections (see Fig. 24-5B).[47,90] Deposition occurs in the cortex and the medulla. Using immunohistochemistry in formalin-fixed, paraffin-embedded tissue, C4d has a similar pattern, although the intensity varies. Glomerular capillary staining also occurs, but it is hard to distinguish from C4d normally found in the mesangium in frozen sections. Formalin fixation eliminates this background staining and shows glomerular C4d in about 30% of acute humoral rejection cases.[286]

Grafts with focal C4d (< 50% of PTC) are of uncertain significance, and the patient should be monitored closely for donor reactive antibodies. Two of three studies have failed to show any significant clinical or pathological difference between cases with focal and diffuse C4d staining.[187,249,273] Antibodies to donor class II antigens were found in two of three patients tested with focal C4d, arguing that this pattern is due to circulating antibodies.[187] C4d deposition can precede histological evidence of acute humoral rejection by 5 to 34 days.[117] C4d in 1-week protocol biopsy specimens was followed by clinical acute rejection in 82% of cases[347] and was associated with donor reactive antibodies.[166]

In acute rejection, C4d is a specific (96%) and sensitive (95%) marker of circulating anti–donor HLA-specific antibodies by the antihuman globulin cytotoxicity test.[195] PTC C4d deposition is associated with concurrent circulating antibodies to donor HLA class I or class II antigens in 88% to 95% of recipients with acute rejection.[27,116,195] False-negative antibody assays are probably most often due to absorption by the graft, as shown by elution from rejected grafts in patients who had no detectable circulating antibody.[191] Alternatively, non-HLA antigens may be the target.[46] $C4d^-$ acute rejection may show flow cytometry evidence of anti–donor reactive antibodies in 50% of cases,[27] owing in part to non–complement fixing antibodies.[368] Cell-based assays have a false-positive rate of less than 10%.[195]

Compared with methods for C4d, the triple-layer immunofluorescence technique[47] proved the most sensitive, although the difference with immunohistochemistry in paraffin-embedded tissue was small.[230] In fixed tissue plasma in the capillaries and interstitium may stain for C4d, which interferes with interpretation.

Other components of the complement system have been sought. C3d, a degradation product of C3, was found in PTCs in 39% to 60% of biopsy specimens from HLA-mismatched grafts with diffuse C4d.[116,127,172,347] C3d was usually,[116] but not always,[172] associated with C4d. C3d correlated with acute humoral rejection in all studies and was associated with an increased risk of graft loss in two series, compared with $C3d^-$ cases, but $C3d^+$ provided no convincing additional risk compared with $C4d^+$. The interpretation of C3d stains is complicated by the common presence of C3d along the TBM.[116]

Even though C3d should indicate more complete complement activation, it added no diagnostic value to C4d in grafts showing histological features of acute humoral rejection except in the setting of ABO-incompatible grafts.[116] Other complement components, such as C1q, C5b-9, and C-reactive protein, are not conspicuous in PTCs in acute rejection.[148,251] Lectin pathway components, which activate C4 by binding to microbial carbohydrates, are sometimes detected.[139,347] Among 18 biopsy specimens with C4d, 16 had diffuse H-ficolin deposition along the PTCs, whereas none of the 42 cases without C4d had H-ficolin. No MBL-associated serine protease (MASP)-1 or MASP-2 was detectable.[139] The significance of this observation is unclear because MASP proteins are required to activate C4 via the ficolins or mannose binding lectin (MBL).

Differential Diagnosis

For differential diagnosis, it is helpful that acute tubular necrosis[296,356] and thrombotic microangiopathy in native kidneys are $C4d^-$. Among 26 cases of thrombotic microangiopathy/hemolytic-uremic syndrome in native kidneys, none was $C4d^+$, including cases with lupus anticoagulant and antiphospholipid antibodies.[296] In five cases of recurrent hemolytic-uremic syndrome in transplant recipients, C4d also was negative.[14] Among native kidney diseases, only lupus nephritis[176,296] and endocarditis[176] have been reported to have PTC C4d. Glomerular C4d deposits are nonspecific because they occur in many forms of immune-complex glomerulonephritis in native kidneys. Arterial intimal fibrosis often stains for C4d, even in native kidneys, and should not be taken as evidence of antibody-mediated rejection.[296]

The comparative features of "pure" humoral and cellular acute rejection are presented in Table 24-4. In acute humoral rejection, neutrophils are the predominant inflammatory cells in PTCs, glomeruli, tubules, and the interstitium, with or without accompanying fibrinoid necrosis. The vascular lesion of acute humoral rejection is fibrinoid necrosis of the wall, whereas in acute cellular rejection, endarteritis is the usual lesion. C4d deposition in PTCs (immunofluorescence microscopy) is typically present only in acute humoral rejection and not in acute cellular rejection.

The prognosis of acute humoral rejection is uniformly worse than acute cellular rejection.[47,121,168,182,357,383] In one series, 75% of the 1-year graft losses from acute rejection

Table 24–4 **Differentiation between Acute Humoral Rejection and Acute Cellular Rejection**

	Acute Humoral Rejection	Acute Cellular Rejection
Interstitium		
Infiltrate	Variable	Moderate-severe
Edema	Present	Present
Peritubular Capillaries	Neutrophils	Mononuclear cells
C4d*	Positive	Negative
Tubules		
Acute tubular necrosis	Can be present	Usually absent
Tubulitis	Neutrophils	Mononuclear cells
Vessels		
Endarteritis	Can be present	Present in type II
Fibrinoid necrosis	Typically present	Present in type III
Glomeruli		
Inflammatory cells	Neutrophils	Mononuclear cells
Fibrinoid necrosis	Can be present	Typically absent

*C4d staining in peritubular capillaries indicates activation of the classical complement pathway by humoral antibody (monoclonal antibody, immunofluorescence microscopy).

were in the C4d+ acute humoral rejection group.[195] Grafts that recover from the acute episode of acute humoral rejection have a similar long-term outcome,[357] however, suggesting that the pathogenetic humoral response can be transient if treated effectively. Current therapies for antibody-mediated rejection are discussed in Chapter 22.

Classification Systems

The most widely used classification system currently is the Banff working schema. Banff started as an international collaborative effort led by Solez and colleagues[335] to achieve a consensus that would be useful for drug trials and routine diagnosis. Banff is still growing and remodeling, undergoing revisions based on data presented and debated at the biennial Banff meetings. These revisions have included restructuring that separated the category of endarteritis, according to the National Institutes of Health Cooperative Clinical Trials in Transplantation criteria[53,280]; the addition of acute[279] and chronic antibody-mediated rejection[336]; and the birth[335] and death[336] of "chronic allograft nephropathy."

Banff scores three elements to assess acute rejection: tubulitis (t), the extent of cortical mononuclear infiltrate (i), and vascular inflammation (intimal arteritis or transmural inflammation) (v). Mononuclear cell glomerulitis (g) is scored but is not yet part of the classification of rejection. Banff recognizes three major categories of acute T cell–mediated rejection (tubulointerstitial, endarteritis, and arterial fibrinoid necrosis) (see Table 24-2). The threshold for type I (tubulointerstitial) acute cellular rejection is greater than 25% cortical mononuclear inflammation provided that tubulitis of at least 5 to 10 cells per tubule is present.[280] Cases with no tubulitis, regardless of the extent of infiltrate, are not considered acute cellular rejection. Biopsy specimens with C4d+ PTCs are considered to have an additional component of antibody-mediated rejection, which occurs in 20% to 30% of cases.[128]

Cases with 10% to 25% infiltrate are termed suspicious for rejection or borderline in the Banff system, as long as tubulitis is present. Many, but not all, of these cases are early or mild acute rejection: 75% to 88% of patients with suspicious/borderline category and graft dysfunction improve renal function with increased immunosuppression,[305,316] comparable to the response rate in type I rejection (86%).[305] A few (28%) untreated suspicious/borderline cases progress to frank acute rejection in 40 days.[206] Almost all patients with suspicious/borderline findings do well provided that there is no element of concurrent antibody-mediated rejection, which commonly has a suspicious/borderline pattern.[286] The suspicious category is not counted as acute rejection in most clinical trials, a major omission in our opinion.

The interobserver reproducibility of the present Banff classification is sufficient but needs improvement. In a Canadian study, the agreement rate for rejection was 74%, but there was only 43% agreement on the suspicious/borderline cases,[112] similar to a European series.[366] Among a group of 21 European pathologists, the agreement rate was poor for all of the acute Banff scores (t, i, v, g) in transplant biopsy slides (all κ values <0.4).[100] Agreement for t and v scores improved significantly when participants were asked to grade a lesion in a photograph (κ values of 0.61 and 0.69), arguing that the challenge is primarily finding the lesion in the glass slide. Lack of improvement in the other categories (g, i) argues that the definitions are faulty. Despite these considerations, Banff is fully accepted as a scoring system of drug trials and is used widely in clinical practice (although not with detailed reporting of the individual scores).

LATE GRAFT DISEASES

Although acute rejection has diminished in clinical importance, allografts are still lost by slow, progressive diseases that cause a 3% to 5% annual attrition rate. The specific causes are many and sometimes difficult to ascertain, particularly if only an end-stage kidney is examined. Two terms—chronic rejection and chronic allograft nephropathy—are widely used in the literature to lump together these myriad diseases. The role of the pathologist in interpreting the biopsy specimen is to provide the most specific diagnosis possible and indicate the activity of the process. Although some authors have argued that the renal biopsy is not useful in analyzing graft dysfunction after 1 year, the data show that in 8% to 39% of patients, the biopsy led to a change in management that improved renal function.[165,267] We discuss here the criteria used to distinguish some of these diseases from rejection. Chronic rejection is best defined as chronic injury primarily mediated by an immune reaction to donor alloantigens. When the cause is unknown, nonspecific interstitial fibrosis and tubular atrophy is preferred to chronic allograft nephropathy. The latter term is often mistaken for a specific diagnosis.

Chronic Antibody-Mediated Rejection

Circulating anti-HLA antibodies have been associated with increased risk of late graft loss.[135,353] Chronic, active antibody-mediated rejection (chronic humoral rejection) has only more recently been recognized as a separate category

in the Banff schema.[336] Chronic humoral rejection differs from acute humoral rejection in the lack of evidence of acute inflammation (neutrophils, thrombi, and necrosis) and the presence of matrix synthesis (basement membrane multilamination and fibrosis in arterial intima and the interstitium). Chronic humoral rejection commonly arises late (>6 months after transplantation), without a history of acute humoral rejection, although C4d or acute humoral rejection in early biopsy specimens is a risk factor for later transplant glomerulopathy with C4d.[285] Many have reduced levels of immunosuppression (absorption, iatrogenic, or noncompliance). In these cases, a combination of chronic humoral rejection and acute humoral rejection may be seen.

The criteria of chronic humoral rejection consist of the triad of (1) one of the following morphological features—transplant glomerulopathy (duplication or "double contours" in GBMs), multilamination of the PTC basement membrane, PTC loss and interstitial fibrosis, or chronic arteriopathy with fibrous intimal thickening (without duplication of the internal elastica); (2) diffuse C4d deposition in PTCs; and (3) circulating donor-specific antibody.[336] If only two elements of the triad are present, the diagnosis is considered "suggestive." Two features point to ongoing immunological activity: the presence of C4d and mononuclear cells in glomerular cells and PTCs. Scoring of multilamination requires electron microscopy, which is not always available in transplant biopsies, and quantitative assessment of the number of layers, because to distinguish from other common causes of lamination, more than six layers have to be present.[144] Duplication of the GBM has many other causes, such as thrombotic microangiopathy and membranoproliferative glomerulonephritis; however, these do not have C4d in PTCs. Also in chronic humoral rejection, GBMs may show multilamination extending completely around the capillary, even between the endothelium and the mesangium, which is rarely, if ever, seen in other conditions.[42]

A sequence of four stages of development of chronic humoral rejection has been shown in protocol biopsy specimens of nonhuman primate renal allografts. The process begins with antibody production, followed by C4d deposition, and, later, morphological and functional changes.[333]

Transplant Glomerulopathy

Transplant glomerulopathy (chronic allograft glomerulopathy) increases in frequency 1 to 5 years after transplantation (5% to 14% of protocol biopsy specimens) and affects graft survival more adversely than does interstitial fibrosis with inflammation.[59] Transplant glomerulopathy is defined as duplication of the GBM with modest mesangial expansion, in the absence of specific de novo or recurrent glomerular disease, revealed best in periodic acid–Schiff or silver stains (Fig. 24-5A). The glomeruli show an increase in mesangial cells and matrix with various degrees of scarring and adhesions. In some cases, mesangiolysis or webbing of the mesangium and segmental or global sclerosis may be prominent. Electron microscopy reveals duplication or multilamination of the GBM (see Fig. 24-5C), often accompanied by cellular (mononuclear or mesangial cell) interposition, widening or lucency of the subendothelial space, and a moderate increase in mesangial matrix and cells.[119] Electron microscopy detects 40% more cases of transplant glomerulopathy than does light microscopy.[144] The GBM typically has rarefactions, microfibrils, and cellular debris, but few deposits.[37,138,277] Endothelial cells may appear reactive with loss of fenestrae, probably undergoing "dedifferentiation."[51,138,277] Podocyte foot process effacement ranges from minimal to extensive,[138] corresponding to the degree of proteinuria. The nonduplicated GBM may become slightly thickened, attributable to compensatory hypertrophy. With immunohistochemical techniques in paraffin sections, C4d is present along the capillary walls in about 10% to 30% of cases.[285,327] Extensive crescents or diffuse immunoglobulin deposits are unusual and suggest recurrent or de novo glomerulonephritis.[108,260,276]

Peritubular Capillary and Tubulointerstitial Lesions

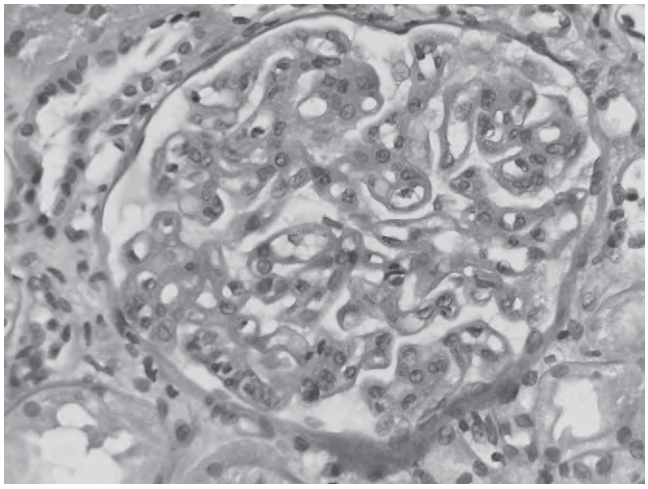
PTCs may be dilated and prominent, with thick basement membranes, or may disappear altogether leaving only occasional traces of the original basement membrane.[24,140] By definition, PTCs have prominent C4d deposition (see Fig. 24-5D), which is associated with circulating anti–donor HLA class I or II reactive antibodies.[196] In our experience, the extent of C4d staining is less than in acute humoral rejection, perhaps as a result of capillary loss or modulation of antigen. Electron microscopy reveals splitting and multilayering of the PTC basement membrane (see Fig. 24-5E), first described by Monga and others.[197,223] Each ring probably represents the residue of one previous episode of endothelial injury going from oldest (outer) to most recent (inner). Quantitation is necessary to establish diagnostic specificity. Three or more PTCs with five to six circumferential layers and one PTC with seven or more circumferential layers were found only in chronic rejection.[144]

PTC lamination correlates with transplant glomerulopathy,[144,197] C4d deposition,[285] and loss of PTCs.[140] In some cases with repeat biopsies, the PTC lesions have been shown to precede the glomerular lesion. Marked multilamination (five to six layers in three capillaries or more than six layers in one capillary) was found in 50% of cases with interstitial fibrosis that lacked arterial or glomerular changes and may point to past episodes of rejection as the cause of the fibrosis.[197]

Tubular atrophy and interstitial fibrosis are regular, but nonspecific, features of chronic humoral rejection and do not serve to distinguish rejection from other causes, such as calcineurin inhibitor toxicity. Atrophic tubules typically have thickened, duplicated TBMs and intratubular mononuclear cells and mast cells.[54] Tubular atrophy should not be confused with the tubulitis of acute rejection. The TBM commonly has C3 deposited in a broad segmental pattern. This deposition is an exaggeration of similar changes found in normal kidneys and probably represents a residue from prior episodes of tubular injury, or possibly a persistent chronic injury. The interstitium typically has a sparse mononuclear infiltrate, with small lymphocytes, plasma cells, and mast cells.[55] Nodular collections of quiescent-appearing lymphoid cells sometimes are found around small arcuate arteries. Abundant plasma cells may be present.

Transplant Arteriopathy

Arterial lesions may be a manifestation of chronic humoral rejection. Alloantibodies to graft class I antigens are a specific



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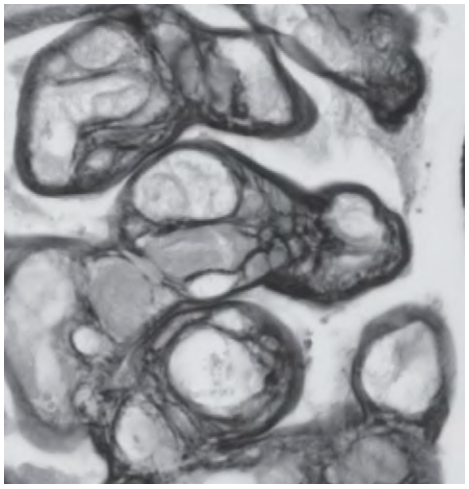

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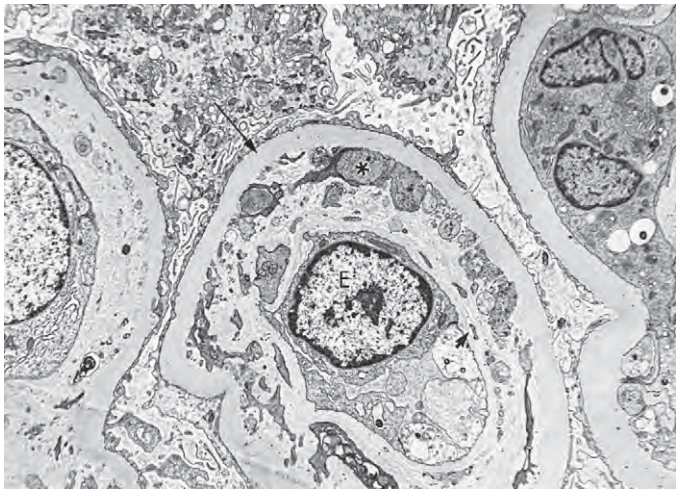

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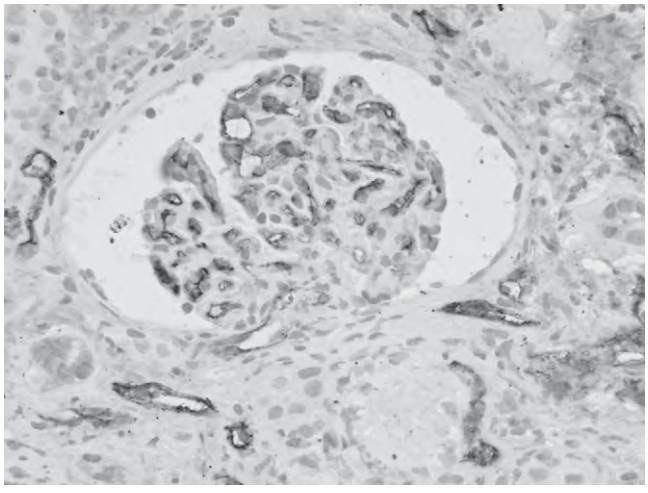

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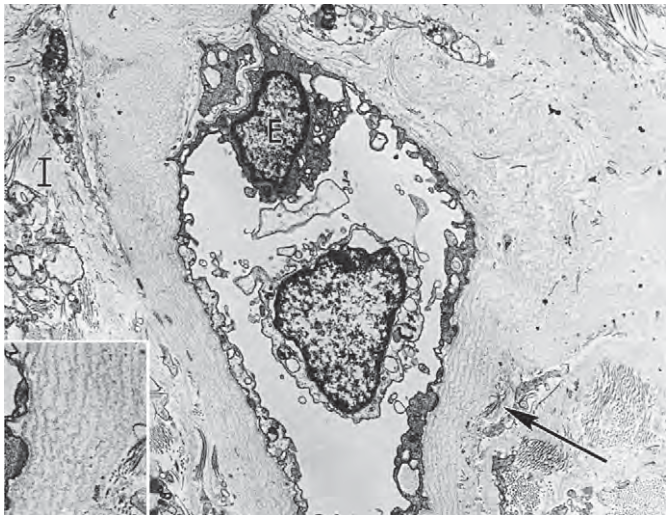


E

Figure 24–5 Chronic allograft glomerulopathy. **A**, Widespread duplication of the glomerular basement membrane (GBM) with mild mesangial hypercellularity and increased mononuclear cells in the glomerular capillaries (periodic acid–Schiff stain). **B**, GBM multilamination at high power in a silver stain. **C**, Electron microscopy. High-power view of a glomerular capillary showing duplication of the GBM; the new or second layer of GBM (*short arrow*) forms underneath the endothelium (E) and is separated from the old GBM layer (*long arrow*) by the cellular (mononuclear or mesangial cell) interposition (∗). **D**, Immunohistochemistry stain for C4d in paraffin sections shows prominent C4d deposition in glomerular and peritubular capillaries. **E**, Electron microscopy. High magnification of a peritubular capillary with multilamination (*arrow*) of the basement membrane. *Inset* is a higher magnification of the area marked by *arrow*. E, endothelium; I, interstitium. (**A**, **B**, and **D**, See color plate.)

risk factor for chronic transplant arteriopathy in human renal allografts.[69,150] Proof that antibody is sufficient to initiate allograft arterial intimal fibrosis has been shown by passive transfer of anti-MHC antibody into immunologically deficient mice (RAG-1 knockout) bearing cardiac allografts.[360] The correlation with C4d in PTCs is not as strong, however, as for transplant glomerulopathy or PTC multilaminated basement membranes.[196,285] Chronic arteriopathy is presented here as a feature of chronic T cell–mediated rejection. This organization is not meant to exclude a synergistic or separate role for antibodies, which would be likely in the setting of $C4d^+$.

Chronic T Cell–Mediated Rejection

Chronic T cell–mediated rejection is a new category and subject to refinement. Using the chronic humoral rejection model, the current Banff classification defines "chronic active T cell–mediated rejection" as showing morphological features of chronicity (arterial intimal fibrosis without elastosis) combined with features indicative of ongoing T cell activity (mononuclear cells in the intima). Interstitial fibrosis with a mononuclear infiltrate and tubulitis in some instances also are probably part of this condition. Other nonspecific features that are commonly present in association with transplant arteriopathy are loss of PTCs, interstitial fibrosis, and tubular atrophy.[140] It is anticipated that gene expression studies will help in the future to document the activity of the infiltrate.

At present, the arterial lesions are the most definitive evidence of chronic cell-mediated rejection, in our opinion. Small and large arteries 1 month after transplantation can begin to develop severe intimal proliferation and luminal narrowing.[36,49] The intimal change is most prominent in the larger arteries, but can be seen at all levels, from interlobular arteries to the main renal artery. The intima shows pronounced, concentric fibrous thickening with invasion and proliferation of spindle-shaped myofibroblasts (Fig. 24-6). This vascular change has been termed chronic transplant arteriopathy and, when combined with an infiltrate of mononuclear cells in the intima, is characteristic of chronic T cell–mediated rejection (see Fig. 24-10). Subendothelial mononuclear cells are one of the most distinctive features and argue that the endothelium itself is a target. T cells ($CD4^+$, $CD8^+$, $CD45RO^+$), macrophages, and dendritic cells infiltrate the intima.[114,258,308] T cells express cytotoxic markers, including perforin[94] and GMP-17,[202] and markers of proliferation (proliferating cell nuclear antigen).[114] No B cells (CD20) are detected.[114] It is hypothesized that this is a dampened version of the endarteritis of acute rejection.

The second distinctive feature is the lack of multilamination of the elastica interna (fibroelastosis), best appreciated in elastin stains. Fibroelastosis, typical of hypertensive, atrophic, and aging arterial changes, provides a useful differential diagnostic feature from rejection. Foamy macrophages containing lipid droplets are characteristically seen along the internal elastica and can be found 4 weeks after transplantation. Fibrin sometimes is deposited in a band-like subendothelial location or mural thrombus. Focal myocyte loss from the media occurs, as shown in mouse and rat studies.[304] Immunofluorescence often shows IgM, C3, and fibrin along the endothelium, in the intima, or in the media, as a diffuse

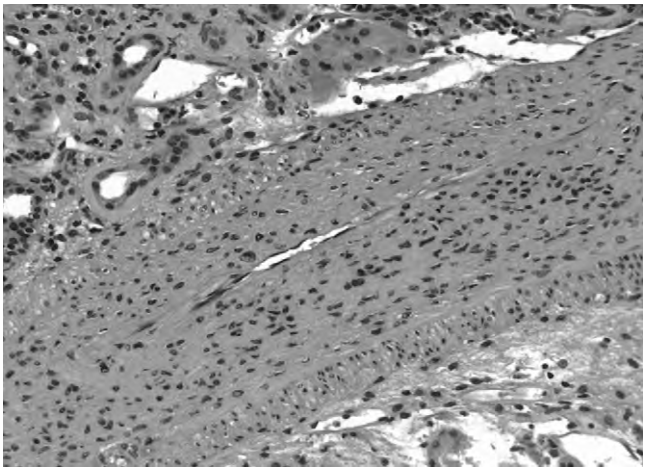


Figure 24–6 Chronic allograft arteriopathy. An interlobular artery with prominent intimal fibroplasia. The presence of scattered mononuclear cells in the intima and the lack of duplication of the internal elastica are characteristic of chronic rejection. This biopsy specimen was positive for C4d. (See color plate.)

blush or focal granular deposits.[10,37,150,199,272] Sometimes these may be accompanied by IgG deposition.

The endothelium expresses increased adhesion molecules, notably ICAM-1 and VCAM-1. Antagonism of ICAM-1 binding and expression inhibits chronic rejection,[301] and in humans certain ICAM-1 genetic polymorphisms (e.g., exon 4, the Mac-1 binding site) seem to confer a higher risk factor for chronic rejection.[200] The endothelium remains of donor origin[137,318]; however, some of the spindle-shaped cells that contribute to the intimal thickening are of recipient origin.[160,258] The myointimal cells stain prominently for α smooth muscle actin, sometimes so strikingly that a "double media" seems to be formed.[306] This phenomenon also has been described as the development of a new artery inside and concentric with the old,[136] with elastic laminae and a muscular media, separated from the old internal elastic lamina by poorly cellular tissue.

By electron microscopy, the thickened intima consists of myofibroblasts, collagen fibrils, basement membrane material, and a loose amorphous electron-lucent ground substance.[275] The matrix consists of collagen, fibronectin, tenascin, proteoglycans (biglycan and decorin), and acid mucopolysaccharides.[50,113,201] Fibronectin has the extra domain (EDA) of cellular fibronectin, typical of embryonic or wound healing fibronectin.[113] Several growth factors/cytokines have been detected. Platelet-derived growth factor (PDGF) A chain protein is primarily in endothelial cells, whereas the PDGF B chain is in macrophages and smooth muscle cells.[8] Enhanced PDGF B-type receptor protein was found on intimal cells and on smooth muscle cells of the proliferating vessels.[89] Fibroblast growth factor-1 and its receptor are present in the thickened intima.[161] TNF-α is in the smooth muscle of vessels with chronic rejection in contrast to normal kidneys.[253]

The T cell–mediated arterial lesions can be divided into three stages, which probably differ in mechanism and reversibility.[51] The stage I lesion is endarteritis, characteristic of type II acute cellular rejection. This lesion lacks matrix formation. This acute stage is believed to be T cell–mediated

endothelial injury. Stage II lesions have intimal matrix production and accumulation of myofibroblasts forming a "neointima." This stage also contains mononuclear cells (T cells and macrophages), which are believed to be active in the intimal proliferation and accumulation of matrix. Intermediate stages between stage I and stage II lesions are sometimes found, with lymphocytes admixed with fibrin and fibromuscular proliferation, well documented in a non-human primate model of chronic rejection.[374] Secondary factors probably become increasingly important as the lesion progresses to stage III, in which the intima is fibrous, and inflammatory cells are scant. A fourth category resembling natural atherosclerosis with cholesterol clefts and calcification also has been proposed.[114]

A large body of experimental evidence supports the concept that the arterial lesions are immunologically mediated[51]: (1) The lesions do not routinely arise in isografts; (2) the target antigens can be either MHC or minor histocompatibility complex antigens[2,63,304]; (3) the specific initiator is probably T cells followed by antibody (antibody is necessary and sufficient for the fibrous lesion in mice); (4) the target cell is probably the endothelium, but the smooth muscle also may be affected; (5) secondary nonimmunological mechanisms analogous to those in atherosclerosis are important in the progression of the lesion; and (6) ultimately the process may be independent of specific antidonor immunological activity. T cells are sufficient to initiate cellular vascular lesions in B cell–deficient mice, but these lesions do not readily progress to fibrosis in the absence of antibody.[302] Fibrous lesions also are markedly reduced in strain combinations that fail to elicit a humoral antibody response. The best evidence for T cell mechanisms of chronic allograft injury in humans is that subclinical or late clinical cellular rejection is associated with progressive graft fibrosis and dysfunction,[58,234,298] and endarteritis is associated with later transplant arteriopathy.[171]

Other Specific Diagnoses

Other conditions that cause slowly progressive graft dysfunction and loss and that can be diagnosed by a renal biopsy are calcineurin inhibitor toxicity, hypertensive vascular disease, polyomavirus infection, recurrent disease, de novo glomerular disease, obstruction, and renal artery stenosis.[57] Chronic calcineurin inhibitor toxicity is most specifically diagnosed by the presence of nodular hyalin replacement of individual smooth muscle cells, which may form distinctive deposits on the outer side of the arteriole, as described by Mihatsch as cyclosporine arteriolopathy.[215-217] Ordinary hyalinosis resulting from diabetes, hypertension, or aging typically is subendothelial. When either form is severe, transmural hyalinosis develops, which is of indeterminant origin.

To distinguish intimal fibrosis resulting from hypertension from that resulting from chronic rejection, an elastin stain is valuable because in hypertension, but not rejection, the elastica interna is multilayered (elastosis), and in chronic rejection, the elastica is not duplicated, but may be fractured. Foam cells and mononuclear cells in the intima also favor rejection. The features that point to a component of chronic antibody-mediated rejection have been discussed previously and include most specifically the presence of C4d in PTCs or glomeruli, or both. Multilamination of the GBM or PTC basement membranes is also typical. Demonstration of polyomavirus by immunohistochemistry in previous biopsy specimens can point to a causal role in the late graft damage, even when the virus is no longer detectable.

Obstruction, usually difficult to diagnose by histology, archetypically shows dilated collecting ducts, especially in the outer cortex; lymphatics filled with Tamm-Horsfall protein; and sometimes ruptured tubules with granulomas. Renal artery stenosis causes tubular atrophy (or even acute injury) accompanied by relatively little fibrosis or intraparenchymal arteriolar/arterial lesions. Recurrent and de novo glomerular diseases are identified by light, immunofluorescence, and electron microscopic criteria in native kidneys.

Chronic Allograft Nephropathy, Not Otherwise Specified

Cases remain with interstitial fibrosis and tubular atrophy in which no specific diagnosis can be made. Some of these cases may be the end stage of active processes in which the causative agent is no longer appreciable (e.g., late effects of polyomavirus or thrombotic microangiopathy). Others may represent burned out or inactive rejection; this might be the case for transplant glomerulopathy or arteriopathy without C4d deposition. Animal studies have shown that limited exposure to anti-MHC antibody can cause long-standing arteriopathy, despite only transient C4d deposition.[360]

The term chronic allograft nephropathy was created in Banff in 1993 to draw attention to the fact that not all late graft injury was due to rejection and that to make the diagnosis of rejection, certain more specific features than interstitial fibrosis and tubular atrophy needed to be present (notably chronic glomerular or arterial lesions). An unintended consequence was, however, that chronic allograft nephropathy itself became a diagnosis that inhibited the search for specific, and perhaps treatable, causes. Chronic allograft nephropathy has been replaced in Banff 2005 with category 5: "Sclerosis, interstitial fibrosis, and tubular atrophy, no evidence of any specific etiology." This category now includes only cases for which no specific causative features can be defined and excludes cases with pathological features of chronic humoral rejection, chronic calcineurin inhibitor toxicity, hypertensive renal disease, polyomavirus infection, obstruction, or other de novo or recurrent renal disease. An alternative term, which we prefer, is chronic allograft nephropathy, not otherwise specified.

Grading Systems for Chronic Graft Damage

The systems for grading chronic rejection generally are based on adding the scores of three component parts: tubulointerstitial, vascular, and glomerular.[141,157,280] The assumption is that these components are part of the same process (i.e., the consequence of chronic endothelial damage); however, many authors would argue that different pathogenetic factors contribute to each lesion. The Banff system grades the different elements into three categories, as various degrees of chronic transplant nephropathy.[334] The chronic allograft damage index[141] has been used and shown to correlate with long-term outcome. The components scored are interstitial fibrosis, tubular atrophy, arterial

intimal thickening, glomerular sclerosis, mesangial expansion, and GBM duplication.[157] The sum score in biopsy specimens taken at 2 years correlated with graft function at 6 years, but there was a fair amount of scatter.[141] Similarly, a chronic graft damage score calculated at 6 months is strongly associated with graft loss 2 to 3 years after transplantation.[74] The chronic allograft damage index can be scored using Banff grades.

PROTOCOL BIOPSY

Protocol or surveillance biopsy specimens taken at predetermined times for evaluation of the status of the renal allograft, independent of renal function, are currently the standard of care at several leading transplant centers[59,159,226,234,298,315] and are widely used in clinical trials to evaluate efficacy.[52] Protocol biopsy specimens have the potential ability to reveal mechanisms of late graft loss and to identify active processes that might be interrupted therapeutically before irreversible injury has occurred. The risk of protocol biopsy is low. There were no deaths or graft losses in the Hannover series of more than 1000 biopsies,[313] and graft loss was 0.04%.[99]

The current interest in protocol biopsies started with Rush and colleagues,[298-300] who observed that 30% of biopsy specimens from stable patients 1 to 3 months after transplantation showed histological rejection,[299] and biopsy specimens with these lesions show later loss of renal function.[298,300] Many other studies have confirmed this result.[59,159,226,234,298,315] Mononuclear inflammation that meets the Banff criteria for acute cellular rejection or borderline acute rejection is found in 5% to 50% of protocol biopsy specimens in the first 12 months, depending on therapy and patient populations.[236] Grafts with inflammation have a higher risk of graft dysfunction or fibrosis at later time points.[59,159,226,234] Grafts with inflammation and fibrosis have the worst prognosis.[59,226,323]

In one study, the best predictor of allograft function 1 year after transplantation was persistent inflammation, of any type, including patterns considered in Banff to be irrelevant to the diagnosis of acute rejection (in areas of interstitial fibrosis, around large blood vessels, in nodules, or in subcapsular areas).[209] Infiltrates in areas of atrophy correlated with chronic allograft nephropathy at 6 months and graft dysfunction at 2 years. These results raise the possibility, or even the likelihood, that these infiltrates are part of the pathogenesis of slow, progressive renal injury.[52]

What differentiates infiltrates in patients with stable and unstable graft function? In stable grafts, endarteritis is found rarely (0.3% in one series)[208] and can herald an impending acute rejection episode.[299] Among interstitial infiltrates, only the diffuse pattern (rich in macrophages and granzyme B CTLs) was more common in biopsy specimens taken for acute dysfunction.[209] In contrast, nodular infiltrates (rich in B cells and activated T cells) were more common in protocol biopsy specimens. Similarly, infiltrates rich in activated macrophages distinguished biopsy specimens with clinical versus subclinical acute rejection.[115] Molecular studies have shown that increased levels of transcripts for T-bet (a T helper type 1 master transcription factor), Fas ligand (cytotoxic mediator), and CD152 (CTLA-4, an inhibitory costimulatory molecule) are associated with graft dysfunction.[132]

Grafts in recipients who are developing tolerance also typically have graft infiltrates, sometimes termed the acceptance reaction,[321] which spontaneously disappears and is followed by indefinite graft survival.[25,303] The acceptance reaction had less infiltration by $CD3^+$ T cells and macrophages, less T cell activation, long-lasting apoptosis of graft-infiltrating T cells, less interferon-γ, and more IL-10 than rejecting grafts.[25,322] More recent evidence shows that regulatory T cells that express the Foxp3 transcription factor infiltrate tolerated grafts in mice treated with costimulatory blockade.[175] Foxp3 cells also can be found in grafts with infiltrates interpreted as acute rejection.[365] Although the significance of $foxp3^+$ cells has yet to be determined, it is likely that high numbers of such regulatory T cells are beneficial, in view of the known suppressor functions of these cells. The hope of much ongoing research is the discovery of markers that predict graft acceptance in a clinical setting.[95]

Subclinical interaction of antibody with graft endothelium (accommodation) has been revealed by showing diffuse C4d in PTCs, found in 2% of routine protocol biopsy specimens[208] and a higher frequency among presensitized patients (17%) or patients with ABO-incompatible grafts (51%).[116] The stability of such accommodation has not been established. In nonhuman primates with MHC-incompatible grafts and no immunosuppression, C4d deposition predicts chronic rejection with glomerulopathy and arteriopathy and ultimate graft loss with a high degree of certainty.[333]

The most important question is whether treatment of subclinical rejection is beneficial (and then what therapy is optimal). No study has dared to randomize treatment in patients with acute rejection on protocol biopsy. The closest to a controlled trial was that of Rush and colleagues,[298] who found that patients with protocol biopsy, who were treated with steroid boluses if they had subclinical rejection, had a better outcome than a group of patients who declined a renal biopsy (and were presumed to have a similar frequency of subclinical rejection). Other diseases revealed by the "eye of the needle" clearly benefit from altered therapy, including calcineurin inhibitor toxicity[234,235] and polyomavirus infection.[35]

ACUTE TUBULAR NECROSIS

The morphological basis of delayed graft function is usually acute ischemic injury (acute tubular necrosis). The most common feature histologically is loss of the brush borders of proximal tubular cells, best shown on periodic acid–Schiff stain with focal interstitial edema and mononuclear cell accumulation (Fig. 24-7). The tubular lumen appears larger than normal and lacks the usual artifactual sloughing of the apical cytoplasm in human renal biopsy specimens (here sloughing has occurred in vivo and has washed downstream) (see Fig. 24-11). The other features of acute tubular necrosis include flattening of the cytoplasm and loss of cell nuclei owing to apoptosis/death of individual tubular epithelial cells and covering of the TBM by the remaining cells. The lumen contains individual apoptotic detached cells ("anoikis") and inflammatory cells.

Reactive changes in the tubular epithelium are seen after 24 to 48 hours, including large basophilic nuclei with prominent nucleoli, increased cytoplasmic basophilia, and occasionally mitoses. Focal interstitial, PTC, and glomerular capillary neutrophils may be seen but are not as prominent

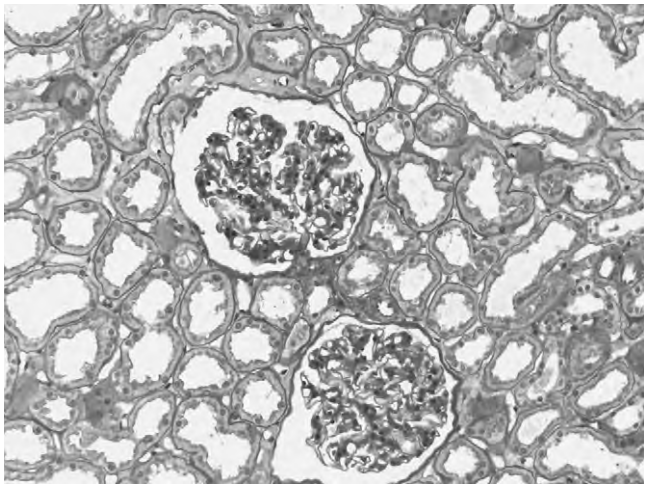


Figure 24–7 Acute tubular necrosis. Dilated, "rigid"–appearing tubular lumens with loss of brush borders, occasional loss of nuclei, and cytoplasmic thinning. Mild edema is present, but there is little inflammation. Glomeruli are normal (periodic acid–Schiff stain). (See color plate.)

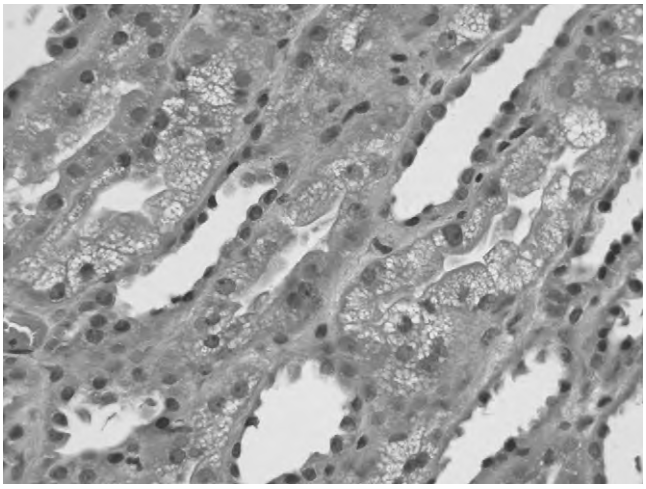


Figure 24–8 Acute calcineurin inhibitor nephrotoxicity with isometric vacuolization of tubular epithelium. This change also can be seen in other causes of tubular injury, including ischemia, osmotic diuretics, and intravenous immunoglobulin. (See color plate.)

as in acute humoral rejection, and C4d is negative. Mechanical flushing of cadaver donor kidneys with organ preservation fluid immediately before transplantation, which has been advocated by some authors, was associated with abnormal cellular debris within the tubules and eosinophilic proteinaceous material within Bowman's capsule and an increased frequency of delayed graft function.[291] Delayed graft function has other causes, and if function has not recovered in 1 to 2 weeks, a diagnostic biopsy is recommended to ascertain the presence of occult acute rejection, found in 18% of patients with delayed graft function at 7 days.[149]

CALCINEURIN INHIBITOR NEPHROTOXICITY

The calcineurin inhibitor class of drugs, including cyclosporine and tacrolimus, causes acute and chronic nephrotoxicity that includes ischemic injury without morphological features, vacuolar tubulopathy, acute endothelial injury (thrombotic microangiopathy), and arteriolar hyalinosis.[219,220] Secondary pathological effects, such as tubular atrophy, interstitial fibrosis, and global or segmental glomerulosclerosis, also occur. As judged by protocol biopsy specimens, chronic calcineurin inhibitor toxicity is universal in renal transplants after about 5 years.[234] Chronic calcineurin inhibitor toxicity also can damage native kidneys in patients with other organ transplants and contributes to the 7% to 21% prevalence of end-stage renal disease in nonrenal transplant recipients after 5 years.[259]

Acute Calcineurin Inhibitor Toxicity

Toxic Tubulopathy

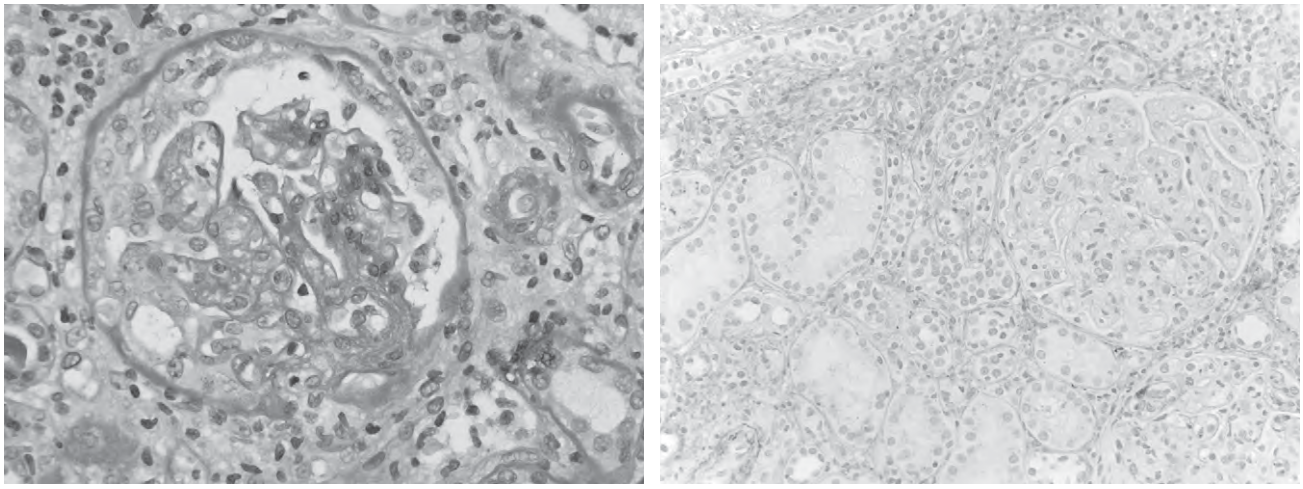
The biopsy features of acute toxicity vary. A normal biopsy specimen is found in functional calcineurin inhibitor toxicity, which is due to reversible vasospasm.[288] In toxic tubulopathy, proximal tubules show the most conspicuous morphological changes with loss of brush borders and isometric (uniformly sized), clear, fine vacuolization (or microvacuoles) in the epithelial cells (Fig. 24-8). The microvacuoles contain clear aqueous fluid rather than lipid. Electron microscopy shows that the vacuoles in cyclosporine toxicity are due to dilation of the endoplasmic reticulum and appear empty.[221] Isometric vacuolization may begin in the straight portion of the proximal tubule,[221] although it can extend to the convoluted portion. The degree of vacuolization does not correlate with drug levels; some patients with calcineurin inhibitor toxicity lack the vacuolar change,[237] and isometric vacuoles can be found in a few patients with stable renal function.[337] Reduction of the calcineurin inhibitor dosage causes disappearance of tubular vacuolization.[367]

Acute Arteriolar Toxicity and Thrombotic Microangiopathy

Arterioles are a significant target of calcineurin inhibitor toxicity. The most characteristic acute changes include individual medial smooth muscle cell degeneration, necrosis/apoptosis, and loss.[221] The apoptotic smooth muscle cells are replaced later by rounded, "lumpy" protein deposits or hyalinosis, which is the beginning of a more chronic arteriolopathy.[221] Accumulation of glycogen (periodic acid–Schiff–positive, diastase-sensitive) in smooth muscle cells has been described on high doses.[174] Endothelial cells can have prominent vacuolization and some swelling. Immunofluorescence microscopy of the vessels often shows deposits of IgM, C3, and sometimes fibrin/fibrinogen, but these changes are nonspecific.[22]

Thrombotic microangiopathy secondary to calcineurin inhibitor was first reported in bone marrow transplant recipients treated with cyclosporine[324] and occurs in about 1% to 4% of renal allograft recipients, even with careful attention to drug levels, suggesting that it is dose independent and probably idiosyncratic.[40,131] Most cases manifest with a delayed onset and a slow loss of function 1 to 5 months after transplantation.[338]

The pathological changes are believed to be an exaggeration of calcineurin inhibitor–induced endothelial and smooth muscle damage. The small arteries and arterioles have mucoid intimal thickening with acid mucopolysaccharides



A B

Figure 24–9 Thrombotic microangiopathy associated with calcineurin inhibitors. **A**, A glomerulus with widespread endothelial swelling, segmental glomerular basement membrane duplication, and focal collapse resembling a crescent. Arterioles show endothelial swelling and occasional peripheral hyaline nodules (periodic acid–Schiff stain). **B**, No glomerular or peritubular capillary C4d deposition is detected in this case (immunohistochemistry for C4d in paraffin, using rabbit polyclonal anti–C4d). (See color plate.)

and extravasated red blood cells and fragments; fibrinoid necrosis and thrombi may be prominent (Fig. 24-9). Apoptosis of endothelial and smooth muscle cells is seen. The medial smooth muscle can develop a mucoid appearance with loss of a clear definition of the cells.[239] The arterioles may show hypertrophy of the endothelial cells and have a "constricted" appearance.[239] The vascular lumens may be partially or completely obliterated by the intimal proliferation and endothelial swelling. The vascular lesions are most severe in the interlobular and arcuate sized arteries and can lead to cortical infarction.[338] By immunofluorescence microscopy, the vessels stain with IgM, C3, and fibrin.

The glomeruli typically have swollen bloodless capillaries with scattered fibrin-platelet thrombi (see Fig. 24-9), particularly in the hilum,[324] the so-called pouch lesion.[216] The endothelial cells are swollen and may obliterate the capillary lumens completely. The GBM is segmentally duplicated with cellular (mononuclear or mesangial cell) interposition best seen by electron microscopy, which also shows the loss of fenestrae and swelling of the endothelial cytoplasm. Variable mesangial expansion, sclerosis, and mesangiolysis[216] may be seen. Marked congestion and focal, global, or segmental necrosis can be present.[362] The affected glomeruli usually are supplied by an arteriole with calcineurin inhibitor arteriolopathy.[216]

Differential Diagnosis

Acute tubular toxicity of calcineurin inhibitors may be indistinguishable from ischemia and tubulopathy from intravenous immunoglobulin and mannitol, which all have vacuoles by light microscopy.[118] By electron microscopy, a coarser and more varied vacuolization is typical of acute tubular necrosis and the periphery of infarcts[215] compared with the isometric (uniform) vacuoles of calcineurin inhibitor toxicity. The vacuoles of osmotic diuretic injury do not involve the endoplasmic reticulum, as do those of calcineurin inhibitor toxicity.[216] Necrosis of tubular cells is more common in acute tubular necrosis (0.5% of tubules), characteristically involving whole tubular cross sections.[337] Acute medial apoptosis/degeneration in arterioles is the only definitive finding favoring acute calcineurin inhibitor toxicity.

Morphology alone cannot distinguish the various causes of thrombotic microangiopathy,[183] which in renal transplants are most commonly calcineurin inhibitor, acute humoral rejection, hepatitis C virus, and recurrent thrombotic microangiopathy. C4d deposition in PTCs is present in acute humoral rejection but absent in calcineurin inhibitor–associated thrombotic microangiopathy (see section on acute humoral rejection). Serum also should be tested for anti-HLA class I, anti-HLA class II, and antiendothelial antibodies. Hepatitis C virus–positive renal allograft recipients may develop thrombotic microangiopathy with associated elevation of circulating anticardiolipin antibody[16]; hepatitis serology and anticardiolipin antibody determination could help distinguish between hepatitis C virus and calcineurin inhibitor in the etiology of thrombotic microangiopathy. Recurrence is the first choice when the recipient's original disease was thrombotic microangiopathy, not associated with a diarrheal illness. The healing phase of thrombotic microangiopathy may leave intimal fibrosis that resembles chronic rejection, even with a few intimal mononuclear cells.

Chronic Calcineurin Inhibitor Toxicity

Irreversible chronic renal failure resulting from calcineurin inhibitor toxicity was first shown in native kidneys of heart transplant patients who received cyclosporine for more than 1 year.[228] Similar lesions arise in patients receiving tacrolimus.[284] Biopsy specimens showed interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, and sometimes focal glomerular scarring. These findings have been confirmed and extended in numerous other studies.[22] Because many features resemble chronic rejection in the kidney, the most convincing pathology data come from nonrenal transplant patients on cyclosporine.[75,252]

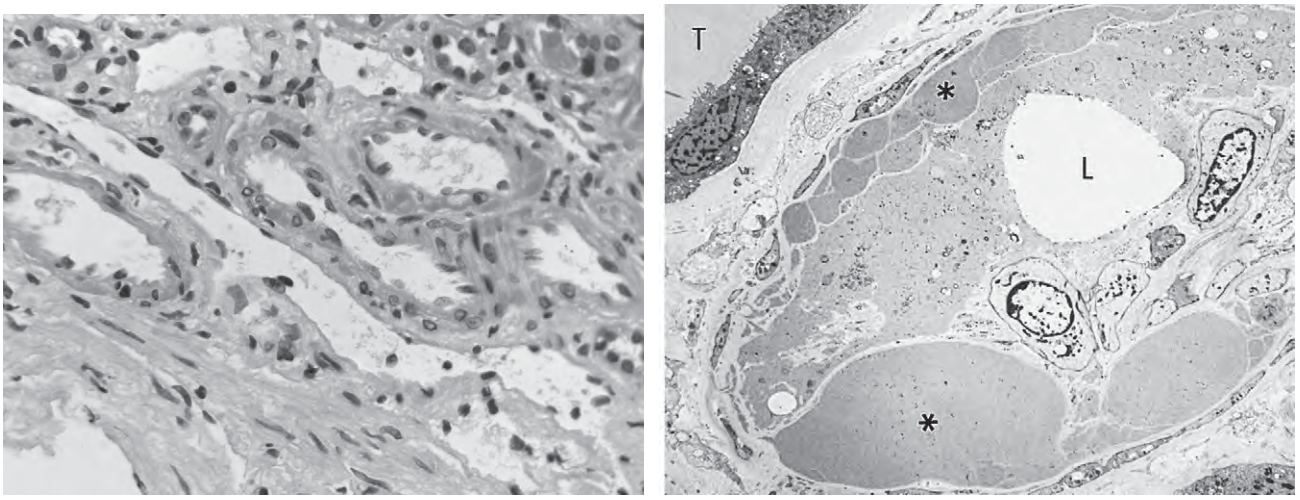


Figure 24–10 Calcineurin inhibitor arteriolopathy. **A**, Several arterioles with peripheral nodular hyalinosis, where hyalin deposits replace necrotic and apoptotic smooth muscle cells in the outermost media. (See color plate.) **B**, Electron microscopy. An artery that has "beads" of hyalin (*) along the outer media (periodic acid–Schiff stain 800×; electron microscopy 2700×). L, arteriolar lumen; T, tubule. (**A**, See color plate.)

Calcineurin Inhibitor Arteriolopathy

The chronic phase of calcineurin inhibitor arteriolopathy is characterized by replacement of the degenerated medial smooth muscle cells with hyaline-like deposits, in a beaded pattern along the peripheral, outer media (Fig. 24-10). This condition has been referred to as "nodular protein (hyaline) deposits"[218] in a "pearl-like pattern"[22] and "peripheral medial nodular hyalinosis" and now is called calcineurin inhibitor arteriolopathy. The current evidence supports the view that this type of arteriolopathy is almost specific for calcineurin inhibitors. In heart and bone marrow transplant recipient autopsy studies, 55% of patients receiving cyclosporine had this type of arteriolopathy in the native kidneys compared with 0% in patients not receiving cyclosporine.[252] Evidence of apoptosis sometimes is found in the form of karyorrhectic debris in the media, but fibrinoid necrosis is not observed.[229] In severe cases, the media is nearly devoid of smooth muscle cells.[229]

Electron microscopy reveals a distinctive replacement of individual smooth muscle cells of afferent arterioles with amorphous electron-dense material that contains cell debris and protrudes into the adventia (see Fig. 24-10B);[22,295,381] this gives rise to the beaded hyalinosis distribution in the outer media noted by light microscopy. The myocyte nuclei are sometimes condensed (apoptotic), or have two nuclei or mitotic figures.[381] The cytoplasm is vacuolated, with dilated endoplasmic reticulum, and has degenerated mitochondria, lipofuscin granules, multivesicular bodies, and a disarray of microfibrils and reduced intercellular junctions. The endothelium sometimes appears "swollen," protruding into and narrowing the lumen, and having reduced cell junctions; aggregates of platelets are rare.[13,381] These findings support the view that the smooth muscle myocyte of the afferent arteriole is a primary target of calcineurin inhibitor injury. Immunofluorescence microscopy shows IgM and C3 in a nonspecific, but conspicuous, sheathing of the arterioles.[22]

Calcineurin inhibitor arteriolopathy begins and predominates in the afferent arterioles, but it may progress to the small arteries and efferent arterioles.[22,381] Decreased renin immunostaining in the juxtaglomerular apparatus suggests that the prime target of calcineurin inhibitor is the renin-producing smooth muscle cell in the afferent arteriole.[345] The frequency of arterioles affected with hyalinosis is typically small (<15%), and the lesions can be overlooked easily.[346] In renal transplant patients receiving cyclosporine, 15% of protocol biopsy specimens at 6 months showed calcineurin inhibitor arteriolopathy; this increased to 45% in 18-month protocol biopsy specimens.[310] "Nonspecific" hyalinosis showed no progressive increase. The arteriolar lesions also develop in native kidneys of patients who receive even low doses of cyclosporine for 2 years.[271,384]

Sis and colleagues introduced a new scoring system of calcineurin inhibitor arteriolopathy with improved reproducibility: grade 1, calcineurin inhibitor arteriolopathy present in one arteriole, no circular involvement; grade 2, calcineurin inhibitor arteriolopathy present in more than one arteriole, no circular involvement; and grade 3, calcineurin inhibitor arteriolopathy with circular involvement independent of the number of arterioles involved.[331] Grading in this manner is valuable to establish therapeutic implications of the various signs of calcineurin inhibitor toxicity.

Glomerular Lesions

After 1 year on cyclosporine, glomeruli show increased numbers with global or segmental sclerosis.[75,252] Focal segmental sclerosis was more common in calcineurin inhibitor–treated bone marrow (13%) and heart transplant (27%) recipients at autopsy than the respective calcineurin inhibitor–free controls (0% and 14%).[252] Heart transplant recipients have an increase in the heterogeneity of glomerular volume and size, with more small and large glomeruli (compensatory hypertrophy), compared with controls (living kidney donors).[229] The shift to smaller glomeruli becomes more extreme with chronic renal failure, and the

hypertrophied glomeruli disappear.[227] Hyperfiltration injury probably causes the progressive glomerular proteinuria and sclerosis.

Bone marrow and heart transplant patients at autopsy show glomerular collapse in 59% of patients receiving calcineurin inhibitors versus 8% of patients not receiving a calcineurin inhibitor.[252] This glomerular collapse can develop into florid collapsing glomerulopathy, attributed to the severe calcineurin inhibitor arteriolopathy.[110] Immunofluorescence findings are nonspecific (IgM and C3 in scarred areas). Electron microscopy in cardiac and liver transplant recipients showed diffuse expansion of the mesangial matrix, with little hypercellularity, GBM lesions, or podocyte lesions.[75,229] Cases with frank collapsing glomerulopathy have podocyte foot process effacement and detachment of podocytes from the GBM.[110] The endothelium shows loss of its normal fenestrae, perhaps reflecting a component of thrombotic microangiopathy (personal observation).

Tubules and Interstitium

Tubular atrophy and interstitial fibrosis were recognized as a feature of calcineurin inhibitor toxicity in early studies.[354] The interstitium had prominent patchy fibrosis, with a scanty infiltrate. Band-like ("striped") narrow zones of fibrosis and tubular atrophy were previously regarded as characteristic of calcineurin inhibitor toxicity[87,294,326]; however, indistinguishable "stripes" occur in patients not maintained on calcineurin inhibitors,[70] casting doubt on the specificity of that pattern. Interstitial fibrosis also develops in native kidneys in patients on calcineurin inhibitors[217,264,384,385] and remains after the drugs are discontinued.[212] Even low doses can cause significant and presumably permanent loss of renal function by inducing chronic tubulointerstitial nephritis.

Differential Diagnosis

Distinguishing between chronic rejection and chronic calcineurin inhibitor toxicity is a challenge (Table 24-5). The finding that favors calcineurin inhibitor toxicity most decisively is arteriolopathy provided that it is distinctive (isolated smooth muscle cell degeneration and string-of-pearls replacement by hyalinosis in the outer media).[219] The arterioles are spared in chronic rejection compared with chronic calcineurin inhibitor toxicity, and the arteries are more affected, with proliferative intimal fibrosis without elastosis.[219] C4d deposits in PTCs or mononuclear cells in the arterial intima are the most useful signs of an active rejection process. An inflammatory infiltrate, including plasma cells, is less common in calcineurin inhibitor toxicity than in rejection.[232] Other features are not decisive. Interstitial fibrosis, tubular atrophy, and glomerular sclerosis are found in either condition. Duplication of the GBM and endothelial dedifferentiation also can be seen in either condition, although perhaps more commonly in chronic rejection.

Table 24–5 Differentiation between Chronic Rejection and Chronic Calcineurin Inhibitor Toxicity

	Chronic Rejection	Calcineurin Inhibitor Toxicity
Interstitium		
Infiltrate	Plasma cells	Mild
Fibrosis	Patchy	Patchy, "striped"
Peritubular Capillaries		
C4d	Often positive	Negative
Multilamination BM	Usual	Absent
Tubules		
Tubular atrophy	Usual	Usual
Vacuoles	Occasional	Occasional
Arterioles		
Smooth muscle degeneration	Absent	Usual
External nodular hyalinosis	Absent	Present
Arteries		
Intimal fibrosis	Usual	Can be present but unrelated
Mononuclear cells intima	Common	Absent
Glomeruli		
Duplication GBM	Usual	Absent
Mesangial expansion	Can be present	Can be present

BM, basement membrane; GBM, glomerular basement membrane.

MAMMALIAN TARGET OF RAPAMYCIN INHIBITOR TOXICITY

Inhibitors of the mammalian Target of Rapamycin (mTOR), including rapamycin, everolimus, and sirolimus, can cause delayed graft function as a result of tubular toxicity that resembles myeloma cast nephropathy. Pathologically, in addition to acute tubular injury, eosinophilic debris and macrophages are present in tubular lumens, which mimic myeloma casts, but the casts stain for keratin, rather than immunoglobulin light chains.[332] mTOR inhibitors also can cause thrombotic microangiopathy, indistinguishable from that caused by calcineurin inhibitor.[289]

Increased proteinuria is common in patients switched from calcineurin inhibitors to mTOR inhibitors because they had developed severe calcineurin inhibitor toxicity. In these patients, glomerular filtration rate improves, but increased proteinuria develops in about 30%, most commonly in patients with more severe preexisting proteinuria or interstitial fibrosis.[177] Calcineurin inhibitor exposure is unnecessary for the proteinuric response to mTOR inhibitors. Conversion from azathioprine to mTOR inhibitors also caused increased proteinuria in all seven patients with preexisting proteinuria and in none of the patients without proteinuria.[361] Patients started on mTOR inhibitors without calcineurin inhibitors had double the risk of proteinuria at 6 to 12 months compared with patients on calcineurin inhibitors.[339]

Few pathological studies have been published. One study reported a variety of glomerular diseases typical of native kidneys (membranoproliferative, membranous, and IgA glomerulonephritis), suggesting recurrent disease.[76] A recipient begun on mTOR inhibitors developed 12 g/day proteinuria in the first week after transplantation, which remitted after the drug was discontinued.[342] A biopsy specimen showed that no obvious glomerular disease was evident by light,

immunofluorescence, or electron microscopy, suggesting that the proteinuria was due to failure of tubular reabsorption. One notable case report described collapsing glomerulopathy in a patient with Kaposi's sarcoma converted to mTOR inhibitors from azathioprine.[147] We have seen two cases of focal segmental glomerulosclerosis in patients started on mTOR inhibitors; one had collapsing glomerulopathy (Cornell LD, et al: unpublished, 2006). More pathology studies are needed, particularly in patients started on mTOR inhibitors.

DRUG-INDUCED ACUTE TUBULOINTERSTITIAL NEPHRITIS

Drug-induced interstitial nephritis in the allograft is similar to that in the native kidney and resembles tubulointerstitial rejection. Both are characterized by an intense mononuclear interstitial infiltrate and tubulitis and have variable numbers of eosinophils. Acute rejection occasionally has a prominent eosinophilic infiltrate[7,120,134,169,352,373]; conversely, drug-induced interstitial nephritis may have no eosinophils, especially when due to nonsteroidal anti-inflammatory drugs.[56] Endarteritis, if present, is unequivocal evidence for rejection. Strong, but not absolute, evidence for a drug cause is the invasion of multiple tubules by eosinophils and eosinophils in tubular casts (personal observation), usually attributed to prophylactic trimethoprim/sulfamethoxazole (Bactrim). We also have seen one case of severe acute interstitial nephritis and serum sickness–like syndrome secondary to horse antithymocyte globulin.

INFECTIONS

Many organisms can infect the transplanted kidney, ranging from *Mycobacterium* and *Candida* species[204] to herpes simplex virus[328] and human herpesvirus type 1.[328] In addition, viruses such as cytomegalovirus and hepatitis C virus can have indirect effects on the transplant promoting rejection or immune-mediated disease.[60,290,359] We discuss here the three most important types of infections—polyomavirus, adenovirus, and bacterial pyelonephritis.

Polyomavirus

Polyomavirus tubulointerstitial nephritis has emerged since 1996 as a significant cause of early and late graft damage.[77,78,193,242,246,265] Among various series of patients taking tacrolimus/mycophenolate mofetil, polyomavirus tubulointerstitial nephritis arises in about 5%, similar to the prevalence of acute rejection. The virus originally was isolated from B.K., a Sudanese patient who had distal donor ureteral stenosis, 3 months after a living related transplant.[105] BK virus is related to JC virus (which also inhabits the human urinary tract) and to simian virus SV40. These viruses are members of the papovavirus group, which includes the papillomaviruses. BK virus commonly infects urothelium, but rarely causes morbidity in immunocompetent individuals. In renal transplant recipients, three lesions have been attributed to BK virus: hemorrhagic cystitis, ureteral stenosis,[45,106,133] and interstitial nephritis.

Polyomavirus tubulointerstitial nephritis is characterized by a patchy mononuclear infiltrate associated with tubulitis and tubular cell injury.[265] The infiltrate often contains plasma cells, which sometimes invade the tubules (Fig. 24-11). Concurrent acute cellular rejection may be present. Tubular cell apoptosis and "dedifferentiation" of tubular epithelial cells, with loss of polarity and a spindly shape, are prominent. Three stages of polyomavirus tubulointerstitial nephritis have been recognized: Stage A has only minimal inflammation; stage B shows marked tubular injury, denudation of the TBMs, and interstitial edema with a mixed, mild-to-marked inflammatory cell infiltrate; and stage C has marked fibrosis and tubular atrophy.[78,79,130,245,246]

The recognition of viral nuclear inclusions is the key step in diagnosis. The affected nuclei are usually enlarged with a smudgy, amorphous lavender inclusion (see Fig. 24-11B). Other nuclear changes found less commonly are eosinophilic, granular inclusions with or without a halo and a vesicular variant with coarsely clumped, irregular basophilic material.[242,243,247] These nuclear inclusions tend to be grouped in tubules, particularly collecting ducts in the cortex and outer medulla, and can often be spotted at low power. Immunohistochemistry and electron microscopy confirm the diagnosis. Monoclonal antibodies are commercially available that react with BK-specific determinants and with the large T antigen of several polyoma species (see Fig. 24-11C). Electron microscopy reveals the characteristic intranuclear paracrystalline arrays of viral particles of about 40 nm diameter (see Fig. 24-11D). Other tests useful for monitoring patients at risk are urine cytology ("decoy cells") and polymerase chain reaction quantitation of virus in the blood, although these are not specific enough to make a diagnosis of polyomavirus tubulointerstitial nephritis.

A newly appreciated feature of polyomavirus infection is that it may cause immune complex deposition along the TBM. This condition was described in 43% of cases in a series from Seattle and was the most common cause of IgG deposits in the TBM of transplants.[28] Granular IgG, C3, and C4d are focally present by immunofluorescence and amorphous electron-dense deposits by electron microscopy. The prognostic significance is unknown, but it is unlikely to be beneficial.

Late graft fibrosis and scarring chronic allograft nephropathy may be caused by polyomavirus, even though the virus is no longer demonstrable. The virus is cytopathic for tubular cells and leads to characteristically destructive tubular lesions, with only TBM remaining. The diagnosis is sometimes possible only by review of prior biopsy samples. Suspicion of polyomavirus tubulointerstitial nephritis is heightened if tubular destruction is severe. The process may be clinically silent; protocol biopsy specimens have shown a subclinical incidence of polyomavirus tubulointerstitial nephritis of 1.2%.[35] Polyomavirus tubulointerstitial nephritis can affect native kidneys of recipients of nonrenal allografts; only a few cases have been reported, but this may be due partly to a presumption of calcineurin inhibitor toxicity and a lack of renal biopsies in this setting.[179] Alternatively, the virus may cause disease by activating rejection or vice versa.

Adenovirus

Adenovirus, most frequently serotype 11, causes hemorrhagic cystitis and occasionally tubulointerstitial nephritis in renal allografts, which may resemble a space-occupying lesion by imaging studies.[178,380] The biopsy specimen shows necrotizing inflammation with neutrophils and tubular

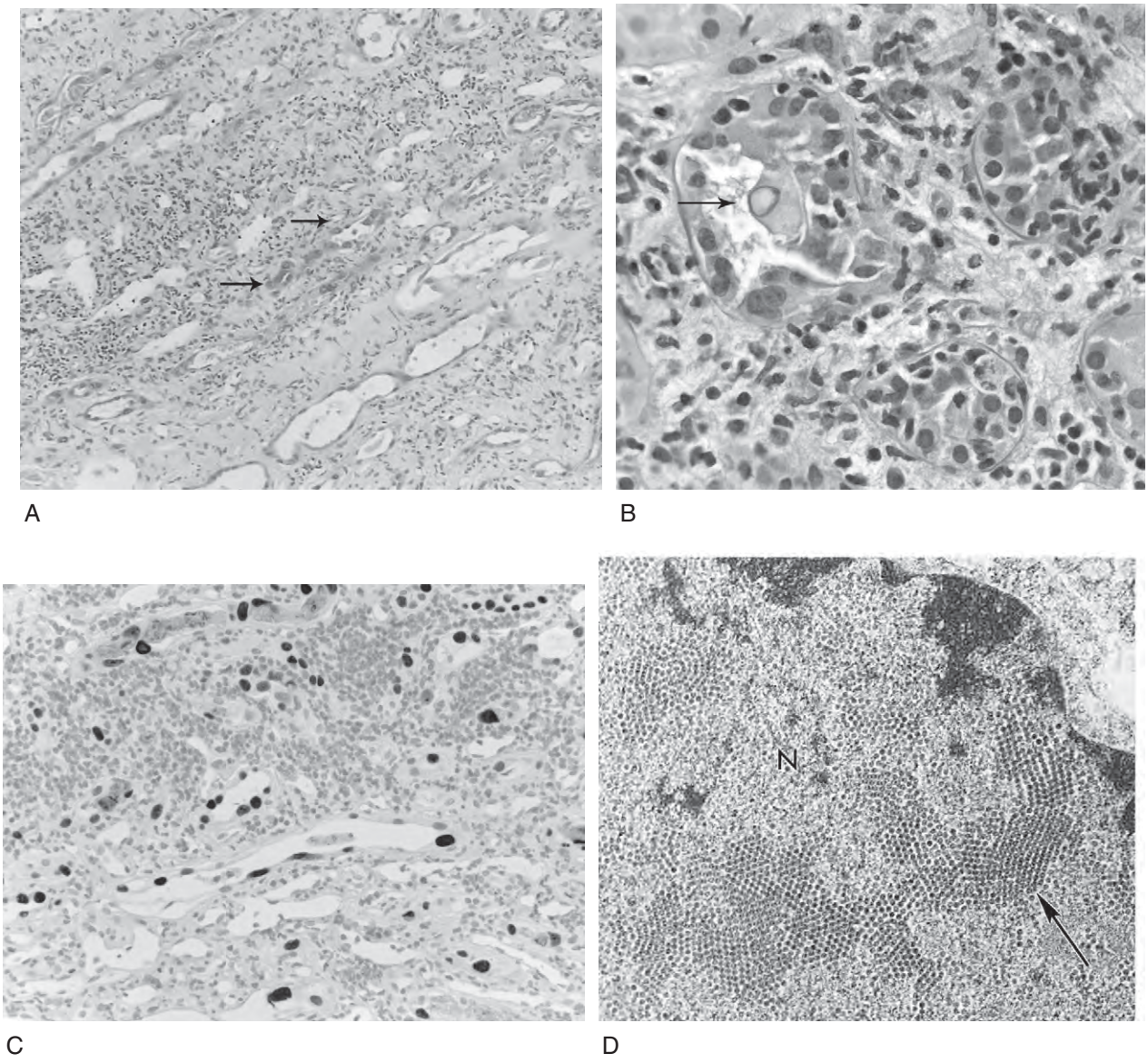


Figure 24–11 Polyoma (BK) virus infection. **A**, Low-power view showing patchy mononuclear inflammation in the medulla with groups of atypical nuclei in tubular epithelium (*arrows*). **B**, Higher power view shows polyomavirus inclusion (*arrow*), marked tubulitis, and tubular cell apoptosis. **C**, Immunohistochemistry. Monoclonal antibody to SV40 large T antigen (homologous to BK, JC, and other polyomaviruses). Many tubular epithelial cell nuclei appear dark brown because of immunoreactivity for polyomavirus. **D**, Electron microscopy. High magnification of a tubular cell nucleus (N) containing polyoma virions (*arrow*), which are rounded, 30 to 35 nm in diameter, and organized in arrays (from Cynomolgus monkey[363]). (**A-C**, See color plate.)

destruction, interstitial hemorrhage and red blood cell casts, granulomatous inflammation,[32,143,241,330] or a zonal inflammation localized to the outer medulla.[192] Tubular cells have intranuclear ground-glass inclusions with a distinct halo surrounded by a ring of marginated chromatin and glassy smudged nuclei. The diagnosis is established by immunoperoxidase stains for viral antigen in tubular cells and electron microscopy to reveal the intranuclear crystalline arrays of 75- to 80-nm viral particles. Immune complexes also may contribute to the injury. Decreased immunosuppression has been followed by recovery.

Acute Pyelonephritis

Pyelonephritis is a potentially devastating complication of transplantation. Pyelonephritis can manifest as acute renal failure[107,382] and cause graft loss.[123,153] Pyelonephritis arises most often 1 year or more after transplantation (80% of episodes).[270] *Escherichia coli* is the most common organism (80%). Acute pyelonephritis is a common finding in renal biopsy specimens, despite the expectation that the process is patchy.[382] Renal biopsy is not the usual method of diagnosis; however, if neutrophils are abundant, especially if they form destructive abscesses and casts in tubules, the diagnosis should be at the top of the list. Other variants are emphysematous pyelonephritis owing to gas-producing organisms,[153] xanthogranulomatous pyelonephritis,[84,152] and malacoplakia.[340]

MAJOR RENAL VASCULAR DISEASE

Most arterial thromboses develop in the early post-transplant period and produce acute infarction with microthrombi and

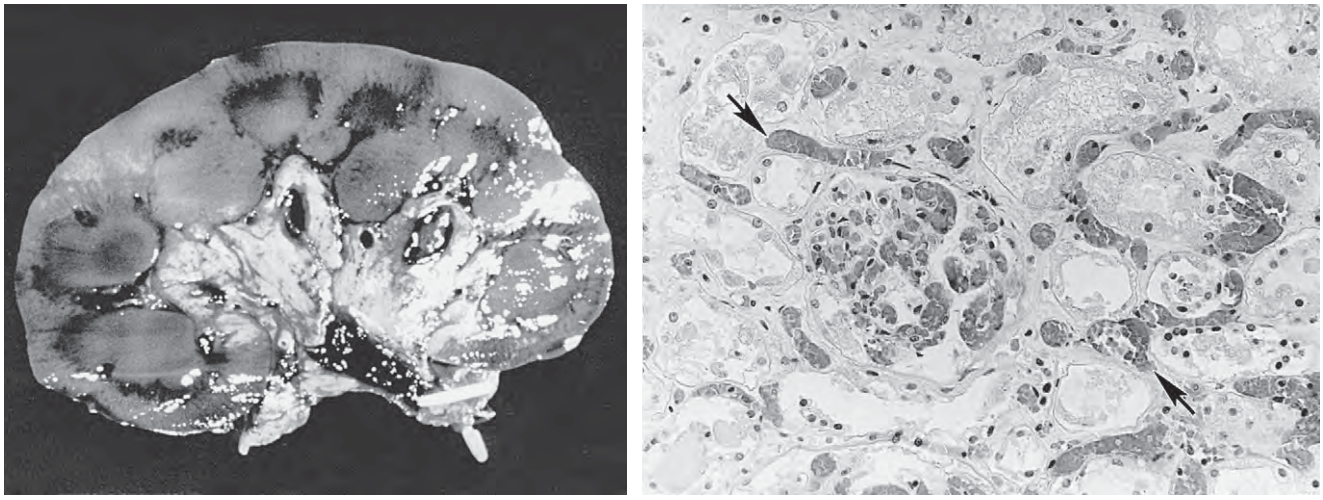


Figure 24–12 Renal vein thrombosis. **A**, Gross specimen of a renal allograft nephrectomy with thrombi in renal veins, hemorrhage, and infarction of the renal parenchyma, including cortex and medulla. **B**, Light microscopy shows cortex, congested peritubular capillaries (*arrows*), necrotic tubules, and congested glomerular capillary loops (hematoxylin and eosin 250×).

scant inflammation.[17] Evidence for underlying rejection should be sought by careful examination of the larger arteries for endarteritis. Renal artery stenosis (typically at the anastomosis site), a cause of late graft dysfunction, can be deceptive clinically and pathologically.[33,329] Biopsy specimens show acute tubular injury or atrophy with relatively little inflammation or fibrosis.

Renal vein thrombosis causes a swollen and purple kidney (Fig. 24-12A). The cortex shows severe hemorrhagic congestion (see Fig. 24-12B) and extensive infarction and necrosis,[211] sometimes with diffuse microcapillary thrombi. Intracapillary leukocytes can be a clue as in native kidneys. Graft rupture may occur.[307] Late renal vein thrombosis is associated with proteinuria secondary to membranous glomerulonephritis or transplant glomerulopathy, sometimes with graft loss.[314] Lupus anticoagulant has been detected in a few patients.[189]

DE NOVO GLOMERULAR DISEASE

Patients without previous glomerular disease occasionally develop lesions in the allograft that resemble primary glomerular disease, rather than the usual chronic transplant glomerulopathy. Although some lesions may be coincidental, at least three are related to an alloimmune response to the allograft: membranous glomerulonephritis, anti-GBM disease in Alport's syndrome, and recurrent nephrotic syndrome in congenital nephrosis. A fourth common de novo glomerular disease, focal segmental glomerular sclerosis, is believed to be related to hyperfiltration injury of the allograft or marked microvascular compromise as a result of calcineurin inhibitor toxicity.

Membranous Glomerulonephritis

De novo membranous glomerulonephritis is typically a late complication, with a prevalence of about 1% to 2%. The risk factors for de novo membranous glomerulonephritis include time after transplant, de novo membranous glomerulonephritis in a first graft,[126] and hepatitis C virus infection.[65,224] Light microscopy usually shows mild GBM changes. Mesangial hypercellularity is found in about 33%. Mononuclear cells can be abundant in glomerular capillaries, raising the possibility of transplant glomerulitis or renal vein thrombosis.[222] Immunofluorescence shows granular deposits along the GBM that stain for IgG, C3, C4d, and factor H[62]; about 35% are more irregular and segmental in distribution than typical primary (idiopathic) membranous glomerulonephritis.[222,358] By electron microscopy, subepithelial electron-dense deposits are present (Fig. 24-13), which are smaller and more irregular in distribution than primary membranous glomerulonephritis.[222,358] Endothelial changes and GBM duplication typical of transplant glomerulopathy are present in half of the cases.[222,358] Repeat biopsy specimens have shown persistence or progression of the deposits in most

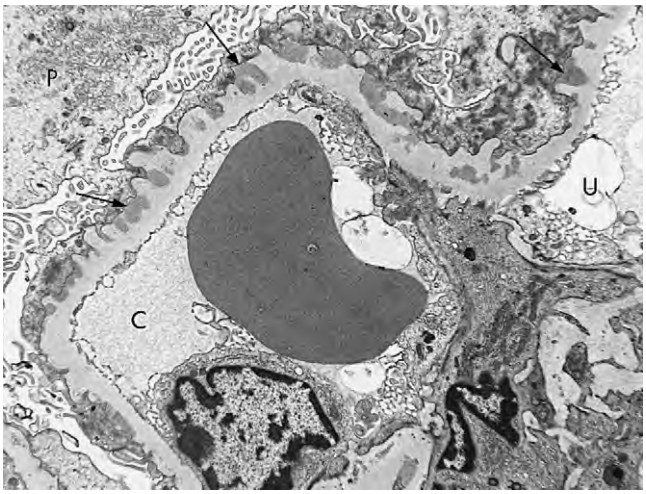


Figure 24–13 De novo membranous glomerulonephritis. Subepithelial electron-dense deposits (*arrows*) along the glomerular basement membrane with intervening basement membrane spikes. Podocyte (P) foot processes are effaced. C, capillary lumen; U, urinary space.

cases and occasionally resolution.[12,222] The pathogenesis of de novo membranous glomerulonephritis has not been established. The literature supports the hypothesis that de novo membranous glomerulonephritis may be a form of antibody-mediated rejection directed at minor histocompatibility antigens in the glomerulus, presumably on the podocyte, or a special type of chronic rejection.[51,355,358] The common presence of transplant glomerulopathy is consistent with this hypothesis.[222,358]

Anti–Glomerular Basement Membrane Nephritis

Patients with Alport's syndrome or hereditary nephritis commonly develop anti-GBM alloantibodies because they genetically lack self-tolerance to GBM collagen components; however, this leads to glomerulonephritis in only a few cases. Overall, de novo crescentic and necrotizing glomerulonephritis secondary to anti-GBM antibodies after transplantation is uncommon, seen in only 5% of adult male renal allograft recipients with typical Alport's syndrome.[155,156] The pathology is similar to that in native kidney with prominent crescents (not a feature of allograft rejection), segmental necrosis, and red blood cell casts. Second transplantations with and without recurrent anti-GBM nephritis have been reported.[73,111,364] The overall 5-year graft survival is equal to that of recipients without Alport's syndrome.[109]

De Novo Podocytopathy in Congenital Nephrosis

Congenital nephrotic syndrome of the Finnish type, an autosomal recessive disease caused by mutations in the nephrin gene *NPHS1*, paradoxically can lead to post-transplant nephrotic syndrome.[188,256] The podocyte pathology resembles minimal change disease and usually responds to cyclophosphamide.[93,173] De novo minimal change disease is thought to be caused by the alloantibodies to nephrin, shown in four of nine patients.[268]

Focal Segmental Glomerulosclerosis

De novo focal segmental glomerular sclerosis has been described in adult recipients of pediatric kidneys,[240,378] in which the presumed pathogenesis is hyperfiltration injury; in long-standing grafts, in which parenchymal loss secondary to calcineurin inhibitor toxicity or chronic rejection leads to hyperfiltration injury of residual glomeruli; and as the collapsing variant of focal segmental glomerular sclerosis, probably related to calcineurin inhibitor arteriolopathy.[205] De novo collapsing glomerulopathy manifests months to years after transplantation with proteinuria (2 to 12 g/day).[205,231,341] Glomerular focal, global, or segmental collapse is evident with prominent hyperreactive podocytes (Fig. 24-14). Arteriolar hyalinosis, arteriosclerosis, and interstitial fibrosis also were present. A rapid progression to renal failure occurred in 80% of the patients (2 to 12 months). The cause is unknown; all patients were negative for human immunodeficiency virus. Collapsing glomerulopathy also can develop in native kidneys in patients receiving calcineurin inhibitors (see Fig. 24-14).[110]

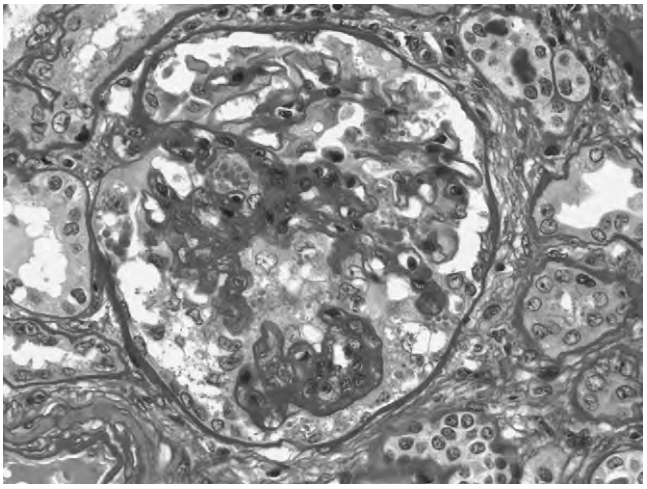


Figure 24–14 De novo collapsing glomerulopathy. Collapsed glomerular capillaries and prominent podocyte proliferation, hypertrophy, and abundant resorption droplets. Severe arteriolar hyalinosis with peripheral nodules typical of calcineurin inhibitor arteriolopathy was present. This is a native kidney in a patient with a heart-lung transplant (periodic acid–Schiff stain).[110] (See color plate.)

RECURRENT RENAL DISEASE

Recurrent disease is a significant cause of allograft failure, estimated to affect 1% to 8% of transplants.[39,92,281] Isografts (identical twins) have the highest recurrence rate attributed to the total lack of immunosuppression.[108] The frequency and clinical significance of recurrence varies with the disease (Table 24-6). At present, only primary focal segmental glomerular sclerosis and membranoproliferative glomerulonephritis recur with sufficient frequency and aggressiveness to affect graft survival.[29] Recurrence may become a greater problem in the future with longer graft survival and development of tolerance protocols that require no immunosuppression. The reader is referred to a comprehensive review for detailed information regarding specific diseases.[57]

Recurrence may be taken as strong evidence for a bloodborne etiological agent. Two idiopathic glomerular diseases were first shown to be caused by bloodborne factors by recurrence in the graft (focal segmental glomerular sclerosis and dense deposit disease). Conversely, failure to recur proves that the disease is intrinsic to the kidney or that the pathogenetic mechanisms are "burnt out" (anti–GBM antibody nephritis, lupus nephritis). For diseases such as anti-GBM disease, recurrence can be avoided by postponing transplantation for 6 to 12 months after the pathogenetic agent disappears from the serum (anti-GBM antibodies).[67] In patients with hemolytic-uremic syndrome, the prime risk factor in recurrence is the causative agent of the original hemolytic-uremic syndrome. Cases caused by infection present the lowest risk.[19,125]

Transplantation also can uniquely illuminate the early pathological events that precede clinical signs and determine the reversibility of preexisting lesions in the donor kidney (e.g., diabetes, IgA nephropathy). In dense deposit disease (Fig. 24-15), the glomerular electron-dense deposits can recur 3 weeks after transplantation, preceding C3 accumulation, and are not always symptomatic. Diabetic nephropathy begins with an increase in allograft glomerular volume at

Table 24–6 **Classification of Recurrent Renal Disease**

Usually Recur (>50% Patients)	
Adverse effect*	Primary hemolytic-uremic syndrome Primary oxalosis Dense deposit disease Collapsing FSGS†
Little or no adverse effect	Immunotactoid/fibrillary affect glomerulopathy† Systemic light chain disease† Diabetes mellitus‡
Commonly Recur (5% to 50%)	
Adverse effect	FSGS Membranoproliferative GN, type I Membranous GN ANCA-related diseases Wegener's granulomatosis Pauci-immune GN Microscopic polyarteritis Progressive systemic sclerosis Sickle cell nephropathy†
Little or no adverse affect	IgA nephropathy Henoch-Schönlein purpura Amyloidosis
Rarely Recur (<5%)	
Adverse effect	Anti-GBM disease
Little or no adverse affect	Systemic lupus erythematosus Fabry's disease Cystinosis
Recurrence Reported§	Thrombotic thrombocytopenic purpura Adenosine phosphoribosyl transferase deficiency Familial fibronectin glomerulopathy Lipoprotein glomerulopathy Malacoplakia
Never Recur (0%)	
Unique complications	Hereditary nephritis/Alport's syndrome (anti-GBM disease) Congenital nephrosis (nephrotic syndrome; nephrin autoantibody)
No unique complications	Polycystic disease (all genetic types) Osteo-onychodysplasia (nail-patella)† Acquired cystic disease Secondary hemolytic-uremic syndrome (infection) Secondary FSGS Familial FSGS† Postinfectious acute glomerulonephritis†

*Adverse effect defined as graft loss of >5% (when disease recurs).
†Limited experience: few cases reported (n <10).
‡Arteriolar and glomerular lesions recur to some degree in most, if not all, cases, but nodular glomerulosclerosis delayed until >5 years.
§Recurrence occurs, but too few cases are reported to classify frequency or consequences.
ANCA, antineutrophil cytoplasmic antibody; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; GN, glomerulonephritis.

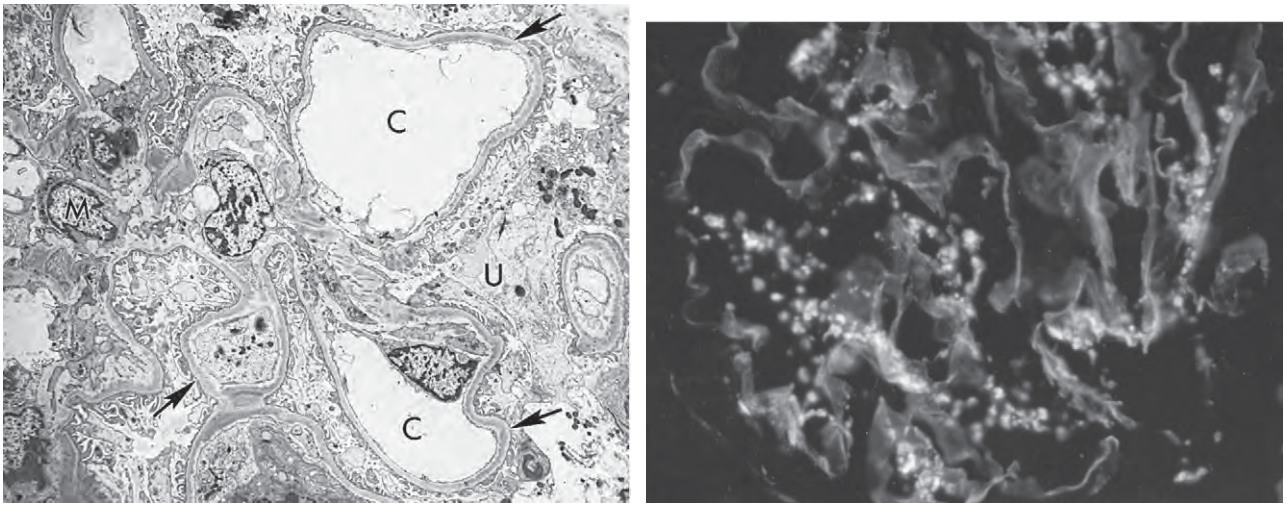
6 months,[261] followed by increases in mesangial volume.[375] Thickening of the GBM is first evident after 2 to 3 years,[26,375] and nodular diabetic glomerulosclerosis is evident at 5 to 15 years after transplantation (Fig. 24-16).[124]

POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISEASE

(see Chapter 33)

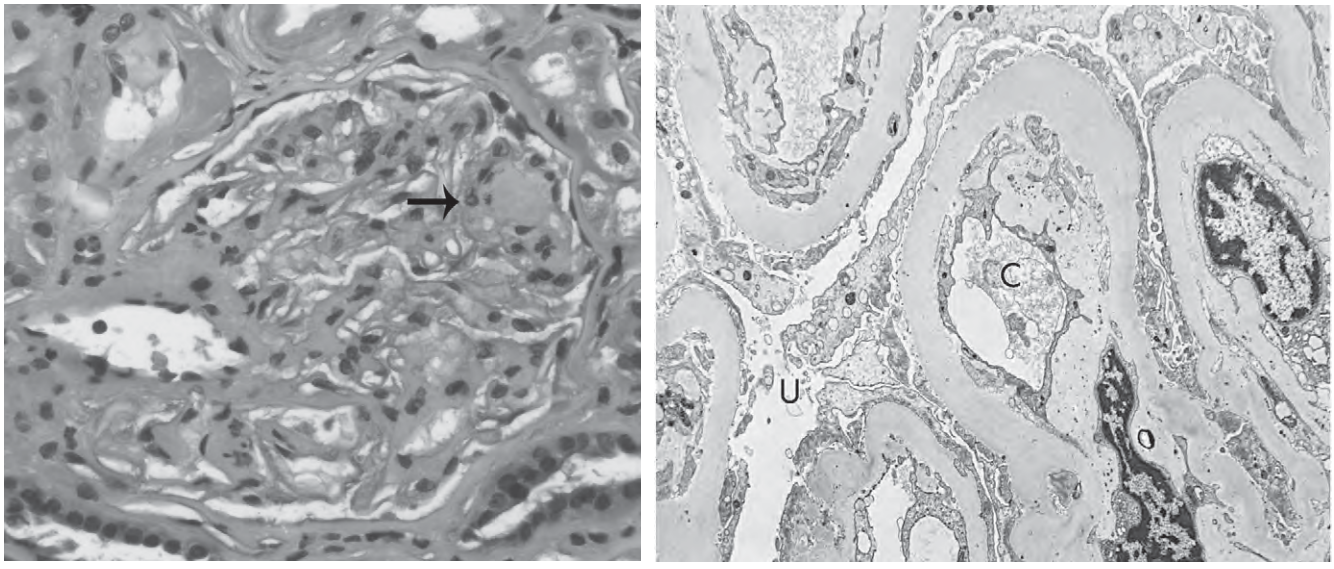
Immunosuppression leads to an increased risk of malignancy, particularly neoplasms caused by viruses and ultraviolet radiation. These malignancies are presumptively suppressed by an immune response that recognizes the viral-derived or mutation-derived neoantigens. The major viral-related tumors are Kaposi's sarcoma (human herpesvirus-8), cervical cancer (human papillomavirus), and PTLD (Epstein-Barr virus). Of these, PTLD commonly affects the kidney, sometimes manifesting as graft dysfunction.

PTLD involving the kidney can resemble acute cellular rejection, in having a widespread mononuclear infiltrate invading tubules and even vessels.[203,282,311] In our experience, a useful clue that favors PTLD is when the infiltrate forms a dense sheet of monomorphic lymphoblasts without edema or granulocytes (Fig. 24-17). Serpiginous necrosis of the lymphoid cells (irregular patches) is distinctive, but not always present.[282] Other features found to be helpful include nodular and expansile aggregates of immature lymphoid cells; the nuclei are enlarged and vesicular with prominent nucleoli that may be multiple. Immunohistochemistry is helpful in identifying the predominance of B cells in the



A B

Figure 24–15 Recurrent dense deposit disease. **A**, Electron microscopy. Widespread, very electron dense deposits that are continuous, linear, and embedded in the glomerular basement membrane proper (i.e., intramembranous) (*arrows*). Similar deposits also are seen in the mesangium (M). C, capillary lumen; U, urinary space. **B**, Immunofluorescence microscopy. Staining for C3 shows broad linear, ribbon-like deposits along the glomerular basement membrane and blob-like deposits in the mesangium (mesangial rings).



A B

Figure 24–16 Recurrent diabetic nephropathy 12 years after transplantation. **A**, Glomerulus with prominent Kimmelstiel-Wilson mesangial nodules (*arrow*) and arteriolar hyalinosis (periodic acid–Schiff stain). **B**, Electron microscopy of another case shows homogeneous thickening of the glomerular basement membrane of 1100 nm. C, capillary lumen; U, urinary space. (**A**, See color plate.)

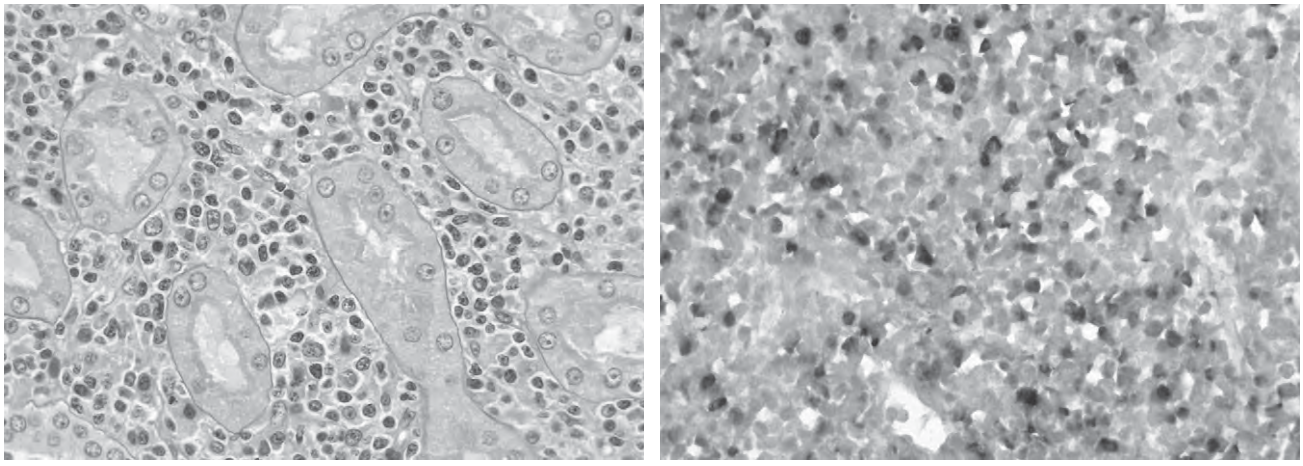


Figure 24–17 Post-transplant lymphoproliferative disease (PTLD). **A**, Dense mononuclear cell infiltrate in the interstitium that permeates between the tubules without tubulitis (although tubulitis may occur in PTLD). The monomorphic infiltrate and the lack of edema distinguish PTLD from the usual cellular rejection. **B**, In situ hybridization. Nuclei of mononuclear cells stain dark, brown-black for Epstein-Barr virus–encoded RNA, which is the definitive test for the diagnosis of PTLD. (See color plate.)

infiltrate, which is never seen in rejection alone. If the cells have a monoclonal K or λ phenotype, the diagnosis is confirmed. The definitive diagnosis of PTLD is made by in situ hybridization for Epstein-Barr virus–encoded RNA (see Fig. 24-17).

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Chapter 25
Chronic Allograft Nephropathy

Brian J. Nankivell

HISTORY

Chronic allograft nephropathy describes the pathology of tubular atrophy and chronic interstitial fibrosis of a chronically impaired renal allograft; the term was agreed on and promulgated by the Banff 1997 expert consensus.[47] The purpose was to provide an accepted universal grading and coding system that was practical and easy to implement, reproducible, and clinically predictive with acceptable sensitivity and specificity. The Banff working classification of renal transplant pathology incorporated the Chronic Allograft Damage Index and Cooperative Clinical Trials in Transplantation classification systems and was subsequently refined and developed (see also Chapter 24). Histological abnormalities within separate anatomical compartments are classified as either acute or chronic lesions (chronic lesions are prefixed by "c") and semiquantitatively scored using standardized definitions. Patterns of scored lesions, when supported by specific pathological features, are classified into a clinicopathological diagnosis, which is graded by severity.

"Chronic allograft nephropathy" was intended to replace the popular but misleading term of chronic rejection and originally designated a nonspecific entity characterized by tubulointerstitial damage. Within the current usage of renal transplant literature, however, its meaning has expanded to that of a collective term describing the overall pathology of a failing allograft. Both definitions are imperfect.

DEFINITION OF CHRONIC ALLOGRAFT NEPHROPATHY

The central histological abnormality defining chronic allograft nephropathy is the presence of chronic interstitial fibrosis and tubular atrophy (Fig. 25-1) (see also Chapter 24). Chronic allograft nephropathy is graded by the extent of the tubulointerstitial damage as follows: grade I—mild, incorporating 6% to 25% of the cortical area; grade II—moderate, incorporating 26% to 50% of the cortical area; and grade III—severe, incorporating greater than 50% of the cortical area. The standard definition of chronic allograft nephropathy recognizes nonspecific interstitial fibrosis and tubular atrophy and specific glomerular and vascular changes that imply an alloimmune cause, but it excludes specific diagnoses, such as calcineurin inhibitor nephrotoxicity, recurrent glomerulonephritis, and donor disease. These processes are recognized in a broad "other" category in the Banff schema; however, they may coexist within any biopsy specimen showing chronic allograft nephropathy.

The 1997 Banff classification broadly divides chronic allograft nephropathy into two subtypes. A nonspecific fibrotic ("sclerosing") subtype is characterized predominantly by tubular atrophy and chronic interstitial fibrosis (chronic allograft nephropathy type a). This is the more common subtype, but is etiologically nonspecific. The second subtype is characterized by additional glomerular and vascular features suggestive of immune-mediated chronic rejection, including glomerular capillary double contours and increased mesangial matrix (typified by transplant glomerulopathy) or fibrointimal hyperplasia in small muscular arteries (associated with intimal mononuclear cell infiltration,

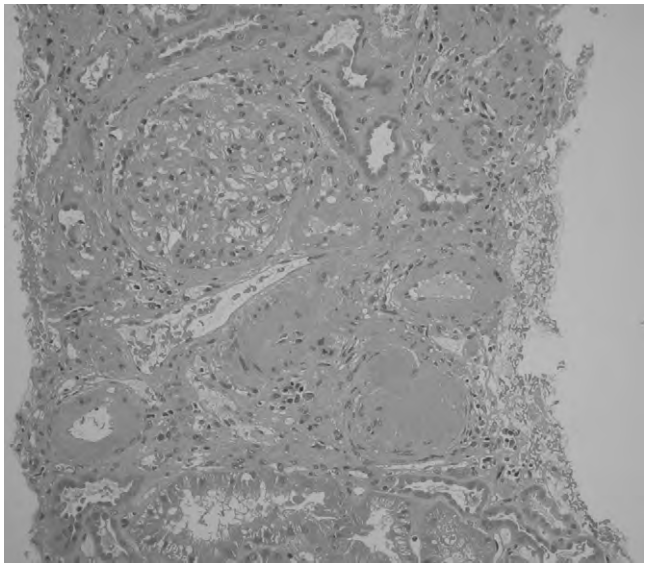

Figure 25–1 Chronic allograft nephropathy showing chronic interstitial fibrosis and tubular atrophy, accompanied by glomerulosclerosis, increased mesangial matrix, and vascular changes. (See color plate.)

neointimal formation, or internal elastic lamina disruption—chronic allograft nephropathy type b).

Several difficulties with the definition of chronic allograft nephropathy contribute to confusion in the interpretation of kidney transplant pathology. The first is that any grouped analysis of transplant pathology depends on the era from which it is derived. Changes with time in the histological material have occurred related to improvements in preservation and surgical techniques, immunosuppressive protocol, type of patient transplanted, proportion of marginal donors, older recipient age, recipient ethnicity, and overall immunological risk. The classic histology of "chronic rejection" was derived from the era when only weak immunosuppression using prednisolone and azathioprine therapy was available. The histology of a chronically failing allograft from that period often showed chronic interstitial lymphocytic infiltration, fibrointimal hyperplasia sometimes progressing to ischemic vascular narrowing of small muscular arteries, and glomerular changes such as double contours. The frequency of these abnormalities has declined with powerful calcineurin inhibitor therapy and a lower incidence of acute rejection and subclinical rejection (SCR), but at the expense of increased nephrotoxicity and infection with polyomavirus. The histological patterns of chronic allograft injury are likely to change further with the introduction and use of newer agents such as the mammalian Target of Rapamycin (mTOR) inhibitors (sirolimus and everolimus) and alemtuzumab.

The second problem is that chronic allograft nephropathy is not a homogeneous entity. It contains the common sclerosing form of tubulointerstitial damage (nonspecific and usually resulting from past injury) and transplant glomerulopathy and arteriopathy (specific as alloimmune markers defining chronic rejection processes). Each form of chronic allograft nephropathy represents distinct pathophysiological processes.

The pathology of a failing allograft often shows mixed histology and pathophysiology. Chronic allograft nephropathy should be understood as a collection of end pathway responses to injury within various anatomical compartments (tubulointerstitial, microvascular, and glomerular), each with a differential occurrence and rate of progression, but expressed by tissues with a limited repertoire of response (Table 25-1). Because tubulointerstitial damage is the final result of multiple previous insults, assigning a specific etiological diagnosis presents a practical difficulty for pathologists—especially if the allograft is approaching end stage. Several drivers of nephron damage and fibrosis may operate simultaneously, although the relative mix alters with time after transplantation.

Nonspecific histology is common. Any kidney transplant pathology may have multiple and overlaid causes, which may be difficult to separate, especially in the absence of prior histology. Despite this, all efforts should be made to evaluate any morphological features leading to a specific etiological diagnosis. Currently, the Banff consensus[48] has moved toward separation of the nonspecific chronic/sclerosing allograft nephropathy (preferably expressed as tubular atrophy/chronic interstitial fibrosis not otherwise specified) from the more specific diagnoses, such as chronic active antibody-mediated rejection (morphologically expressed as transplant glomerulopathy) and true chronic cell-mediated interstitial

Table 25–1 Causes of Allograft Damage (Events and Risks)

Nonimmune
Deceased donor
Older donor age, donor vascular disease, and extended criteria donor
Donor brain death and autonomic storm, inotropic use, donor renal failure
Ischemia-reperfusion injury (warm and cold ischemia times, perfusion and organ transport)
Delayed graft function (clinical) and acute tubular necrosis (biopsy)
Ascending urinary tract infection with allograft pyelonephritis
Transplant ureteric obstruction
Polyomavirus nephropathy
Calcineurin inhibitor nephrotoxicity
Recurrent or de novo glomerulonephritis
Hypertension
Proteinuria
Hyperlipidemia
Recipient smoking
Alloimmune
Young recipient age
Ethnicity
Altered handling of immunosuppressive agents (pharmacokinetics)
Variable trough levels (malabsorption or compliance)
Therapy noncompliance
Histoincompatibility, CREG mismatches
Recipient presensitization (panel-reactive antibodies)
Hyperacute rejection (rare)
Early antibody-mediated acute rejection
Acute rejection (severe or steroid-resistant, vascular, late, or undiagnosed/untreated)
Subclinical rejection
True chronic rejection with fibrointimal vascular hyperplasia
Late de novo anti-HLA antibody formation
Chronic antibody-mediated rejection with transplant glomerulopathy

CREG, cross-reactive groups.

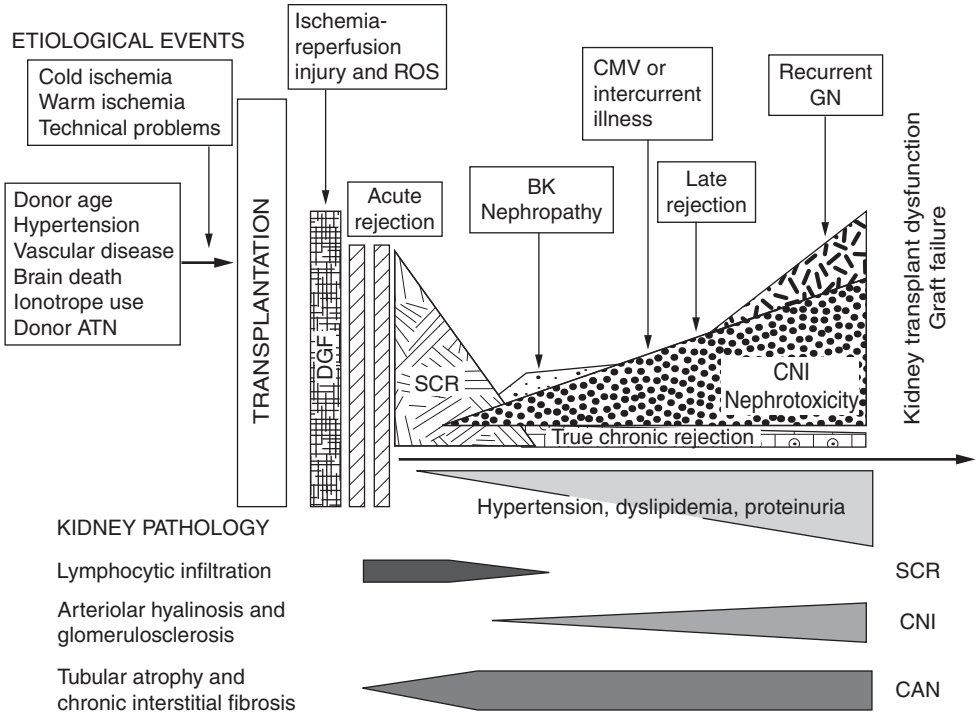

Figure 25–2 Immune and nonimmune events leading to allograft damage and transplant failure. ATN, acute tubular necrosis; CAN, chronic allograft nephropathy; CMV; cytomegalovirus; CNI, calcineurin inhibitors; DGF, delayed graft function; GN; glomerulonephritis; ROS, reactive oxygen species; SCR, subclinical rejection.

rejection, and from other specific processes, such as calcineurin inhibitor toxicity, hypertensive changes, BK viral nephropathy, bacterial infection, and recurrent disease.

PATHOPHYSIOLOGY OF ALLOGRAFT DAMAGE

Chronic allograft nephropathy represents the summated effects of tissue injury from several pathogenic insults combined with the kidney's healing response to injury, which is influenced by alloimmunity and immunosuppression (Fig. 25-2; see Table 25-1). To explain the pathophysiology of chronic allograft nephropathy, several unified hypotheses and specific additional pathophysiological mechanisms supplementing the injury processes have been proposed (although these paradigms are not mutually exclusive).

Major Theories of Kidney Transplant Damage

Chronic Rejection

Originally, allograft damage was thought simply to represent alloimmune injury to the transplanted kidney and correspondingly designated as "chronic rejection." This pattern of lymphocytic infiltration with characteristic vascular and glomerular changes was commonly described in the prednisolone-azathioprine era.[24] Currently, the assumption that immune-mediated injury causes allograft damage is not generally supported by biopsy evidence in compliant patients receiving modern immunosuppression, in whom the risk of acute rejection and SCR has been reduced to less than 15%; by risk factor profiling showing alternative factors are important[42,61]; and by the unchanged long-term graft survival despite lower acute rejection rates and stronger antirejection therapies. True chronic rejection may be relevant, however, in the modern era with immunologically active or noncompliant recipients; with excessive prescribed reductions of immunosuppressive therapy (e.g., following the diagnosis of cancer or late infection); when chronic low-level alloimmune activity is histologically manifested by persistent cellular interstitial inflammation and fibrointimal hyperplasia; or with transplant glomerulopathy associated with circulating donor-specific antibody and tissue C4d.

Input-Stress Model

The input-stress model is a composite model that describes the interaction between the starting "input" of the transplanted kidney (the overall quality or condition of the organ and early events including procurement, preservation, and reimplantation injury) with a series of subsequent immune and nonimmune stresses, including cellular infiltration; antibody-mediated alloimmunity; and other nonimmune ("load") mechanisms, including hypertension, hyperfiltration, proteinuria, dyslipidemia, nephrotoxic drugs, and infection. These stressors have been postulated to drive cells from a normal state into a senescent phenotype, exhaust repair processes, and deplete the finite nephron supply, leading to graft failure.[22]

Cumulative Damage Hypothesis

The cumulative damage hypothesis is based on sequential observational pathology and assumes that chronic allograft nephropathy is the end result of a series of time-dependent immune and nonimmune insults inflicted on the transplanted kidney, resulting in permanent nephron damage.

The number of nephrons within the transplanted kidney is finite, and nephrons, after destruction, cannot be replaced, although hypertrophy of remaining nephrons may occur to compensate partially for losses. Nephron damage results in tubular atrophy with loss of height of the tubular cross section, loss of nuclei, and dilation of the tubular lumen, associated with the deposition of chronic interstitial fibrosis. Activated cellular infiltrate within areas of tubular scarring may aggravate later damage and fibrosis further. The transplanted kidney gradually fails from the summated and incremental loss of individual nephrons, combined with additional internal structural damage leading to overall organ malfunction.

Additional Mechanisms of Injury

Allograft damage is mediated by a multitude of alloimmune, ischemic, and inflammatory stimuli, yielding lethal or sublethal tubular injury with a profibrotic healing response. Tubulointerstitial injury is accompanied by active fibrogenesis or tubular epithelial loss and atrophy with chronic interstitial fibrosis. Multiple pathways and mediators result in cumulative structural damage to all compartments within the transplanted kidney (see Fig. 25-2). Additional mechanisms of injury are outlined next.

Pathophysiological Stressors

Many factors and stressors proven to be important in progression of chronic kidney diseases have been postulated as contributors to progressive transplant damage. These mechanisms include hyperfiltration, proteinuria, hypertension, smoking, hyperlipidemia, reactive oxygen species (ROS) production, and excess cytokine production. Although evidence is largely circumstantial, biological plausibility supports their treatment when appropriate, pending controlled human trials and mechanistic studies.

The cytokine excess theory postulates that chronic allograft nephropathy is due to acute and repeated tissue injury inducing excessive cytokine production (e.g., interferon-γ), leading to interstitial and vascular fibrosis (by transforming growth factor [TGF]-β1). A role for other mediators, such as vascular endothelial growth factor, endothelin-1, plasminogen-activating factor-1, monocyte chemoattractant protein-1, platelet-derived growth factor A and B, RANTES, and advanced glycation end products, is supported by their altered expression in experimental and human chronic rejection or graft fibrosis.[4,13,20,46,62] Similarly, uncontrolled or excessive ROS production from tubular cell mitochondria may cause cellular injury, apoptosis, and expression of a senescent phenotype. Studies have shown that interstitial inducible nitric oxide synthase protein expression, nitrotyrosine, and ex vivo ROS production are increased in chronic allograft nephropathy.[1] The nephrotoxic injury from calcineurin inhibitor therapy also constitutes an important and continuing nonimmune stressor of the kidney allograft.[12,17,38,42,46,61]

The hyperfiltration theory implies that when individual nephrons are progressively lost, the metabolic load and tubular protein reabsorption from the ultrafiltrate falls onto a diminishing number of remaining nephrons. Hyperfiltration with glomerular hypertension can result in further tubular and glomerular damage, although the human evidence is weak. Estimates of single nephron hyperfiltration in transplant recipients are only modestly increased after transplantation, being partially ameliorated by reduced overall transplant glomerular filtration rate (GFR) and single nephron load. Results of graft survival in donor-recipient size mismatch have been contradictory or nonsupportive, with many studies showing no effect. Registry data show no effect on change in calculated GFR and lack of an expected inflexion point of progression of renal dysfunction at lower GFR.[19] The classic hyperfiltration lesions of focal segmental glomerulosclerosis are uncommon. Hyperfiltration may have a deleterious effect only when substantial glomerular loss has occurred, such as in advanced chronic allograft nephropathy or when a small infant donor kidney is transplanted into a large adult, so its overall contribution seems minor.

Proteinuria is a powerful composite risk factor as a marker of kidney damage and has been implicated in tubular injury from ultrafiltration of toxic substances, cytokines, and other mediators. Urinary protein excretion greater than 0.5 g/day has been associated with progressive graft dysfunction and failure[14,66] and may be due to glomerular protein leak (glomerular proteinuria) or failed tubular reabsorption from atrophic tubules (tubular proteinuria), or both.

Hypertension is common before and after transplantation and has been associated with graft failure using registry analysis, although direct histological evidence linking it to chronic allograft nephropathy is limited. Chronic hypertensive changes recognizable in a kidney transplant include fibrointimal thickening with duplication of the internal elastic lamina in small muscular arteries, arteriolar hyalinosis, and ischemic glomerulosclerosis. In transplanted rats with renal artery clips, the induced renovascular hypertension exacerbated vascular intimal thickening with increased TGF-β, platelet-derived growth factor, and tumor necrosis factor (TNF)-α compared with isografted kidneys, which showed only medial hypertrophy.[29]

Failure to Resolve Chronic Inflammation

Normal wound healing after acute injury usually results in self-limited healing with complete resolution of the inflammatory and fibrogenic process. Fibrosis in the allograft differs from normal healing in that repeated episodes of acute injury occur, which may be followed sometimes by a partial resolution of inflammation. An ongoing cycle of nonspecific injury causing tubular inflammation, enhanced allorecognition, and additional immune-mediated injury is created—becoming self-perpetuating and failing to resolve. Persistent chronic inflammatory cells are commonly observed within areas of atrophic tubules and fibrosis and, along with SCR, have been associated with progressive functional impairment, reduced graft survival, and increased tubular damage in sequential biopsy studies.

Epithelial-Mesenchymal Transition–Induced Fibrosis

With the exception of the distal collecting duct, the tubular cells of the kidney are derived from fetal mesenchyme, undergoing transition to cells of an epithelial phenotype during development of the embryonic kidney. These cells retain their ability to back-differentiate or "transition" into mesenchymal cells with the appropriate stimuli, potentially providing a source of interstitial fibroblasts. Sublethal tubular injury or exposure to stimuli such as TGF-β1,

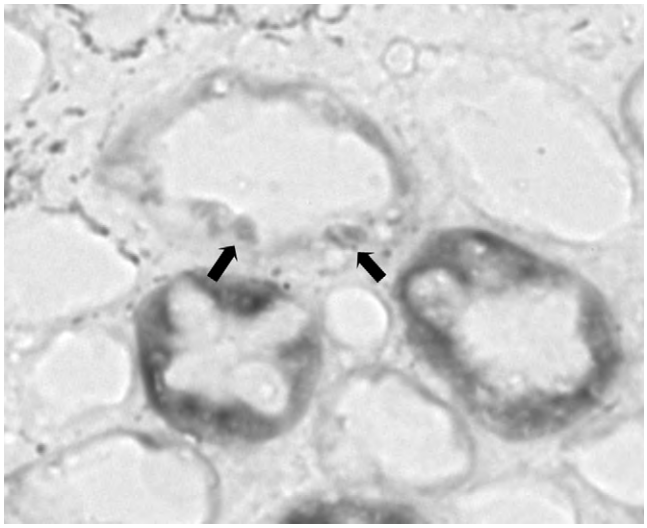

Figure 25–3 Epithelial mesenchymal transition illustrated by dual staining of E-cadherin (blue) and α smooth muscle actin (brown) in a tubular epithelial cell. (See color plate.)

hypoxic injury, or interleukin-1 may be followed by a series of genetically programmed and orchestrated steps initiated by impaired cell-to-cell adhesion and loss of the tubular cell's morphogenetic clues and signals. Transition from tubular epithelial cells into myofibroblasts can begin with loss of tight junctions and adherent junctions, desmosomes, and E-cadherin (an epithelial marker). This is followed by reorganization of F-actin stress fibers and de novo expression of α smooth muscle actin (a mesenchymal marker), filopodia, and lamellipodia for movement controlled by molecular reprogramming of the cell (Fig. 25-3). The metalloproteinases (matrix metalloproteinase-2 and matrix metalloproteinase-9) and membrane assembly inhibitors could disrupt the basement membrane and allow the cell to migrate into the interstitial space, followed by generation of matrix proteins, collagen, and fibronectin. Epithelial-mesenchymal transition may be potentially reversible; surviving cells can repopulate injured denuded tubules with new functional epithelia (so-called mesenchymal-to-epithelial transition). This transition is controlled by a different series of cytokines and growth factors, such as bone morphogenetic protein-7.

Evidence for a role of epithelial-mesenchymal transition in kidney allograft fibrosis is increasing with cross-sectional observational studies.[67] The relative importance of this mechanism of fibrosis remains to be defined, however, against the established contribution of resident or infiltrating fibroblasts. The latter was proved to be important by human studies of sex-mismatch donor-recipient pairs in which interstitial fibroblasts were shown to be of recipient origin by Y chromosomal DNA analysis.[21]

Replicative Senescence

Cellular replicative senescence is the aging process occurring in normal cells that eventually leads to cellular exhaustion. Stress-induced replicative senescence has been considered as a mechanism of graft failure because of the poor actuarial graft survival from older donor kidneys, even when other risk factors are statistically controlled.[22,35,36] Cultured somatic cells usually stop cycling and become senescent after a fixed number of doublings, known as the Hayflick limit. This "mitotic clock" is controlled in humans by telomeres, which are DNA repeats at the ends of chromosomes that shorten with each mitotic division. As the cell repeatedly divides, the telomeres progressively shorten, leading to arrest in the G_1 phase of the cell cycle and a senescent phenotype. The enzyme, telomerase, can maintain telomere length allowing cell immortality, but at the risk of accumulating mutations from genetic mistakes with repeated divisions. Shortened telomeres have been observed in native and transplanted older kidneys (driven by oxidative stress and aging), but with little evidence in human chronic allograft nephropathy.

Senescent cells have altered shape and cytoskeletal collagen, increased tumor-suppressor genes, senescence-associated β-galactosidase activity, and deposition of lipofuscin, an aging pigment. Other markers of the senescent cellular phenotype may be more important and are overexpressed in diseased kidneys and transplants with chronic allograft nephropathy.[36] These markers include cyclooxygenase-1, heat shock protein A5, and the cyclin-dependent kinase inhibitors, $p21^{CIP1/WAF1}$ and $p16^{INK4a}$ within the ATM/p53/p21 and Ras/p38/p16 pathways—predominantly within the tubulointerstitial compartment. Although there is evidence for a senescent phenotype in chronic allograft nephropathy, this is not mediated by telomere shortening and acceleration of the biological clock, but rather by altered expression of cell cycling pathways.

Alternative explanations for the poor outcomes from older donor kidneys include a differential response to injury with age, an impaired ability to withstand stress (e.g., reduced antioxidants and capacity to neutralize ROS), and a limited ability to repair damage once incurred. A final explanation is that preexisting structural abnormalities commonly present in older kidneys amplify external insults, for example, older donor fibrointimal vascular narrowing may exacerbate downstream glomerular ischemia from superimposed calcineurin inhibitor–induced arteriolar hyalinosis and vasoconstriction.

Cortical Ischemia

Tubular cells are downstream from efferent arterioles of the glomerular tuft supplied by the peritubular capillary (PTC) network. Tubular cells are rich in mitochondria powering the electrolyte pumps and endocytotic protein reabsorption machinary. These metabolically active cells are susceptible to ischemia from upstream vascular narrowing—caused by partial or total glomerulosclerosis, arteriolar hyalinosis induced by calcineurin inhibitors and other factors, fibrointimal hyperplasia, hypertension, or donor changes in small muscular arteries.

Injury of the PTCs can be seen with activation and nuclear swelling of endothelial cells, loss of fenestrae, and apoptosis and cellular detachment from the basement membrane, finally leading to collapse and occlusion of the capillary.[25] Cross-sectional studies have associated chronic allograft nephropathy with progressive loss of the PTC network and small muscular arteries, endothelial cell apoptosis, and lamination of the basement membrane.[25] Attenuation of the microvasculature occurred regardless of the cause of chronic allograft nephropathy and was present in chronic cellular rejection, $C4d^+$ chronic rejection, and sclerosing chronic allograft nephropathy. Greater allograft damage paralleled loss of PTC surface area, allograft dysfunction,

and proteinuria.[25] In experimental ischemic acute renal failure, early and permanent rarefaction of the PTC network occurred in the inner stripe of the outer medulla, followed by tubulointerstitial fibrosis and reduced urinary concentrating ability. Although current human evidence is consistent and reproducible, it cannot distinguish cause from effect—whether microvascular loss causes localized tubular ischemia and interstitial fibrosis, whether tubular loss reduces supportive angiogenic factors, or whether angioregression associated with chronic allograft nephropathy is a paraphenomenon reflecting a common insult.

Internal Architectural Degradation

Function within a transplanted organ may be impaired by structural damage at the level of the individual nephron or the intact kidney. Major damage to any component along the nephron causes functional failure of the whole unit. Glomerular damage may manifest as global or partial glomerulosclerosis, transplant glomerulopathy, or the formation of atubular glomeruli, which develop after severe irreversible damage and disconnection of downstream tubules. Tubular malfunction may occur because of localized apoptosis to individual tubular cells, tubular atrophy involving the tubular cross section, or luminal obstruction from cellular debris.

In addition, the transplant kidney may malfunction from internal architectural disruption, leading to loss of ability to modify the tubular ultrafiltrate to form concentrated and acidified urine. Segmentally injured glomeruli may form adhesions attached to Bowman's capsule (synechiae), which potentially can misdirect the glomerular ultrafiltrate into paraglomerular or paratubular channels leading to the interstitial space.[6,28,71] Inflammatory necrosis tends to progress to obliterative fibrosis during healing, with loss of tubular basement membrane integrity and reduced overall functional efficiency. Functional failure of the transplanted kidney is a combination of the summated loss of individual nephrons with additional disturbance of its internal architecture.

PROGRESSION OF HISTOLOGICAL DAMAGE

The pathway of progression from donor kidney to end-stage disease comprises a time-dependent series of pathological insults causing histological injury that is sequentially overlaid on earlier stages of damage (Fig. 25-4). There are two broad phases of allograft damage observed by sequential biopsy studies—starting with early tubulointerstitial injury[30,44,61] followed by later microvascular and glomerular abnormalities and further progressive fibrosis and tubular atrophy.[42]

Most tubular loss and chronic interstitial fibrosis begins soon after transplantation involving mechanisms of ischemia-reperfusion injury, with acute rejection and SCR, and a component of calcineurin inhibitor nephrotoxicity. Later, tubular injury is less intense and may be driven by residual subclinical alloimmune mechanisms, BK virus nephropathy, or calcineurin inhibitor nephrotoxicity accompanied by glomerular, microvascular, and capillary histological changes.

Donor Abnormalities

By definition, inherited donor changes do not constitute chronic allograft nephropathy; however, they strongly influence subsequent allograft structure, graft function, graft response to injury and, ultimately, long-term graft survival. Implantation biopsy histology is needed to define accurately the contribution of donor disease and is recommended as a standard of care. Important donor pathological features include the extent of glomerulosclerosis (>20% is severe, and these kidneys are often discarded), glomerulomegaly (with implied nephron loss and hyperfiltration), and microvascular disease (a persistent histological abnormality associated with donor age, hypertension, and death from cerebrovascular disease).

Early Phase of Tubular Injury and Interstitial Fibrosis

The early changes in the transplanted kidney reflect contemporaneous events inflicted on the donor kidney in situ (e.g., older age, mode of brain death, presence of donor vascular disease, donor renal failure, and use of inotropic agents), at organ procurement (e.g., prolonged warm and cold ischemia times, quality of perfusion, and organ transport), and after implantation (e.g., anastomosis time, recipient sensitization, occurrence of early rejection, delayed graft function, and early immunosuppressive toxicity). In chronic rejection models, cold ischemia causes tubulointerstitial injury, whereas alloreactivity results more in vasculopathy and glomerulosclerosis, illustrating the differential effects on renal structure according to the type and mechanism of injury.

Procurement and Ischemic Allograft Injury

Donor brain death influences graft outcome by nonspecific effects and by potentiation of graft immunogenicity and alloresponsiveness. The importance of brain death is supported by registry data showing excellent and identical survival rates of living unrelated and one haplotype-matched living related donor kidneys, despite genetic and HLA differences, compared with cadaver donor transplants. The transplanted organ is not inert but can be immunologically altered by a cascade of proinflammatory mediators released by brain death, leading to cellular infiltration of the allograft with increased acute rejection episodes.[31] Experimental brain death provokes production of chemokines, cytokines, proinflammatory lymphokines (TNF-α, interferon-γ), and adhesion molecules (intercellular adhesion molecule, vascular cell adhesion molecule, leukocyte function–associated antigen 1), and expression of major histocompatibility complex (MHC) class I and class II antigens, which trigger a more rapid and intense host alloimmune response.

The "autonomic storm" generated by brain death is accompanied by chaotic blood pressure fluctuations—initially with a hypertensive phase from brainstem herniation and massive circulating catecholamine release, followed by hypotension from hypothalamic-pituitary dysfunction, diabetes insipidus, electrolyte abnormalities, reduced thyroid and cortisol levels, hypothermia, core temperature dysregulation, pulmonary changes, and coagulopathies. Systemic hypotension, cardiovascular instability, and adrenergic vasoconstriction may lead to ischemic acute tubular necrosis. Other histological abnormalities associated with brain death include early glomerular hyperemia, glomerulitis, periglomerulitis, endothelial cell proliferation, tubular vacuolation from osmotic agents (e.g., mannitol), and later tubular degeneration

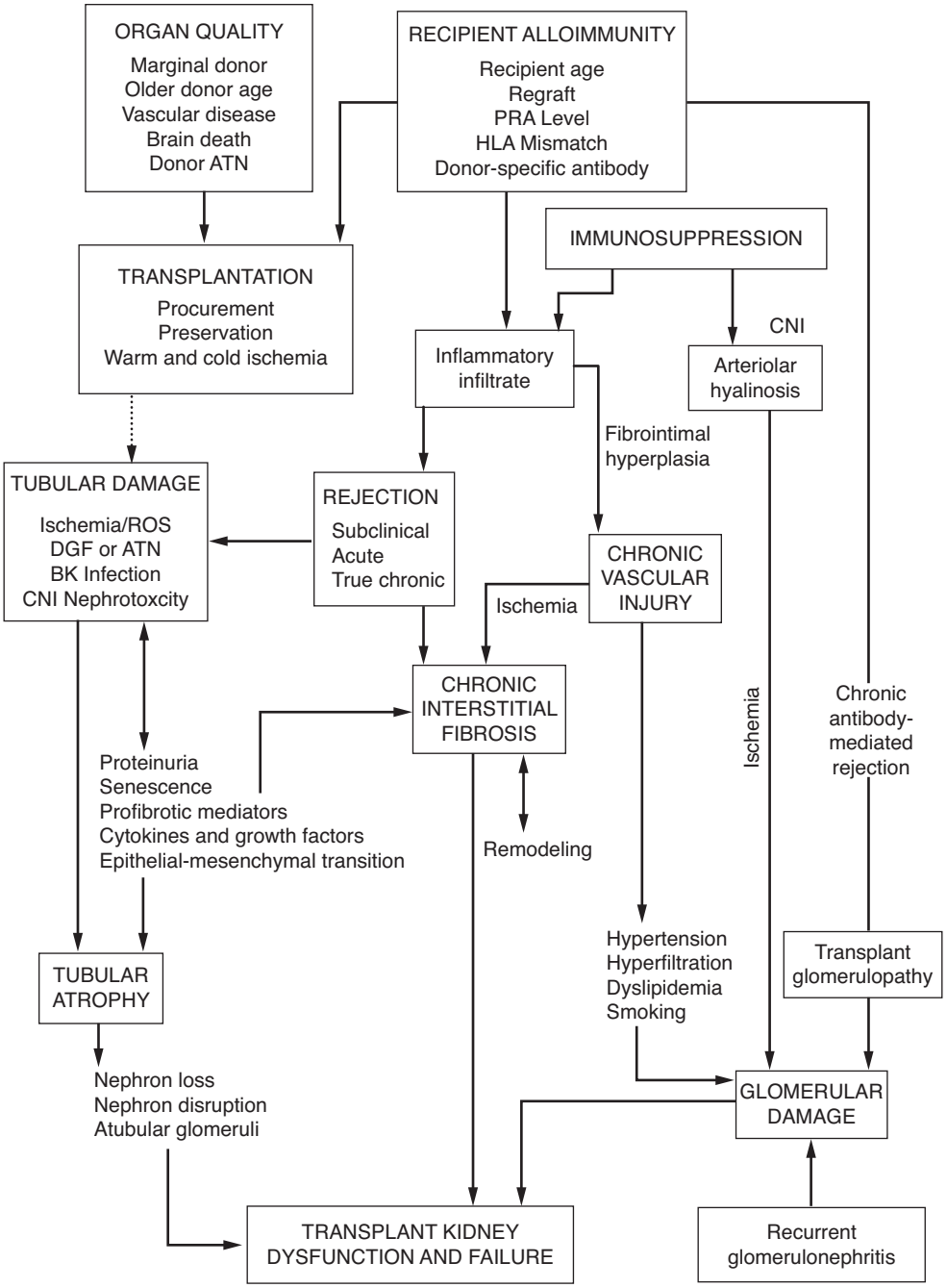

Figure 25–4 Interaction between donor organ quality, transplantation events, and immunosuppression on differing histological compartments leading to allograft damage. ATN, acute tubular necrosis; CNI, calcineurin inhibitors; DGF, delayed graft function; HLA, human leukocyte antigen; PRA, panel reactive antibodies; ROS, reactive oxygen species.

with intracellular biochemical disturbances, necrosis, and atrophy. Transplant dysfunction is greatest from hemodynamically unstable donors experiencing prolonged hypotension after brain death. Strategies to reduce the proinflammatory state and graft immunogenicity may improve transplanted organ quality and function.

Early Tubular Damage

Injury to tubular cells soon after kidney transplantation occurs from many factors, including ischemia-reperfusion injury, acute tubular necrosis, acute rejection and SCR, polyomavirus, and calcineurin inhibitor nephrotoxicity, superimposed on donor disease. Calcineurin inhibitor nephrotoxicity also may contribute to tubular injury with isometric vacuolization, patchy necrosis with microcalcification, and cytoplasmic inclusion bodies that represent giant mitochondria with abnormal cristae. Early interstitial fibrosis may be increased by calcineurin inhibitor therapy compared with sirolimus-treated grafts. Alloimmune mononuclear infiltration increases profibrotic factors including TGF-β and the expression of the tissue inhibitor of metalloproteinases (TIMP) family of enzymes in kidney tissue.

Ischemic tubular injury may recover if the basement membrane remains intact and sufficient residual tubular cells survive to replenish the nephron. Injury beyond this threshold results in permanent tubular damage and nephron loss. Repair of tubular injury is initiated by inflammatory and fibrogenic signaling followed by interstitial infiltration of mononuclear cells and macrophages and variable proliferation of fibroblasts. Tissue remodeling occurs with deposition of extracellular matrix. Chronic allograft nephropathy is the sequela of tubular injury combined with the kidney's interstitial fibrotic response and is clinically accompanied by proteinuria, hypertension, allograft dysfunction, and shortened graft survival.

The extracellular matrix is a dynamic network of proteins and proteoglycans, which accumulate from increased synthesis and decreased breakdown; this is partially mediated by TGF-β1, angiotensin, and the type of immunosuppressive therapy. Cyclosporine generates a profibrotic cytokine profile with increased TGF-β1 and TIMP-1, leading to interstitial fibrosis in humans and experimental models.[3,5,27] Abrogation by angiotensin II blockade suggests renin angiotensin system mediation and a potential treatment modality. In contrast, cell cycle inhibitors, such as mycophenolic acid, reduce interstitial cellular proliferation, myofibroblast infiltration, and collagen deposition in vivo and in experimental chronic rejection. Early evidence shows that sirolimus also limits tubular atrophy, vascular hyperplasia, and possibly the extent of interstitial fibrosis.[16]

Acute Rejection and Alloimmune Mechanisms

Acute rejection episodes have been a constant risk factor for reduced graft half-life and actuarial graft survival (especially cadaver donors). As acute rejection incidence decreases with newer immunosuppression, the individual impact of rejection is enhanced (with more severe rejection remaining), but the overall predictive ability for eventual chronic allograft nephropathy and true chronic rejection becomes diluted. Most recipients with chronic allograft nephropathy have not experienced any clinical acute rejection previously.

Other important alloimmune risk factors for graft loss include recipient sensitization and HLA matching (see Chapter 10). The MHC is the principal target of the alloimmune response, with reduced registry graft survival seen with HLA mismatching, even with modern immunosuppression. Cross-reactive groups share MHC class I antigen epitopes, and mismatch increases acute and chronic rejection (by 62%) and graft dysfunction. Cross-reactive group sharing improves long-term graft survival. Antibodies to HLA antigens may be provoked by blood transfusions, pregnancy or miscarriage, or prior transplantation. These can be tested in serum against a panel of HLA-typed leukocytes (as panel-reactive antibodies). Sensitization against anti–HLA class I and anti–HLA class II increases rejection rates in HLA-mismatched transplants.

After renal transplantation, formation of de novo anti-HLA antibodies has been correlated with subsequent allograft failure from chronic rejection in prospective studies, suggesting a role for antibody-mediated graft loss. This role can be supported by $C4d^+$ biopsy specimens in failing allografts with chronic rejection or transplant glomerulopathy. Antibodies against nonclassic HLA antigens (e.g., endothelial cells, glomerular antigens such as heparin sulfate, and renal basement membrane) also may be important. Younger transplant recipients have a more robust immune system with a greater antibody response to blood transfusion. Increased drug metabolism in pediatric and African American recipients, altered drug dosing schedules, appropriate choice of immunosuppression, and compliance verification are needed to address inferior graft survivals in high-risk groups.

The influence of alloimmune factors contributing to chronic allograft nephropathy depends on the type, timing, severity, and persistence of rejection episodes. When diagnosed and treated promptly, acute interstitial cellular rejection usually resolves without sequelae. In contrast, episodes of vascular or steroid-resistant rejection, recurrent rejection, untreated SCR, true chronic interstitial rejection, or late rejection (usually defined as >3 months after transplantation) can contribute to the burden of allograft damage. Uncontrolled acute or alloimmune inflammation may be followed by chronic damage within the same histological compartment in a later biopsy specimen. Silent interstitial cellular rejection increases later interstitial fibrosis, and episodes of vascular rejection can be followed by later chronic vascular damage.

Subclinical Rejection

SCR is histologically defined acute rejection characterized by tubulointerstitial mononuclear infiltration (Fig. 25-5) without concurrent functional deterioration (variably defined by a serum creatinine <10%, <20%, or <25% of baseline values). It is diagnosed only on biopsy specimens taken per protocol, rather than indication-driven biopsy specimens, and is clinically distinct from acute rejection accompanied by rapid functional impairment. There is substantial variation in the reported frequency of SCR among studies, likely related to differences in patient-recipient immunological risk, HLA mismatch, prior acute rejection episodes, ethnicity, baseline immunosuppression protocol, era, and timing of the biopsy. The prevalence of SCR (acute rejection Banff grade 1a) in 3-month protocol biopsy specimens ranges

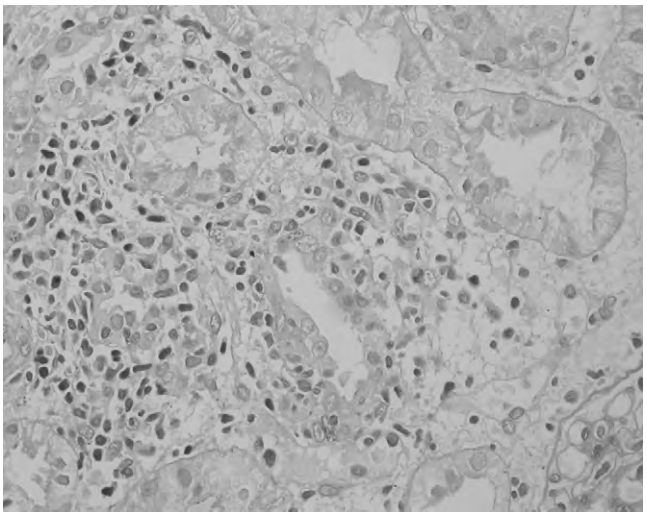

Figure 25–5 Subclinical rejection with interstitial lymphocytic infiltration with low-level tubulitis, but unchanged renal transplant function. (See color plate.)

from 3% to 31%, with borderline SCR ranging from 11% to 41%.[42,59]

Allografts with SCR result in greater histological damage on subsequent biopsy specimens, renal dysfunction, and impaired graft survival.[54,59] SCR is associated with chronic allograft nephropathy, indicating that unsuppressed SCR is followed by tubulointerstitial injury, mediated by several pathways. Lymphocytes, activated macrophages, and inflammatory mediators all can generate interstitial fibrosis, controlled by profibrotic signals including interleukin-1, interleukin-6, TNF-α, adhesion molecules, and TGF-β.[65] Powerful immunosuppression and control of SCR results in less tubulointerstitial damage.

Evidence from a single randomized prospective study of corticosteroid therapy showed that treatment significantly decreased acute rejection episodes and chronic tubulointerstitial scores at 6 months and improved renal function by 2 years after transplantation, with a trend toward better survival by 4 years.[53] Evidence for a role of SCR contributing to chronic allograft nephropathy comes from the compartment-specific nature of histological damage occurring where previous or current subclinical lymphocytic infiltration is colocalized; the temporal sequence, in which SCR occurs before the onset of tubular damage; a dose-dependent relationship, in which the intensity of SCR correlates with the severity of later chronic damage; biological plausibility; and confirmation in several transplant populations.

In sequential biopsy studies, interstitial mononuclear infiltration (coded by the Banff "i" score) usually resolves in a quasi-exponential fashion. In some individuals, however SCR may persist at low levels on repeated biopsy specimens in association with tubulitis and is designated as "true" chronic cellular rejection. In compliant patients at intermediate or low immunological risk using calcineurin inhibitor–based therapy, chronic rejection seems to be uncommon, but it may generate chronic allograft nephropathy in noncompliant or high immunological risk patients, or in patients in whom low-level immunosuppression or steroid withdrawal is used.

Tubulointerstitial Injury from BK Virus Nephropathy (See Chapters 24 and 29)

BK virus is an endemic polyomavirus infection of high prevalence, low morbidity, and long latency that may asymptomatically reactivate in immunocompetent individuals.[23] After primary childhood infection, it usually persists in the renal cortex and medulla and can be transmitted within the transplanted kidney. Asymptomatic reactivation can occur in 10% to 68% of recipients using calcineurin inhibitor–based immunosuppression. Graft dysfunction occurs in 1% to 10% from polyomavirus allograft nephropathy, a term encompassing infection from either the common BK or uncommon JC viruses. Although incipient infection occurs soon after transplantation, asymptomatic BK viremia may occur by 3 months initially without graft dysfunction, and subsequently with clinical renal impairment between 3 and 12 months.

In the early phases of infection, the virus focally replicates in the medulla with mild cytopathic effect and minimal functional impairment. Viral replication within tubules forms intranuclear inclusions, which gradually enlarge with smudgy nuclear chromatin, cellular atypia, and anisocytosis (Fig. 25-6). Tubular epithelial cells degenerate with rounding, detachment, and finally apoptosis or necrosis. As multifocal viral activation advances, a cytopathic inflammatory response of monocytes, polymorphonuclear cells, and plasmacytoid cells is generated, which may resemble acute interstitial rejection (but lacks arteritis, C4d deposition, or HLA-DR expression).[50]

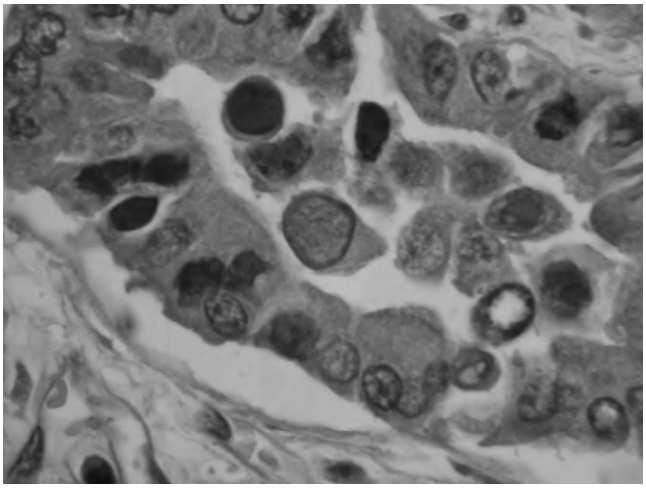

Figure 25–6 BK virus nephropathy infecting a renal tubule. Tubular cells are abnormal with some "ground-glass" nuclear changes, smudging, tubular necrosis, and sloughing into the lumen, eventually forming urinary decoy cells. (See color plate.)

When confronted by suspicious pathology, clarification of BK viral infection should be undertaken by immunochemistry or in situ hybridization for BK virus (Fig. 25-7) and electron microscopy for evidence of the characteristic 35- to 38-nm intranuclear paracrystalline viral arrays (distinguished by size and shape compared with adenovirus at 70 to 90 nm and cytomegalovirus and enveloped herpes simplex at 120 to 160 nm). Viral DNA in the blood can be confirmed by polymerase chain reaction, which also is used for prospective screening. As viral infection progresses, the predominant lesion becomes chronic tubulointerstitial scarring with flattened and atrophic tubules, sometimes associated with dystrophic microcalcification and low-grade chronic inflammation. This final stage of disease may resemble the nonspecific pattern of fibrosis and tubular atrophy of sclerosing chronic allograft nephropathy, although polyomavirus allograft nephropathy remains as a specific differential diagnostic entity.

Progressive and Late Stage Chronic Allograft Nephropathy

As the transplanted kidney ages, damage and injury may appear in the glomerular and microvascular compartments, accompanied by progressive tubulointerstitial damage.[42] Drivers for ongoing tubular injury (see Fig. 25-2) include residual SCR and inflammation, late acute rejection, calcineurin inhibitor nephrotoxicity, BK viral infection, and late acute renal failure secondary to sepsis or cardiac events. Acute late rejection from iatrogenic underimmunosuppression or noncompliance often causes severe tubular damage and initiation of persistent subclinical or chronic rejection, leading to progressive renal dysfunction and early graft failure

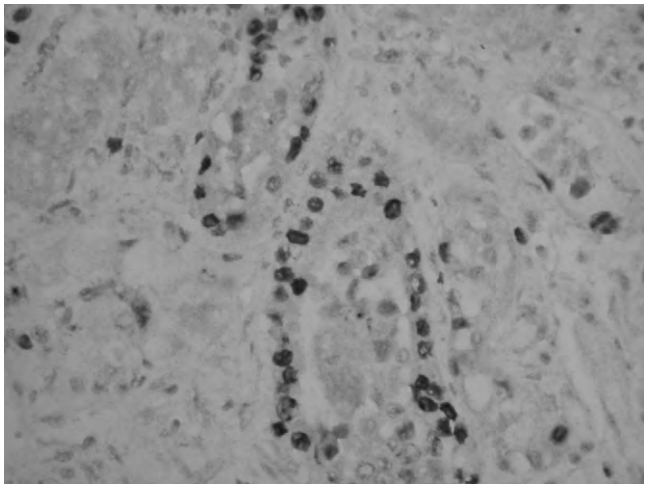

Figure 25–7 Immunoperoxidase stain (SV40T) of BK-infected renal tubular cells (brown)—diagnostic of polyoma viral nephropathy. (See color plate.)

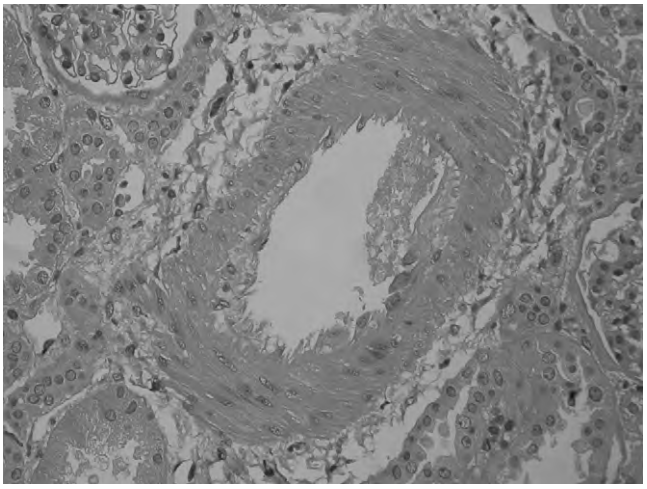

Figure 25–8 Early vascular changes of chronic antibody-mediated rejection in a small muscular artery. Intimal and endothelial cells are abnormal with edema and early neointimal formation present. A small, partially adherent thrombus is seen in the lumen. (See color plate.)

(see Fig. 25-2). Microvascular attenuation and increasing glomerulosclerosis are characteristic of late allograft pathology[25] and have multiple potential causes, including calcineurin inhibitor nephrotoxicity, immune-mediated transplant glomerulopathy, recurrent glomerulonephritis, diabetic microvascular disease, and hypertensive glomerulosclerosis (see Fig. 25-2).

True Chronic Interstitial Rejection

The Banff schema mandates recognition of morphological features of "true" chronic rejection, with arterial and capillary changes being emphasized as discriminating features. Chronic interstitial rejection is less commonly reported in compliant patients with calcineurin inhibitor–based therapy and involves T cells ($CD4^+$ or $CD8^+$) and macrophages. The vascular changes of chronic rejection seen in small muscular arteries include perivascular and intimal inflammation, intimal hyperplasia from smooth muscle proliferation in the vascular media, focal destruction of the internal elastic lamina, infiltration of smooth muscle cells into the neointima, and progression to vascular occlusion (Figs. 25-8 and 25-9). Transplant glomerulopathy is more a reflection of antibody-mediated pathogenesis. Donor disease, prior vascular rejection, hyperlipidemia, hypertension, and smoking also modulate small muscular arterial changes expressed as chronic fibrointimal thickening (reported as the Banff "cv" score) (Fig. 25-10) and should be considered in interpretation.

Calcineurin Inhibitor Nephrotoxicity

The introduction of cyclosporine revolutionized kidney transplantation, progressively increasing the 1-year graft survival beyond 90% and permitting transplantation of nonrenal solid organs. Calcineurin inhibitors are well tolerated and have become the backbone of modern immunosuppression (see Chapters 16 and 17). Calcineurin inhibitors are pleiomorphic nephrotoxins, however, causing transplant abnormalities in all histological compartments, constituting a significant diagnostic and management problem for their use in long-term therapy.

The classic histological features of calcineurin inhibitor nephrotoxicity include de novo or increasing arteriolar hyalinosis (Fig. 25-11) and striped cortical fibrosis (Fig. 25-12), supported by isometric tubular vacuolization (Fig. 25-13) and tubular microcalcification (unrelated to other causes, such as tubular necrosis and hyperparathyroidism) (Fig. 25-14). Other reported diagnostic lesions include peritubular and glomerular capillary congestion (diagnostically unreliable), diffuse interstitial fibrosis (important but nonspecific), toxic tubulopathy (seen predominantly with high-dose cyclosporine therapy), and juxtaglomerular hyperplasia (uncommonly seen and nonspecific). Tacrolimus and cyclosporine are indistinguishable by pathology, although most data come from older studies using cyclosporine. The diagnosis of calcineurin inhibitor nephrotoxicity may be difficult because of the paucity of reliable diagnostic markers and the expression of an incomplete constellation of histological features in any one biopsy sample. The most

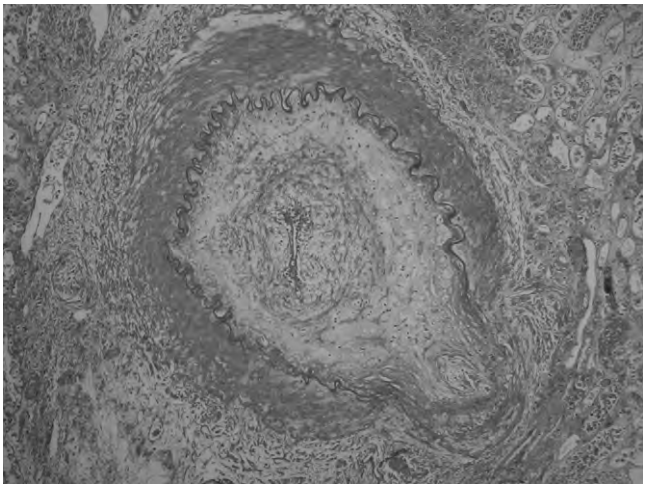

Figure 25–9 More advanced subacute vascular changes with extensive neointimal formation (within the internal elastic lamina boundary), characterized by invading myofibroblasts, deposition of matrix proteins, collagen, and edema—resulting in near-occlusion of the vascular lumen (Masson trichrome stain). (See color plate.)

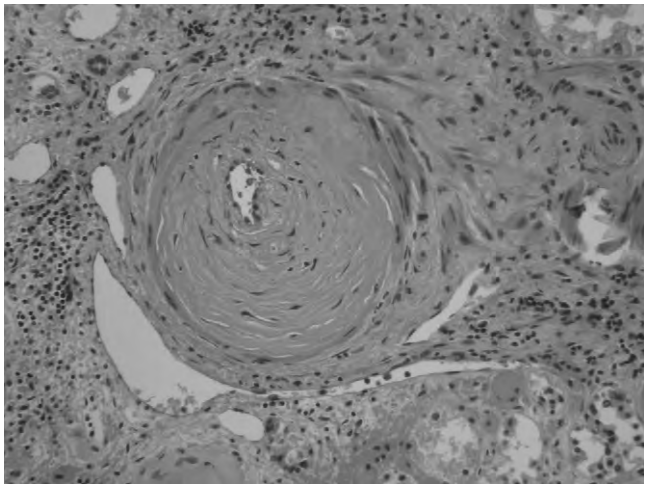

Figure 25–10 Chronic fibrointimal hyperplasia in chronic rejection (severe), with concentric layers of smooth muscle cells and collagen and near-occlusion of the vessel. (See color plate.)

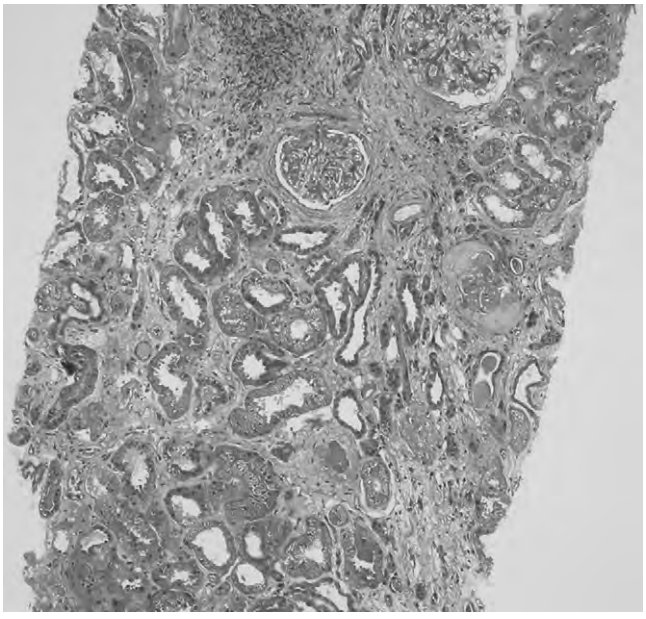

Figure 25–12 Striped fibrosis. Demarked areas of striped fibrosis near adjacent normal cortex, associated with interstitial fibrosis. Masson trichrome stain stains collagen green. (See color plate.)

reliable and specific abnormality is de novo or increasing arteriolar hyalinosis, classically described in a peripheral and nodular pattern (rather than a subendothelial and diffuse distribution) with appropriate clinical exclusions and caveats (see later).

Calcineurin inhibitor–induced arteriolopathy has been attributed to vacuolation and necrosis of arteriolar smooth muscle and endothelial cells, followed by insudation of protein to form (nodular) hyaline deposits. The presence of arteriolar hyalinosis has been associated with acute clinical nephrotoxicity and cyclosporine dose and trough levels. Although the classically described lesions are nodular and peripheral hyaline deposits,[2] potential problems of interpretation include variations of vascular cross section appearance according to the plane of section, lack of definition as to what actually constitutes "nodularity," and early and mild calcineurin inhibitor–related arteriolar hyalinosis manifesting as a circumferential lesion that later progresses to a nodular deposit. Early hyalinosis may be mild and patchy, intermittently observed on sequential biopsy specimens, and is often reversible with calcineurin inhibitor dosage reduction. Later arteriolar hyalinosis lesions have been associated with high-grade and progressive microvascular narrowing, increasing ischemic glomerulosclerosis, and further chronic tubulointerstitial damage; these lesions are less reversible.

When arteriolar hyalinosis occurs in a failing allograft, the diagnosis of calcineurin inhibitor nephrotoxicity is strengthened by evidence of progression of hyalinosis using previous histology and nodularity rather than diffuse hyalinosis (see Fig. 25-11), and exclusion of other alternative explanations, including donor arteriolar hyalinosis (by implantation biopsy), ischemic arteriolar injury, dyslipidemia, hyperglycemia, and hypertensive nephrosclerosis (distinguished histologically by subendothelial hyalinosis, elastic

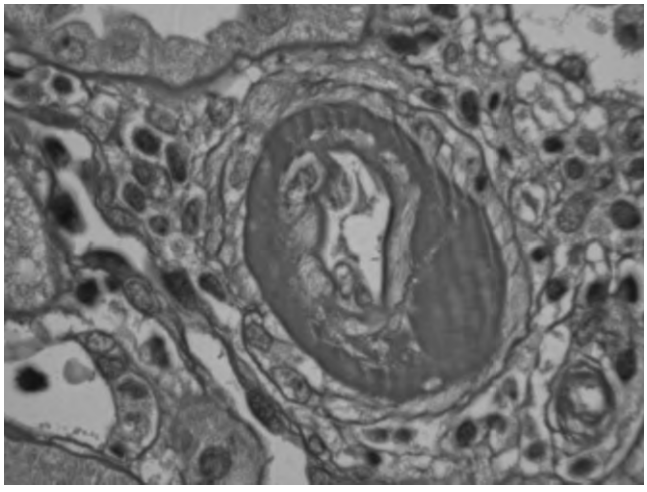

Figure 25–11 Arteriolar hyalinosis, with a large, eccentrically located nodule within the media of the arteriole. (See color plate.)

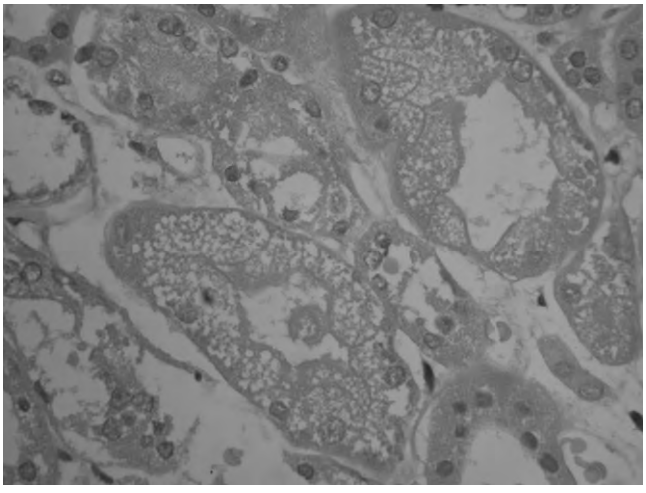

Figure 25–13 Isometric vacuolation in the proximal tubular cells from cyclosporine tubulopathy. (See color plate.)

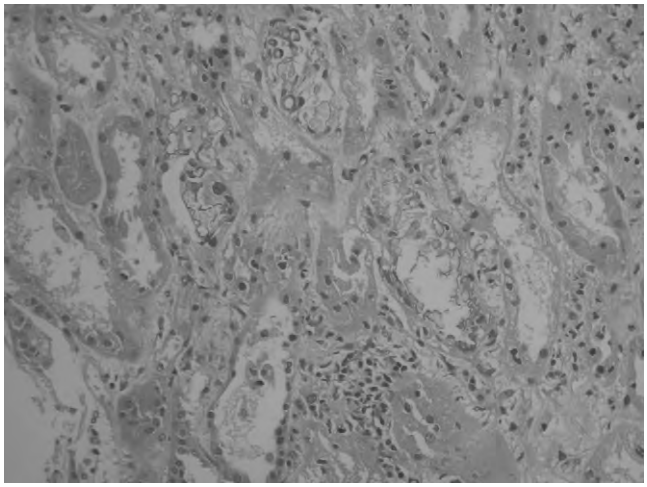

Figure 25–14 Tubular microcalcification (blue staining) within tubular epithelial cells associated with cyclosporine nephrotoxicity. (See color plate.)

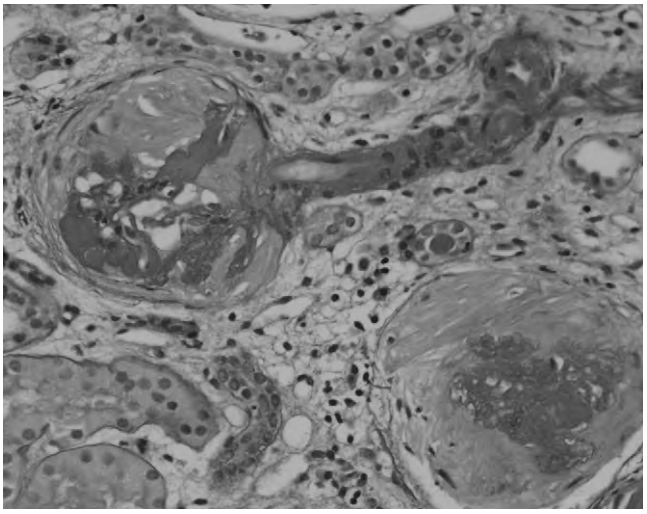

Figure 25–15 Severe arteriolar hyalinosis of the feeding afferent arteriole leading to collapse of the glomeruli from calcineurin nephrotoxicity. (See color plate.)

lamina reduplication, and medial hyperplasia in small arteries and verified by clinical information). Severe arteriolar hyalinosis gradually results in vascular narrowing and downstream ischemic glomerulosclerosis (Fig. 25-15). Arteriolar hyalinosis, especially when progressive, remains the best diagnostic marker of calcineurin inhibitor nephrotoxicity.

Striped fibrosis represents an area of severe tubular damage, subjectively defined by a dense striped cortical fibrosis and atrophic tubules demarcated against areas of normal adjacent cortex (see Fig. 25-12). Striped fibrosis has been usually regarded as pathognomonic of calcineurin inhibitor nephrotoxicity, but lacks sensitivity (repeated biopsy cores may be needed for detection; the "stripe" may be lost in small samples, or obscured where diffuse interstitial fibrosis blurs the margin) and specificity. Striped fibrosis, commonly seen in medullary rays, is probably due to watershed infarction at the level of interlobular or arcuate arteries because the appearance can be reproduced by intra-arterial microsphere injection in experimental kidneys.

Tubular microcalcification can be due to localized cell necrosis from any cause and has been associated with chronic cyclosporine nephrotoxicity (see Fig. 25-14). Proximal tubules are susceptible to calcineurin inhibitor injury, displaying isometric vacuolation in early studies using high-dose cyclosporine therapy (corresponding to dilated endoplasmic reticulum in the proximal straight tubules), tubular cell necrosis, and tubular cytoplasmic inclusion bodies (corresponding to abnormal giant mitochondria with deranged cristae). Because chronic diffuse tubulointerstitial damage may be due to a multitude of causes, tubular microcalcification from calcineurin inhibitors cannot be distinguished from localized immune-mediated tubular damage, residual hyperparathyroidism and hypercalcemia, or previous acute tubular necrosis. Other reported lesions of calcineurin inhibitor nephrotoxicity, such as juxtaglomerular hyperplasia, are uncommon and of uncertain validity in chronic disease. Juxtaglomerular hyperplasia and glomerular capillary congestion are unreliable markers for diagnosis.[43]

LATE GLOMERULAR AND MICROVASCULAR CHANGES

Glomerular Changes

As chronic allograft damage progresses within the microvascular and glomerular compartments, high-grade arteriolar hyalinosis and severe vascular narrowing may be seen not only from calcineurin inhibitor nephrotoxicity but also from hypertension, dyslipidemia, and smoking. Glomerular abnormalities may be secondary to ischemic glomerular loss, formation of atubular glomeruli, recurrent glomerular disease, or chronic transplant glomerulopathy.

Morphometric analysis of chronic allograft nephropathy has identified separate populations of smaller (ischemic) and larger (hyperfiltering) glomeruli, widening the base of frequency histograms of glomerular size. These separate populations of small, ischemic glomeruli are characterized by wrinkling and collapse of the glomerular capillary wall associated with extracapillary fibrotic material, and are contrasted with larger, hyperfiltering glomeruli—representing two distinct pathophysiological processes. Ischemic glomerulosclerosis may occur secondary to early ischemic podocyte injury, resulting in proteinuria and glomerulosclerosis, later vascular or endothelial cell injury from calcineurin inhibitor nephrotoxicity and hypertension, or alloimmune or antibody injury.

Severe arteriolar hyalinosis (Banff "ah" score ≥2) is often followed by progressive glomerulosclerosis, suggesting that vascular narrowing in afferent arterioles causes downstream ischemic glomerulosclerosis or glomerular shrinkage, or both. Hypertension also may result in global glomerulosclerosis and shrunken glomeruli, which can be reduced by therapy with angiotensin-converting enzyme inhibitors.

Formation of Atubular Glomeruli

Severe tubular injury can result in a perfused glomerulus that is functionally disconnected from its downstream proximal tubule. These atubular glomeruli are common in tubulointerstitial kidney diseases, such as chronic pyelonephritis

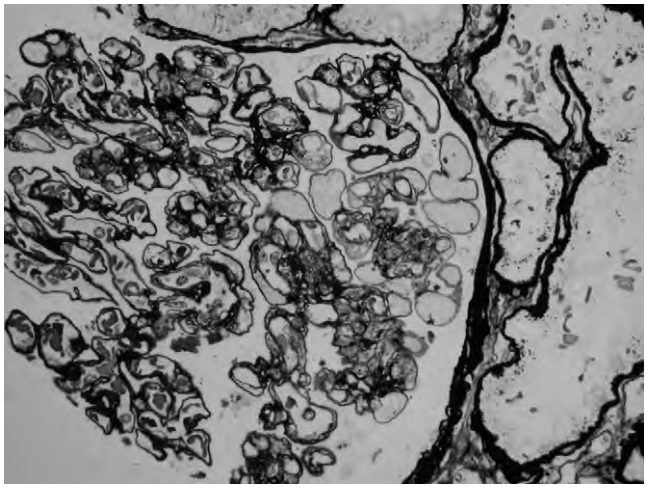

Figure 25–16 Transplant glomerulopathy by methamine silver stain light microscopy, showing double contours of the glomerular capillary loops (seen as a parallel pair of lines).

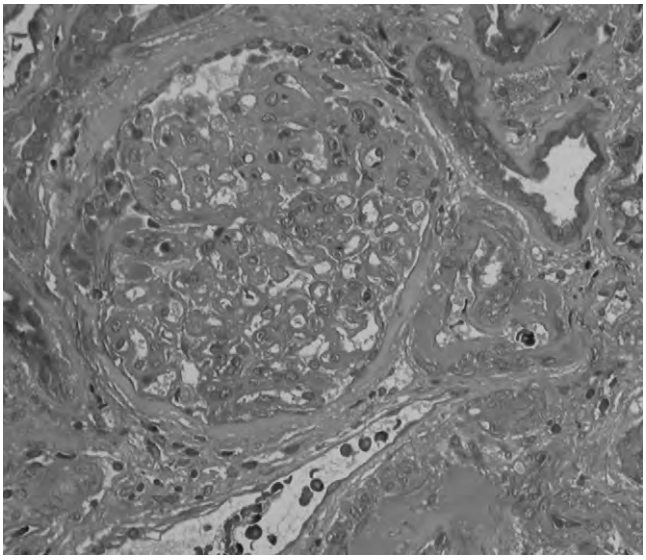

Figure 25–17 Light microscopic appearance of transplant glomerulopathy showing increased mesangial matrix, thickened capillary loops, and partial closure of the capillary loops.

and lithium and cisplatin nephrotoxicity. In normal living and cadaver donor kidneys, 1% to 2% of glomeruli are atubular, increasing to 17% to 18% with chronic allograft nephropathy and 29% with cyclosporine nephrotoxicity.[18] Although atubular glomeruli are a consequence of irreversible obliteration of the tubular lumen, many remain perfused, but nonfunctional, whereas others progress to global glomerulosclerosis after a variable lag period of several years.

Atubular glomeruli are usually smaller than normal or contracted within an enlarged glomerular cyst and may be surrounded by periglomerular fibrosis. Bowman's capsule is lined by abnormal podocytes with intact interdigitating pedicels of uncertain origin. Bowman's space is filled by inspissated proteinaceous material from residual glomerular filtration and local reabsorption.[18] The presence of tubular glomeruli may be inferred from light microscopic features of a small contracted glomerular tuft and periglomerular fibrosis, although freeze-fracture scanning electron microscopy and serial sections with three-dimensional reconstruction are the diagnostic methods used in research settings.

Transplant Glomerulopathy

Chronic transplant glomerulopathy comprises a spectrum of abnormalities, which include chronic glomerular changes of thickening or duplication of the glomerular capillary basement membrane, double contour formation, and mesangial interposition (Figs. 25-16 and 25-17). Chronic glomerulopathy scores (designated as Banff "cg") are determined by the extent of peripheral capillary loop involvement of the most affected of nonsclerotic glomeruli, preferably using periodic acid–Schiff stains.[47] A score of cg0 is no glomerulopathy, cg1 is 10% to 25% of the most affected peripheral capillary loops, cg2 is 26% to 50%, and cg3 is greater than 50% of affected.

Associated histological features include deposition of subendothelial flocculent or fibrillary material (Fig. 25-18); mesangial cellular proliferation with matrix expansion; multilamination, or multilayering, of the PTC basement membrane (Fig. 25-19); and C4d deposition in glomerular capillaries or PTCs (Fig. 25-20).[26,51] Transplant glomerulopathy implies chronic endothelial injury of the glomerular capillary loops and is clinically accompanied by substantial or nephrotic-range proteinuria, renal functional impairment, and reduced transplant survival.

The likely pathophysiology encompasses chronic alloimmune mechanisms involving B cell and persistent humoral rejection,[10] suggested by the association with circulating anti–donor HLA antibodies, endothelial C4d deposition in glomeruli or PTCs, or both (as a surrogate marker of classic complement activation by antibody), glomerular infiltration of activated T cells, and presence in human SCR and experimental chronic rejection.[51,60] Complement-fixing alloantibodies that bind endothelial cell targets may result in endothelial cell lysis or stimulation, with activation of coagulation and local complement pathways and later macrophage and neutrophil recruitment. The prevalence of C4d deposition ranges from 91% in biopsy specimens with

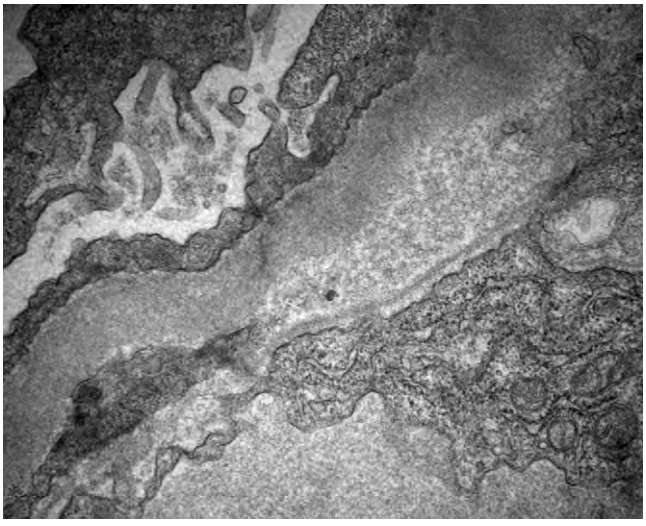

Figure 25–18 Subendothelial fibrillary material in transplant glomerulopathy by electron microscopic examination.

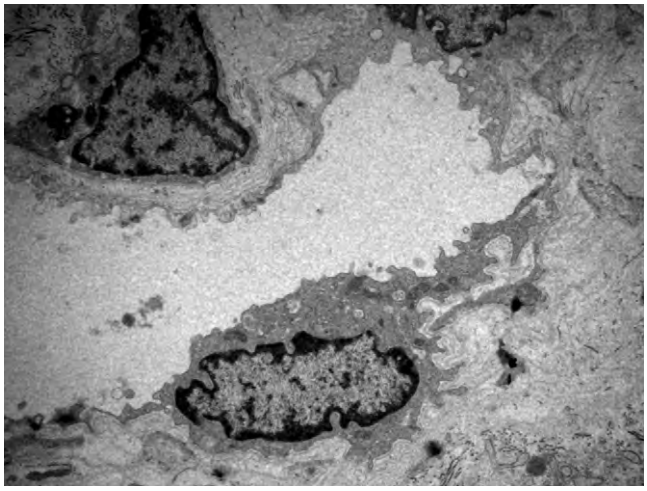

Figure 25–19 Peritubular multilamination of the basement membrane in chronic antibody-mediated rejection (transplant glomerulopathy). Note multiple layers of reduplicated basement membrane.

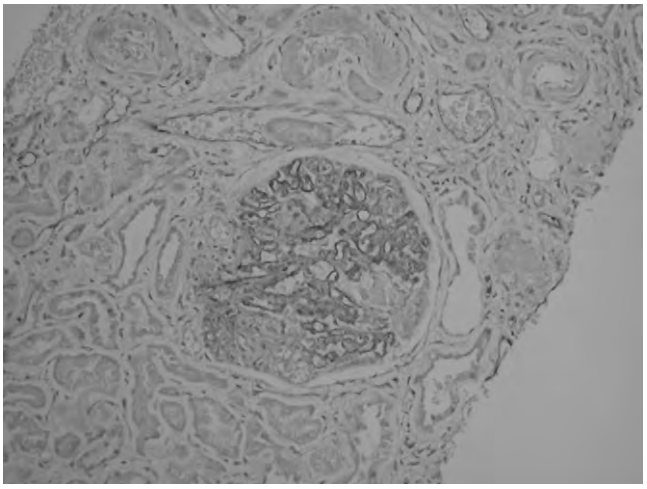

Figure 25–20 Immunoperoxidase stain for C4d in glomerular capillary loops and peritubular capillaries in chronic antibody-mediated rejection. (See color plate.)

transplant glomerulopathy, 12% to 61% in biopsy specimens with chronic rejection and renal dysfunction,[34,51] to only 2% in well-functioning protocol biopsy specimens.[37] The prevalence varies by center, clinical scenario, methodology, and definition of C4d positivity (Fig. 25-20).

A role for non-HLA immunity is suggested by inferior late graft survival of HLA-identical siblings who have panel-reactive antibodies that are potentially directed against minor histocompatibility complex antigens or other antigens. Because the principal target of alloantibody is the endothelium, injury occurs predominantly in small glomerular and peritubular capillaries. Injuries manifest as transplant glomerulopathy and PTC basement membrane multilamination, which are frequently correlated with each other.

PTC basement membrane multilamination and splitting are defined by electron microscopy and probably indicate past or recent endothelial cell injury with subsequent repair. Some regression also may occur. Moderate (five to six layers) or severe (seven or more layers) multilamination may be present in 38% of failed transplants ascribed to chronic rejection. PTC basement membrane multilamination has been associated with C4d deposition in PTCs, transplant glomerulopathy on light microscopy, and circulating donor-specific antibody, consistent with chronic antibody-mediated pathophysiology. Smaller amounts of multilamination (generally average two to three layers or less) are seen in kidney disease from obstructive uropathy, analgesic nephropathy, radiation nephritis, immune-complex glomerulonephritis, diabetes, and hypertension and in transplanted kidneys with other types of glomerulopathies. Moderate disease with five to six layers is an acceptable positive cutoff level for transplanted kidneys.

Diagnosis of Chronic Antibody-Mediated Rejection

The diagnostic triad of chronic (or late) antibody-mediated rejection includes the following:

1. Morphological features of transplant glomerulopathy (Banff score ≥cg1, with double contours on light microscopy), supported by PTC basement membrane multilamination by electron microscopy, and possibly PTC loss
2. Diffuse C4d deposition in PTCs (defined subsequently) or in glomeruli (assessable only by paraffin sections), or in both
3. The presence of donor-specific antibody to donor HLA or endothelial antigens

Mononuclear inflammatory cells within the PTCs, transplant glomerulitis, chronic arteriopathy with fibrous intimal thickening and splintering of elastica, or a plasma cell interstitial infiltrate also may be supportive. A diagnosis "suggestive of chronic antibody-mediated rejection" can be made in cases in which chronic capillary changes are associated with either C4d or donor-specific antibody.

Thrombotic microangiopathy may produce similar glomerular histology and requires clinical exclusion by blood film examination, haptoglobin, and lactate dehydrogenase levels (differential diagnosis includes infection, recurrent hemolytic-uremic syndrome, and anti-cardiolipin antibody thrombotic microangiopathy). Other causes of $C4d^-$ transplant glomerulopathy include technical error; failed recognition in cases in which damaged PTCs disappear with advancing chronic allograft nephropathy, clinical inactivity, or disappearance or absorption of circulating antibody; failed recognition in cases in which residual chronic glomerular morphological changes remain from a previous antibody-mediated episode; or a T cell–mediated glomerular process. Similarly, marked absorption of antibody directed to the kidney transplant may result in negative circulating donor-specific antibody.

Recurrent Glomerulonephritis and Glomerular Disease

Because glomerular disease (including diabetes) accounts for most end-stage renal failure, some recipients develop recurrence of their original disease in the allograft. Recurrent glomerulonephritis is diagnosed by exclusion of donor-transmitted disease and de novo glomerulonephritis. It has a negative impact on graft survival and causes 8.4% of

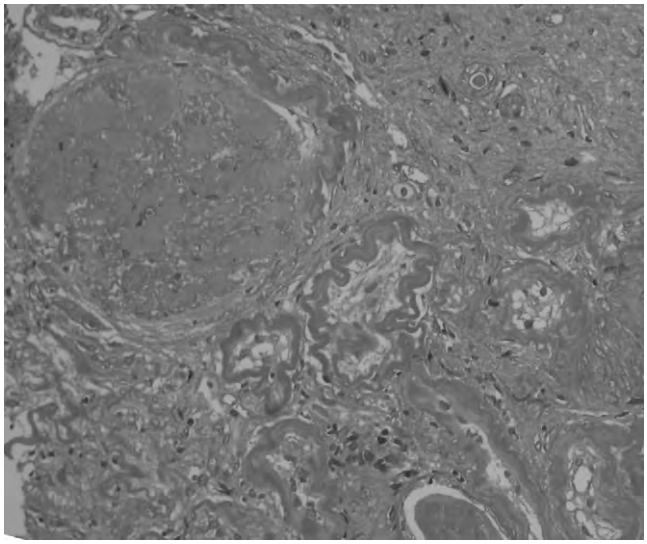

Figure 25–21 Recurrence of diabetic nephropathy in a renal transplant showing thickened tubular basement membranes and diffuse diabetic glomerulopathy with massively increased mesangial matrix and thickened glomerular basement membrane (green). (See color plate.)

allograft losses by 10 years in recipients with renal failure from glomerulonephritis.[7] The relative impact of recurrent glomerulonephritis increases as graft survival lengthens, or in some populations in whom primary glomerulonephritis is prevalent or severe. The clinical course and severity of recurrent glomerular disease often copies that of the patient's original disease,[8] except for patients with vasculitis or lupus nephritis; these conditions are usually controlled by transplant immunosuppression. Focal segmental glomerulosclerosis (20% to 50% recurrence rates) and dense deposit disease (50% to 90% recurrence) have the worst prognosis and together constitute 55% to 60% of all recurrent glomerulonephritis. Membranous glomerulonephritis recurs in 29% to 50%, membranoproliferative glomerulonephritis type 1 recurs in 20% to 33%, and IgA nephropathy recurs in 58%, although with limited early (but increased later) clinical impact.[8] Diabetic glomerulopathy also may recur, but with variable clinical effect (Fig. 25-21).

Focal Segmental Glomerulosclerosis

Focal segmental glomerulosclerosis has the greatest clinical impact of the recurrent glomerular diseases because of its high recurrence rate, poor intermediate outcome, and the number of young patients with focal segmental glomerulosclerosis who undergo transplantation. Proteinuria may recur within hours, but usually is seen by 1 to 2 weeks after transplantation. Recurrence risk is increased in younger recipients (partially explained by the proportion of children transplanted for primary focal segmental glomerulosclerosis); in white recipients; and in recipients with mesangial hypercellularity, steroid resistance, or aggressive glomerular disease (defined by time of initial diagnosis to native renal failure ≤3 years). Nephrotic proteinuria, hypertension, and hematuria are seen in 80% of recipients, and by 5 years, 50% of grafts are lost. Graft loss from recurrent focal segmental glomerulosclerosis predicts recurrence in 70% of subsequent allografts, and most of these fail, possibly precluding that individual from future transplantation.

Podocyte injury is the key abnormality in focal segmental glomerulosclerosis, resulting in loss of the glomerular charge barrier and proteinuria, followed by collapse of the underlying glomerular capillary with sclerosis. In primary focal segmental glomerulosclerosis, a circulating permeability factor capable of altering the glomerular albumin reflection coefficient has been postulated (measurable in vitro by incubated isolated glomerular volumes). A circulating recipient factor explains early recurrent proteinuria after transplantation and a therapeutic response to plasmapheresis. Mutations of podocyte-specific genes also may cause proteinuria and focal segmental glomerulosclerosis. Nephrin, a component of the glomerular slit diaphragm, absent in Finnish congenital nephrotic syndrome, and other defective podocyte proteins, such as podocin, α-actin 4, and CD2AP, all may result in glomerular proteinuria. Many "idiopathic" cases of focal segmental glomerulosclerosis are actually genetic abnormalities of podocytes. Why transplanting a genotypically normal kidney into these recipients results in recurrent focal segmental glomerulosclerosis in one third is unexplained. Secondary segmental glomerulosclerosis occurs as a result of glomerular hyperfiltration and hemodynamic mechanisms from reduced nephron number or from other proteinuric glomerular diseases.

Membranous Glomerulonephritis

Membranous glomerulonephritis recurs in 10% to 30% of patients and is a common de novo glomerular disease. Recurrent disease occurs slightly sooner (1 to 2 years) than de novo membranous glomerulonephritis (2 to 3 years),[11] and both usually manifest as nephrotic syndrome. The 10-year graft loss rate is approximately 50%, with increased risk in male recipients, recipients with aggressive original disease, and recipients of living related transplants. Subepithelial immune complexes, containing terminal complement, insert into podocyte membranes, causing sublytic cellular activation, oxidant and protease production, and damage to the underlying GBM. Target antigens are unknown in humans (except one case of neutral endopeptidase expressed on podocyte cell membrane)—precluding screening of prospective recipients. Immunosuppression with mycophenolate mofetil or azathioprine and corticosteroids to reduce antibody formation, or with rituximab to eliminate CD20 B cells (but not plasma cells), may have a role.

ASSESSMENT OF A FAILING GRAFT

Renal Function and Chronic Allograft Nephropathy

Transplant renal function depends predominantly on the extent of tubulointerstitial damage, with a contribution from sclerosed glomeruli and glomerular abnormalities. Serum creatinine and calculated GFR formulas, although inexpensive and simple, are imperfect compared with the more expensive and accurate isotopic GFR methods. Errors are related to differential creatine generation (e.g., muscle loss from corticosteroids, malnutrition, and sepsis), the variable tubular secretion of creatinine and nonlinear relationship with GFR, and inaccuracies and laboratory differences in biochemical measurement. Serum creatinine underestimates the extent of tubulointerstitial damage, and early biopsy should be considered before the occurrence of severe renal dysfunction.

Table 25–2 **Clinical Scenarios and Kidney Transplant Pathology**

Clinical Scenarios	Key Defining Features	Associated Features
Extended or marginal donor	Arterial (cv) and arteriolar (ah) disease, glomerulosclerosis	Interstitial fibrosis
Early ischemia-reperfusion injury	Tubular necrosis or interstitial edema or both	Tubular atrophy and chronic interstitial fibrosis
Subclinical rejection	Interstitial infiltration of mononuclear cells and tubulitis	Tubulointerstitial damage
Chronic interstitial rejection	Interstitial cells and tubulitis, fibrointimal hyperplasia	Tubulointerstitial damage
Chronic antibody-mediated rejection	Transplant glomerulopathy, C4d$^+$ (PTC) donor-specific antibody Mesangial matrix	Double contours, PTC-BM ML by EM mesangial matrix Proteinuria, decreased GFR
Calcineurin inhibitor nephrotoxicity	Progressive arteriolar hyalinosis, striped fibrosis	Microcalcification, diffuse fibrosis, tubulopathy
Polyomavirus nephropathy	Inflammatory tubular necrosis, viral nuclear changes, histochemistry (SV40T antigen) Urinary decoy cells Blood BK virus PCR	Tubular cell virus by EM, urinary decoy cells, blood BK PCR
Hypertensive nephrosclerosis	Arterial vascular changes, IEL reduplication	Glomerulosclerosis, arteriolar changes

ah, arteriolar hyalinosis; cv, chronic vascular changes; EM, electron microscopy; GFR, glomerular filtration rate; IEL, internal elastic lamina; PCR, polymerase chain reaction; PTC, peritubular capillary; PTC-BM ML, peritubular capillary basement membrane multilamination.

Kidney Transplant Biopsy

Principles Guiding Clinical Biopsy

Chronic allograft nephropathy is diagnosed by histology (Tables 25-2 and 25-3; see also Chapter 24). Patients with progressive chronic allograft dysfunction usually need evaluation by renal biopsy with the following caveats:

1. Transplant biopsy should be considered after clinical exclusion of obvious causes of dysfunction, such as ureteric obstruction, acute calcineurin inhibitor nephrotoxicity, dehydration, transplant hypoperfusion, uncontrolled hypertension, and sepsis.
2. Biopsy should be done early before substantial deterioration in transplant function because late histology with significant damage is often nonspecific, the damage is less responsive to therapy, and it is more difficult to define an etiological diagnosis.
3. Biopsy samples containing at least 10 glomeruli and 2 arteries are needed to fulfill the Banff adequacy criteria. Samples also should include arterioles (defined as fewer than 3 medial muscle layers and absent or incomplete internal elastic lamina) for assessment of calcineurin inhibitor–induced hyalinosis and small muscular arteries for assessment of immune-mediated fibrointimal hyperplasia (scored as Banff "cv"). Tubulointerstitial damage can be appreciated easily on small histological samples; however, assessment of glomerular and microvascular changes provides important etiological clues. Some pathological features are patchy, so 2 cores of cortex are recommended. Care should be taken with older transplants; a dense surrounding fibrotic capsule may need careful penetration to obtain adequate cortical tissue.
4. Fibrosis may be difficult to appreciate, standardize, and quantify, especially if it is patchy, as with striped fibrosis, or variably diffuse between tubules. Objective assessment using trichrome or Sirius Red staining linked to a validated image analysis system may be preferable. These techniques usually detect collagen and early fibrosis, and other matrix proteins may not be stained. Biological variability and sampling errors occur because of inadequate sample size and differences in pathologist's scores using the Banff schema. This variability reduces the diagnostic reliability of histology to reflect accurately the extent of chronic allograft nephropathy and provide a specific etiological diagnosis. Reproducibility between pathologists is imperfect, with consistent undergrading or overgrading of scores. Interobserver agreement for major chronic scores (e.g., ci and ct) are generally good compared with alloimmune markers and acute rejection parameters.
5. Implantation or postperfusion biopsy specimens are important to distinguish preexisting donor pathology from newer changes and allow comparison of changes over time. If a temporal sequence of histology can be created from the implantation biopsy specimen with other interval biopsy specimens, contemporary histology can be compared with interval clinical events and therapy to aid the interpretation and the etiological assessment of graft dysfunction.
6. The biopsy specimen from a chronically failing graft should be processed similarly to a specimen from native kidney disease. Light microscopy assesses the presence, extent, and grade of chronic allograft nephropathy, along with any accompanying specific diagnoses, such as calcineurin inhibitor nephrotoxicity, hypertensive vascular disease, BK virus nephropathy, or transplant glomerulonephritis. Periodic acid–Schiff stain highlights basement membranes and arteriolar hyalinosis, silver stains allow identification of double contours in transplant glomerulopathy, and trichrome stains are used for collagen deposition and the extent of chronic fibrosis. Immunofluorescence or immunoperoxidase techniques are usually negative or nonspecific in most biopsy specimens with chronic allograft nephropathy, but are helpful to diagnose recurrent or de novo glomerulonephritis, allograft viral infection (e.g., BK virus or cytomegalovirus stains), or chronic

Table 25–3 **Kidney Transplant Diagnostic Pathology**

Banff Qualifier (Banff code)	Interstitial Mononuclear Infiltration (i)	Tubulitis (t)	Chronic Interstitial Fibrosis (ci)	Tubular Atrophy (ct)	Fibrointimal Thickening or Glomerulopathy (cv or cg)	Arteriolar Hyalinosis (ah)	Glomerular Sclerosis	Comments
Acute tubular injury	0 to +			(+ some acute tubular loss)				Tubular injury with necrosis, nuclear changes or tubular dilation; changes may be minimal
Acute cellular rejection	++ to +++	++ to +++						Acute renal dysfunction; i1 and t1 is borderline; occasionally arteritis with i0 and t0
Subclinical rejection	+ to +++	+ to +++						Normal renal function; acute or borderline; rarely arteritis
Sclerosing CAN "TA/IF not otherwise specified"			+ to +++	+ to +++			0 to +++	Nonspecific tubulointerstitial damage; often cellular inflammation in areas of damage; very common
Chronic (interstitial or cellular) rejection	+ to +++	+ to +++	+ to +++	+ to +++	cv 0 to +++ (cg variable)		Variable	Fibrointimal hyperplasia, neointima and neomedia formation, internal elastic lamina disruption, and intimal inflammation as defining features; may be $C4d^+$
Chronic antibody-mediated rejection with glomerulopathy	Variable		Variable	Variable	cg + to +++	(+)	+ to +++	Capillary loop double contours, capillary interposition, increased mesangial matrix, PTC multilamination by EM; usually $C4d^+$ and donor antibody positive
Hypertensive nephrosclerosis					cv + to +++	0 to ++	+ (wrinkled)	Internal elastic lamina reduplication, hyperplastic small arteries, small arterial hyaline may be present
Recurrent glomerulonephritis			Variable	Variable			0 to +++ common late	Proliferative glomerular changes; diagnostic IF and EM needed
Chronic CNI toxicity	(+)		+ to +++ (striped or diffuse)	+ to +++ (vacuolation)	cv 0 to + (myxoid)	+ to +++ nodular	±0 to +++ (±wrinkled)	±Microcalcification and isometric vacuolation of tubules; rarely acute thrombotic microangiopathy and juxtaglomerular hyperplasia

CAN, chronic allograft nephropathy; CNI, calcineurin inhibitor; EM, electron microscopy; IF, immunofluorescence; i1, Banff acute mild interstitial inflammation; PTC, peritubular capillary; T1, mild tubulitis; TA/IF, tubular atrophy/interstitial fibrosis.

antibody-mediated rejection (for peritubular C4d deposition). Electron microscopy can detect early transplant glomerulopathy before light microscopy or can detect electron-dense deposits to confirm transplant glomerulonephritis.

7. Adequate clinical information should be available to the interpreting pathologist, including current transplant function; donor quality; previous events, such as delayed function, acute rejection, immunosuppression, and suspected noncompliance; and the cause of recipient end-stage renal failure. A collaborative clinicopathological diagnosis is the optimal way to interpret transplant histology (see Tables 25-2 and 25-3).

Risk and Safety of Transplant Biopsies

Core needle biopsy has an excellent risk profile with a low risk of graft loss and minimal risk of morbidity. The risk of major complications, such as substantial bleeding, macroscopic hematuria with ureteric obstruction, peritonitis, or graft loss, is approximately 1%. Minor complications reported are gross hematuria in 3.5%, perirenal hematomas in 2.5%, and asymptomatic arteriovenous fistulas in 7.3%.[58] The risk of graft loss from protocol biopsy is 0.03%, although risk is increased with indication-driven procedures, when adult kidneys are placed in either an extraperitoneal or a transperitoneal position in infants, or when a needle exceeding 18-gauge is used. Safety should be maximized by use of a skilled operator employing ultrasound guidance and an automated gun.

NONINVASIVE DIAGNOSIS OF CHRONIC ALLOGRAFT NEPHROPATHY

Imaging

Two-Dimensional Diagnostic Ultrasound

Diagnostic ultrasonography is often used to evaluate transplant size (often comparable to implantation length despite chronic allograft nephropathy), ureteric obstruction as a cause of dysfunction, and vascular supply by Doppler to detect any areas of cortical infarction (e.g., from a thrombosed polar artery) or to exclude renal artery stenosis. Ultrasound is excellent for diagnosis of surgical complications, but suboptimal for either acute rejection (the features of which include increased renal volume, reduced cortical echogenicity, loss of the corticomedullary differentiation, and splaying of the medullary pyramids) or chronic allograft nephropathy. The chronic parenchymal changes of irregular cortical outline, reduced cortical width, increased echogenicity, and loss of corticomedullary junction differentiation are seen only after significant damage has occurred, making it insensitive for the early diagnosis of chronic allograft nephropathy.

Doppler Ultrasound Assessment

The resistance index (RI) of the kidney transplant is a noninvasive measure of intrarenal compliance; the RI is determined by averaged measurements in the early segmental arteries branching off the main renal artery. It is calculated from the index of peak systolic blood velocity (Vmax) relative to the minimal diastolic velocity (Vmin), expressed as $1 - (Vmin/Vmax)$. Higher RI values imply decreased diastolic blood flow and reflect augmented downstream vascular resistance. RI is correlated with many factors, including the site of measurement, intra-abdominal pressure (e.g., Valsalva maneuver), older age, and pulse pressure profile and inversely with pulse rate. An RI exceeding 0.80 is an adverse prognostic indicator accompanied by decreased creatinine clearance of 50% and increased graft failure from 2.5 to 23.3 years.[49] A high RI (>0.80) also is associated with fractional interstitial fibrosis by PicroSirius-Red (9.5% versus 5.2%) and a positive predictive value for 2-year renal dysfunction of 67%. RI is insensitive to detect early chronic allograft nephropathy, becoming abnormal only after substantial allograft damage has occurred. RI also predicts mortality, probably explained by its relationship to the recipient's mean arterial blood pressure and vascular compliance.

Renal transplant angiography of chronic rejection classically shows severely "pruned" vessels from vascular attenuation associated with chronic interstitial fibrosis. Noninvasive Doppler techniques have been developed to quantify intragraft blood flow.[25] Using Doppler cineloop imaging, which acquires and quantifies systolic pulsatile blood flow, allograft perfusion decreases with parenchymal damage—yielding a positive predictive value of 86% for chronic allograft nephropathy grade II.[41] Similar methods of pulsatility index (a reciprocal measure related to RI) also have been used. Contrast-enhanced phase inversion Doppler ultrasound employs a pulse of ultrasound energy to destroy microbubbles of an injected contrast agent (burst imaging), which is followed by low frame rate imaging during reperfusion, which is substantially reduced with abnormal tissue structure of chronic allograft nephropathy, although further clinical data are required.[69] Because renal fibrosis alters the elastic properties of the allograft, measuring mechanical deformation using a phase-sensitive, two-dimensional speckle tracking technique allows evaluation of internal tissue characteristics, but its sensitivity remains to be defined.[68]

Magnetic Resonance Imaging and Nuclear Imaging

Magnetic resonance imaging (MRI) is capable of quantifying kidney transplant volume loss accurately, but relies on detecting microstructural changes and blood flow alterations secondary to parenchymal damage. Moderate chronic allograft nephropathy must occur before detection. T1-weighted pulse sequences can distinguish acute rejection from calcineurin inhibitor nephrotoxicity using intensity differences between the cortex and the medulla (corticomedullary demarcation) as a sensitive indicator of parenchymal disease. The MRI appearance of acute rejection is similar to chronic allograft damage, however, regardless of cause.[64] The loss of corticomedullary demarcation was poorly correlated with biopsy diagnosis (acute cellular rejection, acute vascular rejection, and chronic vascular rejection all gave similar patterns), making it a nonspecific and insensitive marker of early calcineurin inhibitor toxicity or chronic allograft nephropathy.

Superparamagnetic contrast MRI enhances corticomedullary demarcation. Using gadolinium-enhanced dynamic turbo fast low angle shot (FLASH) imaging, the arterial signal intensity ratio between medulla and cortex and cortical peak became indistinct with severe renal dysfunction; however, overlap between diagnostic groups limited its clinical applicability.[40] Gadolinium MRI perfusion normally shows a moderate increase in the signal intensity of

renal cortex and medulla, which becomes attenuated in acute rejection, but not with acute tubular necrosis, in which a uniphasic medullary enhancement pattern is seen.[63] Similarly, marked changes in intrarenal oxygenation occur during acute transplant rejection, allowing techniques of blood oxygenation level–dependent (BOLD) MRI in the transplant medulla to distinguish rejection from acute tubular necrosis.[55] Although data regarding acute rejection show promise, studies in chronic rejection and chronic allograft nephropathy are lacking.

Allograft perfusion assessed by isotopic (Tc 99m diethylenetriamine pentaacetic acid) perfusion scintigraphy is reduced in chronic allograft nephropathy. Although it may be helpful in distinguishing acute tubular necrosis from rejection, it is nonspecific and insensitive for chronic damage.

Urinary Diagnostics

The urine contains exfoliated tubular epithelial cells, cytokines, and growth factors and offers a potential diagnostic window into the intragraft environment. The integrity of cells in the urine depends on the physiochemical environment, including the urinary pH, osmolality, and temperature and, for a measurable biomarker, on the time elapsed until testing or inactivation (e.g., by snap freezing). Quality control is essential to ensure the purity and integrity of any measured substrate. Quality control requires optimal urinary collection, storage, isolation, concentration techniques, and appropriate validation.

Markers of Tubular Injury

Urinary excretion of low-molecular-weight proteins, including β_2-microglobulin and the tubular enzymes (alanine aminopeptidase, γ-glutamyl transpeptidase, and alkaline phosphatase), is a sensitive parameter for proximal tubular injury; these markers increase in aminoglycoside nephrotoxicity, preeclampsia, and chronic pyelonephritis. Similarly, α_1-microglobulinuria and *N*-acetyl-D-glucosaminidase have been found to be useful in native and transplant tubular injury. None of these markers has yet evolved into having a clinical role for the diagnosis of chronic allograft nephropathy.

Proteomic Markers of Rejection

Acute tubulointerstitial renal allograft rejection may be recognized from urinary protein peaks derived from non-tryptic-cleaved forms of β_2-microglobulin, split in acidic urine by aspartic proteases (cathepsin D). Patients with acute tubulointerstitial rejection displayed lower urinary pH and greater aspartic protease concentrations and intact β_2-microglobulin—leading to more cleaved urinary β_2-microglobulin by mass spectrometry.[57] Matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) mass spectroscopy also has identified an 11.7-kD urinary protein peak, confirmed as β_2-microglobulin by enzyme-linked immunosorbent assay and strongly associated with acute rejection.[45] Glomerular filtration and intrarenal catabolism of β_2-microglobulin has an impact on its urinary excretion, which is influenced by functioning kidney mass.

Proteomic analysis of urinary samples using capillary electrophoresis coupled to mass spectrometry can detect distinct urinary polypeptide patterns of acute or subclinical tubulointerstitial rejection distinct from urinary tract infection.[70] Urinary candidate biomarkers detected from spectra derived from surface-enhanced laser desorption/ionization (SELDI) mass spectrometry with bioinformatic analysis potentially may distinguish acute rejection in renal transplant recipients; combinations or panels of biomarkers may be used to enhance diagnostic performance.[9] Magnetic resonance or infrared spectra of urine also have been suggested as a low-cost, rapid-turnaround diagnostic tools, potentially reflecting subclinical inflammation, although this also remains at the research stage.

Markers of Allograft Inflammation

Generally, lymphocytes present in the renal transplant urine have traversed the kidney. Transcriptional profiling of the urinary sediment cells has been suggested as a marker of alloimmune intragraft pathology. Urinary mRNA levels of FOXP3, a specific marker for regulatory T lymphocytes, were increased with acute rejection compared with chronic allograft nephropathy and normal biopsy specimens. They were inversely correlated with serum creatinine in acute rejection and predictive of severe rejection poorly responsive to antirejection therapy. Low expression of FOXP3 identified patients at risk for graft failure, although there was considerable overlap between groups.[39] Gene expression of other molecules from urinary cells, including cytotoxic T lymphocyte markers, CD3 (a T cell marker), CD103 (CD8 cytotoxic T lymphocyte intraepithelial homing marker), perforin and CD25 (both T cell activation markers), interferon-inducible protein 10, and chemokine receptor CXCR3, has been correlated with acute rejection, but not with chronic rejection or normal biopsy specimens.[39]

Urinary TGF-β, detected by coculture with luciferase-expressing cells, is increased with renal dysfunction from acute or chronic renal allograft rejection, but not in stable kidney function.[52] Similarly, fresh urinary cell TGF-β1 mRNA levels measured using real-time quantitative polymerase chain reaction also are higher in long-term patients with chronic allograft nephropathy compared with stable renal function, independent of proteinuria.[33]

Serum Neopterin and Soluble CD30

Neopterin indicates activated macrophages and is easily measured in serum, plasma, or urine. Although serum neopterin is a sensitive marker for acute immunologic activity (increased in early or severe rejection), it is nonspecific (being elevated in cytomegalovirus infection), and levels require serial measurement and adjustment for kidney function for appropriate interpretation. In adults, levels are usually very high in acute rejection, moderate in acute tubular necrosis and decreasing with resolution, and low with cyclosporine nephrotoxicity. In pediatric studies, serum neopterin has not been shown reliably to differ between chronic transplant dysfunction and stable function, and it failed to delineate a low-risk population who might be spared biopsy. Although serum neopterin levels are slightly higher in chronic allograft dysfunction compared with stable function, neopterin does not have a role in long-term alloimmune monitoring. Alternative immune markers, including serum soluble CD30 levels (a T cell T helper type 2 immune response marker), also have been correlated with subsequent chronic rejection; however, these also are increased by infection (including cytomegalovirus), and are influenced by the type of calcineurin inhibitor therapy, limiting their clinical specificity.

Proteinuria

Proteinuria is a powerful and independent risk factor for graft survival (and patient survival) because it represents a composite number of adverse diagnostic groupings (e.g., transplant glomerulopathy, recurrent focal segmental glomerulosclerosis and glomerulonephritis causing glomerular proteinuria, and severe nonspecific chronic allograft nephropathy with tubular proteinuria). Urine protein excretion may increase with hypertension, hyperfiltration, obesity, and mTOR inhibitors and be reduced by renin-angiotensin blockade, calcineurin inhibitor therapy, ischemia, and poor transplant function. Persistent proteinuria has an adverse impact on 5-year graft survival (93% versus 31% with transient proteinuria); even modest levels of 0.5 g/day increase risk. Proteinuria from native kidneys may obscure interpretation; however, this usually declines rapidly by 1 month, and decreases further to low levels by 1 year after transplantation. Proteinuria that fails to decrease or increases (quantified by serial urine protein-to-creatinine ratios) portends a worse prognosis. Persistent, high-grade, increasing, or de novo proteinuria or hematuria with proteinuria should prompt diagnostic biopsy.

Molecular Markers in Kidney Tissue and Blood

New technologies, including DNA microarrays, transcriptome gene chips, proteomics, and metabolomics, are exceptionally powerful and potentially useful techniques, capable of generating vast quantities of information on tissue, blood, or urine samples. Pattern analysis generates a distinct "footprint" potentially indicative of a specific diagnostic pathological process. Potential applications of array-based data include definition of the mechanisms of chronic allograft nephropathy, identification of targets for pharmacological intervention, and development of new monitoring and diagnostic systems. Of these "high-throughput" arrays, gene expression microarrays are the only systems approaching clinical diagnostic utility, but they still require appropriate clinical validation.

Gene expression profiles generated from kidney biopsy specimens and peripheral blood lymphocytes using DNA microarrays may be analyzed by expression signal determination, hierarchical clustering, and class analysis to yield distinctive signatures. These patterns have been correlated with clinical diagnoses (usually proved by biopsy) and seem to discriminate reliably between different patient groups, enabling the diagnosis of acute rejection, acute renal dysfunction without rejection, or a normal graft.[15] Gene expression profiles may be able potentially to differentiate high and low immunological risk groups. Relevant key markers that discriminate between diagnoses on the basis of differentially expressed genes may be extracted to form limited diagnostic arrays (of ≤100 genes). These are cheaper and may be more diagnostically useful by allowing rapid turnaround times. Molecular screening of blood and urine may provide an alternative to invasive biopsy for surveillance of early acute rejection or SCR, although the discriminatory power for the definition of preexisting disease, ischemia-reperfusion injury, and other (inflammatory) causes of acute allograft dysfunction remains to be validated.

Although subtypes of acute rejection (tubulointerstitial versus vascular, cell-mediated versus antibody-mediated) have been subcategorized by gene expression profile, chronic allograft nephropathy and chronic allograft fibrosis appear as a homogeneous entity, with no obvious differential gene expression defined according to different etiological causes.[56] Because differential gene expression by microarray may not accurately reflect the intracellular protein concentration (which depends on post-translational events, such as degradation and phosphorylation), array data need confirmation with reverse-transcriptase polymerase chain reaction and Western blots. Small cohort studies producing high-throughput data may be unreliable and lack reproducibility. Exclusion of patients with systemic infections or inflammatory processes limits their extrapolation to wider populations and produces false estimates of their true specificity. Discrimination between the various causes of fibrosis in the renal allograft is limited, and inactive fibrosis without a cellular component and nuclear material may limit DNA/RNA available for complementary DNA microarrays. Kidney transplant biopsy remains the "gold standard" for definitive allograft assessment, although supplementary data from these new techniques are likely to improve diagnostic assessment and therapeutic response.

TREATMENT

General Principles

1. Chronic allograft nephropathy is the end result of multiple pathophysiological pathways of injury (see Fig. 25-2). No single "magic bullet" is likely to be sufficient for its treatment, but rather several therapies and approaches would be needed to counteract the specific and varied etiological insults (Table 25-4). These potentially could include specific antagonists targeted at fibrogenic mechanisms[32] or indirect therapies, such as treatment of hypertension, lipids, infections, and smoking.
2. Drivers of injury are time dependent, and therapy ideally should be initiated before or during periods of ongoing injury. Experimental and clinical data suggest that treatments have different windows of benefit: Some may help early after transplantation only, and others may be detrimental if used late. Therapeutic flexibility of immunosuppression should be maintained. An example would be potent front-loaded calcineurin inhibitor therapy to suppress early rejection, followed by minimal levels to limit nephrotoxicity or infective complications, including BK nephropathy.
3. Prevention is better than cure. Chronic allograft nephropathy and allograft fibrosis reflect the later expression of prior pathogenic insults. Treatment options need to be exercised early to prevent permanent nephron destruction and to minimize early tubulointerstitial damage and nephron loss from ischemia and alloimmune insults.
4. Therapy should be tailored according to individual requirements and immunological risk and adjusted for different and changing clinical scenarios. Examples would be calcineurin inhibitor minimization strategies with delayed graft function or late calcineurin inhibitor nephrotoxicity, or conversely, strengthening of immunosuppression when subclinical or late rejection has occurred.

Table 25–4 **Management of Chronic Allograft Nephropathy and Chronic Allograft Damage**

Prevention and Screening
Minimize ischemia-reperfusion damage (shortest ischemic times, optimal procurement and transport)
Minimize donor-recipient histoincompatibility
Rapid diagnosis and effective treatment of acute rejection
Early optimal immunosuppression (including early CNI and interleukin-2 receptor antibody in recipients with medium to high immune risk)
Control of early subclinical rejection
Prophylaxis for CMV with valganciclovir or valaciclovir
Early BK virus screening (especially with high-dose immunosuppression)
Monitoring of renal function, urinalysis (for glomerulonephritis), and imaging (for ureteric obstruction)
Regular compliance review
Control of Progression Factors
Control hypertension (ACE inhibitor and ARB preferred to limit scarring, calcium channel blocker or β blocker may be added, diuretic may often be needed)
No added salt, stop smoking, control lipids, limit weight gain
Control diabetes and urinary tract infections (if present)
Reduce (eliminate or substitute) long-term CNI in recipients with low to medium immune risk (if chronic allograft nephropathy or CNI nephrotoxicity develops)
Avoid late underimmunosuppression (risk of subclinical rejection)
Match immunosuppression to immunological risk and rejection history
Clinical management of acute interval recipient events (e.g., sepsis, acute tubular necrosis) with restitution of appropriate immunosuppression with stability of acute insult
Monitoring and preventive strategies for neoplasia and cardiovascular risk factors in patient

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CMV, cytomegalovirus; CNI, calcineurin inhibitor.

5. Technical advances, such as gene complementary DNA microarrays, proteomics, and metabolomics, are expected to yield diagnostic advances. Transcriptional changes can be detected before histological fibrosis. Potential applications of new bioinformatics would include discrimination of inflammatory infiltrates according to the constellation of expressed genes and cellular expression profiling. Improved diagnostics may allow optimization of treatment strategies.

Specific Treatment Approaches

Multiple induction and maintenance regimens are available, and validation of the ideal combination of immunosuppressive agents is being undertaken by long-term clinical studies (see Table 25-4; see also Chapters 15 through 21). Most units use calcineurin inhibitor–based triple therapy; some withdraw corticosteroids routinely and often use dosage adjustment and switching of therapy according to changing clinical scenarios. Specific approaches include the following:

1. Limit early damage before it occurs by procuring an optimal donor organ, limiting ischemia-reperfusion injury, controlling ROS generation, using optimal initial immunosuppression, and implementing appropriate surveillance (e.g., biopsy in delayed graft function).
2. Prevent alloimmune injury by selecting immunosuppression appropriate to each individual patient's immunological risk category and implementing early (biopsy) diagnosis and adequate treatment for severe or resistant rejection. Severe rejection may result in persistent SCR, so follow-up biopsy may be considered.
3. Control ongoing damaging processes, including SCR or calcineurin inhibitor therapy according to individual cases by appropriate prevention, detection, and therapeutic strategies. When persistent SCR is evident, strengthen immunosuppression by conversion of azathioprine to mycophenolate mofetil, addition of corticosteroids to dual therapy, and continued use of calcineurin inhibitors.
4. Implement early BK virus surveillance and treatment.
5. Limit long-term calcineurin exposure, especially in low and medium immunological risk recipients, with low-dose calcineurin inhibitors, calcineurin inhibitor elimination with caution and monitoring for acute rejection (rates range from 10% to 40%), or replacement of calcineurin inhibitors with an alternative agent (at present mTOR inhibitors are available, but options should expand with further drug development).

Long-Term Immunosuppression

The ideal long-term immunosuppressive agents should be effective, be well tolerated, and have minimal side effects. Desirable properties include the following:

1. Alloimmune effectiveness—to be able to provide adequate immunosuppression to avoid SCR, true chronic rejection, or chronic antibody-mediated rejection
2. Lack of nephrotoxicity or even renoprotective properties
3. Few or minimal cosmetic and subjective side effects to optimize compliance (especially in young women and adolescent recipients)
4. Antineoplasic properties (e.g., mTOR inhibitors)—as opposed to some properties of calcineurin inhibitors that may promote cancer
5. Minimal or absent enhancement of comorbidity (e.g., lipids, post-transplant diabetes mellitus, cardiovascular disease)

SUMMARY

Chronic allograft nephropathy is the generic term to describe chronic interstitial fibrosis and tubular atrophy commonly seen in kidney transplants, which is responsible for most allograft losses, excluding recipient death. Chronic allograft nephropathy is neither a synonym for chronic rejection (implying ongoing immunological activity) nor chronic allograft dysfunction (a functional definition without regard of transplant histology). Despite improvements in immunosuppression and the control of acute rejection, it remains an important clinical challenge. Progressive late allograft failure and chronic allograft nephropathy is no longer believed to simply represent chronic rejection, but instead is best conceptualized as the consequence of cumulative transplant damage from time-dependent immune and nonimmune mechanisms resulting in a final common pathway of nephron loss and its fibrotic healing response.

Chronic allograft nephropathy is common, progressive, time-dependent, and clinically important. An early phase of tubulointerstitial damage occurs soon after transplantation, secondary to ischemia-reperfusion injury, acute tubular necrosis, acute rejection and SCR, polyomavirus in some cases, and calcineurin inhibitor tubular nephrotoxicity, which are superimposed on any preexisting donor disease. Subsequently, cellular infiltration and alloimmune injury gradually lessen and are progressively supplemented by microvascular and glomerular abnormalities from causes including calcineurin inhibitor nephrotoxicity, hypertension, immune-mediated fibrointimal vascular hyperplasia, transplant glomerulopathy and capillary injury, and recurrent or de novo glomerulonephritis.

Additional pathogenic mechanisms of underlying progressive damage include disruption of the internal architecture of the transplanted kidney, cortical ischemia from microvascular attenuation, persistent chronic inflammation that fails to resolve, the onset of replicative senescence and evolution to a senile cellular phenotype, cytokine and growth factor excess promoting fibrosis, and epithelial-to-mesenchymal transition of tubular cells. Accelerating factors such as hypertension, proteinuria, dyslipidemia, and smoking also are likely to contribute. Understanding the causes and mechanisms of injury may provide targeted strategies to prevent the initiation or progression, or both, of chronic damage.

Acknowledgments

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Chapter 26

Vascular Complications after Kidney Transplantation

Richard D. M. Allen

The last 3 decades have seen a dramatic improvement in kidney graft survival as a result of better immunosuppression and focused greater emphasis and importance on minimizing technical causes of kidney graft loss. Surgical misadventure after kidney transplantation previously ranked low as a cause of graft loss in the first 6 months after transplantation in proportion to loss from acute rejection approaching 20% and death with a functioning graft of around 10%. In the current transplant era, all three of these causes of graft loss contribute almost equally to the total overall graft loss, however, which is about 5% at 6 months in Australia (Fig. 26-1). The enduring techniques of vascular anastomoses described by Carrel more than a century ago have not changed significantly (see Chapter 1). His simple test of satisfactory anastomoses was observation of a viable kidney transplant producing urine within minutes of completion.

The transplanted kidney is a highly vascular organ. Ten percent to 15% of cardiac output at rest, accounting for perhaps 500 to 800 mL/min, passes through the kidney. A graphic example of the magnitude of the renal blood flow is the simple temporary occlusion of the transplant renal vein with a pair of forceps at the time of surgery, described clinically as the Hume test, which results in rapid and pulsatile engorgement of a well-perfused kidney transplant. Equally, a breach in the continuity of the transplanted artery or vein can result in catastrophic blood loss and circulatory failure within minutes, particularly in the presence of a recipient left ventricle already compromised by coexisting coronary artery disease, long-term effects of systemic hypertension, or uremic cardiomyopathy.

The kidney is unforgiving of interruption of blood flow, with the cortex more sensitive to hypoxia than the medulla. The magnitude of the effect of acute and complete interruption of blood flow during the transplantation procedure depends on the quality of the donor kidney, length of ischemia time, temperature of the kidney, and extent of intrarenal thrombosis during the period of stasis of renal blood flow. In some circumstances, irreversible cortical necrosis can occur within minutes and even in the most favorable situations is inevitable by the 20-minute mark.

Incomplete interruption of blood flow has a more subtle effect. Arterial pressure sensors within the kidney detect pressures below which a cascade of autoregulatory changes are set in place to increase systemic pressures to satisfy the requirements of the kidney, usually at the expense of the recipient's well-being. Impaired venous drainage is probably better tolerated, although sudden occlusion of a previously well-perfused kidney can lead to dramatic rupture of the cortex with uncontrolled bleeding from intrarenal veins.

TECHNICAL COMPLICATIONS AND THEIR PREVENTION

Vascular complications during and soon after kidney transplantation are common. Describing the possible complications of kidney transplantation to a patient

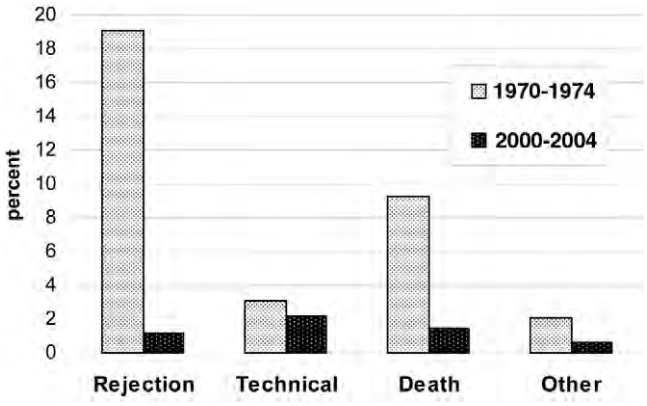


Figure 26–1 Causes of graft loss in first 6 months after kidney transplantation in Australia reported to ANZDATA Registry, living and cadaver donor, comparing 5-year time periods 1970 through 1974 (n = 1118 transplants) and 2000 through 2004 (n = 2868 transplants).

before surgery without causing alarm can be difficult. Kidney transplantation is not a straightforward ablative surgical procedure, but rather one that involves placing a kidney in a nonanatomical heterotopic position. By comparison, cardiothoracic and liver transplant surgeons have a much easier technical task, placing size-matched donor organs into an orthotopic position after removal of the failed recipient organ.

In deceased donor kidney transplantation, the transplant kidney surgeon must cope at short notice with whatever computer-allocated pairing of the donor kidney and recipient turns up at any hour of the day or night. Donor kidneys, particularly from the increasingly common marginal deceased donors, are not new engine parts that can be taken off a spare parts shelf. They are preowned, cannot be preordered, and have no regenerative capacity. Equally, the potential kidney recipients are not mass-produced engines. They come in different shapes and sizes, and many have cardiovascular systems that are less well cared for and more compromised than others. By the end of the transplant operation, the kidney has to fit into its designated foreign position and have the potential to work immediately or soon thereafter. The good surgeon is one who appreciates that there exists little margin for error and who avoids the difficult situations by careful preparation and anticipation of the potential pitfalls. When the sometimes inevitable complications do occur, the surgeon must salvage the situation, balancing risks to recipient and kidney, by responding quickly and appropriately. No two kidney transplant procedures are the same.

The incidence of vascular complications depends to a great extent on the careful evaluation of the recipient, the donor kidney, and the surgical technique of implantation. These are discussed in detail in Chapters 4 and 11, but some of these points are worth reiterating here.

Preoperative Assessment

Evaluation of the recipient arterial and venous systems by history, examination, and imaging is discussed in Chapter 4. An accessible patent iliac artery and vein with unimpeded proximal blood flow and that are able to be sutured are essential. Access can be difficult because of morbid obesity or a preexisting kidney transplant. Extensive mural arterial calcification can make clamping and suturing impossible without disruption of the artery. The extent of the surgical evaluation is the same for recipients of living and deceased donor kidneys. In both, correctable problems are sorted out beforehand. What is different is the need for ongoing assessment of the recipient on a deceased donor kidney waiting list and ready availability of documentation for other surgeons who are not involved in the assessment but who are part of the on-call roster. These requirements may be a logistic and communication challenge for large, regionally based transplant units with many rostered surgeons. Placement of a patient on a kidney transplant waitlist without surgical assessment and lack of systems in place to ensure access to results of the assessment at all times could be considered medically negligent in the event of a subsequent and attributable vascular complication.

Right or Left Donor Kidney

Despite evidence that the results of transplantation of the right kidney are the same as those for the left, the transplant surgeon when given a choice takes the left donor kidney over the right because it has a longer renal vein.[36] When given the choice of a living left donor kidney with two arteries or a right kidney with one, most surgeons choose the former.[31] The longer left renal vein is less fragile and more easily sutured to the more deeply situated external iliac vein. In contrast, with a short right renal vein, the longer right renal artery anastomosis is more difficult to site correctly because of the propensity of the renal artery to kink if the deceased donor aortic patch is used for the arterial anastomosis. For this reason, the venous anastomoses should be performed before the renal artery anastomoses. Anastomosis of the deceased donor right renal vein can be facilitated by vein elongation using the adjacent inferior vena cava or a donor iliac vein extension graft. Alternatively, as is frequently the need in living donor right kidneys, the recipient external iliac vein can be mobilized by dividing the internal iliac veins. All of these maneuvers are undertaken before a vascular clamp is placed on a recipient vessel.

Right-sided or Left-sided Surgery

All else being equal, it is conventional to place a right-sided donor kidney in the left iliac fossa and vice versa, allowing the urinary collecting system to be on the medial side of the kidney. This placement facilitates easier corrective surgery for common ureteric complications. This orientation also is advantageous for end-to-end anastomosis of the donor renal artery to the recipient internal iliac artery. It can be argued, however, that the reverse is the case if the external iliac artery is the favored vessel for placing the arterial anastomosis. Relative contraindications to the use of one side over the other include the presence of an ipsilateral thigh arteriovenous fistula (because of the potential for vascular steal from the transplanted kidney) and ipsilateral lower limb amputation.

Back Table Preparation

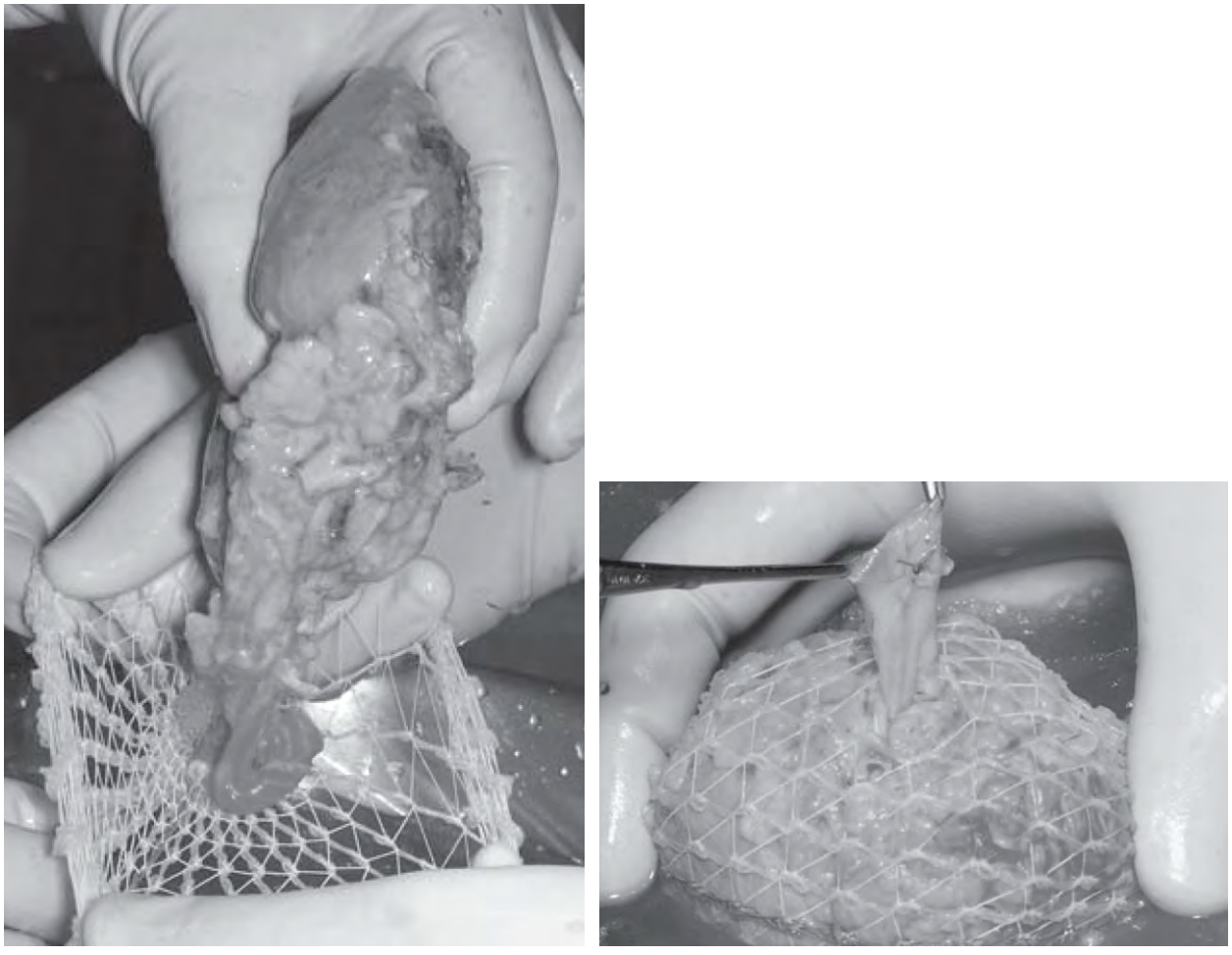
All donor kidneys require back table preparation. Failure to look at the deceased donor kidney before starting the

recipient procedure can create problems if the kidney is not as "advertised" by the donor surgeon's description. Accessory arteries may have been missed or divided. Atheromatous plaque, clot, or an intimal flap may be impinging on the lumen of the renal artery. Inadvertent traction or a donor surgeon's wayward scissor may have torn or injured the donor renal vein. If problems are identified and corrected before beginning surgery, recipient operating and anastomosis times are kept to a minimum and surgical options are retained, such as preservation of the inferior epigastric artery for anastomosis to a lower pole artery. For living donor kidneys, a missed accessory artery in the living donor kidney is apparent at the time of initial cool perfusion at the back table. This is not the case for the in situ cool perfused deceased donor kidney. Donor artery and vein are mobilized as necessary, with perirenal adipose tissue trimmed, gonadal vein removed and, in the case of a deceased donor kidney, adrenal gland removed. Hemostasis after revascularization of the transplanted kidney is easier if vein tributaries and small hilar vessels associated with trimmed tissue are ligated.

Repeat flushing of a deceased donor kidney with a small volume of preservation solution has several advantages. Residual venous blood, if present, can be cleared. Leaking vessels can be identified and ligated before revascularization. There is clinical evidence that the subsequently "freshened" deceased donor kidney is more likely to avoid primary nonfunction.[53] Finally, the kidney vasculature is accurately oriented. The superior and inferior margins of the artery and vein can be marked to reduce the risk of twisting the vessels at the time of anastomosis. To reduce handling of the donor kidney during the surgical procedure and for ease of surgery, the kidney can be placed in a temporary stocking, surgical glove, or pack (Fig. 26-2). Ice saline slush for the back table dissection should be available until the vascular anastomoses are completed in case it is necessary to cool the kidney again.

Venous Anastomosis

The external iliac veins in an obese recipient and a short male patient with a deep pelvis and almost vertically disposed external iliac vein can be challenging, particularly for right-sided donor kidneys. It is tempting to place the venous anastomosis close to the inguinal ligament. This placement may be feasible if a long left renal vein is available, but it is often a mistake, with subsequent compression of the renal vein occurring during wound closure. A better but



A B

Figure 26–2 **A**, Placement of a donor kidney in an elastic stocking to assist with handling of the kidney during vascular anastomoses. **B**, A hole is made in the stocking to expose the renal vein. The stocking is removed before revascularization of the kidney transplant. (See color plate.)

sometimes tedious and difficult alternative is to mobilize the external iliac vein by dividing the internal iliac vein tributaries. This mobilization should be done with great care and only with an experienced assistant. The surgeon should ensure that long stumps of the ligated veins are left on the external iliac vein side. Loose ligatures can make control of bleeding almost impossible as the large and thin-walled labyrinth of pelvic and presacral veins retracts into the depths of the surgical wound. Massive blood loss can occur within minutes and is best managed by carefully packing the depths of the wound and applying pressure. The surgeon should call for the cell saver and blood products and systematically gain control by application of metal clips or polypropylene (Prolene) sutures.

A thrombosed or stenosed external iliac vein is best identified before surgery and should be considered in patients with a history of deep vein thrombosis (DVT), previous transplant surgery, unilateral leg swelling, and emergency dialysis access via the femoral vein (Fig. 26-3). When encountered at the time of surgery, the common iliac vein, which usually has a preserved lumen, can be dissected, or, alternatively, the surgeon can close the wound and transplant the kidney into the opposite iliac fossa.

Unless there is a recipient history of factors predisposing to venous thrombosis, systemic heparinization for the vascular anastomoses is unnecessary. The site of the iliac vein anastomosis is marked with a sterile surgical marking pen before applying the venous clamps because of their inherent tendency to rotate the alignment of vein one way or the other during application (Fig. 26-4). Accurate sizing of the venotomy length prevents stretching of the end of the transplant renal vein to accommodate a venotomy that is too long. Stretching leads to a long stenosed anastomosis. After opening the vein, the surgeon searches for pairs of valve cusps and disrupts them if they are adjacent to the anastomosis. A stay suture is applied to the midpoint of at least one of the sides of the venotomy to reduce the risk of catching the opposite wall of the anastomosis with the continuous running vein suture. The orientation of the kidney should be reviewed.

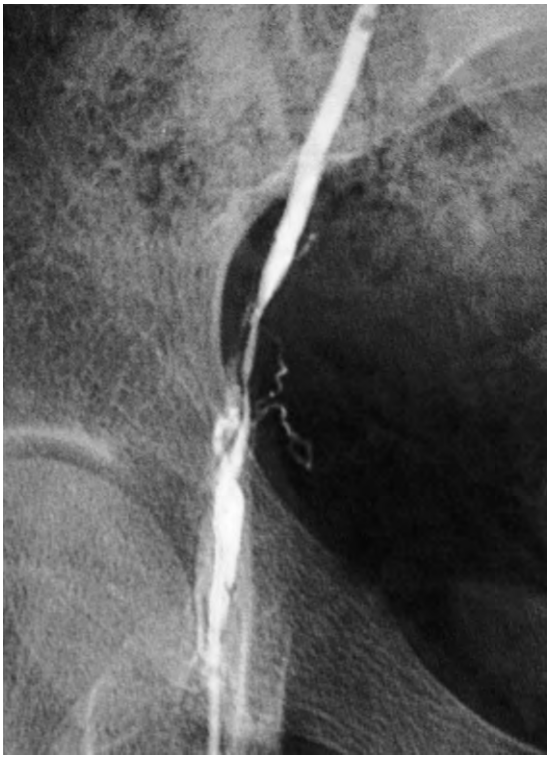


Figure 26–3 Ascending venogram shows a long stenosis of the right iliac vein in a patient with previous thigh arteriovenous fistula and temporary hemodialysis cannulas.

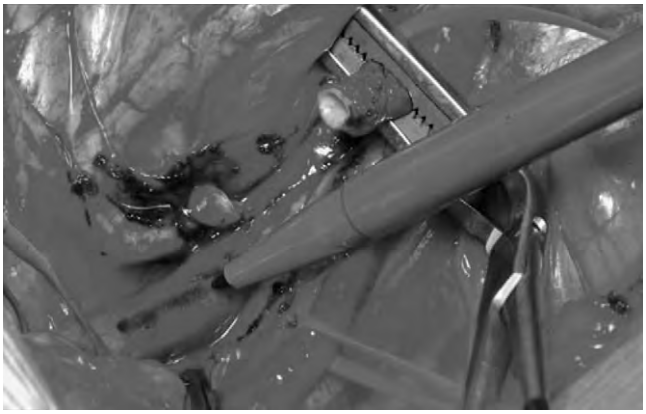


Figure 26–4 Marking of the position of the vein anastomosis site before placing vascular clamps on the external iliac vein. (See color plate.)

Arterial Anastomosis

The arterial anastomosis is generally placed more proximally than the vein for a left or a right kidney. Limiting the extent of the dissected iliac artery limits disruption of adjacent lymphatic channels. If the internal iliac artery is to be used, the surgeon fully mobilizes the bifurcation of the common iliac artery and carefully examines the origin for atheromatous plaque. Use of the internal iliac artery should be avoided if it already has been used on the opposite side for another transplant. The bifurcation or trifurcation of the internal iliac artery should be preserved to reduce the risk of buttock claudication. Claudication is inevitable, as is impotence in a man, if both internal iliac arteries have been used for transplantation.

The arterial clamps are applied with care. Clamps with silicone inserts applied horizontally are less likely to disrupt calcified plaque commonly on the posterior aspect of the artery. Endarterectomy often can be avoided by carefully selecting a soft segment of artery and, if necessary, adjusting the length of the renal artery by resecting the donor aortic patch. This resection may be necessary with a right-sided kidney to avoid kinking of a proximally placed anastomosis during wound closure. Equally, the shortened artery of the right kidney can be anastomosed to the end of the internal iliac artery; this has the added advantage of deeper placement of the transplant anastomoses, less tension on the short right renal vein, and easy positioning of the kidney after revascularization. Alternatively, the aortic patch of a right-sided kidney, transplanted into the left iliac fossa, can be anastomosed to the medial side of the iliac artery, subsequently providing space for the artery to curve gently medial to the hilum of the kidney positioned on its side.

Multiple renal arteries are encountered more commonly with the increasing popularity of laparoscopic living

kidney donation and the preference for the left kidney.[31] At least 20% of left kidneys have more than one artery after living donation. They present their own challenges, and a meta-analysis has shown marginally poorer outcomes for living donor kidneys with multiple arteries.[37] Precluding the use of living donor kidneys on the basis of multiple arteries alone is unfair to the potential recipient. Small accessory renal arteries, particularly at the upper pole, can be ligated without problems.[20] Anastomosis of two arteries close together on an aortic patch of a left-sided deceased donor kidney is comparatively straightforward. If they are more than 2 cm apart, consideration could be given to performing two separate anastomoses, one on either side of the renal vein. Dual arteries to a right-sided kidney make positioning of the kidney difficult without kinking one or the other artery, usually adjacent to the patch.

Individual transplant surgeons will have their own views about how best to manage multiple arteries of a living kidney donor. Despite longer anastomosis times, the author's preference is for two separate anastomoses in most instances, particularly if the arteries are of nearly equal size. This approach avoids a complex anastomosis with at least a theoretical increased risk of thrombosis. The exception is a small upper pole or lower pole accessory artery in close proximity to, and that can be anastomosed to, the side of a main renal artery, away from the end of the renal artery, on the back table.

Reperfusion

Reperfusion is the high point of the transplant procedure; there is no turning back. The combined total anastomosis time should be 20 to 40 minutes. Any longer suggests difficulty and increases the probability of primary nonfunction. Before completing the arterial anastomosis, the surgeon should exclude air from the clamped vessels by injecting heparinized saline. The surgeon should ensure that fixed retractors are not compressing the proximal iliac vessels. The individual anastomoses should be tested before revascularization of the transplanted kidney (Fig. 26-5). Control of imperfect anastomoses is managed more easily before rather than after revascularization of the transplanted kidney. The arterial clamp is released first. The last clamp removed is the distal iliac artery clamp when systemic blood pressure has stabilized after reperfusion of the kidney. Observation of urine within a couple of minutes is a reassuring sight; a pink, firm, and well-perfused kidney is the next best thing. If these are not observed, the surgeon should actively look for problems. Kidneys from marginal donors or with long renal ischemia times may have a "blotchy" or mottled appearance with dark, less well-perfused areas. An encouraging sign is the gradual reduction in extent of the dark areas until the kidney is uniformly pink (Fig. 26-6).

A flaccid, poorly perfused kidney is reason for concern. Modern tissue typing and crossmatching techniques have essentially excluded hyperacute rejection as a cause (see Chapter 10). The surgeon should start with inspection of the renal artery to exclude kinking, the most likely cause, or twisting and resolve this by repositioning the kidney if possible. Next, the surgeon should assess the pulsatility of the iliac artery proximal to the anastomosis and into the renal artery to the hilum of the kidney looking for evidence of interruption of flow. If surgery has been careful, an intimal flap is unlikely, but nevertheless possible, particularly in recipients with underlying arterial disease. Management is not easy. The most likely site of an intimal flap would be at the anastomosis.

A difficult decision sometimes needs to be made between revising the arterial anastomosis or the "safety first option" of removing the transplanted kidney, reperfusing with preservation solution, and starting all over. The now warmed kidney may not tolerate more than 15 minutes of warm ischemia. When revising the anastomosis, the kidney artery is flushed with at least 50 mL of heparinized saline, and the renal vein is clamped. If one is confident of the cause, another option is to transect the external iliac artery distal to the anastomosis and perform a blind eversion endarterectomy of the iliac artery with an arterial forceps followed by end-to-end repair of the artery.

If no problem can be identified, and systemic blood pressure is satisfactory, the surgeon should be patient, particularly if the kidney increases in size and becomes pulsatile

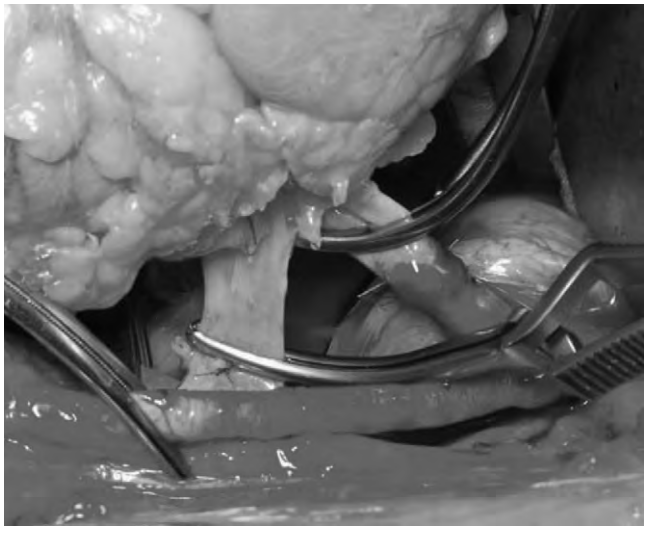


Figure 26–5 Testing the integrity of the end-to-end right internal iliac artery to transplant renal artery anastomosis before revascularization of the kidney transplant. (See color plate.)

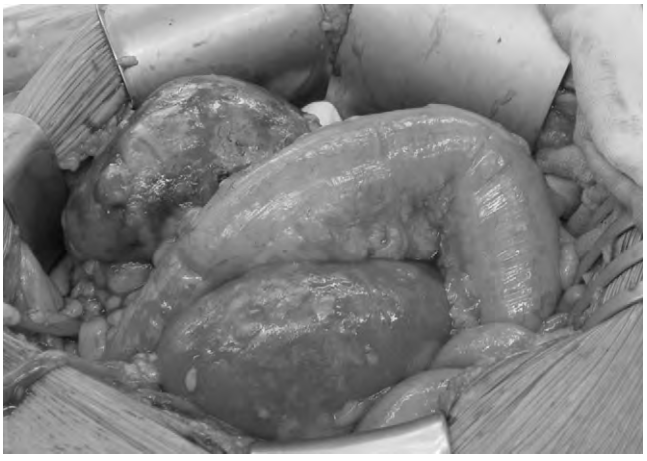


Figure 26–6 Sigmoid colon separating the dual kidney transplants from a marginal cadaver donor. The left-sided kidney was transplanted first and is of uniform appearance. The right kidney is of mottled appearance 10 minutes after revascularization. Ten minutes later, it had the same appearance as the left kidney transplant. (See color plate.)

when the renal vein is temporarily occluded. A small incision into the capsule of the kidney followed by evidence of bright arterial bleeding also can be reassuring. Extrarenal arterial spasm is a frequent finding and probably the result of undue traction on the renal artery during donation or implantation surgery. In such situations, the kidney usually is not discolored. Placement of a swab generously soaked in papaverine may help. Spasm is usually self-limiting.

Catastrophic bleeding after removing all vascular clamps is unlikely to occur if the anastomoses have been assessed before revascularization. If present, however, bleeding is usually venous in nature and either from a tributary vein or, much worse, from a disrupted venous anastomosis because of traction on a thin-walled, usually right-sided renal vein. Because of the continuous nature of the suture, simple repair is usually impossible and, if attempted, results in extensive blood loss or anastomotic stenosis, or both. In contrast to arterial inflow correction, the strong recommendation is to remove the donor kidney, reperfuse with preservation solution, and start all over after trimming the end of the renal vein. The living donor kidney is resilient to warm ischemia or prolonged anastomosis times compared with the deceased donor kidney.

The observation of a tense, engorged, and pulsatile kidney, usually associated with anuria, is an indication of venous outflow obstruction. The surgeon should look for a twisted renal vein, or close apposition of the sides of the venous anastomosis because of imperfect suturing. Because of the relatively controlled situation, revision of the anastomosis usually can be undertaken within 10 minutes after systemic heparinization, clamping the renal artery, and exsanguination of the transplant. An uncommon cause is compression of the left common iliac vein as it passes under the right common iliac artery, described as the May-Thurner syndrome.[4] Presumably, the extra 500 mL of blood per minute from the transplanted kidney is enough to compromise the narrowed iliac vein at that point. It is managed by stenting the iliac vein depending on the time of diagnosis after transplantation.

A disappointing observation on completion of the vascular anastomoses would be finding the transplant ureter pointing in the wrong direction. The transplanted kidney otherwise has a healthy appearance. If the kidney is turned 180 degrees in either direction, it does not look healthy, even if the ureter does seem to be pointing in the correct direction toward the bladder. The vein is twisted near the hilum, and the artery is wrapped around it. If the kidney is turned back to the starting position, it looks healthy again. The obvious conclusion is that the kidney has been transplanted upside down. This error is more likely to occur with a living donor kidney in the absence of the full length of the renal vein and the absence of the aortic patch on the artery. One tedious option, particularly with a marginal donor kidney, is to remove the kidney, reperfuse with preservation solution, and start again. Alternatively, the kidney can be left as is, and the ureter can be provided with a more circuitous route to the recipient bladder. Reports (personal communications from several surgeons) suggest that the latter option is reasonable, albeit with stories of recipients finding that they pass more urine when in a supine position. A better option is to ensure that this error does not happen.

Positioning the Kidney and Wound Closure

The ureterocystoneostomy, the anastomosis of the ureter to the bladder, should be the relaxing part of the kidney transplant operation. There is not the same intensity of time constraint and the lack of margin for error associated with the vascular anastomoses. All being well, urine is being produced. The abdominal wall retractors have been removed, and the kidney may be out of sight during much of the ureter anastomosis. Positioning of the kidney during this stage is important, but not as crucial as when the kidney tries to find its own position as the wound is being closed. If a suction drain is to be inserted, it is done with assistance to protect the kidney. Avulsion of a tenuous venous anastomosis with lateral movement of the surgeon's hand is not easy to cope with at this stage of the operation.

During apposition of the abdominal wall muscles, the potential for kinking of the kidney transplant vasculature is considerable. At this stage of the transplant procedure, the surgeon most appreciates that a kidney does not naturally fit into an iliac fossa. Although the transplanted artery and vein may appear to be in a satisfactory position with the wound open, wound closure tends to compress the transplant in an anteroposterior direction, either compressing the vasculature or repositioning the kidney such that its planar axis is at right angles to that of the vasculature. Difficulty with wound closure is more likely to occur in thin patients with large kidneys and male patients with narrow deep pelves. Wound closure is made more difficult if the venous anastomosis is too close to the inguinal ligament or the incision is too close to the anterior superior iliac spine. Dividing the internal iliac veins at this stage would not help and would be hazardous.

The "surgical escape" can be to place the kidney into the peritoneal cavity by creating a longitudinal window in the peritoneum. The kidney is positioned anterolateral to the cecum on the right side or the sigmoid colon on the left side. If possible, the greater omentum can be used to separate the bowel from the kidney. These maneuvers usually solve the problem, and percutaneous biopsy is still feasible after placement of a local anesthetic agent at the level of the peritoneum. Fixation of the kidney by sutures usually is not required, although avoidance of mammalian Target of Rapamycin (mTOR) inhibitor–based immunosuppression in the initial months after transplantation is advisable because of its inhibition of adhesion formation (see Fig. 26-24 later in this chapter).

Postoperative Recovery

The surgeon or a senior member of the surgical team should remain with the recipient in the early recovery phase until there is conclusive evidence of satisfactory perfusion of the transplanted kidney. If there is a problem, it requires urgent resolution with return to the operating room. Most of the time, there is not a problem. Transplanted kidneys producing urine at the end of the surgical procedure are easier to manage, particularly if urine is being produced in volumes that could not be achieved by residual native kidney function. The better the urine volume, the less likely that clots will form in the bladder.

The bladder catheter bag is placed in a position where it can be readily observed. If no urine production has been seen on the operating table or in the initial recovery area, and the recipient is hemodynamically stable with a central venous pressure of at least 5 cm H_2O, a duplex ultrasound examination may be indicated. Ultrasound is best performed before the recipient leaves the operating room, particularly if there is reason to be concerned on the basis of difficulty in finding a satisfactory position for the kidney during wound closure. Because of time constraints, particularly after hours, it is advantageous for the transplant surgeon to be adept at the use of an ultrasound machine dedicated to the kidney transplant unit.

The need for concern about the transplant vasculature is even greater if urine was being produced on the operating table. The surgeon should look out for a restless recipient awakening from the anesthetic and drawing up the knees because of pain, intolerance of the urinary catheter, or hypoxia. The surgeon also should beware the radiographer determined that the recipient sit upright for a chest radiograph to check the position of the central venous line. A 45-degree angle is sufficient. If there is unexpected anuria or oliguria, a bladder washout should be performed. If the anuria or oliguria is unresolved, an ultrasound examination should be done. An inadequate arterial signal and significant collections are indications for an immediate return to the operating room.

Patency of an accessory renal artery is difficult to determine in the early postoperative phase by observation of urine output alone. These smaller vessels are more prone to thrombosis or kinking, and longer term consequences include poor graft function and hypertension. An avascular segment of kidney can occur, at least initially, without noticeable effect. The need for an extra-arterial anastomosis is a relative indication for an early duplex ultrasound examination of the transplanted kidney in the presence of the operating surgeon with knowledge of the surgical vascular anatomy. An indication of segmental infarction is a lactate dehydrogenase level greater than 500 IU/L.[38]

Because of the quality of duplex ultrasonography, indications for formal angiography in the early phase after kidney transplantation are few. Indications are limited to perhaps the suspicion of proximal iliac artery disease or clamp injury and an obese recipient in whom visualization of the renal artery and iliac vessels is not technically feasible. Helical computed tomography (CT) angiography is usually easier to arrange and faster to obtain.

Compartment Syndrome

All can be well with a transplanted kidney while the recipient is in a supine or near-supine position. Duplex ultrasound also is performed with the recipient in a supine position. When the patient is placed in a sitting position, however, all can change with the downward movement of a large polycystic kidney or heavy small bowel mesentery and greater omentum in a patient with truncal obesity. External compression of a mobile transplanted kidney can change its position or reduce perfusion. A hematoma or paralytic ileus or an early lymphocele could do likewise. The contribution of the compartment syndrome to initial poor kidney transplant function should not be underestimated (Fig. 26-7). Reversible factors should be resolved.

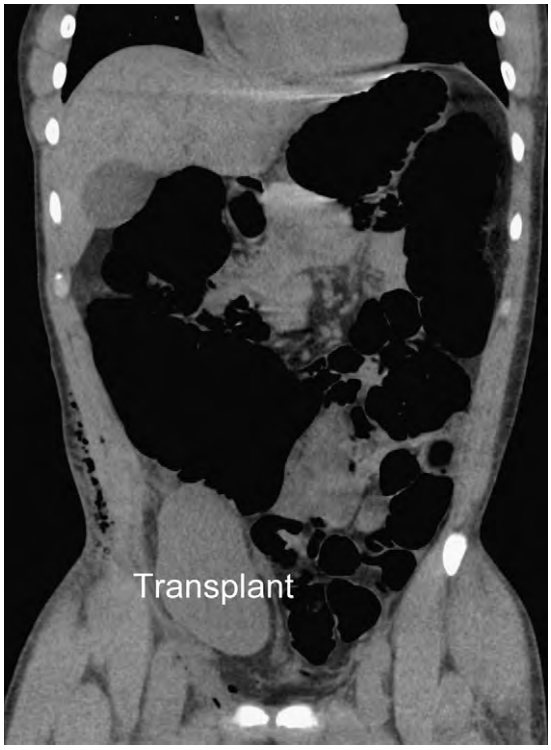


Figure 26–7 CT scan with coronal view of abdomen 24 hours after kidney transplantation. The perfusion of the kidney transplant in the right iliac fossa was compromised by gross pseudo-obstruction of the large and small bowel.

Drain Tube Removal

If suction drainage has been used, removal of the drain should be a straightforward task. Suction is removed, and the drain is withdrawn slowly with a twisting motion to dislodge fatty tissue that might be lodged in the small side holes of the drain as a result of the suction. Small pediatric kidneys have been known to undergo torsion of the vascular pedicle on removal of the drain with resultant loss of graft function.

The timing of drain tube removal varies according to volume and nature of the drained fluid. It is not unusual to record 100 to 200 mL of heavily blood-stained drainage in the first few hours of transplantation. The bleeding usually stops spontaneously. Brisk bleeding (e.g., from an imperfect anastomosis) is often difficult for a suction drain to cope with because of clot formation. Drainage volume can be an unreliable gauge of active bleeding. Patient discomfort, tachycardia, hypotension, and abdominal findings of an enlarging mass around the transplant are indicators of a significant bleed requiring urgent surgical exploration. Large volume drainage of less heavily blood-stained fluid generally indicates residual peritoneal dialysate, lymph, or urine. Urine is excluded by biochemical analysis or absence of glucose on dipstick testing. If the drainage is not urine, and it persists beyond the first day, it is most likely lymph.

HEMATOMA

Hematoma formation is a common finding after kidney transplantation in the immediate postoperative period. Hematomas also can occur spontaneously in an anticoagulated recipient and after percutaneous transplant biopsy.

Most hematomas are small and insignificant ultrasound findings that resolve spontaneously. Some are not, however, and are associated with recipient discomfort, abdominal swelling, and decreasing hemoglobin. The hematoma is able to expand progressively in the retroperitoneal space. This space is limited, however, and a pressure effect on the transplanted kidney, to a greater or lesser extent, is inevitable with an adverse effect on arterial blood flow into the kidney. Hematomas also can cause hydronephrosis by compression of the renal pelvis or ureter. Hydronephrosis is evident by an increasing serum creatinine level or by comparative duplex scan imaging against a baseline study; both are nonspecific, but nevertheless reliable, markers of a compromised kidney. The extent of the hematoma in the retroperitoneal space is best shown, often as a heterogeneous crescentic peritransplant collection, by CT without vascular contrast (Fig. 26-8). The appearance of the CT findings varies with time.

Percutaneous drainage of the hematoma may be diagnostic but does not suffice as treatment because of the mixed nature of the hematoma. Surgical exploration in the first 24 hours or so after transplantation and evacuation of the hematoma might locate bleeding from a hilar vessel, a retroperitoneal vein, or abdominal wall muscle. Thereafter, a more common finding is a stable hematoma without obvious cause. Great care is taken to remove the hematoma, always being alert to the possibility of dislodging clot that is providing tenuous hemostasis at the site of a vascular anastomosis. Invariably, transplant function improves after evacuation of the hematoma. Bruising in dependent subcutaneous areas lateral to and below the transplant, such as the labia or the scrotum, is often seen several days later.

The risk of hematoma formation is increased by the use of anticoagulants, particularly in patients receiving heparin by infusion for prophylaxis against vascular thrombosis.[25,40,49] Careful titration of heparin infusion rate to maintain an activated partial thromboplastin time of 60 seconds is not easy. The reported risk of need for surgical intervention in patients heparinized after transplantation is 30% to 60%. Heparinized patients positive for lupus anticoagulant are especially difficult to manage with heparin.[45] Greater safety can be achieved with the use of thromboelastography to direct judicious use of heparin, during and after transplant surgery, in patients at risk.[17] Anecdotally at least, the same problem seems to occur with the use of antiplatelet agents, increasingly prescribed on a long-term basis by cardiologists and nephrologists in patients at risk for coronary artery disease or in the belief, for which there is as yet no evidence, that fistula patency would be improved.

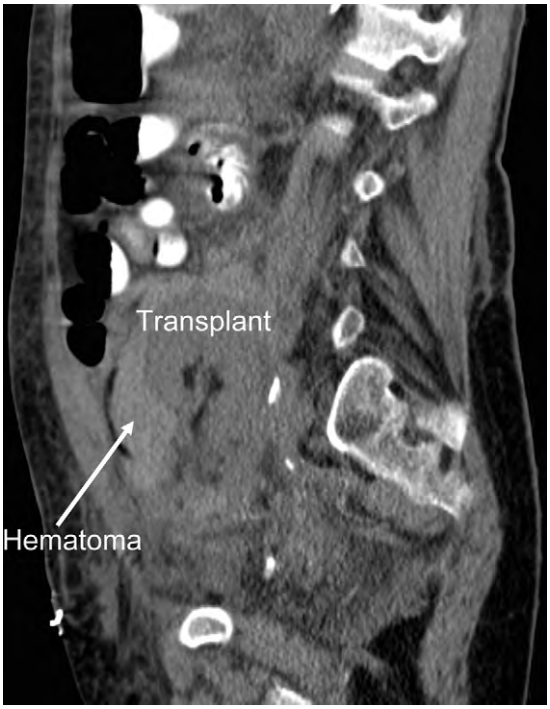


Figure 26–8 CT scan (without vascular contrast material) with sagittal view of abdomen showing compression of the kidney transplant by an anteriorly placed hematoma.

VASCULAR THROMBOSIS AND THROMBOPHILIA

Early kidney transplant loss as a result of acute thrombosis of the artery or vein remains a constant and devastating complication with an incidence of 2%.[24,32] Compared with other forms of vascular surgery, the incidence of thrombosis is low and supports the classic view that renal failure is associated with a bleeding tendency secondary to platelet and clotting factor dysfunction.[21] Arterial thrombosis or infarction of a denervated kidney is often painless and heralded only by loss of graft function. By the time the diagnosis is confirmed by one of the several appropriate imaging modalities, kidney salvage is not a practical option (Fig. 26-9). The more common interruption of the venous drainage can be spectacular with graft rupture and bleeding. It has an equally disappointing prospect for kidney salvage because of the rapidity of the process when occlusion of the renal vein has occurred. Identification of risk factors and preventive management undertaken at the time of transplantation are indicated to minimize thrombotic complications.

Thrombosis of the kidney vasculature is the end result of stasis, endothelial changes, and procoagulant factors. The cause is often multifactorial. Causes of stasis are largely technical in nature, as described earlier, and should

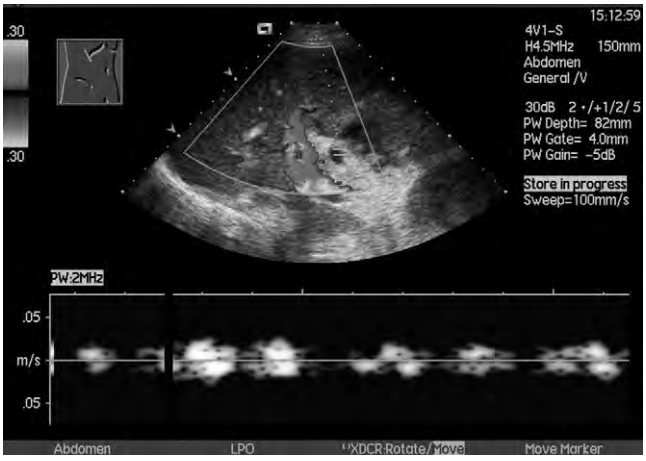


Figure 26–9 Color duplex ultrasound shows minimal blood flow into kidney transplant as a result of almost complete occlusion by thrombus of the transplant renal artery 5 days after transplantation. The transplanted kidney was not viable when explored soon afterward. (See color plate.)

be preventable. They are readily identifiable at the time of transplant exploration and are due to poorly constructed anastomoses, malpositioning of the transplant, rotation of the kidney, or external compression. Recipient hypovolemia and inadequate cardiac output, for whatever reason, can be contributory factors. The contribution of intrarenal causes, such as acute vascular rejection and acute tubular necrosis (ATN), is less quantifiable, but can be diagnosed by histological examination, provided that viable cortical tissue can be obtained. Because this is often not the case, intrarenal causes are probably underestimated and underdiagnosed.

Epidemiological studies have attempted to identify other risk factors, particularly those amenable to preventive strategies.[35] Risk factors that cannot be modified are recipient and donor age, recipient and donor vascular pathology, diabetes mellitus and, at least in the view of some recipients, morbid obesity. A large registry-based and case-matched study has shown that half of all cases of kidney transplant vascular thrombosis occurred in repeat transplant recipients.[54] The implication is that transplanted kidneys in the setting of retransplantation are more likely to have endothelial inflammation and development of microthrombi after exposure to the recipient immune system. Strategies exist to minimize this risk in selected, highly sensitized recipients with a negative donor lymphocytotoxicity crossmatch (see Chapter 10).

Recipients treated with peritoneal dialysis before transplantation are more likely to have thrombotic complications than recipients who were on hemodialysis.[51] The reason for the increased risk associated with peritoneal dialysis, particularly in children, is unclear.[19] It may be due to intravascular hypovolemia and may be preventable by aggressive volume loading at the time of transplantation. ATN is associated with increased intrarenal pressures, making perfusion of the transplanted kidney more difficult, and nonspecific endothelial changes attributable to the reperfusion injury after revascularization.

The introduction of recombinant human erythropoietin (rEPO) revolutionized the treatment of anemia associated with end-stage renal disease, both reducing the need for routine blood transfusion and improving dialysis, patient survival, and quality of life. The dose of rEPO is titrated to provide recipient hemoglobin in the range of 100 to 120 g/L. With higher hemoglobin values, there is an increased risk of adverse cardiac events.[12] Despite the current widespread use of rEPO in patients presenting for kidney transplantation, this has not equated to an increased risk of complications related to vascular thrombosis,[21,50] which is perhaps a reflection of appropriate monitoring of rEPO dose by nephrologists.

Six months to 2 years after successful kidney transplantation, erythrocytosis after transplantation, defined as a hematocrit greater than 51% or hemoglobin greater than 160 g/L, occurs in 10% to 15% of recipients.[67] About a quarter regress spontaneously, with the remainder persisting for several years, remitting as graft function diminishes. Because of the 30% incidence of thromboembolic events and symptoms of lethargy, malaise, and headache, repeated venipuncture is often necessary in these patients. The problem is more common in male patients, smokers, and patients with a rejection-free course. Erythropoietin levels are usually in the normal range. Patients introduced to small doses of angiotensin-converting enzyme inhibitor for management of hypertension were serendipitously noted to have progressive reduction of hematocrit to more normal levels. Angiotensin II receptor blocking agents have the same effect, suggesting that angiotensin II may be a growth factor for red blood cells.

Thrombophilic Factors

Often there is no factor identified as a cause of vascular thrombosis after exclusion of technical possibilities in a hemodynamically stable kidney transplant recipient. Thrombosis may be explainable, however, by numerous hypercoagulable or thrombophilic states identified more recently, many of which are inherited, but are more frequently acquired.[3,35,39] These include deficiencies of antithrombin III, protein C, and protein S, each occurring in less than 1% of the population. When a thrombotic event of any kind occurs in a patient older than 45 years and in the absence of a family history, these deficiencies are unlikely.

Inheritance of factor V Leiden (FVL) or prothrombin G20210A mutations can increase the risk of thrombosis, usually venous, of the transplant vasculature by at least threefold. FVL mutation is present in 2% to 5% of the normal population and is not more common in patients with kidney disease. It is found, however, in 15% to 20% of patients with venous thromboembolism and 60% of patients with a family history of thromboembolism. When FVL or prothrombin G20210A mutations are present in kidney transplant recipients, the risk of major thrombotic events, particularly renal vein thrombosis (RVT), is 40%.[71] The presence of FVL or prothrombin G20210A mutations also is associated with shorter graft survival, probably as a result of the greater risk of microvascular thrombosis and vascular rejection.[30] A case could therefore be made for routine genetic screening for these polymorphisms in patients awaiting a renal transplant, especially if there is a history of thromboembolism.

The presence of acquired antiphospholipid antibodies (APAs), including anticardiolipin antibody and lupus anticoagulant, is common in patients awaiting kidney transplantation. Although APAs are present in about 10% of patients, related clinical events are less common. When there is a history of thrombotic events, patients are labeled as having APA syndrome, which is more common in patients with systemic lupus erythematosus. These patients have a universal incidence of graft loss to thrombosis when prophylaxis is not employed.[64] The presence of APAs without a history of thrombosis is seemingly not a problem. Equally, anticoagulation after transplantation offers protection against graft loss.[1]

Contribution of Immunosuppressive Agents

The introduction of cyclosporine to clinical practice, usually at doses of 15 mg/kg or more, was associated with an increased incidence of graft thromboses, particularly RVT, in the first week after transplantation.[57] Cyclosporine subsequently was shown to have procoagulant properties, increasing factor VIII and release of tissue factors from monocytes and von Willebrand factor and P-selectin from endothelium. Circumstantial evidence suggests that this is probably a dose response because the incidence of RVT has decreased substantially with the much lower cyclosporine doses and serum levels used in current practice. This suggestion is supported

indirectly by the findings of the Euro-SPK trial comparing cyclosporine and tacrolimus in combined pancreas and kidney transplant recipients. The Euro-SPK trial showed a significantly worse pancreas graft survival in cyclosporine patients because of venous thrombosis, all in patients with trough levels of cyclosporine greater than 300 ng/L.[10] There also was significantly more kidney rejection in the patients receiving cyclosporine. mTOR inhibitors are not thought to contribute to thromboembolic events after kidney transplantation.[42]

Hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura is an infrequent, but well-described complication of cyclosporine use.[73] The diagnosis, seen soon after transplantation, is based on deteriorating renal function, decreasing platelet count, and characteristic glomerular thrombi seen on core biopsy specimen. Most cases resolve with discontinuation of cyclosporine and conversion to tacrolimus. Reports also describe the same presentation with tacrolimus, which responds with conversion to cyclosporine. The alternative would be to introduce an mTOR inhibitor.

Renal Vein Thrombosis

Occlusion of the renal vein by thrombus at the time of surgery or soon after is an unusual event and invariably associated with a technical problem. More common, at least in past years and with an incidence of 6%, is the seemingly spontaneous event of RVT occurring classically toward the end of the first week of transplantation in an otherwise uncomplicated transplant kidney.[59] Witnessing the dramatic presentation over a couple of hours is an unforgettable experience. Rapid onset of oliguria and hematuria is accompanied by graft enlargement and rupture associated with extreme patient discomfort and life-threatening bleeding. RVT can happen during the course of a morning ward round.

The duplex ultrasound findings are of a swollen graft with a crescent of clot along the convex margin of the kidney and covering a longitudinal rupture of the cortex. In this clinical setting, the appearance of the arterial waveform is virtually diagnostic with marked reverse diastolic flow (Fig. 26-10). If the transplant is to be saved, an early clinical diagnosis must be made, and the patient taken directly to the operating room by the surgical team in the hope that an empty room, along with an understanding anesthesiologist, can be located. The operative findings match those of the ultrasound description together with active arterial bleeding from the ruptured cortex. The findings are similar to the description of graft rupture associated with severe ATN seen in the era before brain death legislation. Surgeons of that era reported performing prophylactic division of the kidney capsule to allow the kidney to cope better with the parenchymal swelling associated with tubular necrosis.

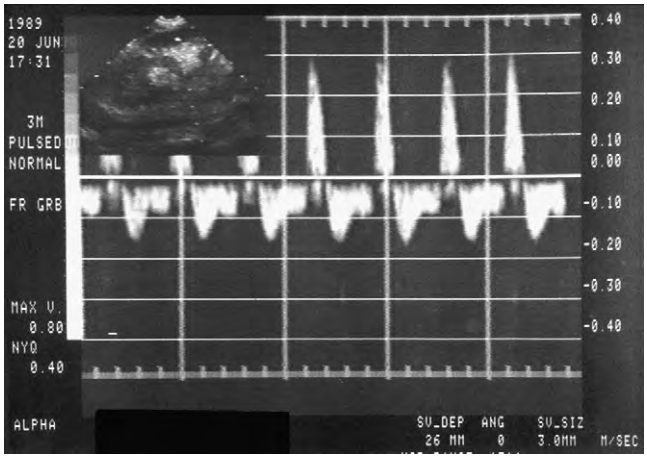


Figure 26–10 Duplex scan of interlobular renal artery shows reverse diastolic flow consistent with renal vein thrombosis.

The author has been associated with five cases of RVT, two of which showed the value of prompt diagnosis by allowing salvage of the transplanted kidney despite rupture. All occurred more than 15 years ago and fit the previous description. All were left-sided donor kidneys in patients receiving comparatively high doses of cyclosporine. Three of the patients experienced thrombosis of the arteriovenous access in the preceding days. Two kidneys were saved, both in patients diagnosed on a morning ward round. At the time of urgent exploration, the patients were heparinized before clamping of the transplant renal artery, which controlled bleeding from the ruptured transplant. Fresh clot was removed from a transverse renal vein venotomy, and flow was restored within minutes. A technical cause was not identified in any of the cases, but some evidence of endothelitis was seen in either a core biopsy specimen or the removed transplant in three cases. Assays for thrombophilic risk factors were unavailable. One or more may have been present, particularly in patients with fistula thrombosis. Equally, other contributory thrombogenic factors in this small personal series, including a long renal vein, high-dose cyclosporine, and rejection, were present and underscore the likelihood of multiple factors contributing to thrombosis.

Thereafter, the Westmead Transplant Unit protocol included routine shortening of the left renal vein at the time of surgery and introduction of low-dose subcutaneous heparin sodium for 5 days in all patients shortly after surgery. No instances of RVT have been seen at Westmead since 1989. The approach of the Oxford Transplant Unit was to introduce daily aspirin from the time of surgery, the effect of which was to decrease the incidence of RVT from 5.6% to 1.2%.[59]

Surgical management of an acutely occluded short right renal vein, particularly from a living donor, is more difficult. If identified in the early period after transplantation, simple reopening of the wound and making more space for the transplanted kidney may be associated with a rapid improvement in appearance of the kidney. If so, placement of the kidney in the peritoneal cavity may prevent the same from happening again. If thrombus is present in the renal vein of a right-sided donor kidney, removal of the kidney and reperfusion with preservation solution may be the only practical option. The donor kidney is retransplanted with consideration given to provision of greater mobilization of the iliac vein and more proximal siting of the venous anastomosis.

Beyond the early period after transplantation, reports of RVT are uncommon, but it can be seen in a subacute situation associated with secondary causes, such as iliofemoral vein thrombosis, de novo membranous nephropathy, glomerulonephritis, and thrombophilic

states. Because of the time of presentation many months after surgery, percutaneous combined mechanical and chemical thrombolysis is feasible.[46] Reports of RVT limited only to case studies suggest that the transplant renal vein is both comparatively tolerant of external compression and resistant to anastomotic stenosis, perhaps because of the high renal vein blood flow.

Renal Artery Thrombosis

Thrombosis of the renal artery occurs as a result of a reduction of the cross-sectional area of the renal artery, usually for technical reasons, and can occur at any time. Contributory factors include poor cardiac output, thrombophilic states, and increased intrarenal pressure as seen with ATN or acute rejection. The renal arteries of kidneys that have failed because of chronic rejection often remain patent for many years. Apart from loss of graft function, the signs and symptoms are negligible. The diagnosis is made by duplex ultrasound or at the time of surgical exploration. Arterial thrombosis is a terminal event and can be averted only if arterial inflow is considered as a cause of poor graft function, and immediate intervention is undertaken. By the time of diagnosis, it is too late to save the kidney transplant.

Thrombosis Prevention Strategies

Acknowledging that vascular thrombosis is a multifactorial event, prevention necessitates the need for a combination of general and specific measures. ATN can be minimized by avoiding prolonged cold and warm ischemia. It also is probable that the current preservation solutions used for multiorgan retrieval procedures, such as University of Wisconsin solution, provide better organ preservation than the solutions developed for kidneys in the 1970s (see Chapter 9). The combination of careful attention to surgical technique and recipient fluid status with early biopsy diagnosis and aggressive management of vascular and antibody-mediated rejection should minimize the contribution to vascular thrombosis of stasis and endothelial damage. The value of this broad strategy is highlighted by the excellent results in pediatric kidney transplantation and transplantation of pediatric kidneys into adults in which there are the added variables of small size of donor or recipient vasculature.[29]

The recognition of thrombophilic states as the major contributor to vascular thrombosis after kidney transplantation has introduced the possible need for routine screening and directed therapy to reduce the risk of thrombosis and graft loss.[49] There is no consensus for either strategy. Universal screening is expensive, and most thrombophilic states are rare. The most common are APAs, but in the absence of a previous thrombotic event, the risk of allograft thrombosis is low. It would be reasonable to limit laboratory investigation to potential recipients with a previous history or family history of thrombotic events, including deep and superficial vein thromboses, pulmonary emboli, thrombosed fistulas, multiple occlusions of central venous dialysis catheters, and thrombosed kidney transplant. To this list could be added patients undergoing preemptive transplantation with a living donor kidney.

Management of thrombophilic states also is not well defined. For known thrombophilia and a history of clinical events, perioperative heparinization followed by long-term anticoagulation with warfarin has proven efficacy, including successful retransplantation.[25,32,49] The risk of bleeding and hematoma formation seems acceptable in view of the incidence of thrombotic complications. Shorter term use of warfarin has been considered in some circumstances, depending on the history and number of risk factors. Thereafter, recommendations are difficult to make, and prospective trials are warranted. Known thrombophilia in the absence of a positive history might be managed by long-term low-dose aspirin alone. A role of other platelet inhibitors has yet to be defined.

DEEP VEIN THROMBOSIS

The hypercoagulable state persists for 4 weeks after major surgery and is no different in patients undergoing kidney transplantation. The early Oxford study, based on clinical findings in a kidney transplant population in which specific DVT prophylaxis was not used, showed an incidence of 8.3% in 480 patients. The peak incidence was in the fourth month after surgery and was usually associated with another event necessitating bed rest or involving pelvic pathology, such as a lymphocele (Fig. 26-11).[2] The implication was that kidney transplant recipients are at low risk in the early weeks after transplantation because of the protective bleeding tendency afforded by end-stage kidney disease and the preceding hemodialysis. The subsequent absence of reports of change of early DVT incidence in the rEPO era suggests that the protective effect is not related to anemia.

Stable kidney transplant function places recipients at the same risk as the general population with, apart from a lymphocele, no unique risk factors (Fig. 26-12). The physical presence of the kidney transplant itself, situated in the iliac fossa, does not seem to be a risk factor. Equally, proximal extension of an iliofemoral DVT is an uncommon event, probably because of the volume of blood entering the iliac vein from the transplanted kidney. It is nevertheless possible and is associated with a poor outcome.[56]

Adoption of universal measures for DVT prophylaxis has merit in a kidney transplant unit, despite the low incidence of DVT in the early period after kidney transplantation and

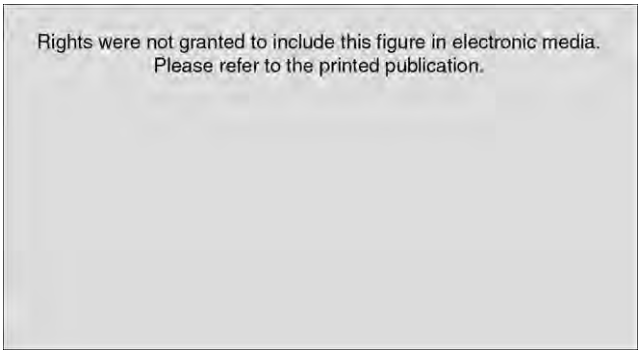


Figure 26–11 Time of diagnosis of thrombotic events after kidney transplantation. (From Allen RD, Michie CA, Murie JA, et al: Deep venous thrombosis after renal transplantation. Surg Gynecol Obstet 164:137-142, 1987.)

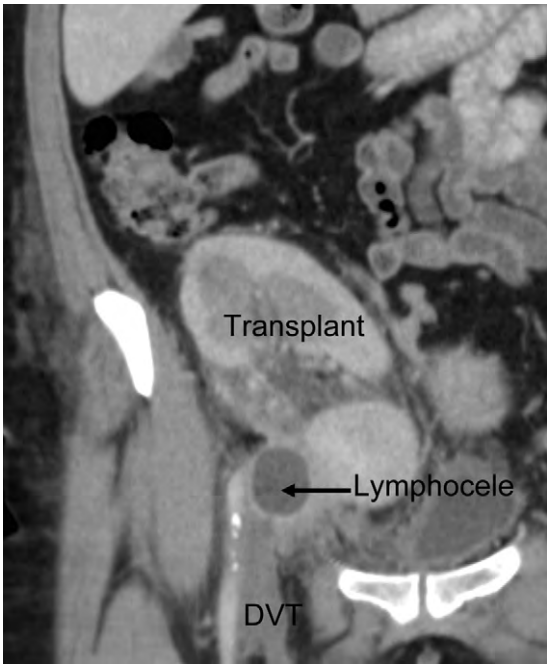


Figure 26–12 CT scan (coronal view) with vascular contrast material shows small lymphocele compression of the distal external iliac vein and thrombosis of the more distal vein.

the reassuring absence of reports showing an increase in incidence in the rEPO era. These prophylactic measures include the fitting of below-knee antithrombosis stockings before surgery and the use of intermittent mechanical calf compression during the transplant surgical procedure. They are considered to be as effective as subcutaneous heparin, provided that the stockings are worn throughout the inpatient stay, and early ambulation and calf exercises are undertaken. If these low-risk measures become routine practice, they are less likely to be overlooked in higher risk patients with a history of pulmonary emboli or DVT and obese patients. In these patients, subcutaneous heparin can be added, with unfractionated preferred to long-acting fractionated heparin because of the ability to reverse activity if necessary in situations such as troublesome hematuria or the need to obtain a biopsy specimen of the kidney transplant.

LYMPHOCELE

A lymphocele is defined as a collection of lymph that accumulates in the postoperative field in a nonepithelialized cavity. In kidney transplantation, lymphocele occurs as a result of divided recipient lymphatics accompanying the iliac vessels. The frequency of detection has increased with the routine surveillance of the kidney transplant by ultrasound and more recently with the introduction of mTOR inhibitors as part of maintenance immunosuppression regimens. Lymphoceles are usually innocuous and asymptomatic but can equally cause dramatic presentations as a result of external pressure on the transplant and its adjacent structures, or when complicated by infection involving the transplant wound. The best approach to treatment of a symptomatic lymphocele is not well defined.

Incidence

Lymphatic channels are inevitably divided when the iliac vessels are mobilized for arterial anastomosis. Considering the frequency with which these vessels are exposed in routine vascular operations and the rarity of lymphatic complications, it came as a surprise to vascular surgeons when the severity of lymphatic leakage after renal transplantation was first appreciated.[43] Early reports after kidney transplantation, based on clinical presentation, variously estimated the incidence in large series to be around 2% and reflecting the clinical significance of lymphocele. The advent of ultrasound for routine graft surveillance, together with the realizations that most lymphatic collections remain subclinical and that most resolve spontaneously,[5,55] caused the figure to be revised to about 50%.

Etiology

The obvious suspected source of lymphatic leakage after kidney transplantation would be the graft itself, and occasionally, this may be the case, when 1 L of clear fluid with biochemical analysis similar to serum is drained from the transplant surgical site in the first 24 hours after transplantation. A normal kidney has well-developed lymphatic drainage that is generally left unligated when transplanted. However, it is estimated that 300 mL of lymph per day passes through the external iliac lymph channels. Subsequently, studies of injected radiopaque dyes and

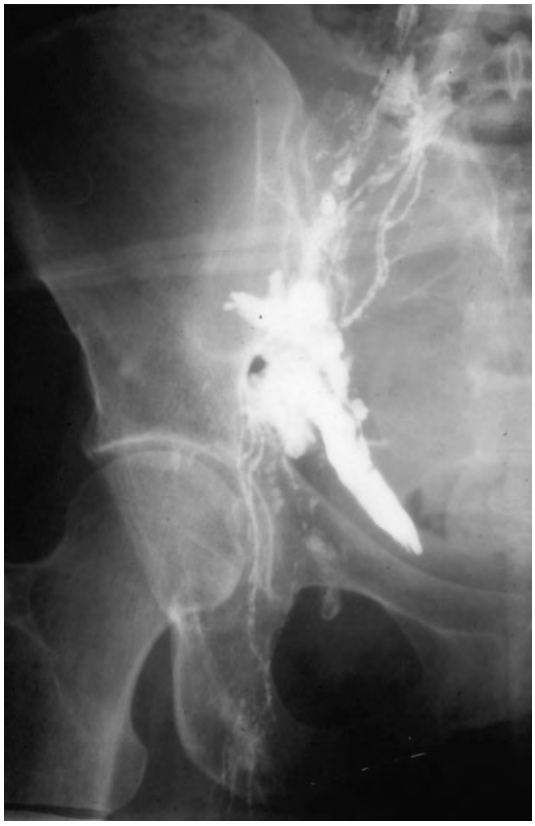


Figure 26–13 Lymphangiogram shows leakage of lymph from external iliac lymph channels causing a lymphocutaneous fistula through the transplant wound.

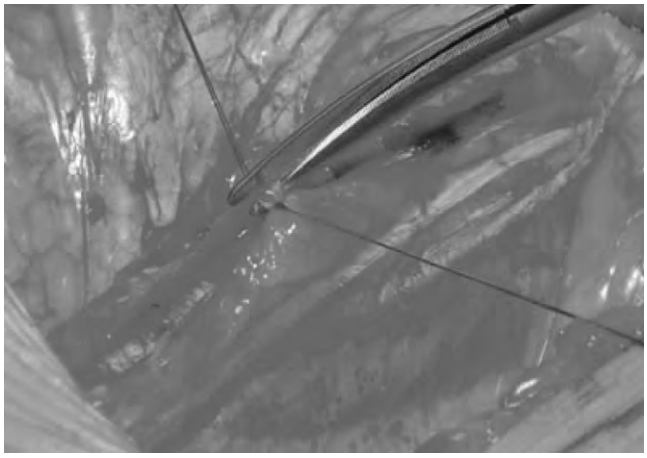


Figure 26–14 Division of external iliac lymphatics after ligation. (See color plate.)

radiolabeled substances showed that most lymphoceles originate from iliac vessel lymphatics of the recipient (Fig. 26-13).

Meticulous ligation of even the smallest lymphatic trunk with nonabsorbable or slowly absorbed ligature material during mobilization of the iliac vessels is crucial in the prevention of lymphoceles (Fig. 26-14). Why the transplant kidney lymphatics contribute so little, if any, to the presence of a lymphocele remains unexplained. The author's observation is that more surgical care than usual is required when encountering large, fleshy external iliac lymph nodes. Use of high suction wound drains also might encourage open lymphatics to remain open. Based on their own experience, Sansalone and colleagues[61] proposed that transplant lymphoceles could be preventable if the vascular anastomoses were to the common iliac vessels, where fewer lymphatics and lymph nodes are encountered during dissection.

The only differences between a routine retroperitoneal vascular procedure on the iliac vessels and kidney transplantation are the physical presence of the kidney, an alloimmune response, and immunosuppression. Potentially, the kidney could create areas of dead space, particularly near the lower pole, and into which open lymph channels could drain. Immunosuppression also may have a role in preventing the normal healing processes from sealing the lymphatic vessels and is the more likely explanation for the difference in the transplant setting. Macrophage function is adversely affected by steroids, and there is some evidence that the incidence of lymphocele has decreased since the introduction of low-dose steroid regimens. The more recent strong association of mTOR inhibitors with problematic lymphoceles is attributed to their powerful antifibroblastic activity, particularly in obese patients being treated for rejection (body mass index >30 kg/m^2).[27,65,66] Lymphoceles are more common in obese recipients, probably because the lymph channels are more difficult to identify during dissection of the iliac vessels. Aggressive use of diuretics also has been implicated, but it could equally be argued that diuretics are more likely to be used in an edematous transplant recipient, who is more likely to have greater lower limb lymph flow.

Presentation

Most lymphoceles are less than 3 cm in diameter, contain less than 100 mL of lymph, are clinically silent, and resolve spontaneously with time. Larger collections may become apparent clinically and usually do so at 2 weeks to 6 months after transplantation; the peak incidence is 6 weeks.[55] Most lymphoceles are situated adjacent to the lower pole of the kidney and posterolateral to the transplant ureter. Although intralymphocele fluid pressure measurements have not been reported, they must be considerable. The most common presentation is sleep disturbance owing to urinary frequency as a result of compression of the bladder and often associated with a sense of fullness in the pelvis. Ipsilateral painless leg edema is often present. The presentation of greatest clinical concern is deteriorating renal function, either due to compression of the ureter or to the direct effect of pressure on the kidney.

The timing of clinical presentation of a lymphocele soon after removal of a transplant ureteric stent 1 month after transplantation is expected. In contrast, the most challenging clinical presentation to resolve is an infected lymphocutaneous fistula through the transplant wound. A less common presentation is DVT as a result of compression of the external iliac vein, a diagnosis that requires exclusion in the common finding of the ipsilateral swollen leg (see Fig 26-12). Bladder outlet obstruction has also been reported.[33]

Diagnosis

Ultrasound examination is the key to diagnosis. Ultrasound usually can distinguish a lymphocele from hematoma collections on the basis of characteristic homogeneity and distinctive shape and position (Fig. 26-15).[1] Most lymphoceles are adjacent to, but clearly separate from, the bladder. They can be multilocular and multiple in number (Fig. 26-16); this can be confirmed easily, if necessary, by passage of a urinary catheter and repeat ultrasound. The examination also may show hydronephrosis with obstruction of the ureter with dilated calices. The diagnosis can be confirmed by ultrasound-guided or CT-guided drainage, allowing biochemical and cytological analysis of the fluid consistent with the presence of lymph. If emptied, resolution of the hydronephrosis

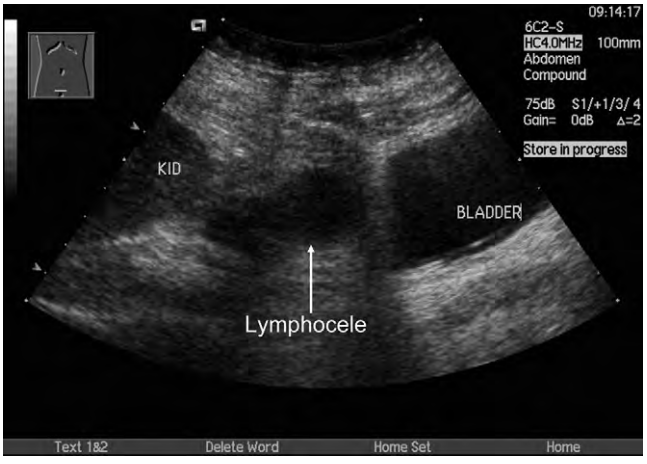


Figure 26–15 Ultrasound shows lymphocele between kidney transplant and bladder. Note the indentation of the bladder by the lymphocele.

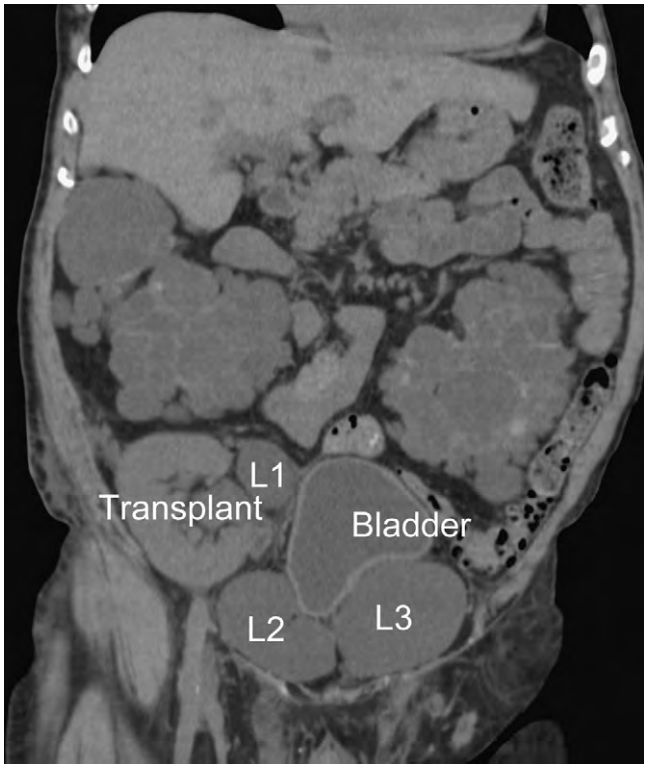


Figure 26–16 CT scan (coronal view) shows three lymphoceles (L) compressing bladder and kidney transplant in a patient with polycystic kidneys.

is seen. Adjunctive radiological procedures, such as CT, are usually unnecessary except in complicated cases or planning before surgery. The use of vascular contrast medium helps with localization of the ureter in the excretory phase.

Treatment

Small and symptom-free collections are common and may resolve spontaneously if left alone. Unnecessary intervention may lead to infective complications. Otherwise, ultrasound-guided drainage confirms the diagnosis and provides initial treatment. The possible urgency of the situation is resolved by relief of urinary obstruction and restoration of renal function. Although simple aspiration is sometimes curative and may be repeated on several occasions, the likelihood of spontaneous resolution becomes small after three aspirations followed by recurrence.[55] Every aspiration brings a small risk of infection. Symptoms and signs can recur within days.

Prolonged external drainage through a percutaneously inserted catheter has been advocated by some authors and is possible in an outpatient setting (Fig. 26-17). Injection of sclerosants has been described. Tetracycline did not seem to be effective,[55] but the injection of povidone-iodine in association with external drainage has been claimed to be effective with a low failure rate.[58] Chandrasekaran and coworkers[22] have recommended that povidone-iodine be instilled into the surgical site soon after transplantation to reduce the incidence of lymphocele formation. The drawback of prolonged drainage is that it takes 20 to 30 days before drainage ceases, during which time the risk of infection remains. Of further concern is the observation of acute renal failure as a result of the direct nephrotoxic effect of povidone-iodine.[44]

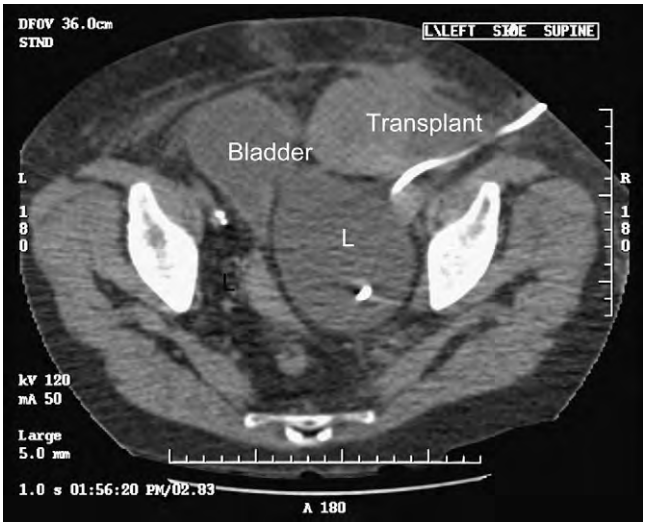


Figure 26–17 CT scan with axial view of lymphocele (L) with percutaneous drain below the lower pole of the kidney transplant. Note displacement and compression of the bladder.

If simple percutaneous aspiration fails on two occasions, the author's preferred treatment is a simple surgical procedure. The principle of the surgical procedure is to drain the potential 300 mL/day of lymph into the peritoneal cavity, where it is absorbed by the peritoneum. The operation of choice has been called incorrectly "marsupialization"; it might be described more correctly as unroofing or fenestration. This operation can be done either laparoscopically or through a lower midline abdominal incision and a transperitoneal approach to the lymphocele, depending on its relationship to the kidney. Sometimes, the previous transplant wound needs to be reopened to achieve access.

The least invasive surgical technique is a laparoscopic approach.[23,47,63] A planning CT scan is obtained to provide information about position and presence of loculi. The surgery is scheduled when the lymphocele cavity is full and not the day after drainage. Otherwise, the lymphocele can be difficult to locate. The surgeon should ensure that the recipient has an indwelling catheter and the bladder is empty. The lymphocele usually is seen at operation bulging into the peritoneal cavity. Localization with intraoperative ultrasound can be of assistance, particularly for obese patients and deeply situated lymphoceles. It is sometimes easy to confuse the swelling made by the extraperitoneal kidney with that made by the lymphocele. The role of intraoperative ultrasound in avoiding confusion has been stressed, allowing a perfect outcome record.[47] A 5-cm opening between the lymphocele and the peritoneal cavity is made, taking care to avoid damage to any structures that may be running between the wall of the collection and the peritoneum, particularly the ureter. The most difficult lymphocele position to treat is the one situated deep in the pelvis lateral to transplant artery and vein. These are more safely treated by an open operative approach.

To avoid recurrence, various authors have recommended maneuvers such as excision of a 5-cm disc of the wall of the lymphocele, oversewing the edges, and mobilizing the omentum, which is then stitched down into the cavity.[16] Routine fenestration at the end of the transplant operation potentially could be performed in high-risk recipients as has been suggested in children.[72]

Lymphocutaneous fistulas through the transplant wound are invariably infected and often associated with wound dehiscence in obese patients in the first weeks after transplantation. Prolonged use of appropriate antibiotics and free drainage is advocated along with all possible measures that might improve wound healing. This suggested regimen tests the patience of the nursing staff and patient alike. In such situations, the transplant wound can be reopened, and a large peritoneal fenestration can be created. Sometimes it is possible to see the offending leaking lymphatic channel at the base of the lymphocele cavity, anterior to the external iliac artery. It can be suture ligated and, in the author's experience, this is invariably successful. The risk of this procedure is introduction of infection to the peritoneal cavity and the need for ongoing antibiotic coverage.

TRANSPLANT RENAL ARTERY STENOSIS

Transplant renal artery stenosis (TRAS) is probably the most common vascular complication after kidney transplantation, with the incidence varying widely from 1% to 23% depending on the definition and, more recently, the availability of less invasive diagnostic imaging.[24] The true incidence is probably somewhere between these two figures, with the remaining key determinants of variability being the experience and skill of surgeons in avoiding the problem and the current comparative ease and safety of interventional radiology techniques to correct the problem.

The safety and low cost of Doppler ultrasound examination has made it an indispensable tool in the transplant clinic. It has taken away much of the "guess work" from clinical management of the complicated kidney transplant recipient. Although difficult to quantitate, color flow duplex ultrasound has made a major contribution to the continuing improvement in graft survival, particularly in the first 3 months after transplantation. It also is used as a screening tool and has raised awareness of previously unappreciated arterial pathology that might be a contributing cause to the common diagnosis of hypertension.

Having identified flow disturbance in the transplant renal artery, there are several key unresolved questions that can be answered only by standardized reporting and management of those findings.[18] Is the stenosis progressive in the long term? Is hypertension alone an indication for intervention? How do we determine a hemodynamically significant stenosis? When and how do we intervene? There are only observational studies to provide answers for these questions, and the studies have varying reporting criteria and methodology. The natural history of TRAS is uncertain, and the long-term benefit of intervention is unknown. Adoption by the transplant community of the reporting guidelines established by the American Heart Association for native renal artery stenosis would be reasonable.[60]

Definition and Incidence

There is no consensus definition of TRAS. At one end of the spectrum is the classic presentation of a bruit over the transplant, refractory hypertension, deteriorating renal function, life-threatening congestive cardiac failure secondary to fluid retention, and dramatic reversal by correction of the stenosis.[26] It occurs most commonly 3 months to 2 years after transplantation and is caused by activation of the renin-angiotensin system. At the other end of the spectrum is the incidental finding of a stenosis on color flow duplex ultrasound examination of 50% or greater in a normotensive patient in the absence of graft dysfunction—a definition akin to the "drive-by" diagnosis of a native renal artery stenosis by an interventional cardiologist. Intervention to correct a clinically insignificant stenosis would have inherent risks and provide no measurable benefit for the kidney transplant recipient, at least not in the short to medium term. It may provide benefit in the long term, however.

Hypertension in kidney transplant recipients is multifactorial, common, and an independent risk factor for long-term graft survival (see also Chapter 28).[74] Any measure to improve blood pressure control may be valid. The decision to intervene on the basis of imaging findings, and hence perhaps the "local definition," also would depend on factors of local clinical expertise and other individual patient variables.

The author's suggested clinical definition of TRAS is one based on a diagnosis of hypertension requiring increasing amounts and numbers of antihypertensive agents, with or without deterioration in graft function and in the presence of renal artery stenosis, which, when corrected, results in improvement of blood pressure control or renal function, or both. If such a definition were used, the incidence of TRAS would probably be closer to 1% than to 23%. There are many possible and plausible variations to this definition—hence the variation in reported incidence.

Pathogenesis

The stenosis usually is situated near the anastomosis of the renal artery to an iliac artery. It can be short, diffuse or at multiple sites, and occur at different times, suggesting that there are several causes for TRAS. In a comparatively large series of TRAS, Voiculescu and coworkers[69] reported that most stenoses are identified in the first 6 months. Fibrosis accounted for 40%, donor artery atherosclerosis accounted for 27%, and renal artery kinking accounted for 21%. Stenoses at the anastomosis site are more likely to be technical and apparent from time of transplantation and probably stable. End-to-side anastomoses may be more of a problem than end-to-end anastomoses.[48] Progressive anastomotic stenosis, particularly involving the end of the renal artery, as is the case for living donor kidneys, probably represents fibrosis and intimal hyperplasia in response to damage to the renal artery at the time of donation or implantation surgery (Fig. 26-18). Another precipitating factor may be a subintimal dissection or flap created at the time of instrumentation.

For reasons discussed earlier, kinking or twisting of the renal artery with placement of the kidney at time of wound closure probably occurs more frequently than appreciated. The kink occurs either at the apex of the curve of the artery or near the anastomosis where the artery is comparatively fixed in position (Fig. 26-19). A twist can be difficult to detect at the time of surgery because it is usually situated in, and hidden by, the adipose tissue of the hilum of the kidney.

The long and more diffuse stenoses tend to occur later and have been attributed to immune-mediated endothelial injury with progressive intimal proliferation, particularly if concentric in nature. Multiple stenoses, often associated with renal artery branching, probably fit into the same

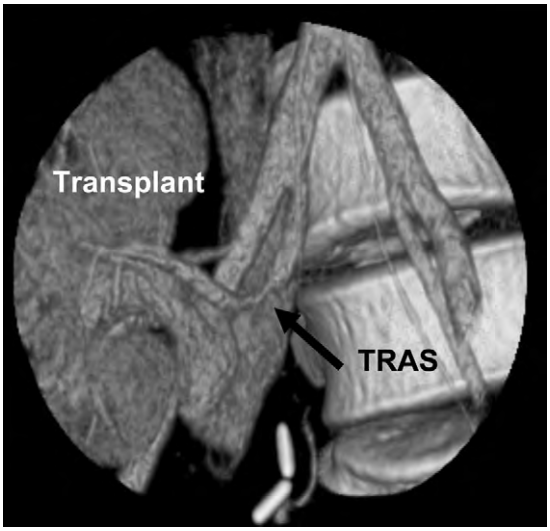


Figure 26–18 CT scan with vascular contrast material and three-dimensional reconstruction showing transplant renal artery stenosis (TRAS) distal to anastomosis to the internal iliac artery, 2 months after living donor kidney transplantation. The stenosis is probably due to intimal fibrosis. (See color plate.)

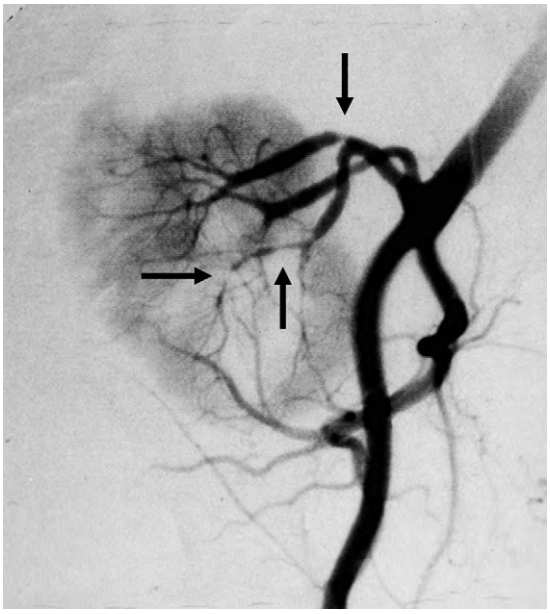


Figure 26–20 Angiogram shows multiple stenoses of the renal artery branches (*arrows*) in a kidney transplanted 2 years previously and complicated by rejection.

category (Fig. 26-20). The reported temporal association with vascular rejection and subsequent stenosis is inconclusive.[6,70] A single-center study of 27 patients with TRAS showed a significant association by multivariate analysis with cytomegalovirus infection and delayed graft function.[6] Cytomegalovirus infection is thought to trigger smooth muscle cell proliferation and induce endothelial damage. It has a similar or same effect in the development of cardiac allograft vasculopathy. Delayed graft function is more likely to occur in poorly preserved donor kidneys and may explain why TRAS is more common in deceased donor kidneys.

Progressive atherosclerosis, occurring either de novo or already present in the donor renal artery, can cause a diffuse stenosis, particularly if eccentric in distribution. Equally, an arterial stenosis or obstruction anywhere in the arterial tree upstream from the transplanted kidney could produce the same clinical presentation as TRAS.[68] Many transplant recipients, particularly smokers, patients with kidney disease secondary to type 2 diabetes mellitus, and older patients, have significant diffuse arterial disease at time of transplantation. Immunosuppression after transplantation influences progressive atherosclerotic peripheral vascular disease further. An iliofemoral bruit may be present together with a weak or absent femoral pulse (see Fig. 26-21). Of clinical relevance in this setting is a history of claudication in recipients capable of exercise.

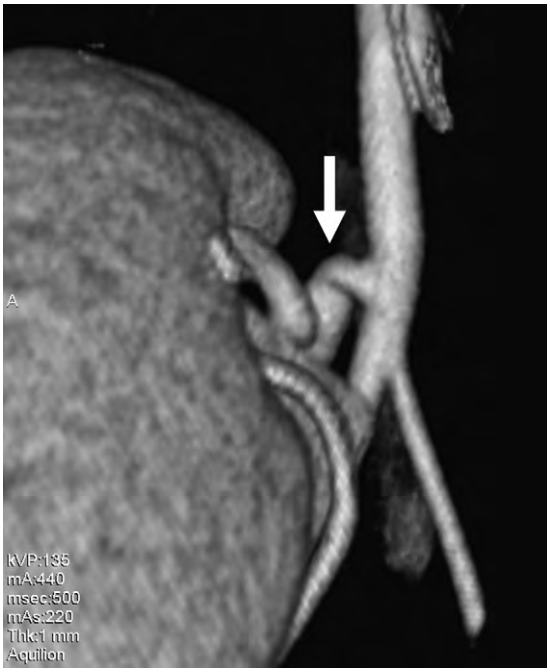


Figure 26–19 CT scan with three-dimensional reconstruction showing kinking of the transplant renal artery (*arrow*). The patient was normotensive and had stable renal function. No intervention was undertaken. (See color plate.)

Pathophysiology—"One Kidney, One Clip"

In 1934, Goldblatt and colleagues[28] published their seminal study on the hypertensive effect of partial reduction of the blood flow to a kidney in dogs by applying a silver clip to one of the two renal arteries. These investigators proposed the existence of a pressor substance released by the ischemic kidney. Over the next 25 years, other investigators subsequently defined the renin-angiotensin system, with renin being the hormone released from the ischemic kidney.[7] Renin is measurable in elevated levels in the venous blood of the ischemic kidney. Its pressor effect follows the release of angiotensin by enzymatic processes from the circulating substrate, angiotensinogen. Angiotensin is an octapeptide with wide-ranging effects, including vasoconstriction, renal sodium retention, aldosterone secretion, and hypertrophy of myocardium and arteries.[14,26] Blood pressure in this model is driven by the direct pressor effect of angiotensin II, with excess salt and water excreted by the nonischemic good

kidney, which can be treated by inhibitors of the renin-angiotensin system.

Goldblatt and colleagues[28] also studied the effect of applying one clip to the renal artery in a dog with one kidney ("one kidney, one clip")—an analogous situation to the transplanted kidney with a hemodynamically significant renal artery stenosis. Hypertension also results, but may not be renin dependent as is the case for the "two kidney, one clip" model, but rather reflect a balance between the angiotensin-dependent system and volume-dependent mechanisms based on salt and water retention, which otherwise would be excreted by a normal contralateral kidney. The perfusion pressure to the single ischemic kidney is maintained by the high circulating volume and not the direct pressor effect of angiotensin. Renal vein renin levels are near-normal and sufficient to maintain the elevated circulating volume and with it, normal glomerular filtration rate and renal function. If renin-angiotensin system inhibitors are prescribed, however, the existing drive for salt and water retention is removed, causing reduction in perfusion to the solitary kidney and deterioration in kidney function. The diagnosis of TRAS sometimes is made by observation of rapid deterioration in allograft function with the introduction of renin-angiotensin system inhibitors.

Dogs also have been used to determine the minimal degree of renal arterial stenosis needed to cause hypertension. Imanishi and colleagues[34] concentrically constricted the left renal artery of anesthetized dogs using a radiolucent constrictor device and evaluated the stenosis by cine-angiography. With the kidney either innervated or denervated, systemic blood pressure began to increase when the stenosis was more than 70% of the diameter of the renal artery. Renal blood flow decreased when the stenosis was more than 75% of the diameter. Using magnetic resonance imaging (MRI) and an implanted inflatable arterial cuff and flow probe, Schoenberg and associates[62] showed in dogs that stenoses of 30% to 80% gradually reduced early systolic peak, but only minimally affected peak mean flow. At 50% stenosis, the pressure decrease across the stenosis was recorded at about 10 mm Hg, and at 80% stenosis, it was 28 mm Hg. At 90% stenosis, mean flow was decreased by greater than 50%. An equivalent study in humans is impossible. Knowledge of the relationship between the magnitude of the pressure gradient across a TRAS required before better blood pressure control can be achieved after correction of that stenosis by angioplasty or stenting would be relevant, however. Results could be correlated with spiral CT angiography assessment of the cross-sectional area of the stenosis before angioplasty. Such a study has not been reported.

Imaging

Contrast angiography has long been the "gold standard" investigation of TRAS. In a 1975 article reporting Hamburger's 14-year experience from 1959 of TRAS, Lacombe[41] concluded that angiography was so valuable that it should be performed at routine intervals in all transplant recipients. Although angiography still might be the "gold standard," and was responsible for Lacombe providing the often quoted TRAS incidence figure of 23%, color flow Doppler ultrasound has become the imaging modality to enable routine surveillance of transplant renal arteries. It provides an instantaneous assessment of intrarenal vasculature and a global impression of transplant perfusion (Figs. 26-22 and 26-23).[8]

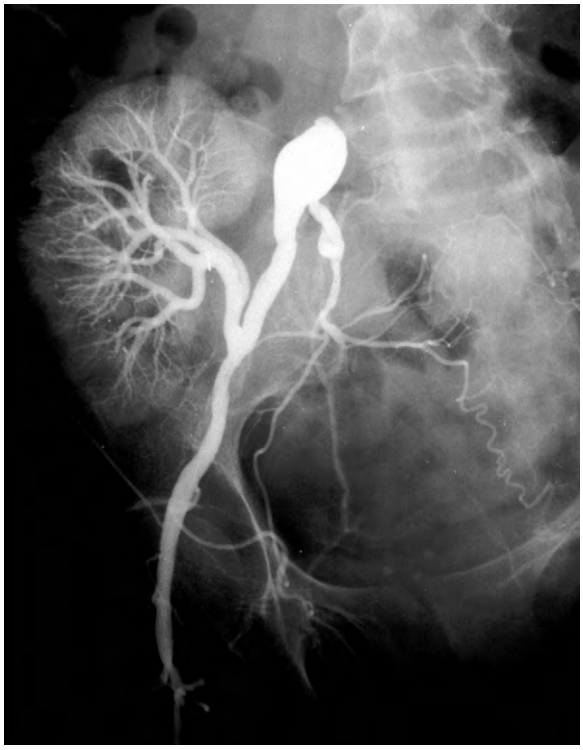


Figure 26–21 Angiogram shows occlusion of an aneurysmal right common iliac artery proximal to the kidney transplant. At the time of angiography and before proximal arterial bypass surgery, the patient had been receiving hemodialysis for 2 months. After bypass surgery, normal kidney transplant function returned.

There are two ultrasound approaches to the diagnosis of TRAS. The extrarenal approach involves scanning the renal artery from the hilum to the anastomosis and beyond to the proximal iliac artery. The peak systolic velocity is measured along the whole course of these vessels. A hemodynamically significant stenosis has a peak systolic velocity of greater than 2.5 m/sec.[9] The degree of stenosis and the site can be reported with a high degree of accuracy in the hands of an experienced ultrasonographer. Secondary spectral findings

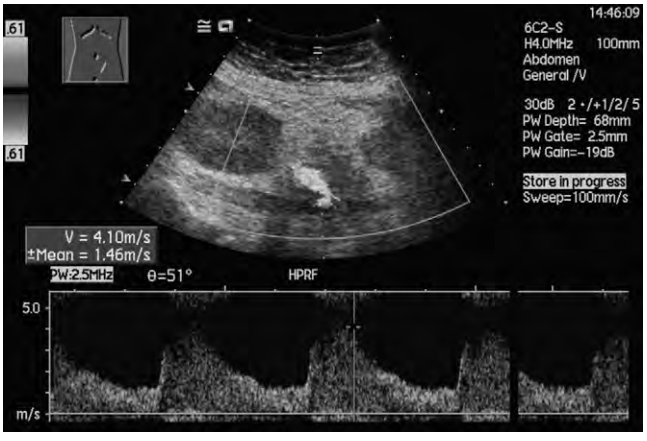


Figure 26–22 Color duplex scan of a kidney transplant shows peak systolic velocity in the main renal artery of 4.10 m/sec, diagnostic of transplant renal artery stenosis. (See color plate.)

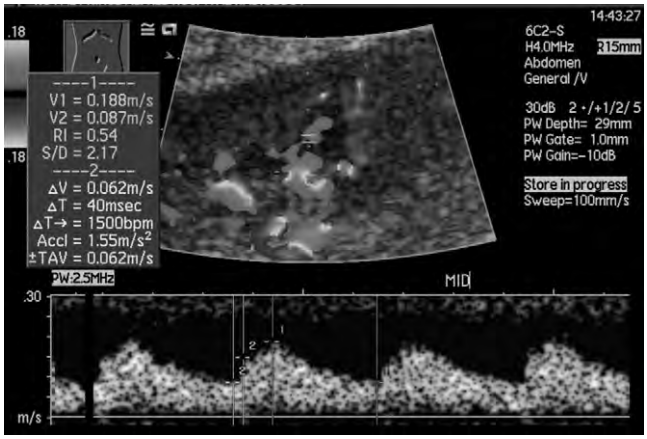


Figure 26–23 Color duplex scan of a kidney transplant (same kidney as in Fig. 26-22) shows arterial waveform of the intrarenal arcuate artery, showing the features of "parvus-tardus," also indicative of transplant renal artery stenosis. Note the comparatively low resistance index of 0.54. (See color plate.)

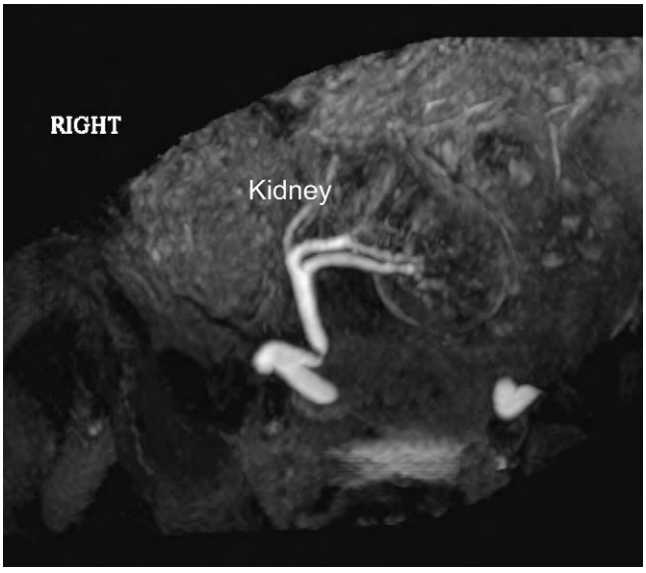


Figure 26–24 MRI angiogram shows torsion of a right-sided donor kidney with its long renal artery. The kidney had been placed in an intraperitoneal position 3 months earlier in a patient receiving mTOR inhibitor immunosuppression. The kidney transplant was subsequently lost despite exploration soon after MRI angiography.

of downstream turbulence and spectral broadening increase the confidence of the diagnosis of TRAS. The disadvantage of this technique is that it is operator dependent and more time-consuming. The transplant renal artery can have numerous twists and turns, making it difficult to obtain the accurate angle of correction necessary for precise spectral quantification. Distinguishing a focal stenosis from a tortuous renal artery can be problematic, and reporting can err on the side of false-positive findings. A careful diagram and direct communication with the ultrasonographer can increase the value of the report.

The intrarenal approach has the advantage of being less operator dependent, more reproducible, and easier to perform. It relies on the intrarenal downstream assessment of the effects of a TRAS. The early systolic peak is flattened and delayed, the so-called parvus-tardus pattern. It is associated with a low resistive index of 0.5. The disadvantages of the intrarenal technique are that it can only diagnose high-grade stenoses of greater than 75%, and it cannot localize the stenosis. The preferred approach is to combine both, but this request may not be well received in a busy ultrasound laboratory on short notice.[8] Nevertheless, routine evaluation for TRAS at designated time points after transplantation as recommended by Hamburger and colleagues in 1975 remains appropriate.[41]

Having identified a TRAS on ultrasound examination, the next decision is whether or not to proceed with vascular contrast studies. The risk is contrast nephropathy in an already compromised kidney.[52] Of greater concern would be loss of the kidney transplant. The decision is not difficult. The risk can be reduced by adequate hydration with normal saline and perhaps the use of oral *N*-acetylcysteine before and after injection of contrast material.

Newer generation multislice helical CT permits accurate assessment of the site and degree of TRAS and, at the very least, provides imaging that is valuable in planning subsequent intervention. Advocates state that helical CT requires less volume of iodinated vascular contrast medium than formal angiography. There may be less toxicity associated with intravenous rather than intra-arterial infusion of contrast medium. The nature of the vascular contrast medium may be a more important consideration, however, than the volume. A meta-analysis has shown that the risk of contrast nephropathy is not related to the volume of contrast medium or the degree of renal failure.[13] Protection of the transplanted kidney is recommended at all times when vascular contrast medium is injected, regardless of renal function and contrast volume.

The alternative is to perform helical CT or MRI with gadolinium, a noniodinated contrast medium. Reports of nephrogenic systemic sclerosis with use of gadolinium are concerning, however. Also, definition is less satisfactory because of its lower density, and therapeutic intervention is impossible. Good screening images nevertheless can be achieved, as shown in Figure 26-24 of a twisted intraperitoneal kidney transplant 3 months after transplantation in a patient receiving immunosuppression with an mTOR inhibitor.

Conventional angiography remains the gold standard investigation because of the quality of definition, the ability to measure pressure gradients across the stenosis, and the potential to intervene at the same visit to the angiography suite. The contralateral femoral artery approach is used for kidneys transplanted to the internal iliac artery by end-to-end technique. Otherwise, an ipsilateral approach is used first to complete an aortoiliac run using 20 to 30 mL of iodinated vascular contrast medium. This step can be avoided if satisfactory helical CT or MRI images exist. Selective runs are performed with oblique or other views as necessary using about 10 mL of contrast medium with each run. False-negative examinations can occur if insufficient views are obtained. To this extent, multislice helical CT angiography with reconstructions has investigational advantages over conventional angiography.

Conservative Treatment

If stenosis is not greater than 60% on ultrasound examination, kidney function is satisfactory, and the recipient is not

hypertensive or has stable blood pressure readings on minimal treatment, continued observation with repeat ultrasound examination is a practical option. Nevertheless, there are no reports of the long-term safety of this line of management, and the natural history of a 60% TRAS is unknown. Anecdotal evidence suggests that for a kinked transplant renal artery, this is probably safe. Based on longitudinal ultrasound evaluation, Buturovic-Ponikvar[18] believes that conservative treatment is safe provided that there is no deterioration of kidney function.[18] For other causes, such as intimal hyperplasia, this may not be the case, and the indication for angiography is graft deterioration in the absence of other causes of graft dysfunction.

One would be more nervous about continued observation of a stenosis of 80% on ultrasound examination, with or without a high degree of clinical suspicion of a hemodynamically significant TRAS. Based on the previously described dog studies, significant flow disturbance exists. Even if not causing clinical problems, such a stenosis would be more susceptible to occlusion in the presence of periods of dehydration or cardiovascular instability, and intervention should be considered.

Angioplasty and Stenting

Percutaneous transluminal angioplasty (PTA) is recognized as the initial treatment of choice for TRAS.[11] Technical success has been reported at greater than 80% with clinical success, as judged by treatment of hypertension and improvement of allograft function, being proportionately less. Clinically insignificant stenoses can be judged only on radiological success. Intervention would have inherent risks, and it can be argued that unless a significant pressure decrease exists across the TRAS, PTA should not be undertaken. There is as yet no agreed-on value of stenosis measurement beyond which intervention is warranted, however. If the dog studies of Schoenberg and colleagues[62] are to be believed, the pressure decrease should be at least 10 mm Hg across the stenosis.

In the presence of a satisfactory radiological result and no improvement in clinical parameters, other underlying causes of hypertension and graft pathology should be sought. To this extent, PTA could be performed as an investigation of exclusion if the complication rates were acceptable. As with other forms of interventional angiography, most of the complications relate to puncture site problems in the groin. The skills of the local clinicians may dictate the wisdom of this line of management. The success of interventional angiography is probably influenced by cooperative decision making by the radiologist and transplant surgeon. If intervention goes wrong, the surgeon may be required at short notice to salvage the allograft, and for this reason it is wise to have a vascular transplant surgeon "on standby" when radiological intervention is taking place. Increasingly, with newer premounted stents deployed by balloons, complications leading to graft loss are unusual.[11] Equally, it can be argued that when thrombosis does complicate PTA, an experienced interventional radiologist using urokinase and further stenting usually benefits the recipient much faster and more efficaciously than the surgeon who must find an emergency operating room and rapidly undertake a difficult dissection and vascular reconstruction (Fig. 26-25).

The restenosis rates are reported to be 10% to 60% and are probably influenced by cause of the stenosis, length of follow-up, and use of stents.[6,69] Data on long-term effects of PTA on kidney allograft survival after PTA are scarce and tend to be uncontrolled, perhaps understandably so. For ethical reasons, a trial might be feasible only in patients with stable function and blood pressure control, in which case the measure of success of the procedure would be graft survival. Such a trial might take a decade or more to complete.

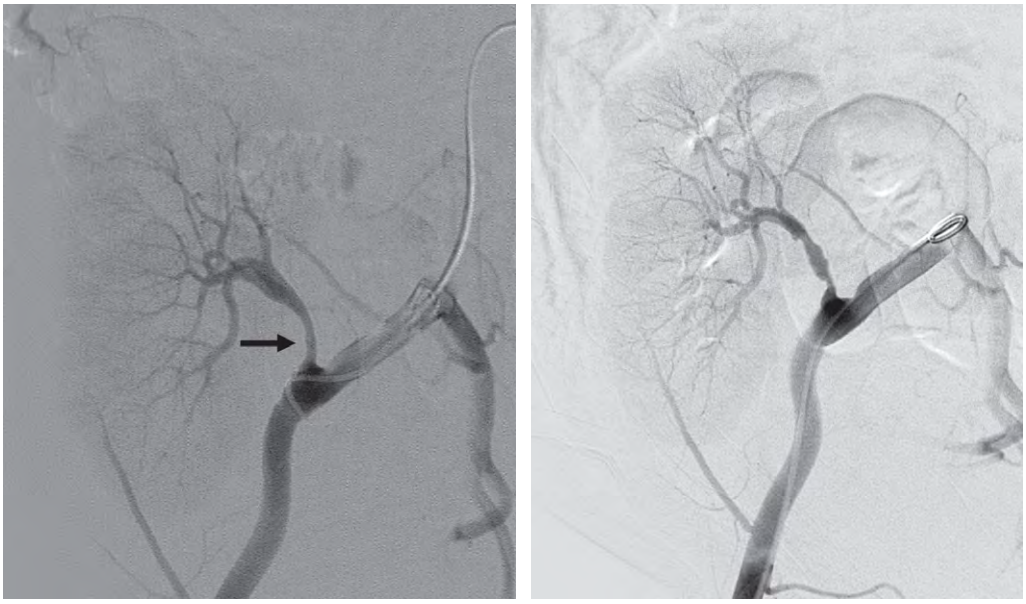
Surgical Correction

Historically, correction of TRAS by surgery is seen as a difficult operation with graft loss rates of 20%.[15,41] The risk to the transplanted kidney is irrelevant, however, provided that it is not to the patient, if return to dialysis is the only other option. Surgery is now considered rescue therapy for cases unsuitable for PTA. These include TRAS caused by kinking and complex atherosclerotic disease. Options include excision of the stenosis with direct anastomosis to the external iliac artery and grafting with saphenous vein, recipient internal iliac artery, and preserved ABO blood group compatible deceased donor artery. The United Network for Organ Sharing guidelines recommend that deceased donor artery grafts be used within 7 days of donation.

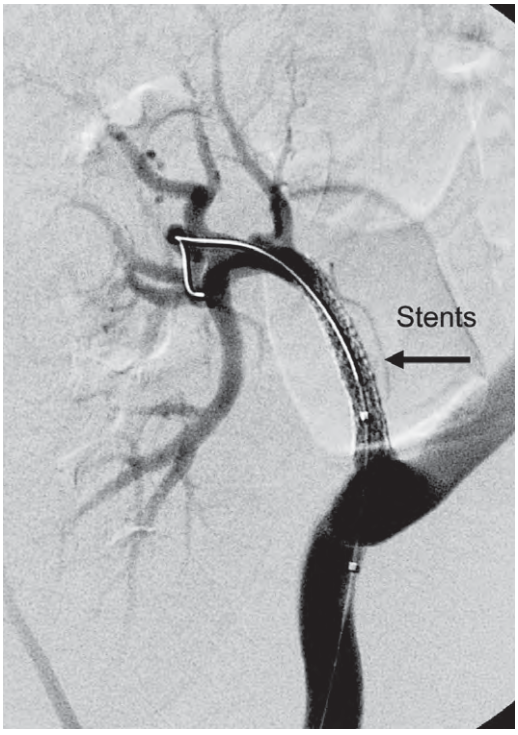
The limiting factors for surgical correction of TRAS are access to the artery and the warm ischemia time. A heparinized kidney allograft might tolerate warm ischemia of 60 minutes because of the preexisting diminished blood flow, albeit with increasing risk of ATN and cortical necrosis as the minutes tick away. An infrequently used option is autotransplantation of the kidney after back table reconstruction of a complex arterial problem. Figure 26-26 illustrates one such case of the author, in which a deceased donor artery and vein were used successfully to replace an aneurysmal transplant renal artery. The donor kidney came from an 8-year-old child with brain death resulting from rupture of an intracerebral artery aneurysm. The recipient was 14 years old at the time of transplantation and presented 6 years later with sudden onset of severe graft dysfunction after introduction of an angiotensin II blocking agent to control hypertension. The TRAS was caused by kinking secondary to distortion caused by the enlarging aneurysm, off which came four branches of the renal artery.

BIOPSY-RELATED COMPLICATIONS

Small false aneurysms and arteriovenous fistulas within the transplant kidney are common, with risk increasing with each needle core biopsy. They are readily shown by duplex ultrasonography (Fig. 26-27). A regimen of bed rest, intermittent ultrasound and local compression, and temporary cessation of aspirin, antiplatelet agents, and heparin is usually successful in managing a false aneurysm. Occasionally, duplex scanning may detect a fistula between the main vessels. The widespread introduction of smaller gauge needle core biopsy systems using spring-loaded biopsy machines has probably lowered the incidence of this complication. Arteriovenous fistulas within the kidney are usually asymptomatic, although an impressive bruit may be present on auscultation. In most cases, conservative management is advocated, even for large fistulas, as shown in Figure 26-28.

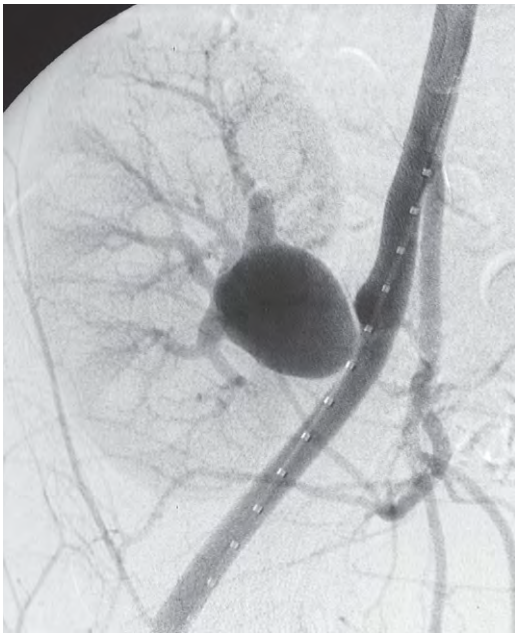


A B



C

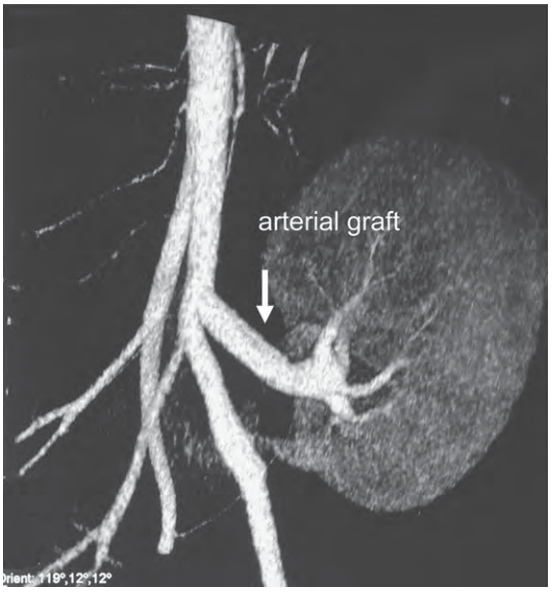
Figure 26–25 **A**, Angiogram of a kidney transplant 3 months after transplantation. The mean arterial pressure gradient across the stenosis (*arrow*) in this symptomatic patient was 16 mm Hg. **B**, Appearance of the renal artery 24 hours after percutaneous transluminal angioplasty. **C**, Appearance of transplant renal artery after insertion of two self-expanding stents.



A

B

C



D

Figure 26–26 **A**, Digital subtraction angiogram shows a 4-cm aneurysm of the transplant renal artery of a right-sided donor kidney 6 years after transplantation into a 14-year-old boy. Note the kinking of the artery proximal to the aneurysm. The donor kidney came from an 8-year-old child who sustained brain death after bleeding from a cerebral artery aneurysm. **B**, Complete mobilization of the kidney transplant, before removal for ex situ reconstruction of the renal artery using cadaver donor vessels. **C**, View of the inside of the thin-walled aneurysm showing four branches with takeoff from the aneurysm of the transplanted renal artery. **D**, CT angiogram with oblique view of arterial reconstruction 2 weeks after replacement of the transplant renal artery and vein with deceased donor iliac vessels and autotransplantation. (**B** and **C**, see color plate.)

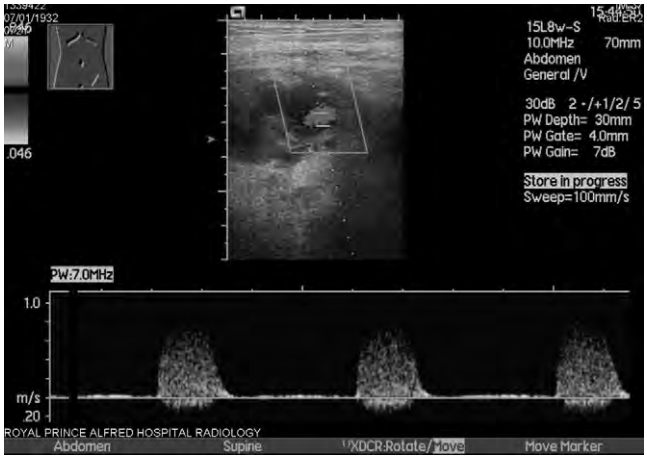


Figure 26–27 Color Doppler ultrasound of a 3.2-cm false aneurysm in the lower pole of a kidney transplant. The aneurysm subsequently was thrombosed by careful direct ultrasound-guided compression after cessation of intravenous heparin initially begun to protect a compromised coronary artery. (See color plate.)

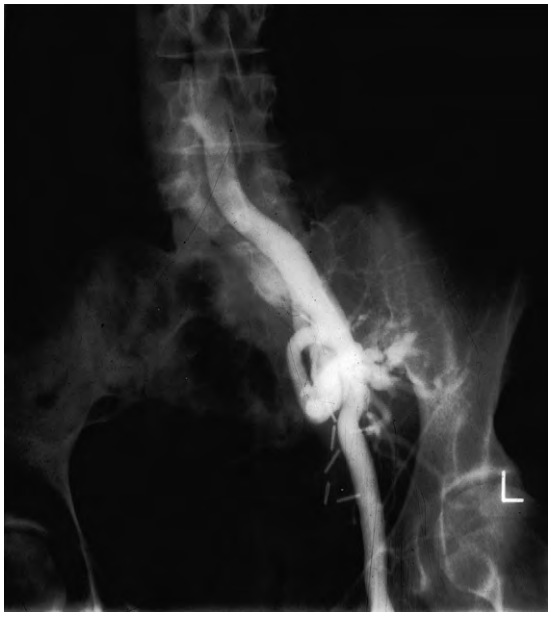


Figure 26–28 Angiogram of a left-sided kidney transplant shows rapid passage of vascular contrast material into the common iliac vein, consistent with a large intrarenal arteriovenous fistula secondary to a percutaneous 14-gauge core biopsy of the kidney. The fistula was treated conservatively.

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Chapter 27

Urological Complications after Kidney Transplantation

Daniel Shoskes • David Cranston

Urological complications are inevitable in renal transplantation. Their incidence and impact on graft survival can be minimized, however. This chapter reviews the types of urological complications that may occur, maneuvers to prevent them, when to suspect and how to diagnose them, and treatment options and algorithms to maximize long-term outcome.

Retrospective series quote an incidence of urological complications of 1% to 15%.[27,31,37,40] The incidence depends on many factors, in particular duration of follow-up and how broadly urological complications are defined. Some studies include hematuria, urinary tract infection, and urinary retention; others are confined to ureteric strictures or leaks. There also is an era effect, with a higher incidence in studies that go back to the 1970s and 1980s.[37] This chapter discusses the following urological complications: ureteral leak, ureteral obstruction, urinary calculi, urinary retention, and erectile dysfunction.

URETERAL COMPLICATIONS

Ureteral leak or obstruction is typically caused by either technical errors or ischemia. The native ureter derives its blood supply from renal and pelvic sources, but the transplant ureter must rely on branches from the anastomosed renal artery. The ureter becomes more ischemic the more distal it is from the kidney. One of the advantages of placing a renal allograft into the pelvis is the short distance to the bladder, which allows a minimal length of transplant ureteral length. The other surgical principle to ensure optimal ureteral perfusion is preservation of the blood supply. This is accomplished during procurement by removing the ureter complete with a significant margin of periureteral tissue, avoiding a "stripped" ureter. During the back table preparation of the kidney, it is important to preserve the perirenal fat bordered by the ureter and lower pole of the kidney (the "golden triangle") as seen in Figure 27-1. All attempts should be made to preserve or repair a lower pole renal arterial branch because this commonly (although not invariably) is the end artery supplying the ureter. Ureteral complications may be more common in kidneys with multiple ureters,[17] and in such cases small upper pole arteries should be preserved as well if possible.

Ureteral Leak

Ureteral leaks are reported in 1% to 3% of renal transplants.[27,40] The two most common causes are ureteral ischemia with necrosis and surgical technical error. Technical errors include misplacement of ureteral sutures and insufficient ureteral length with tension on the anastomosis. Other rare causes of urine leak include outflow obstruction (blocked Foley catheter or urinary retention) with disruption of an otherwise perfused and technically perfect anastomosis, unrecognized surgical laceration of the ureter or renal pelvis, acute ureteral obstruction with perforation through a renal calyx, and protrusion of a ureteral stent. Leaks resulting from technical errors often occur within the first 24 hours, whereas leaks from necrosis usually occur within the first 14 days. Kidneys with delayed graft function may not have an evident leak until a suitable diuresis ensues. Delayed graft function and older donor age are risk factors for ureteral necrosis.[20]

Because the risk factors for ureteral leak are known, the incidence can be reduced by preventive measures. Preservation of periureteral tissue is essential, especially in living donors procured laparoscopically. The early experience with laparoscopic donor nephrectomy was associated with high rates of urinary leaks, but this rate has declined with improved technique to be almost as good as open donors.[30] A ureter that appears compromised at the time of surgery—because of a transected lower pole artery, obvious "stripping," or failure to become pink and bleed after reperfusion—should be cut as proximally as necessary to reach well-perfused tissue. This may necessitate an alternative technique to achieve urinary continuity, either by anastomosis to the ipsilateral native ureter or by an extension technique of the bladder (psoas hitch, Boari flap; see later). In this situation, a stent also is advisable.

The clinical presentation of ureteral leaks can be obvious or subtle. The clearest clinical scenario is a patient with excellent early function whose urine output suddenly decreases or stops completely, associated with lower abdominal or scrotal swelling and seepage of fluid through the wound or drain

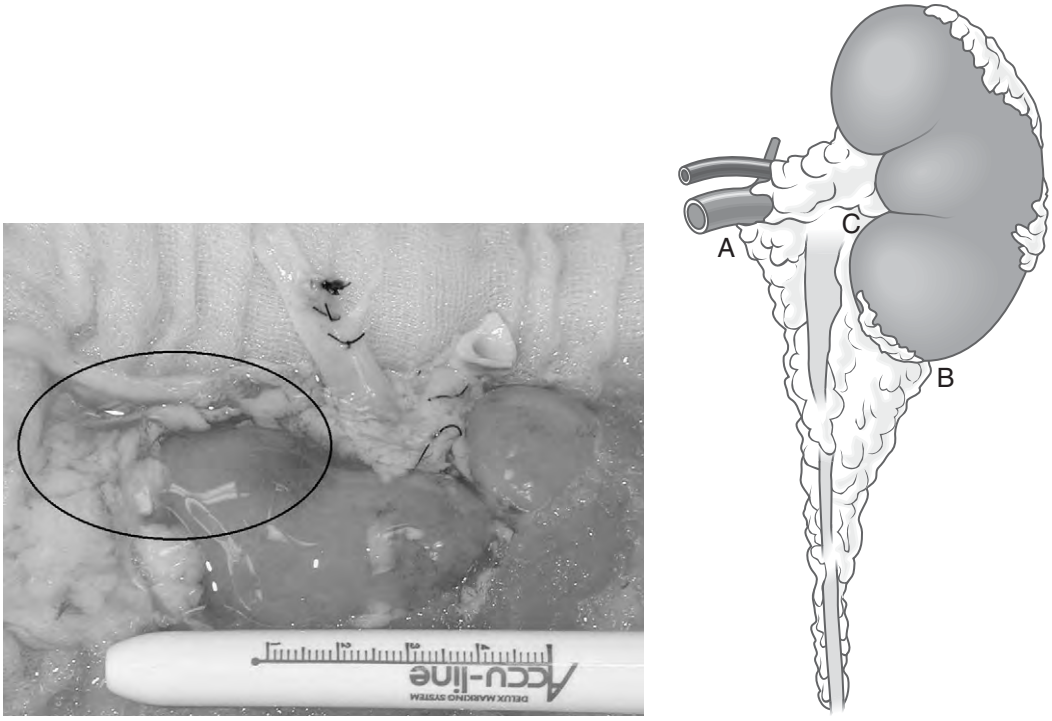

Figure 27–1 **A**, Cadaver donor kidney after back table bench cleaning. Note preservation of the tissue between the lower pole of the kidney and the ureter (*circled*), which typically contains the blood supply to the ureter and must be preserved. **B**, The golden triangle (as outlined by *A, B*, and *C*). Dissection in this area should be avoided during removal and preparation of the kidney for transplantation.

with a creatinine value several times the current serum creatinine. More often, however, the presentation is more subtle; there may be high output maintained from the native kidneys, delayed graft function may limit the urine output, and seroma or lymph already may be draining from the wound or surgical drain. Urine leak should be part of the differential diagnosis in the early post-transplant period whenever there is poor urine output, new fluid collection, new wound drainage, or delayed graft function (see Chapter 14). Any new fluid drainage (or aspirated fluid collection) should be sent for creatinine measurement, and the value should be compared with serum. Several imaging studies may be diagnostic. A Tc 99m MAG-3 renal scan may show tracer outside the anatomical confines of the urinary tract (Fig. 27-2). A cystogram may show the leak, particularly if it is located at the ureterovesical junction. Ultrasound may show a fluid collection, but not its source.

Management of a ureteral leak is endoscopic or operative. If a patient already has an indwelling ureteral stent and no Foley catheter, replacing the Foley catheter often stops the leak, unless the entire distal ureter is necrotic. If this is effective, leaving the Foley catheter in for at least 2 weeks, followed by a confirmatory cystogram, often solves the problem. If there is no ureteral stent, the choice is between stenting and immediate surgical exploration. Placement of a retrograde stent in a transplant ureter can be technically challenging because of the ectopic position of the orifice and lack of periureteral supports, although some groups report high success rates.[38] Percutaneous nephrostomy with antegrade stenting also can be challenging because there is rarely hydronephrosis associated with a urine leak. In the case of ureteral necrosis, open repair is likely inevitable. For those reasons, we prefer to explore and repair these early leaks as an open procedure, unless the patient is clinically unstable.

There are multiple surgical options to repair a ureteral leak depending on the location and extent of ureteral necrosis. We prefer to use a three-way Foley catheter connected to irrigation that can intermittently fill and empty the bladder to identify the leak better. If the ureter is well perfused, and a leak at the ureterovesical junction is clearly due to a technical problem with the anastomosis, the leak can be repaired with additional interrupted sutures. Otherwise, the transplant

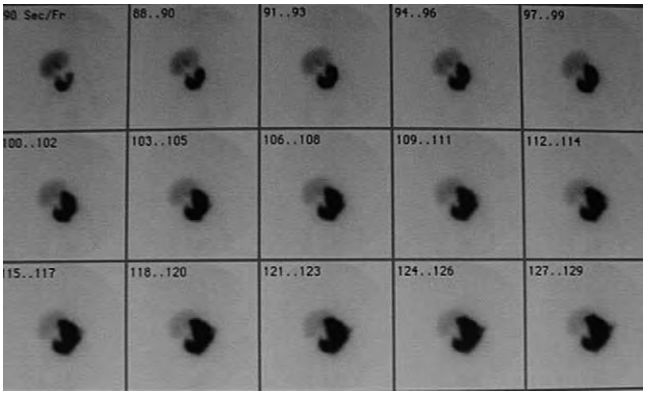

Figure 27–2 MAG-3 renal scan of a patient with transplant urine leak. Note as time progresses how nuclear tracer is seen outside the confines of the urinary bladder.

Table 27–1 **Surgical Techniques to Bridge Gap between Transplant Ureter and Bladder**

Technique	Advantages	Disadvantages
Direct reanastomosis	Simple, quick	Limited by length of well-perfused ureter
Psoas hitch	Bladder reconfigured; no loss of volume	Must mobilize bladder; limited distance for small bladder
Boari flap	Can bridge large distance; well vascularized	Loss of bladder volume
Ureteroureterostomy	Simple; bladder not entered; well vascularized	Ureter may be absent or atretic
Pyelovesicostomy	No need for donor or recipient ureter	May be difficult to reach, especially if renal pelvis is anterior (e.g., left kidney in right iliac fossa); free reflux
Ileal ureter	Can bridge large gap; large lumen in case of stone formation	Need for bowel anastomosis; free reflux

ureter should be cut back to where it is clearly healthy. If the ureteral loss is minor, a simple reimplant of the transplant ureter is usually sufficient. Because the leak of urine often makes the local tissue edematous and inflamed, we recommend doing the repair or reimplantation of the ureter over a stent.

If a tension-free anastomosis cannot be achieved because of limited ureteral length, several options are available (Table 27-1), which also can be used in cases of ureteral stenosis (see later). The bladder may be brought closer to the ureter by mobilizing its attachments and in particular severing the contralateral obliterated umbilical artery. In the psoas hitch, the bladder is incised in the same line as the ureter and reconfigured by closing the bladder incision in line with the ureter (Fig. 27-3).[28] This bladder, now elongated in the direction of the ureter, can be fixed to the ipsilateral psoas muscle to allow a tension-free ureteral reimplant. A small atrophied bladder may not give sufficient length with this technique, however. Alternatively, or in addition to the psoas hitch, a Boari flap of bladder can be raised to bridge the gap for an anastomosis either to the transplant ureter or to the transplant renal pelvis (Fig. 27-4).[13]

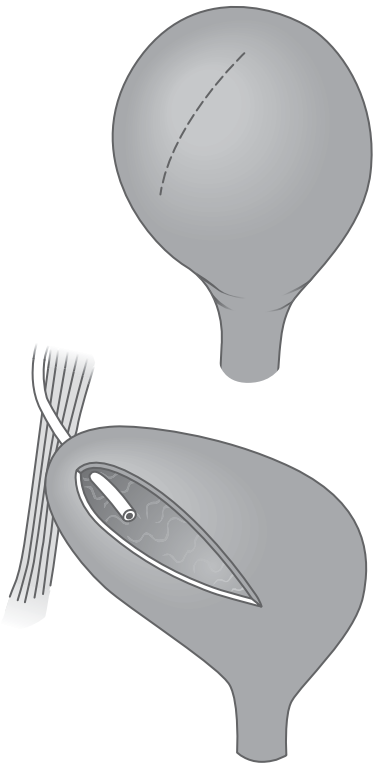

Figure 27–3 Psoas hitch, enabling implantation of a short transplant ureter.

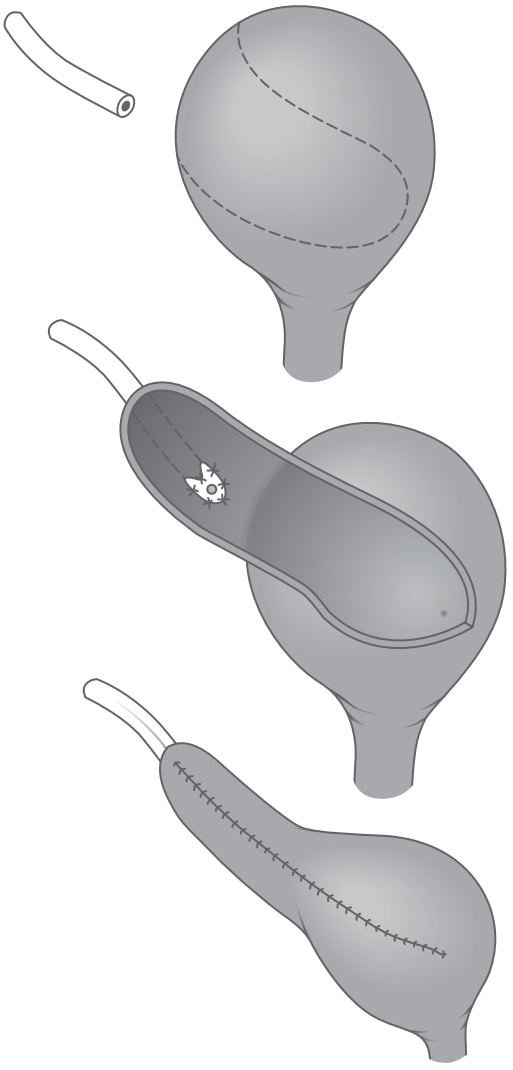

Figure 27–4 Creation of a Boari flap to enable implantation of a short transplant ureter.

A Boari flap reduces the total bladder volume, so it may be inappropriate for a small "disuse atrophy" bladder of a previously anuric patient. The preferred technique here is to use the ipsilateral ureter (if present) to anastomose either to the transplant ureter or directly to the transplant renal pelvis (Fig. 27-5). Typically, the proximal native ureter can be tied off without the need for ipsilateral native nephrectomy.[16] The advantages of this last technique include excellent ureteral blood supply, a large segment of native ureter that can be repositioned without tension, and no compromise of bladder volume. If native urothelium is unavailable, an ileal ureter can bridge the bladder and renal pelvis.[36] The use of completely synthetic conduit material also is being explored.[2]

Ureteral Stenosis

Stenosis of the transplant ureter occurs in approximately 3% of transplant recipients.[25,37] The obstruction can be extraluminal (compression from lymphocele or spermatic cord), ureteral (ischemia), or intraluminal (stone, fungal ball, sloughed renal papilla, foreign body). Ureteral stenosis may occur months or years after an otherwise successful transplant. The rate of ureteral stenosis was high in the early experience with laparoscopic kidney retrieval, but it has decreased more recently to the rate of open surgery.[6] Risk factors for late ureteral stenosis include advanced donor age, delayed graft function, and kidneys with more than two arteries.[19] Although initial ureteral stenting reduces the incidence of early stenosis, there is no impact on the rate of late ureteral stenosis.[35] The emerging problem of polyomavirus (BK virus) can produce ureteritis and ultimately ureteral stenosis.[11]

The clinical presentation of ureteral stenosis can vary according to its location, degree, and speed of onset. Most commonly, ureteral stenosis is gradual and asymptomatic, with an unexplained increase in creatinine leading to discovery of hydronephrosis on ultrasound or computed tomography (CT) scan. Pain over the allograft is rare, unless the obstruction is sudden and high grade. Hydronephrosis is not synonymous with obstruction; dilation of the renal pelvis and calices can occur without obstruction in the setting of prior obstruction (e.g., long-standing ureteropelvic junction obstruction in the donor), reflux, or loss of renal cortex parenchyma in chronic allograft nephropathy. Patients with new-onset hydronephrosis also should be screened for urinary retention by ultrasound.

After establishing hydronephrosis, the two potential confirmatory tests are a diuretic (furosemide) nuclear renogram or a percutaneous antegrade nephrostogram (Fig. 27-6). A diuretic renogram, usually performed with Tc 99m MAG-3 and furosemide, suggests obstruction if the urinary transit time is prolonged, or if the clearance curve shows pelvicaliceal holdup, especially after the diuretic.[29] False-negative results can occur in patients with poor renal function, and false-positive results can occur with bladder outflow obstruction or reflux. Antegrade pyelography is the preferred test when obstruction is strongly suspected. A hydronephrotic transplant kidney is easily accessible with a small spinal needle to inject contrast medium and diagnose obstruction.[3] If obstruction is confirmed, the needle can be converted to a nephrostomy tube over a wire, and antegrade stenting can be performed immediately or after the renal function improves, and ureteral edema lessens (see Fig. 27-6).

Endoscopic management of transplant ureteral strictures is preferable to surgery, which can be difficult when done months or years after the original transplant surgery. The stricture can be accessed in an antegrade fashion as described earlier or retrograde via the bladder.[5] Retrograde stenting is possible,[38] but often difficult because of the ectopic position of the ureteral orifice and the lack of strong tissue supports of the transplant ureter. If a stent does not pass easily over a wire, the stricture can be balloon dilated[4] or incised with a holmium:YAG laser[23] or knife.[7] The initial

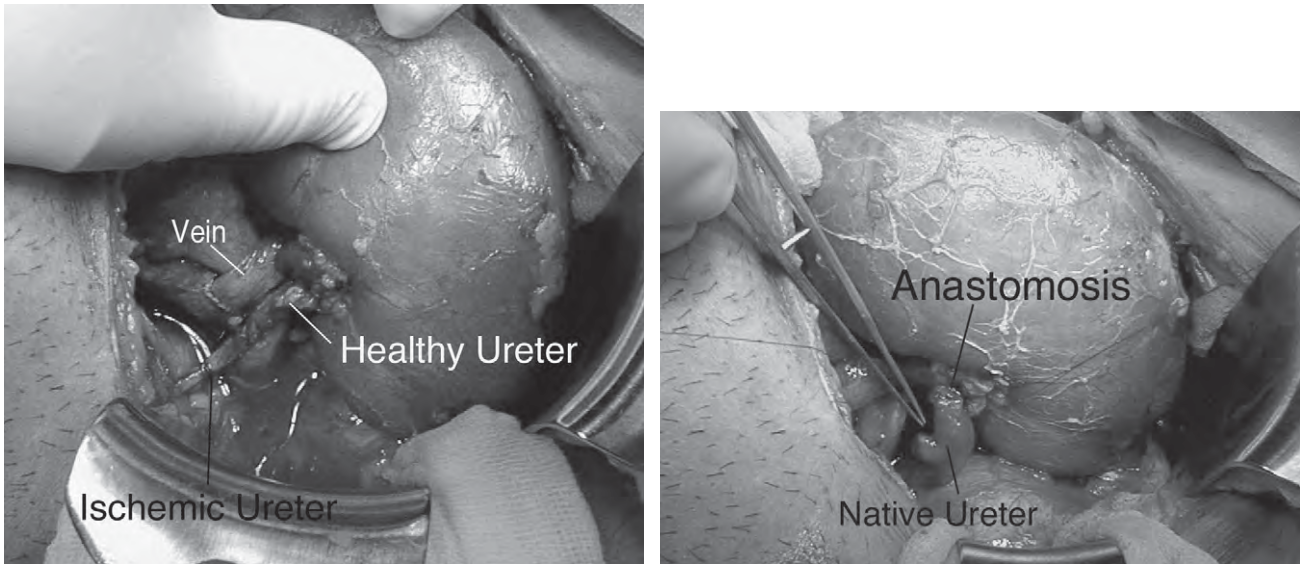

Figure 27–5 Repair of transplant ureteral necrosis by ureteroureterostomy. **A**, Distal ureteral necrosis. Note the distal ureter, proximal ureter, and accumulation of urine in the wound. **B**, After repair. The native ureter was transected and rotated to the proximal transplant ureter. Anastomosis was end-to-end over a double-J stent using 5-0 PDS suture. The proximal native ureter was tied off without native nephrectomy. (**B**, See color plate.)

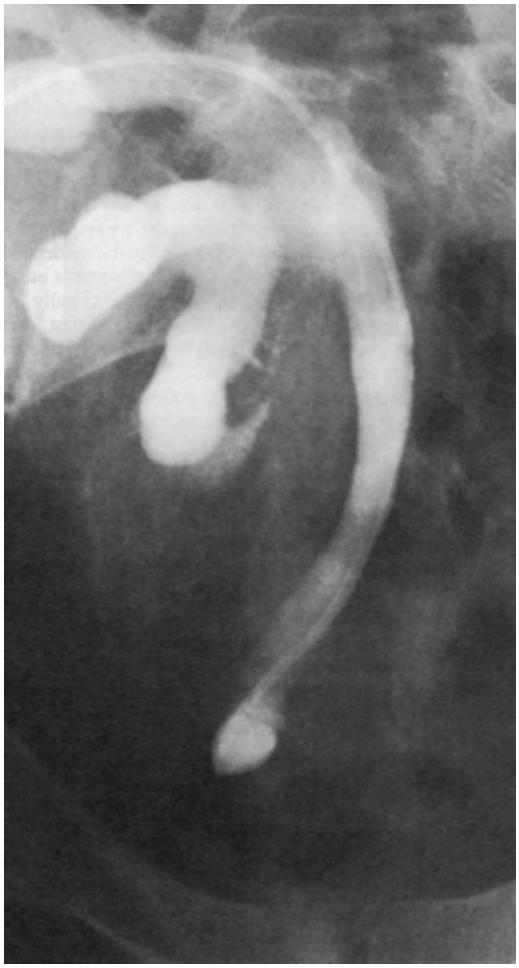

Figure 27–6 Antegrade study in a transplanted kidney showing an obstructed lower ureter.

procedure is successful in about 50% to 65% of cases. Recurrent strictures may result from inadequate primary therapy or extensive ischemia that does not respond durably to dilation. Occasionally, patients have been managed with long-term stents.[8] Typically, recurrent strictures are managed with open surgery, however. When the site of obstruction is identified, and the diseased segment of ureter is excised, any of the operative approaches discussed previously for ureteral leak may be used (e.g., psoas hitch, Boari flap, ureteropyelostomy, pyelocystostomy, ileal ureter). Successful treatment of transplant ureteral stenosis results in long-term graft survival.[19]

USE OF PROPHYLACTIC URETERAL STENTS

The routine use of double-J ureteral stents (Fig. 27-7) at the time of kidney transplantation has been controversial. Table 27-2 lists the pros and cons. In some series, stents can reduce the incidence of ureteral leaks and early ureteral stenosis[35] and make the early management of leaks easier. Other reports, including prospective randomized trials, have shown no impact.[15] Even if stents do reduce the incidence of complications, in at least 95% of patients their use would be unnecessary. Especially in busy programs, there is the danger of a forgotten stent turning up calcified months or years later (Fig. 27-8).

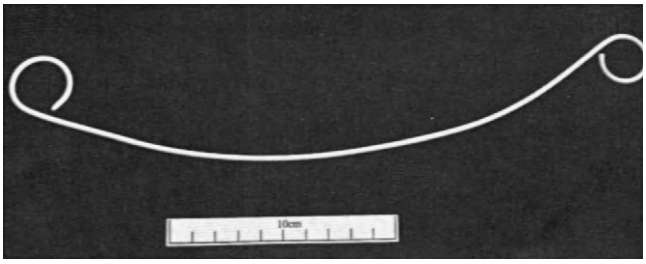

Figure 27–7 Double-J stent.

Two meta-analyses have addressed the issue of prophylactic routine stenting in renal transplants. Mangus and Haag[26] performed a meta-analysis of 49 published studies, including randomized controlled trials and case studies. These investigators found a significant reduction in ureteric complications with stents in randomized (from 9% to 1.5%; $P < .0001$) and case series (from 4.8% to 3.2%; $P = .007$) data. In a separate study, Mangus and coworkers[27] found stenting to be cost-effective. Wilson and colleagues[42] analyzed data in the Cochrane Register of Controlled Trials. They found the relative risk of major urological complications with stents to be 0.24 (95% confidence interval 0.07 to 0.77; $P = .02$). Although urinary tract infections were more common in the stented group, this increase disappeared in patients receiving routine antimicrobial prophylaxis.

The optimal duration of prophylactic stenting has not been determined. Based on local center preference, it is usually 2 to 6 weeks. Some surgeons tie the stent directly to the Foley catheter, which eliminates the need for cystoscopic removal, but also risks early removal if the catheter requires changing. If a stent is used, it is important that the case notes are flagged and the patient is told that he or she has a stent in place that must be removed.

URINARY CALCULI IN TRANSPLANT RECIPIENTS

Urolithiasis in renal transplant recipients is uncommon. Incidence ranges from about 1%[10] to 5%[21] of transplants performed. In the United States, only 1 in 1000 transplanted patients had a hospital admission for stones,[1] with the strongest risk factors being female sex and prior history of stone disease. As more centers transplant kidneys from living donors with known asymptomatic renal stones, this incidence may increase.[14] Other causes of stones include the use of

Table 27–2 **Advantages and Disadvantages of Routine Prophylactic Ureteral Stenting in Renal Transplants**

Advantages	Disadvantages
Reduction in ureteric complications	95% of patients have unnecessary stent
Urine leak easier to manage	Increased risk of urinary tract infection
Cost-effective	Risk of stent migration or stone encrustation
	No evidence for patient or graft survival benefit
	Patient discomfort from bladder spasm

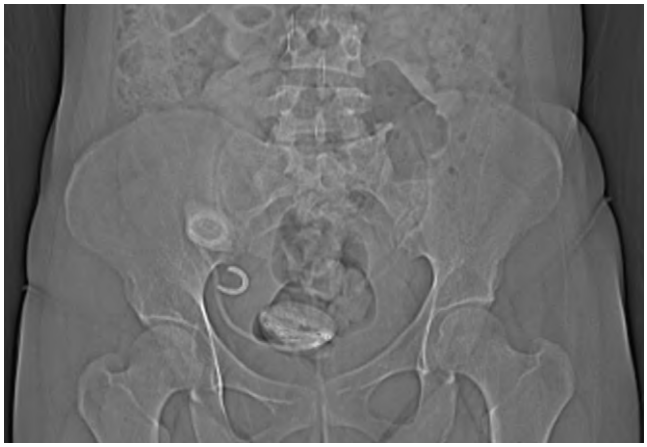

Figure 27–8 Plain radiograph of a retained stent. The patient had stent placement at the time of transplant, but moved to another country before the stent was removed. The patient presented to our institution 2 years later with stones in the kidney and bladder.

nonabsorbable suture in the urinary tract, foreign body (e.g. retained stent), persistent urinary tract infection, ileal conduit diversion, and incomplete bladder emptying. Metabolic evaluation of transplant recipients who form stones most commonly reveals hypocitraturia, hyperparathyroidism, hypophosphatemia, and hypercalcemia.[18] Hypocitraturia has been linked with the use of calcineurin inhibitors.[39]

The clinical presentation of transplant urolithiasis varies, in keeping with the denervated state of the transplant kidney. Patients may complain of pain over the graft, hematuria, or reduced or absent urine output. Asymptomatic stones may be discovered as part of routine imaging or as part of investigating an increasing creatinine. Anuria requires emergent intervention, usually with a percutaneous nephrostomy. Otherwise, stone number and location are best delineated by CT scan. Bladder calculi also should be evaluated by cystoscopy to assess outflow obstruction. All patients with stones also should have a urine culture performed.

Therapy of stones in a kidney transplant is similar to therapy in native kidneys, with the exception that antegrade techniques are easier because of the ready accessibility of the kidney in the pelvis, and retrograde techniques are more difficult because of the ectopic position and course of the ureter. When a stone is identified in a living donor, the kidney with the stone is always the one transplanted. Successful stone retrieval has been reported using ureteroscopy on the back table[32] or ultrasound-guided nephrolithotomy.[14] Many of these small stones pass spontaneously without intervention. Larger stones and stones causing obstruction or symptoms can be managed by extracorporeal shock wave lithotripsy, antegrade or retrograde ureteroscopic stone extraction (with laser fragmentation if necessary), or rarely open surgery.[10] Bladder calculi can be managed endoscopically with fragmentation via electrohydraulic lithotripsy or holmium:YAG laser. Large stones may be best managed by open cystolithotomy.

URINARY RETENTION

After renal transplantation, urinary retention may be due to bladder outflow obstruction or a neurogenic noncontractile bladder. In anuric patients, these problems may not be identified until after the transplant Foley catheter is removed. Patients with a noncontractile bladder usually have a preexisting history of voiding problems or neurological disorders, such as Parkinson's disease, multiple sclerosis, or diabetes with peripheral neuropathy. When bladder pathology is suspected, urodynamics can make the diagnosis. Immediate therapy is clean intermittent self-catheterization, which is safe and effective in transplant recipients.[9]

Bladder outflow obstruction after transplantation is almost exclusively seen in men and may be due to urethral stricture, benign prostatic hypertrophy, or bladder neck contracture, or, more rarely, foreign body, persistent posterior urethral valves, or an ectopic ureterocele. Anuric men with benign prostatic hypertrophy should not be offered surgical relief before transplantation because transurethral prostatic surgery in a "dry urethra" has a high incidence of stricture formation. After transplant in men with significant bladder outflow obstruction from benign prostatic hypertrophy, therapy should be started with an α-blocker (e.g., terazosin, tamsulosin, alfuzosin) and a 5α-reductase inhibitor (e.g., finasteride, dutasteride). Men in retention despite medications should start intermittent self-catheterization and delay definitive prostatic surgery for at least 3 months. Although transurethral resection of the prostate can be done in the immediate post-transplantation period,[22] significant morbidity[33] and mortality[37] have been reported. Although there are no publications on minimally invasive therapies for benign prostatic hypertrophy in transplant recipients, we have anecdotally used transurethral needle ablation and photoselective vaporization of the prostrate (PVP) ("green light" PVP) with success.

ERECTILE DYSFUNCTION

With an aging transplant population, erectile dysfunction is a prevalent and increasingly identified problem. In one study of 113 male transplant recipients, 53% reported erectile dysfunction.[34] Factors contributing to erectile dysfunction are often the same factors responsible for renal failure, including diabetes, hypertension (and its medical treatment), and vasculopathy. Dialysis patients may have elevated serum prolactin, which can depress testosterone and lead to erectile dysfunction; this may explain partly the 20% of patients whose erectile dysfunction improves after transplant.[34] Although the internal iliac artery is less commonly used for renal artery anastomosis than previously, it should be avoided in men receiving a second transplant, in whom vasculogenic impotence can occur as a result in 25%.[41]

Given the multifactorial nature of erectile dysfunction in this population, there is limited value in an extensive workup, beyond measuring testosterone and prolactin. Treatment is symptom-oriented. Transplant patients seem able to tolerate phosphodiesterase 5 inhibitors well, with good efficacy for sildenafil and no impact on calcineurin levels.[43] For patients who fail oral therapy, intracorporeal injection with agents such as prostaglandin E_1 or papaverine is effective in transplant recipients.[24] Finally, penile prostheses have been used safely and successfully in transplant patients. If an inflatable prosthesis is desired, it would be better to use a "two-piece" model rather than the more common "three-piece" model, which uses a fluid reservoir that is placed in the retroperitoneum. This fluid reservoir is prone to damage in transplant recipients owing to the proximity to vascular and urinary anastomoses, which results in device failure.[12] In a patient about to receive a kidney transplant who has a

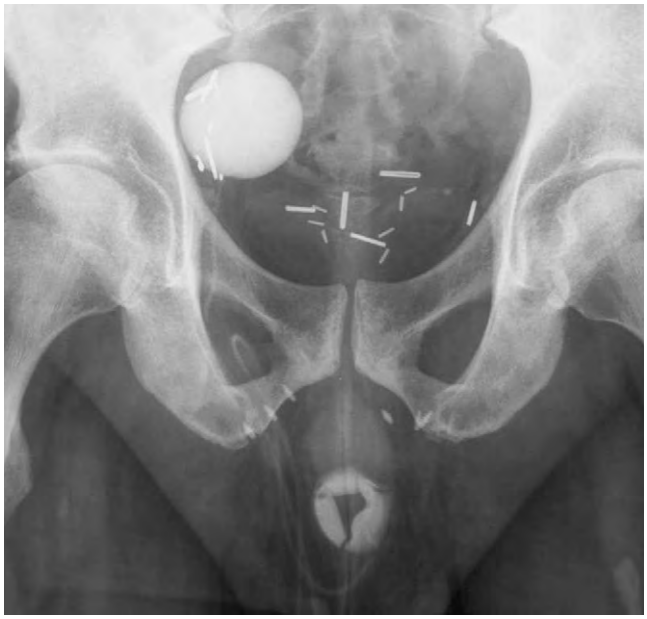

Figure 27–9 Plain radiograph of a patient with an artificial urinary sphincter. Note the position of the fluid reservoir in the lower right pelvis, where it could be damaged during transplant recipient dissection. A "three-part" inflatable penile prosthesis would have a similar reservoir but would be filled with water and be radiolucent. Pretransplant imaging with noncontrast CT scan can confirm the location and direct the incision to the contralateral side.

penile prosthesis and does not know of what type, it is worth checking a preoperative CT scan to ensure that there is no pelvic fluid reservoir or to choose to operate on the contralateral side, if possible. This is true for patients with an artificial urinary sphincter as well (Fig. 27-9).

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Chapter 28

Cardiovascular Complications after Renal Transplantation*

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Successful kidney transplantation was first achieved in the 1960s with immunosuppressive drug regimens that included azathioprine, prednisone, and often polyclonal antibodies to lymphocytes administered immediately after transplantation. In the 1970s, 1-year graft survival (patient survival with a functioning kidney) was 50% in many centers, and most patients had one or more acute rejection episodes during the first year. This situation improved with the adoption of cyclosporine in the 1980s, but even then the major threat to long-term graft survival continued to be the loss of the kidney to rejection.

In the 1990s, there were remarkable improvements in 1-year graft survival attributable to new immunosuppressive drug regimens. Currently, 1-year graft survival exceeding 90% is common, despite the fact that transplant candidates are at increasingly higher risk for graft failure. This remarkable improvement in short-term graft survival has shifted the focus from preventing short-term rejection to maintaining long-term patient and graft survival. Improvements in outcomes for patients who survive beyond the first year with a functioning kidney have not been as dramatic as improvements in short-term outcomes. (See also Chapter 37.)

Some kidneys are lost to acute rejection even after the first post-transplant year because of noncompliance with immunosuppressive medications. Most kidneys are now lost to either chronic graft dysfunction or premature death with function, however. The causes of chronic graft dysfunction are poorly understood but include calcineurin inhibitor toxicity, de novo or recurrent glomerular disease, and a poorly defined entity called chronic allograft nephropathy. Since the 1990s, the rate of patients returning to dialysis or needing another transplant has been gradually declining, while the rate of graft failure owing to death with a functioning kidney has not changed (Fig. 28-1). Death with a functioning kidney is approaching return to dialysis or retransplantation as the most common cause of graft failure (see Fig. 28-1). Although the goal of transplantation is to have every patient die with a functioning kidney, most deaths after transplantation are still occurring prematurely. The fact that these deaths are premature is widely accepted, albeit poorly documented, in the medical literature.

Although graft dysfunction undoubtedly contributes to mortality, not all deaths would be prevented by improving graft function. An important task for clinicians caring for increasing numbers of transplant recipients is to reduce mortality. Although the cause of death for many transplant patients is unknown, many deaths are directly or indirectly related to immunosuppression; these include deaths resulting from infection and malignancies, which account for more than one third of mortality in transplant recipients (Fig. 28-2). Cardiovascular disease (CVD) is the most common cause of death after kidney transplantation, however; this includes deaths resulting from strokes (embolic/thrombotic and

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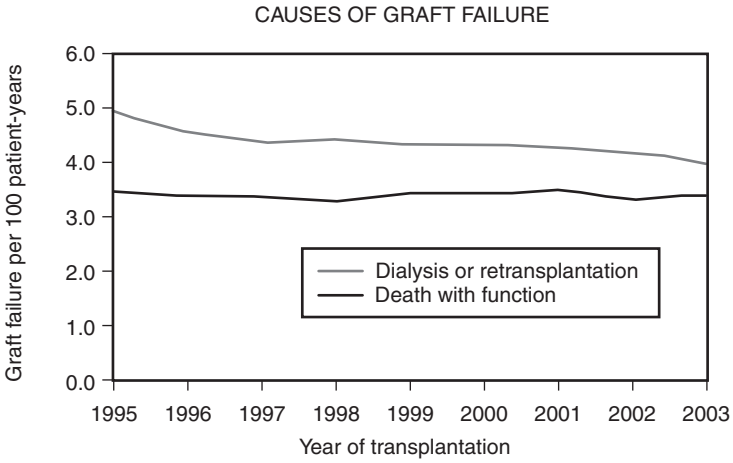

Figure 28–1 Causes of graft failure per 100 patient-years of a functioning graft, by year of transplantation. (Data from the United States Renal Data System Annual Data Report 2005 [www.usrds.org].)

hemorrhagic), peripheral arterial disease (e.g., ischemic extremities that become infected, ruptured abdominal aortic aneurysms), and heart disease.

The most common cause of heart disease after kidney transplantation is ischemic heart disease (IHD). Structural heart disease also may contribute to mortality by causing arrhythmias or congestive heart failure (CHF). As in the general population, hypertension is the major cause of structural heart disease after kidney transplantation. Patients with chronic kidney disease (CKD) also are prone to vascular calcification, which may diminish the elastic properties of arteries and contribute to hypertension and structural heart disease. Valvular calcifications also are common in patients with CKD, and valvular heart disease may be an underestimated cause of mortality after kidney transplantation.

Even before full understanding of the pathogenesis of CVD in kidney transplantation, the clinician's immediate goal should be to prevent CVD. Clinical studies of prevention strategies can improve understanding of the pathogenesis of CVD and vice versa. No matter what causes CVD after transplantation, many modifiable risk factors have been identified. These modifiable risk factors should be the targets of clinical trials and best-practice interventions pending the results of clinical trials.

Because the number of transplant recipients is small, the best evidence for preventing CVD in kidney transplant recipients often comes from large studies in the general population. The development of evidence usually follows a sequence of (1) noting an association between a putative risk factor and CVD in the general population; (2) establishing the risk factor in large, well-designed, prospective, observational studies in the general population; (3) proving in randomized trials that reducing the risk factor safely reduces CVD in the general population; (4) showing the same association between the risk factor and CVD in kidney transplant recipients; and (5) showing that an intervention can reduce the risk factor safely in kidney transplant recipients. This chain of evidence may be completed by conducting a randomized trial in kidney transplant recipients. It is usually not feasible, and it is sometimes not ethical, to conduct a randomized trial of CVD prevention in the small kidney transplant population, however.

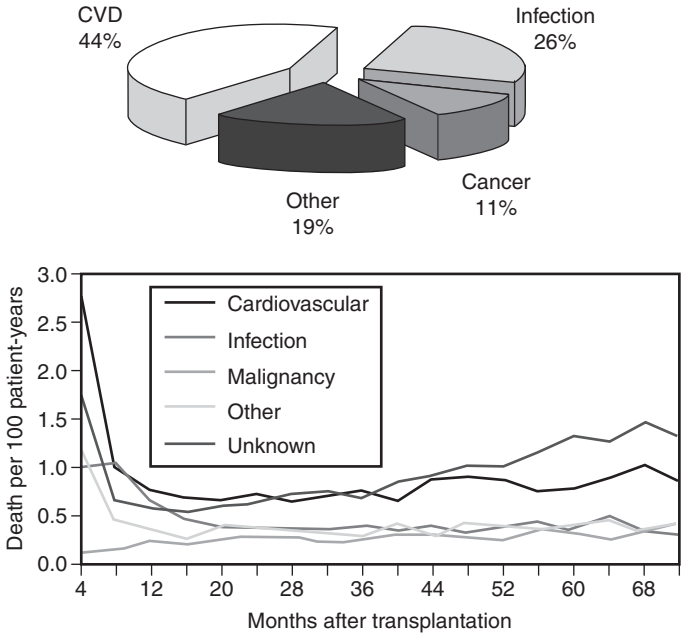

Figure 28–2 *Upper panel*, Causes of death after kidney transplantation for adult, first-time, kidney-only transplant recipients, 1995 through 2003, who died with functioning graft ($N = 10{,}648$). *Lower panel*, Differences in cause-specific death rates by time after transplantation. CVD, cardiovascular disease. (Data from the United States Renal Data System Annual Data Report 2005 [www.usrds.org].)

In the absence of evidence from large randomized trials, data from trials in the general population and from observational studies in kidney transplant recipients can be used to develop a comprehensive clinical strategy to prevent CVD after kidney transplantation (Fig. 28-3). Early referral and pretransplant screening for IHD may help prevent post-transplant IHD events. Perioperative β blockade also may be effective. The management of traditional CVD risk factors before and after transplantation includes aspirin prophylaxis, cigarette abstinence, treatment of hypertension and dyslipidemias, and intensive blood glucose control. Although the risk for CVD can be reduced by minimizing the use of corticosteroids, calcineurin inhibitors, and sirolimus, the management of CVD risk factors also must include a strategy of optimal immunosuppression to prevent acute rejection and maximize long-term kidney function. Finally, numerous lifestyle modifications may favorably affect CVD risk factors and should be encouraged.

INCIDENCE OF CARDIOVASCULAR DISEASE IN KIDNEY TRANSPLANTATION

It is generally acknowledged that the incidence of CVD is higher after kidney transplantation than in the general population, but it is lower than for comparable patients treated with dialysis. Retrospective studies published in the 1990s included patients transplanted before the cyclosporine era, who were often treated with high doses of prednisone. In a Scandinavian study of 1347 transplants over 5 years, IHD accounted for 53% of deaths. Deaths from IHD in nondiabetic patients 55 to 64 years old were 6-fold higher than in the general population, and among diabetics, deaths were 20-fold higher than in the general population.[104] In the Netherlands, age-adjusted and sex-adjusted CVD mortality was 12-fold higher in the first year after transplantation and 9-fold higher in subsequent years compared with the general population.[11]

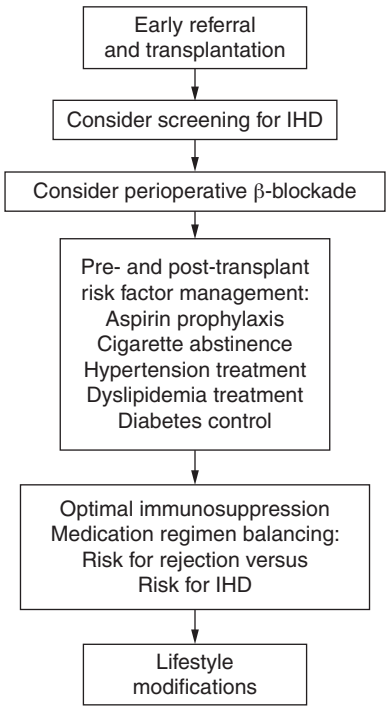

Figure 28–3 Approach to the management of ischemic heart disease (IHD) risk in kidney transplant recipients.

In a study from the United States, 23% of patients who underwent transplantation during the period 1976 through 1991 developed IHD by 15 years after transplantation, defined as acute myocardial infarction (AMI), revascularization, or death attributable to IHD.[81] In the same study, 15% developed cerebral vascular disease (strokes or transient ischemic attacks), and 15% developed peripheral arterial disease (nontraumatic amputations or revascularizations) by 15 years after kidney transplantation.[81] In a more recent study from the same center, the adjusted relative risk of de novo IHD occurring more than 12 months after transplantation declined; compared with transplants done during the period 1963 through 1985, the relative risk for IHD was 0.60 (95% confidence interval [CI] 0.39 to 0.92; $P = .019$) for transplants done during 1986 through 1992, and 0.27 (95% CI 0.11 to 0.63; $P = .002$) for transplants done during 1992 through 1997.[80] In United States Renal Data System (USRDS) registry analyses, the adjusted incidences of post-transplant acute coronary syndromes and death resulting from CVD also have declined[4,116]; however, the adjusted incidence of post-transplant AMI has not changed.[98]

The incidence of CVD seems to be lower for transplant recipients versus comparable patients on the waiting list for a deceased donor kidney. In a USRDS registry analysis, after the first 3 months after transplantation, CVD mortality rates among 60,141 first kidney transplant recipients during the period 1995 through 2000 were lower than CVD mortality rates among 66,813 patients on the waiting list.[117] Table 28-1 shows the CVD mortality rates for deceased and living donor transplant recipients and patients on the waiting list.[117]

Similarly, the incidence of AMI seems to be lower for transplant recipients versus comparable patients on the waiting list for deceased donor kidneys. In a study of 53,297 U.S. Medicare beneficiaries placed on the transplant waiting list for a deceased donor kidney in 1995 through 2002, the Kaplan-Meier cumulative incidence of AMI was 8.7% by 3 years.[84] This incidence was higher than the 6.1% 3-year incidence for de novo AMI for recipients of deceased donor kidney transplants and the 4.2% 3-year incidence for living donor kidney transplants.[84] Compared with the deceased donor waiting list, the adjusted relative risk of AMI for a deceased donor kidney transplant recipient was 3.57 (95% CI 3.21 to 3.96; $P < .0001$) in the first 3 months after transplantation but 0.45 (95% CI 0.41 to 0.50; $P < .0001$) thereafter.[84] The relative risk of AMI for a living donor transplant was 2.81 (95% CI 2.31 to 3.42; $P < .0001$) in the first 3 months after transplantation, and 0.39 (95% CI 0.33 to 0.47; $P < .0001$) thereafter.[84] Lentine and coworkers[98] reported a higher 3-year cumulative incidence of post-transplant AMI of 11.1% among 35,847 Medicare beneficiaries transplanted in 1995 through 2000, but they did not exclude patients with prior IHD. They reported that the 3-year incidence of AMI on the waiting list was 16.7% (adjusted by average demographic characteristics).[98]

CVD seems to be much more common after kidney transplantation than it is in the general population. The incidence of CVD events is lower after kidney transplantation than among comparable patients on the deceased donor waiting list. In some, but not all, studies, the incidence of CVD events after kidney transplantation seems to be declining in recent years.

Table 28–1 **Cardiovascular Disease Mortality for Renal Transplant Recipients (per 1000 Patient-Years) versus Patients on the Waiting List for a Deceased Donor Kidney**

Months after Transplantation	Deceased Donor	Living Donor	Waiting List
0-3	20.7	8.1	3.4
3-6	6.4	3.7	4.7
6-12	5	2.8	8
12-24	4.8	2.6	16.5
24-36	6.7	3.3	28.4
36-48	7	3.6	36.2
48-60	11.2	4.7	40.7
>60	10.3	6	25

From Meier-Kriesche HU, Schold JD, Srinivas TR, et al: Kidney transplantation halts cardiovascular disease progression in patients with end-stage renal disease. Am J Transplant 4:1662-1668, 2004.

PATHOGENESIS OF CARDIOVASCULAR DISEASE

There is growing evidence that, compared with the general population, a greater proportion of CVD in stage 5 CKD may be structural, and not simply due to atherosclerotic plaque formation. Structural heart disease is more common in patients with CKD than in the general population, and it is more common after kidney transplantation. Patients with CKD have increased vascular calcification. This is likely partly due to abnormalities in calcium, phosphorus, and parathyroid hormone that begin in early stages of CKD.[24] Vascular calcification eventually occurs in most stage 5 CKD patients[56] and is associated with CVD and all-cause mortality.[147] Vascular calcification is medial and intimal.[105] Vascular calcification may contribute to changes in the compliance of arteries, which may contribute to hypertension and left ventricular hypertrophy. Decreased arterial compliance may be reflected in the increased pulse wave velocities measured in patients with CKD.[106] These changes in arterial compliance can lead to left ventricular hypertrophy and ultimately CHF. In dialysis patients, intimal and medial calcifications are associated with all-cause and CVD mortality.[105]

In a histological study of iliac arteries at the time of transplantation, Vincenti and coworkers[165] reported that 31 of 50 patients (62%) had arterial disease, and that its severity was associated with previous hypertension. There was fragmentation of the internal elastic lamellae, smooth muscle proliferation, and intimal fibrosis, but little lipid deposition. Other investigators have reported similar findings.[47,68]

Nonatherosclerotic alterations in large and small arteries in CKD may explain why so-called traditional risk factors do not seem to predict CVD mortality in stage 5 CKD dialysis patients as well as they do in the general population. Low rather than high cholesterol is associated with increased mortality in dialysis patients.[107] A more recent randomized trial in diabetic dialysis patients failed to show that lowering cholesterol with atorvastatin reduced major CVD events.[170] Similarly, obesity and hypertension seem to have inverse relationships with mortality in stage 5 CKD dialysis patients.[97,179]

There is some evidence that valvular heart disease may be more common in CKD than in the general population. In a study of Medicare patients, hospitalization for valvular heart disease was more common among dialysis patients compared with the general population.[3] Similarly, in a case-control autopsy study, heart valves from hemodialysis patients showed significantly more inflammation than heart valves from matched controls.[75] Valvular calcification is common in hemodialysis patients, and clinical correlates to valvular calcification include older age, longer hemodialysis duration, elevated blood pressure, and high calcium-phosphorus product.[157] Valvular calcification is associated with increased mortality in hemodialysis patients.[130,163] This association does not prove that there is a causal relationship between valvular calcification and mortality. Other investigators have shown that hemodialysis patients with valvular calcification also are more likely to have atherosclerotic vascular disease.[168]

There are few studies of valvular heart disease after kidney transplantation. In a study of USRDS patients, valvular heart disease was more common in patients on the waiting list than after transplantation.[5] It is difficult to exclude selection bias, however, in patients who underwent transplantation compared with patients on the waiting list. It is difficult to conclude with certainty that transplantation decreases the incidence of valvular heart disease.

ROLE OF TRANSPLANTATION IN REDUCING CARDIOVASCULAR DISEASE

Cardiovascular Disease in Chronic Kidney Disease

Even in its early stages, CKD is associated with an increased incidence of CVD.[55,111,112,123,146] The incidence of CVD increases in proportion to the severity of kidney dysfunction, or clinical stage of CKD. The highest incidence is seen among patients in stage 5 CKD (estimated glomerular filtration rate $\leq$15 mL/min/1.73 m^2 or requiring renal replacement therapy).[51] Most of the data examining the relationship between the stage of CKD and the incidence of CVD have been cross-sectional and collected retrospectively. Nevertheless, it is probably reasonable to infer that CVD progresses with duration and severity of CKD.

Early Referral and Transplantation

Mortality is lower in patients after kidney transplantation than in patients on the waiting list for deceased donors.[174] This difference is undoubtedly due partly to a reduction in deaths from CVD. As noted earlier, the incidence of CVD events is lower in patients after transplantation compared

with patients on the waiting list.[84,98,117] It is reasonable to conclude that the sooner a patient can be transplanted, the lower the risk of IHD. In addition, the high incidence of AMI in the first 3 months after transplantation suggests that effective screening and management for IHD as part of the transplant evaluation could be beneficial.

RISK FACTORS FOR CARDIOVASCULAR DISEASE

Numerous single-center and multicenter observational studies have been conducted to define risk factors for CVD after kidney transplantation (Table 28-2). Generally, these studies have been limited by small numbers of CVD events during follow-up. Most were retrospective. Nevertheless, the studies have identified several traditional risk factors for CVD, including age,* male sex,[1,2,4,81,143] diabetes,[2,7,44,70,80,81,143,152] cigarette smoking,[7,44,80,152] total cholesterol,[70,80] low-density lipoprotein (LDL) cholesterol,[7] high-density lipoprotein (HDL) cholesterol,[2,44,81] obesity (measured as body mass index),[7] and blood pressure.[1,2,143] In addition, several nontraditional risk factors for CVD have been identified, including using a deceased (versus living) donor,[143] pretransplant splenectomy,[81] pretransplant bilateral native kidney nephrectomy,[80] anemia,[143] triglycerides,[80] C-reactive protein,[44] homocysteine,[44] low serum albumin,[80,81,143] proteinuria,[80] acute rejection,[70,80,81,143] serum uric acid,[7] and serum creatinine.[70]

*References 1, 2, 4, 7, 44, 70, 80, 81, 116, 143, 152.

Table 28–2 **Individual Center Analyses of Cardiovascular Disease Risk Factors**

First Author, Year	Study Population	End Point	Risk Factors ($P < .05$)
Kasiske, 1996[81]	*N* = 706 Inception cohort Transplanted 1976-1991 Graft survival >6 mo	IHD[a] (*n* = 85)	Age Diabetes Male Splenectomy Acute rejection HDL cholesterol Pretransplant IHD[b] Post-transplant PAD[c] Post-transplant cerebral VD[d]
Kasiske, 1996[81]	*N* = 706 Inception cohort Transplanted 1976-1991 Graft survival >6 mo	Cerebral VD[d] (*n* = 54)	Diabetes Smoking Splenectomy Acute rejection Low serum albumin Pretransplant IHD[b] Post-transplant IHD[a] Pretransplant cerebral VD[d]
Kasiske, 1996[81]	*N* = 706 Inception cohort Transplanted 1976-1991 Graft survival >6 mo	PAD[c] (*n* = 71)	Diabetes Male Smoking Serum albumin Pretransplant PAD[c] Post-transplant IHD[a]
Aker, 1998[7]	*N* = 427 Inception cohort Transplanted 1987-1992	CVD[e] (*n* = 50)	Age Diabetes Smoking Body mass index LDL cholesterol Uric acid
Aakhus, 1999[1]	*N* = 406 Cross-sectional cohort	PAD[f] (*n* = 18)	Age Male Systolic blood pressure
Sung, 2000[152]	*N* = 664 Inception cohort Transplanted 1985-1995	PAD[g] (*n* = 29)	Age Diabetes Smoking Pretransplant PAD[g]
Kasiske, 2000[80]	*N* = 1124 Inception cohort Transplanted 1963-1997 IHD before 1 yr excluded	IHD[a] (*n* = 123)	Age Diabetes Smoking Year of transplant Native nephrectomy Acute rejection Low serum albumin Proteinuria Cholesterol Triglycerides

Table continued on the following page

Table 28–2 **Individual Center Analyses of Cardiovascular Disease Risk Factors—cont'd**

First Author, Year	Study Population	End Point	Risk Factors ($P < .05$)
Rigatto, 2002[143]	$N = 638$ Inception cohort Transplanted 1969-1999 IHD before 1 yr excluded	IHD[h] ($n = 61$)	Age Male sex Diabetes Diastolic blood pressure Acute rejection
Rigatto, 2002[143]	$N = 638$ Inception cohort Transplanted 1969-1999 IHD before 1 yr excluded	CVD death[i] ($n = 67$)	Age Diabetes Anemia Systolic blood pressure Deceased donor Acute rejection
Rigatto, 2002[143]	$N = 638$ Inception cohort Transplanted 1969-1999 IHD before 1 yr excluded	CHF[j] ($n = 63$)	Age Diabetes Anemia Low serum albumin Systolic blood pressure Deceased donor
Aakhus, 2004[2]	$N = 406$ Cross-sectional cohort	IHD[k] ($n = 96$)	Age Diabetes Systolic blood pressure HDL cholesterol Total cholesterol Congestive heart failure Cerebral VD[l]
Aakhus, 2004[2]	$N = 406$ Cross-sectional cohort	IHD death[m] ($n = 56$)	Age Diabetes Systolic blood pressure HDL cholesterol Congestive heart failure
Aakhus, 2004[2]	$N = 406$ Cross-sectional cohort	Cerebral VD[l] ($n = 23$)	Age Male Sedentary lifestyle
Ducloux, 2004[44]	$N = 344$ Inception cohort Graft survival >1 yr Prior CVD[o] excluded	IHD[n] ($n = 27$)	Age Diabetes Smoking HDL cholesterol C-reactive protein Homocysteine
Jardine, 2005[70]	$N = 1052$ Cross-sectional cohort Placebo arm of ALERT[p]	Nonfatal AMI ($n = 66$)	CHD Total cholesterol Acute rejection
Jardine, 2005[70]	$N = 1052$ Cross-sectional cohort Placebo arm of ALERT[p]	Cardiac death[q] ($n = 54$)	Age Diabetes ECG ST-T changes Serum creatinine

[a]Defined as AMI, coronary revascularization, or death attributable to IHD.[81]

[b]Defined as any clinical evidence of coronary artery disease, including angina, AMI, coronary lesions on angiogram, revascularization, or death attributable to IHD.[81]

[c]Defined as amputation (resulting from vascular insufficiency) or a peripheral revascularization procedure.[81]

[d]Defined as stroke or transient ischemic attack.[81]

[e]Defined as coronary disease on angiography, AMI, transient ischemic attack, stroke, or intermittent claudication.[7]

[f]Defined as intermittent claudication with objective signs of peripheral arterial occlusive disease.[1]

[g]Defined as bypass, major amputation, claudication, or percutaneous angioplasty.[152]

[h]Defined as AMI or revascularization.[143]

[i]Defined as death from AMI, revascularization procedure, cardiogenic shock, arrhythmia, stroke, or ruptured abdominal aortic aneurysm.[143]

[j]Defined as dyspnea plus at least two clinical findings.[143]

[k]Defined as a major IHD event (i.e., death from or onset of IHD), where IHD was defined as the presence of angina pectoris or AMI or both determined by the local nephrologist.[2]

[l]Defined as typical history of transient ischemic attack or stroke with or without clinical sequelae.[2]

[m]Defined as death from AMI, congestive heart failure, or sudden death.[2]

[n]Defined as AMI, coronary revascularization, or typical history of angina with abnormal coronarography.[44]

[o]Defined as a "past history of vascular complication."[44]

[p]Placebo arm from a randomized trial examining the effects of fluvastatin on CVD.[70]

[q]Cardiac deaths included sudden death, death caused by AMI, and death caused by heart failure.[70]

ALERT, Assessment of Lescol in Renal Transplantation; AMI, acute myocardial infarction; CHD, coronary heart disease; ECG, electrocardiogram; CVD, cardiovascular disease; HDL, high-density lipoprotein; IHD, ischemic heart disease; LDL, low-density cholesterol; PAD, peripheral arterial disease; VD, vascular disease.

It is important to understand the relationship between different risk factors and to be able to assess the overall risk for CVD in individuals and populations. Many formulas have been developed to determine the risk for CVD in the general population by combining several risk factors. The formula that has arguably been most extensively studied is the one developed, and modified over the years, by the Framingham Heart Study. This formula has been found to be associated with IHD events after kidney transplantation.[44,80] In these studies, the Framingham equation underestimated the absolute risk for IHD events after kidney transplantation, however, suggesting that other risk factors may be important.[44,80]

An important predictor of post-transplant CVD is the presence of CVD at the time of transplantation. In a retrospective study, we examined pretransplant and post-transplant clinical correlates of subsequent CVD events among 706 consecutive patients who underwent transplantation between 1976 and 1991, who survived with a functioning graft for at least 6 months.[81] One of the strongest risk factors for IHD was a history of pretransplant IHD. Patients who developed cerebral vascular disease or peripheral arterial disease also were more likely to have subsequent IHD. Similarly, Jardine and coworkers[70] reported that a prior history of CHD or ST-T wave changes on a baseline electrocardiogram were associated with subsequent CVD events. Aakhus and colleagues[2] also reported that a prior history of cerebral vascular disease or CHF was associated with subsequent IHD events. Finally, Sung and colleagues[152] reported that pretransplant peripheral arterial disease was a risk factor for post-transplant peripheral arterial disease.

Registry analyses, which have included many more CVD events than single-center or multicenter studies, also have identified risk factors for CVD (Table 28-3). Registries generally have few data that were accurately and systematically collected to measure traditional CVD risk factors, however, such as cigarette smoking, dyslipidemias, and hypertension. Nevertheless, risk factors identified in these registry analyses include age,[4,98,99,116] male sex,[4,98,99] African-American ethnicity,[98,116] Hispanic ethnicity,[98] obesity,[99] employment status,[98,99] end-stage renal disease (ESRD) secondary to hypertension,[99] ESRD secondary to diabetes,[98,99] pretransplant diabetes,[4,98,99,116] new-onset diabetes after transplantation,[98] pretransplant CVD,[98,99] pretransplant anemia,[99] pretransplant dyslipidemia,[98] pretransplant hypertension,[116] pretransplant smoking,[99] duration of pretransplant ESRD,[116] post-transplant anemia,[99] post-transplant hypertension,[99] post-transplant AMI,[99] use of a deceased donor,[98,116] donor age,[98,99] donor CVD death,[99] delayed graft function,[98] graft function at 1 year (serum creatinine),[116] year of transplantation,[99] and graft failure.[4,98]

Several traditional and nontraditional risk factors have been found to be associated with CVD after kidney transplantation. Among nontraditional risk factors, it is becoming increasingly clear that the level of graft function is an important predictor of CVD. Patients who have older donor kidneys, delayed graft function, acute rejection episodes, proteinuria, and higher serum creatinine are more likely to have CVD. Graft failure also is associated with subsequent CVD mortality. The message is clear. Transplantation reduces CVD by restoring kidney function, and the better the kidney function, the lower the risk for CVD events.

RISK FACTORS FOR CONGESTIVE HEART FAILURE

Left ventricular hypertrophy (LVH) and CHF are common after kidney transplantation. Many of the same risk factors for IHD also are risk factors for LVH and CHF. Many risk factors for LVH and CHF are unique, however, and the pathogenesis of these CVD outcomes may be different than those of IHD.

Lentine and coworkers[99] used registry data from the USRDS to examine risk factors for de novo CHF ascertained from billing records. They studied 27,011 Medicare beneficiaries who underwent their first kidney transplantation between 1995 and 2001 and did not have evidence of pretransplantation CHF. The cumulative incidences of CHF after transplantation were very high: 7.8% (95% CI 7.6% to 8.3%) at 6 months, 10.2% (95% CI 9.8% to 10.6%) at 12 months, and 18.3% (95% CI 17.8% to 18.9%) at 36 months. Independent risk factors for CHF included age; female sex; obesity (increased body mass index); employment status (lower risk if working full-time); ESRD secondary to diabetes; ESRD secondary to hypertension; comorbidities at transplant (from the Medicare 2728 registration form) including diabetes, anemia, IHD, peripheral arterial disease, and smoking; older donor age; donor CVD death; year of transplantation (lower risk more recently); delayed graft function; post-transplant hypertension; post-transplant anemia; new-onset diabetes after transplantation; graft failure; and post-transplant AMI. Most of these risk factors also were risk factors for AMI, CVD death, and acute coronary syndromes (see Table 28-3). Obesity and anemia figured more prominently as risk factors for CHF, however, than for other CVD (see Table 28-3). Also unique was the higher risk for CHF among women compared with men.[99]

In a two-center study, all consecutive kidney transplants between 1969 and 1999 were included if the recipients survived with a functioning graft for at least 1 year (see Table 28-2).[143] Among 638 patients, de novo CHF occurred as frequently as de novo IHD. De novo CHF was defined as dyspnea plus two other findings of increased jugular venous pressure, bibasilar crackles, chest x-ray evidence of pulmonary venous hypertension, or pulmonary edema. The cumulative incidence of CHF was 3.6%, 12.1%, and 21.6% at 5, 10, and 20 years after transplantation. Statistically independent clinical correlates of de novo CHF were age, diabetes, lower hemoglobin, lower serum albumin, higher systolic blood pressure, and deceased (versus living) donor.[143] In univariate analysis, there was a 50% higher risk of de novo CHF for women, but this was not statistically significant ($P = .1$). The effect of obesity was not studied.[143]

Similar to IHD, CHF may be less common after kidney transplantation compared with dialysis.[143] There are numerous anecdotal reports of improvement in cardiac function after kidney transplantation.[21,50,131] In a retrospective cohort study, 103 kidney transplant recipients with pretransplant left ventricular ejection fraction 40% or less and CHF were reassessed at 12 months after transplantation. After transplantation, 70% of patients had left ventricular ejection fraction 50% or greater.[167] Most dialysis patients with CHF, especially patients who had not been on dialysis for a long time, had improved cardiac function with transplantation.

Similarly, LVH seems to improve after kidney transplantation. In a prospective cohort study of 433 dialysis patients,

Table 28–3 **Registry Analyses of Cardiovascular Disease Risk Factors**

First Author, Year	Study Population	End Point	Risk Factors ($P < .05$)
Abbott, 2002[4]	$N = 14,237$ Transplanted 1995-1998 Excluded ACS pretransplant USRDS Registry	ACS[a]	Age Male ESRD diabetes Earlier transplant era Time after transplantation (↓)[d] Graft loss Diabetes in women
Meier-Kriesche, 2003[116]	$N = 58,900$ First transplant Transplanted 1988-1998 USRDS Registry	CVD death ($n = 1797$)	Age African-American (↓)[d] ESRD due to hypertension ESRD due to diabetes Pretransplant ESRD duration Deceased donor Earlier transplant era Serum creatinine at 1 yr
Lentine, 2005[99]	$N = 27,011$ First transplant Medicare beneficiaries Inception cohort Transplanted 1995-2001 Followed 3 yr USRDS Registry	CHF[b]	Age Female Obesity (body mass index) Employment status ESRD due to diabetes ESRD due to hypertension Anemia (2728 form)[c] Diabetes (2728 form)[c] Pretransplant AMI (2728 form)[c] Angina (2728 form)[c] Arrhythmias (2728 form)[c] PAD (2728 form)[c] Smoking (2728 form)[c] Donor age Donor CVD death Year of transplant (↓)[d] Post-transplant anemia Post-transplant hypertension NODAT Graft failure Post-transplant AMI
Lentine, 2005[98]	$N = 35,847$ First transplant Medicare beneficiaries Inception cohort Transplanted 1995-2000 Followed 3 yr USRDS Registry	AMI[e]	Age Male African-American (↓)[d] Hispanic (↓)[d] Unemployed ESRD due to diabetes Diabetes (2728 form)[c] Pretransplant AMI (2728 form)[c] Angina (2728 form)[c] PAD (2728 form)[c] Dyslipidemia (2728 form)[c] Arrhythmia (2728 form)[c] Deceased donor Donor age DGF NODAT Graft failure

[a]Medicare claims with ACS diagnosis International Classification of Diseases 9th Modification Diagnosis Codes 410.x or 411.x.

[b]Medicare claims with CHF diagnosis International Classification of Diseases 9th Modification Diagnosis Code 428.x.

[c]Risk factors from the Center for Medicare Services form #2728 filled out at the time of ESRD registration and often incompletely reported.

[d]Each risk factor was associated with an increased risk except as indicated by (↓).

[e]Post-transplant AMI by Medicare claims or death from AMI.

ACS, acute coronary syndrome; AMI, acute myocardial infarction; CVD, cardiovascular disease; DGF, delayed graft function; ESRD, end-stage renal disease; NODAT, new-onset diabetes after transplantation; PAD, peripheral arterial disease; USRDS, United States Renal Data System.

143 underwent kidney transplantation.[131,142] After transplantation, echocardiographic left ventricular mass and left ventricular volume indices declined.[131,142] There are many reasons why LVH may improve after transplantation, such as less anemia and better volume control. In some patients, closure of a hemodialysis arteriovenous fistula was associated with a reduction in left ventricular mass.[160]

LVH on echocardiography is an independent risk factor for CHF and death after kidney transplantation.[141] Only a few studies have examined clinical correlates of echocardiographic LVH in kidney transplant recipients, however. In a prospective study, 67 kidney transplant recipients had an echocardiogram 1 and 2 years after transplantation.[142] Most had improvement in LVH between years 1 and 2, although some did not. Among all of the traditional CVD risk factors (obesity was not studied), a failure of regression in LVH between years 1 and 2 correlated with older age, duration of hypertension, the number of antihypertensive medications, LVH at baseline, and (counterintuitively) low pulse pressure. That low, rather than high, pulse pressure was associated with a failure for LVH to regress was thought to be a phenomenon of "reverse causality," whereby heart failure might be expected to cause a low pulse pressure even if a high pulse pressure was a bona fide risk factor for LVH. That this was the case was suggested by the observation that a high pulse pressure was associated with an increase in LVH between years 1 and 2 among recipients without LVH at baseline.[142]

Many of the risk factors for atherosclerotic CVD also are risk factors for LVH and CHF (see Tables 28-2 and 28-3). These include age, diabetes, and especially hypertension. Anemia and obesity have been more readily identified as risk factors for CHF, however, than for atherosclerotic CVD. There was some indication that although men are more likely to develop atherosclerotic CVD, women are more likely to develop CHF. These observations need confirmation in additional studies. There is general agreement, however, that the cardiac function, similar to the risk for atherosclerotic CVD, improves after kidney transplantation.

PRETRANSPLANT MEASURES TO REDUCE CARDIOVASCULAR DISEASE

Screening for Ischemic Heart Disease before Transplantation

The high incidence of AMI in the first 3 months after kidney transplantation suggests that the stress of surgery, delayed graft function, acute rejection, and high doses of immunosuppressive drugs may precipitate AMI.[84] As a result, guidelines for the evaluation of kidney transplant candidates generally recommend screening for IHD and performing prophylactic coronary artery angioplasty or bypass grafting in asymptomatic individuals who are discovered to have significant coronary artery occlusions.[79] There is no evidence that screening reduces the risk for perioperative cardiac events either for patients in the general population undergoing noncardiac surgery or for patients undergoing kidney transplantation. Guidelines of the American College of Cardiology and the American Heart Association do not recommend screening asymptomatic patients before noncardiac surgery.[46]

Some observational studies examining outcomes after preoperative screening in the general population have cast doubt on the effectiveness of this strategy in reducing perioperative IHD events. The potential for bias in observational studies is great, however. McFalls and associates[115] conducted a large, multicenter, randomized controlled trial to examine the benefit of coronary artery revascularization before major elective, noncardiac, vascular surgery. They randomly allocated 510 patients with significant coronary artery disease to undergo revascularization or not before surgery. After a mean 2.7 years of follow-up, there were no differences in mortality in the revascularization group (22%) versus the no-revascularization group (23%) ($P = .92$). Similarly, there was no difference in postoperative AMI: 12% in the revascularization group and 14% in the no-revascularization group ($P = .37$). This study suggests that the recommendations of the American College of Cardiology and the American Heart Association to avoid screening asymptomatic patients for coronary artery disease before major surgery are justified.[46]

It is possible that the higher incidence and severity of IHD in stage 5 CKD may render pretransplant screening more effective than screening for IHD before major surgery in the general population. Manske and coworkers[113] randomly allocated asymptomatic diabetic patients with significant coronary artery occlusions to revascularization versus medical management. The Data Safety Monitoring Board halted the study prematurely. After only 26 patients had been enrolled, the number of IHD events was significantly higher in the group allocated to revascularization compared with patients who received medical management.[113] The number of events in the medical management arm of this study is much higher than is generally seen today, and the number of patients in this study is too small to allow firm conclusions. Medical management has changed since this trial was conducted. A larger, randomized, controlled trial is needed to determine if screening asymptomatic patients with advanced CKD reduces CVD events.

Currently, most transplant centers screen high-risk patients (e.g., patients with prior CVD, diabetes, multiple CVD risk factors, or older age) with a noninvasive cardiac stress test.[35] Patients with a positive stress test undergo coronary angiography and revascularization if there are significant occlusive coronary lesions. Some centers have examined the results of this strategy and have reported that 50%,[96,100] 39%,[101] 71%,[133] and 44%[85] were considered low risk and did not undergo cardiac stress testing. Of the high-risk patients who underwent noninvasive stress testing, only 3.2%,[101] 2.9%,[133] and 9%[85] had coronary artery revascularization procedures as a result of screening. With less than 10% of patients screened undergoing revascularization, the revascularization would need to be very effective in reducing IHD events to make screening beneficial and cost-effective for the pretransplant evaluation.

Current guidelines in the general population do not recommend screening asymptomatic patients for IHD before major, noncardiac surgery. Nevertheless, many transplant centers routinely screen transplant candidates with a noninvasive cardiac stress test. A randomized trial is needed to determine whether screening is effective in this setting.

Perioperative β Blockade

Many randomized controlled trials in the general population have examined whether perioperative β blockade

reduces IHD events. A meta-analysis of 22 randomized controlled trials of β-blocker treatment in patients having noncardiac surgery included 2437 patients.[39] There was a 56% (95% CI 3% to 80%; $P = .04$) reduction in the composite outcome of cardiovascular mortality, nonfatal myocardial infarction, and nonfatal cardiac arrest. There was more than a twofold increase in the risk of bradycardia requiring treatment. The authors concluded that the "evidence that perioperative β-blockers reduce major cardiovascular events is encouraging but too unreliable to allow definitive conclusions."[39]

In an observational study of 663,635 patients with no contraindications to β-blockers, 122,338 (18%) received β-blockers during the first 2 hospital days after noncardiac surgery.[103] The benefit of perioperative β blockade was proportional to the risk, as assessed by the Revised Cardiac Risk Index (RCRI). Among the 580,665 patients with an RCRI score of 0 or 1, treatment was associated with no benefit, whereas patients with an RCRI score of 2, 3, and 4+ had adjusted odds ratios for death of 0.88 (95% CI 0.80 to 0.98), 0.71 (95% CI 0.63 to 0.80), and 0.58 (95% CI 0.50 to 0.67). The authors concluded that perioperative β blockade was associated with a reduced risk of death among high-risk, but not low-risk, patients undergoing major noncardiac surgery.[103]

Evidence from the general population suggests that perioperative β blockade may be beneficial in high-risk patients undergoing major noncardiac surgery. Because many kidney transplant candidates are high risk, perioperative β-blocker therapy may be beneficial. There are no randomized trials in patients with stage 5 CKD, however, and complications of β-blocker prophylaxis in this population also may be greater than the general population.

POST-TRANSPLANT MEASURES TO REDUCE CARDIOVASCULAR DISEASE

Prophylactic Anticoagulation

Approximately 2% to 5% of kidney transplants are lost to perioperative graft thrombosis.[15,135,161] There have been anecdotal reports that hereditary risk factors for venous thrombosis, or "thrombophilia," also are risk factors for renal allograft thrombosis.[59,126] It has been suggested that transplant candidates should be screened for gene polymorphisms associated with an increased risk of venous thrombosis, and that prophylactic heparin (or low-molecular-weight heparin fractions) could reduce the incidence of graft thrombosis in high-risk individuals. Observational studies in the general population also have linked some of the same hereditary risk factors for venous thrombosis to CVD events.[175] Theoretically, the incidence of perioperative IHD events also could be reduced by using prophylactic anticoagulation in high-risk individuals. Anticoagulation is associated with a higher risk of perioperative bleeding, however, and there are no randomized trials examining the risk-to-benefit ratio in this setting.

Aspirin Prophylaxis

Randomized controlled trials in the general population have shown that low-dose aspirin is effective in reducing IHD events in patients with known IHD. In high-risk patients without IHD, low-dose aspirin also has been shown to reduce the risk for first AMI. As a result, the United States Preventive Services Task Force concluded that "the balance of benefits and harms is most favorable in patients at high risk for coronary heart disease (those with a 5-year risk ≥3%), but it is also influenced by patient preference."[158] In a randomized trial comprising 39,876 healthy women 45 years old and older, 100 mg aspirin every other day failed, however, to reduce first major CVD events (nonfatal AMI, nonfatal stroke, or death from CVD).[140] Some doubt has been raised over whether the benefits of aspirin are the same in men and women.

Patients who are taking aspirin before transplant surgery generally do not need to discontinue it. In a meta-analysis of perioperative bleeding in 41 observational and randomized trials, aspirin increased the risk of bleeding by 50% but did not lead to a higher level of the severity of bleeding complications.[20] The authors concluded that low-dose aspirin should be discontinued before surgery only if it is expected to cause bleeding with increased mortality or sequelae comparable to the observed cardiovascular risks without aspirin.

There have been no controlled trials of aspirin prophylaxis in kidney transplantation. Kidney transplant recipients have been shown, however, to have increased platelet aggregability,[13,26,69] fibrinogen,[102] C-reactive protein,[33,44] antiphospholipid antibodies,[43] and homocysteine,[44] all of which could predispose transplant patients to graft thrombosis and IHD events. It is an intuitively compelling argument that low-dose aspirin might be beneficial. The risk of gastrointestinal and other bleeding also is likely to be increased in kidney transplant patients, however.

In a retrospective study, 105 deceased donor kidney transplant recipients treated with prophylactic aspirin (150 mg/day) for the first 3 months after transplantation had no episodes of primary allograft thrombosis compared with 6 of 121 (5%) episodes in untreated controls ($P = .03$).[154] Similarly, a study of 830 kidney transplant recipients found that aspirin prophylaxis (100 mg/day used in 205 patients) was associated with improved kidney allograft survival.[58] These studies provide only marginal, circumstantial evidence that aspirin prophylaxis may prolong graft survival. The evidence from the general population that aspirin prophylaxis reduces IHD events in high-risk individuals provides a more compelling reason to use aspirin in patients at high risk for IHD after kidney transplantation. A randomized controlled trial of aspirin prophylaxis in kidney transplant recipients at increased risk for graft thrombosis or IHD or both is warranted.

Aspirin prophylaxis seems to be effective in reducing IHD in the general population, although there is some debate over the relative benefit in men versus women. There are no randomized controlled trials of aspirin prophylaxis in transplant patients, and whether the risk-to-benefit ratio warrants treatment with low-dose aspirin in this population is unclear. Given the fact that the risk for thrombosis is higher in kidney transplant recipients than in the general population, however, and that many markers of inflammation are also abnormal, aspirin prophylaxis seems warranted when there are no contraindications.

Cigarette Abstinence

Most of the risk of IHD in the general population is attributable to a few traditional risk factors.[178] Cigarette smoking has repeatedly been one of the strongest predictors of IHD.

In one case-controlled study from the general population, the odds of AMI for current smokers versus never-smokers was 2.87, and the population attributable risk of smoking (percent of cases explained by smoking) was 35.7%.[178]

Smoking also is associated with CVD in kidney transplant recipients.[7,44,80] In one retrospective analysis, the risk associated with cigarette smoking for IHD more than 1 year after transplantation was greater than that predicted by the Framingham Heart Study.[80] Smoking also is associated with all-cause mortality and increased risk of graft failure. In a study of first deceased donor transplants that were performed during 1984 through 1991 and functioned for at least 1 year, cigarette smoking was associated with all-cause mortality. The magnitude of the effect of smoking was similar to that of diabetes.[28]

Similarly, in a study of 1334 patients transplanted during 1963 through 1997, 24.7% smoked at the time of transplantation (similar to the smoking prevalence in general population).[83] Smoking was associated with a higher risk of graft failure secondary to all-cause mortality. Smoking also was associated with CVD and malignancies. This study did not find an association between smoking and death-censored graft failure.[83]

Other investigators have reported an adverse effect of smoking on death-censored allograft failure. In a retrospective study of 645 patients who underwent transplantation between 1985 and 1995, 24% smoked at the time of transplantation.[153] Of these, 90% continued to smoke after transplantation. Smoking was associated with a 2.3-fold increased risk for graft loss. Death-censored graft survival rates of deceased donor and living donor transplants were adversely affected by smoking. In contrast, graft survival was improved for patients who quit smoking before transplantation. Smoking may be a marker of noncompliance, but in this study the incidence of acute rejection was not different in smokers and nonsmokers (64% versus 61%; $P = .35$).[153]

It seems reasonable to conclude that abstinence from cigarette smoking should be strongly encouraged. Evidence-based guidelines on effective smoking cessation methods have been developed.[72,156,172] An effective smoking cessation effort should include several basic elements, as follows: (1) There should be readily accessible records on current smoking status. (2) At least once a year physicians should advise smokers to stop smoking and should document this effort in the medical record. There is evidence that repeated efforts at smoking cessation are warranted. (3) Pharmacotherapies for tobacco dependence are effective and should be used. These include sustained-release bupropion hydrochloride, nicotine gum, nicotine inhaler, nicotine nasal spray, and nicotine patches. There is no clear evidence that one therapy is better than any other, and multiple therapies may be effective. There is evidence, however, that treating patients with structured smoking cessation programs that provide social support and pharmacotherapy can increase the rates of smoking cessation by twofold.[72] (4) Help from trained health care professionals specializing in smoking cessation should be made available. Three types of counseling have been found to be effective: practical counseling, social support as part of treatment, and social support arranged outside of treatment.

There is strong evidence that cigarette smoking contributes to CVD after kidney transplantation. There also is evidence that smoking may increase all-cause mortality and may have a negative impact on graft survival, independent of its effects on mortality. Every effort should be made to encourage patients to quit smoking. Identifying patients who smoke and providing counseling and a structured smoking cessation program should be an integral part of routine post-transplant care.

Hypertension

Incidence

The incidence of hypertension defined as blood pressure 140/90 mm Hg or greater is 60% to 80%, and the incidence of hypertension defined as blood pressure 120/80 mm Hg or greater may be 80% to 90%, after kidney transplantation.[78,127] In an analysis of the Collaborative Transplant Study Registry, only 9.8% of 28,509 patients had systolic blood pressure less than 120 mm Hg 1 year after transplantation.[127] In the Collaborative Transplant Study, 55.5% of patients had systolic blood pressure 140 mm Hg or greater at 1 year.[127]

We conducted a retrospective, single-center study of 1660 consecutive patients transplanted during the period 1976 through 2002.[78] Blood pressure was recorded during routine clinic visits at weeks 1, 2, 4, 8, 12, 26, and 52, and annually thereafter in all patients. Systolic blood pressure was highest immediately after transplantation and declined during the first year (Fig. 28-4). We classified blood pressure in accordance with the Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure.[25] Among 1295 patients with a functioning graft and complete data at 1 year, only 12.4% had normal blood pressure (<120 mm Hg systolic or <80 mm Hg diastolic), 36.3% had prehypertension (120 to 139 mm Hg systolic or 80 to 90 mm Hg diastolic), 34.2% had stage 1 hypertension (140 to 159 mm Hg systolic or 90 to 99 mm Hg diastolic), and 17.1% had stage 2 hypertension (≥160 mm Hg systolic or ≥100 mm Hg diastolic), despite treatment with antihypertensive medications.[78] Of patients with normal blood pressure at 1 year, only 28.1% were not receiving antihypertensive medications, so overall, only 3.5% had truly normal blood pressure without antihypertensive medications at 1 year after transplantation.[78]

The control of blood pressure improved only slightly in 1993 through 2002 compared with 1976 through 1992, and this improvement was confined to the first year (see Fig. 28-4). The lack of improvement in blood pressure control was despite a substantial increase in the number of antihypertensive medications used (Fig. 28-5). Patients not taking any antihypertensive medications 1 year after transplantation declined from 26.7% in 1976 through 1992 to 5.2% in 1993 through 2002. The proportion of patients taking two or more antihypertensive medications increased from 43.5% in 1976 through 1992 to 54.6% in 1993 through 2002.[78]

Altogether, these results suggest that the incidence of hypertension is high after kidney transplantation. It is possible that the incidence is lower in patients treated without prednisone or without calcineurin inhibitors; however, to date, there are few epidemiological studies documenting this. In the meantime, more needs to be done to control blood pressure.

Pathogenesis

The pathogenesis of hypertension is likely multifactorial. We determined clinical correlates to systolic blood pressure at

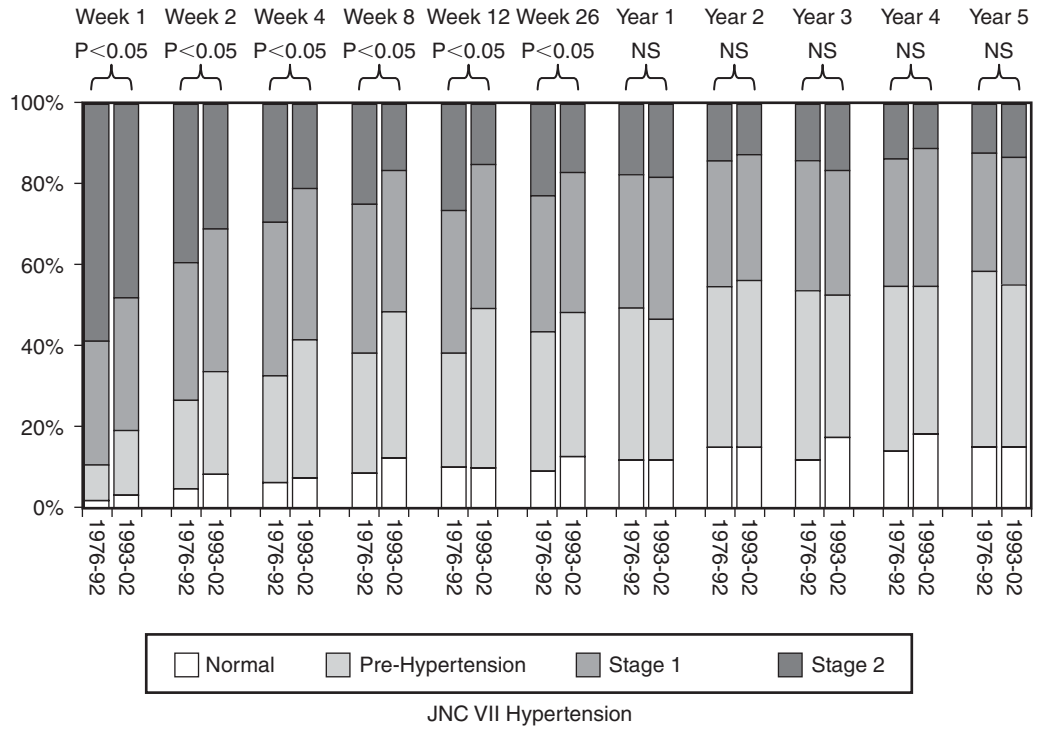

Figure 28–4 The percent of patients in each blood pressure category at different times after transplantation. *P* values (χ^2) compare eras at each time. JNC-7, Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure; NS, not significant. (From Kasiske BL, Anjum S, Shah R, et al: Hypertension after kidney transplantation. Am J Kidney Dis 43:1071-1081, 2004.)

weeks 1, 2, 4, 8, 12, 26, and 52 and annually thereafter using multiple linear regression analyses.[78] Male sex, recipient age, and body mass index were associated with higher blood pressure. Patients with primary ESRD owing to type 1 diabetes had higher blood pressure, and, to a lesser extent, so did patients with ESRD owing to type 2 diabetes. Patients with ESRD secondary to hypertensive nephrosclerosis had higher blood pressure early after transplantation. Donor age and delayed graft function also were associated with higher blood pressure, but only in the first few weeks after transplantation. Patients who had been undergoing treatment for ESRD for a longer time before transplantation had lower

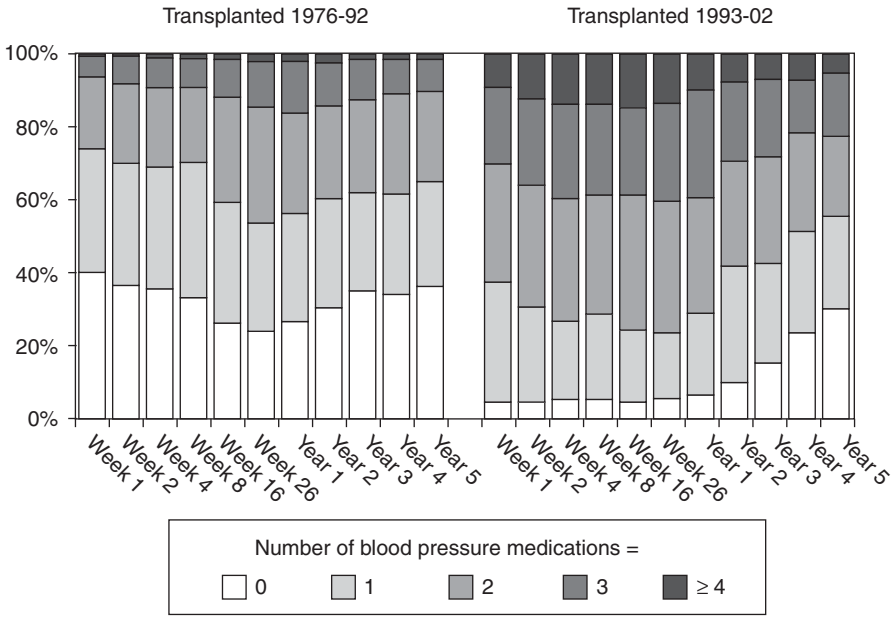

Figure 28–5 The number of antihypertensive medications per patient at different times after transplantation: 1976 through 1992 (*left panel*) and 1993 through 2002 (*right panel*). (From Kasiske BL, Anjum S, Shah R, et al: Hypertension after kidney transplantation. Am J Kidney Dis 43:1071-1081, 2004.)

blood pressure, possibly because patients who survived longer with ESRD did so because they had less vascular disease (reverse lead-time bias).[78]

In our study, pretransplant bilateral native kidney nephrectomy was associated with lower blood pressure at most times after transplantation.[78] Several other studies have found that hypertension is less common after transplantation among patients who have their native kidneys removed.[67,136] Other studies have failed to confirm this association, however.[36,90] Still others have reported that the removal of both native kidneys after transplantation improves blood pressure.[34,52] The morbidity of native kidney nephrectomy is arguably less in the era of laparoscopic surgery.

Having a previous acute rejection was associated with higher blood pressure at virtually all times after transplantation, and these associations were independent of estimated creatinine clearance.[78] Independent of acute rejection, patients who had a higher creatinine clearance also had lower blood pressure early after transplantation. In this center, cyclosporine was routinely discontinued 1 year after transplantation if patients were stable. Similarly, stable patients received alternate-day prednisone. Patients who were still receiving cyclosporine and patients who remained on higher doses of prednisone after the first year after transplantation had higher blood pressure.[78]

Transplant renal artery stenosis (TRAS), or obstruction of the iliac artery above the anastomosis, can cause hypertension and is often associated with graft dysfunction. The incidence of transplant renal artery stenosis varies depending on how often diagnostic tests are ordered to detect this condition. In most series, the incidence of transplant renal artery stenosis that prompted intervention was approximately 5%.[16,60,134] Reported predisposing factors include donor age,[16] recipient weight,[16] acute rejection,[12] cytomegalovirus infection,[12] and delayed graft function.[12] Angioplasty with placement of a stent is the most common treatment and results in improved blood pressure and graft function, at least in the short term.[16] Long-term outcomes may be worse in patients with transplant renal artery stenosis, however, despite treatment.[16] In summary, causes of hypertension include the use of corticosteroids, calcineurin inhibitors, allograft dysfunction, the presence of diseased native kidneys, and occasionally allograft renal artery stenosis.

Association with Cardiovascular Disease and Other Outcomes

Numerous clinical trials in the general population have shown that treating high blood pressure reduces the incidence of AMI, CHF, and strokes. This finding has led to widely accepted guidelines for the treatment of hypertension in the general population.[25] There has been controversy over what the best, first-line agents are for treating blood pressure,[22,23,169] but there has been no controversy over the need to treat hypertension.

Limited data suggest that the relationship between blood pressure and IHD is similar in kidney transplant recipients as it is in the Framingham Heart Study.[80] There are few reasons to believe that treating hypertension would not reduce the incidence of IHD, heart failure, and strokes in kidney transplant recipients. Because reducing blood pressure retards the progression of CKD in nontransplant patients,[23] it also is possible that treating hypertension would reduce the incidence of kidney allograft failure.

It has been reported that hypertension precedes, and could cause, acute rejection.[30,155] Analyzing blood pressure and allograft function (creatinine clearance) as time-dependent covariates in a Cox analysis of acute rejection, the association between blood pressure and acute rejection was entirely explained, however, by reduced graft function.[78] It is possible that undiagnosed rejection, or other factors associated with poor allograft function, could explain the apparent relationship between blood pressure and subsequent acute rejection, and that increased blood pressure per se does not cause acute rejection.

Several studies have reported an association between blood pressure and allograft failure. Even after controlling for allograft function, blood pressure is associated with decreased graft survival.[16,78,109,110,121,127] In one study, the association between hypertension and graft failure was seen in African Americans but not whites.[27] We found, however, that blood pressure also was associated with graft failure in whites (Fig. 28-6).[78] Although the number of African Americans in our study was small (n = 96), the relative risk of graft failure associated with systolic blood pressure was greater among African Americans (relative risk 1.32 [95% CI 1.11 to 1.54]) than whites (relative risk 1.12 [95% CI 1.09 to 1.16]).[78] Altogether, these studies suggest that hypertension may contribute to mortality and allograft failure. Nevertheless, without randomized controlled intervention trials, it is difficult to prove that hypertension causes graft failure.

Treatment

No antihypertensive agent is absolutely contraindicated after kidney transplantation. Some clinicians have been reluctant to use angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers because it has been reported that these drugs can cause acute renal allograft failure.[6,54,162,173] This allograft failure is presumably from vascular disease that reduces blood flow to the allograft and makes glomerular filtration rate more dependent on angiotensin II. In our study, among patients who underwent kidney transplantation between 1993 and 2002, 24% were treated with angiotensin-converting enzyme inhibitors at 1 week after transplantation and 30% were treated at 1 year.[78] Similarly, large numbers of patients were treated with β-blockers, diuretics, and agents from most other major classes of antihypertensive agents.

Although hypertension is another reason to minimize the use of corticosteroids and calcineurin inhibitors, it is rarely the sole reason for discontinuing these agents. When blood pressure is difficult to control with antihypertensive agents, particularly when lowering blood pressure reduces kidney function, consideration should be given to screening for compromised blood flow to the allograft. Occasionally, correcting iliac artery or graft renal artery stenosis improves graft function and reduces blood pressure. When all else fails, removal of the patient's native kidneys should be considered.[34,52]

The goal of treatment should be to reduce blood pressure to less than 120/80 mm Hg if possible, but certainly to less than 140/90 mm Hg. Although the incidence of adverse effects from antihypertensive agents may be different in kidney transplant recipients compared with the general population (Table 28-4), no antihypertensive agents are contraindicated. Most often, more than one agent is needed.

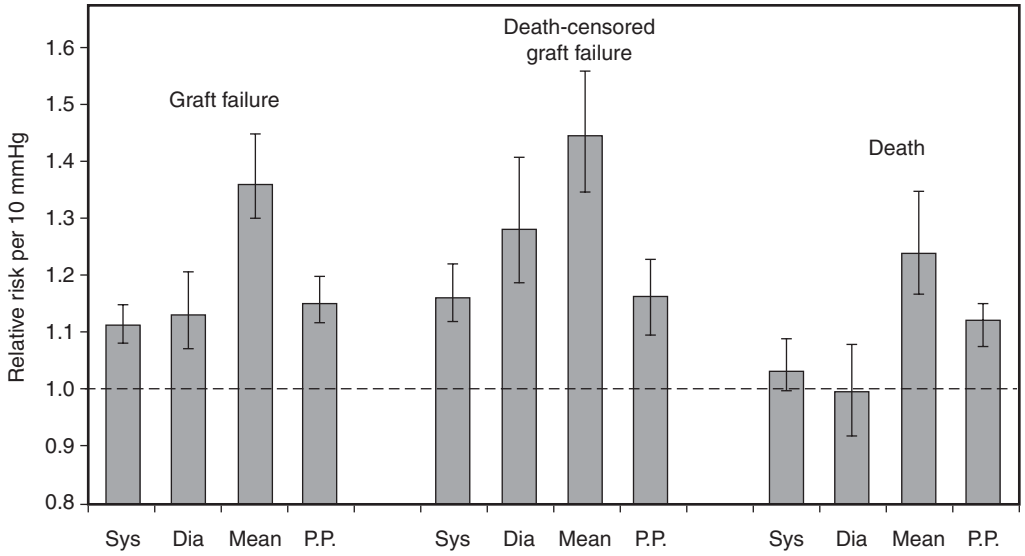

Figure 28–6 Relative risks (and 95% confidence intervals) for different time-dependent measurements of blood pressure for graft failure (*left*), death-censored graft failure (*middle*), and death (*right*). Failure of the 95% confidence interval to include 1.00 (*dashed line*) indicates $P < .05$. Each analysis was adjusted for multiple variables, including time-dependent covariates for acute rejection and estimated creatinine clearance. Dia, diastolic; P.P., pulse pressure; Sys, systolic. (From Kasiske BL, Anjum S, Shah R, et al: Hypertension after kidney transplantation. Am J Kidney Dis 43:1071-1081, 2004.)

Summary

There is compelling evidence from randomized trials in the general population that treating blood pressure prevents CVD; there is no reason to believe that this would not also be the case for kidney transplant recipients. Kidney transplant recipients may benefit further from hypertension treatment if treatment slows the progression of chronic allograft dysfunction. Although the risk of adverse effects may be higher than in the general population, no antihypertensive agents are contraindicated after kidney transplantation, and combination therapy is often required to achieve goals.

Dyslipidemias

Incidence

The incidence of hypercholesterolemia (≥200 mg/dL [≥5.18 mmol/L]) and increased LDL cholesterol (≥100 mg/dL [≥2.59 mmol/L]) is probably 60% to 80% after kidney transplantation, but this depends on the type of immunosuppressive agents that are used.[77] Generally, HDL is only modestly reduced, at least in patients treated with corticosteroids. Triglycerides are frequently elevated.

Pathogenesis

Many clinical factors have been associated with elevated lipid levels after kidney transplantation, including obesity, diabetes, reduced kidney function, and proteinuria (particularly if it is nephritic range). The type of immunosuppressive medication used is undoubtedly the major cause, however, of the high incidence of dyslipidemias after kidney transplantation. Corticosteroids, cyclosporine, sirolimus, and, to a lesser extent, tacrolimus all can cause dyslipidemias. In contrast, azathioprine and mycophenolate mofetil do not seem to affect the lipid profile adversely.

Association with Cardiovascular Disease and Other Outcomes

Reducing LDL has been convincingly shown to lower the risk of IHD and strokes in the nontransplant, general population.[14,149] The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, or statins, are most effective in lowering LDL and in safely reducing IHD events and all-cause mortality.[14,149] The role of fibrates, which more effectively reduce triglycerides and increase HDL, is less certain.[14,89,149]

Limited data tend to confirm that increased cholesterol,[2,70,80] increased LDL,[7] low HDL,[2,44,81] and high triglycerides[80] are risk factors for IHD in kidney transplant recipients, similar to in the general population. In the Assessment of Lescol in Renal Transplantation (ALERT) trial, Holdaas and coworkers[65] randomly allocated 2102 stable kidney transplant recipients to either placebo or up to 80 mg of fluvastatin. A 17% reduction in the primary end point (major adverse cardiac events) in the fluvastatin group was not significantly different from placebo ($P = .139$). There was a 38% reduction in mortality ($P = .031$), however, and a 35% reduction in cardiac death or nonfatal AMI ($P = .005$) in the fluvastatin group compared with controls.[65] The ALERT trial, although "negative," nevertheless provided suggestive evidence that lipid lowering with a statin might be beneficial in kidney transplant recipients as it is in the general population. Altogether, observational data associating dyslipidemias with IHD and the results of the ALERT trial provide at least some evidence that dyslipidemias may be contributing to IHD after kidney transplantation.

Because statins have anti-inflammatory properties, it was natural to speculate that statins may reduce the incidence of acute kidney allograft rejection. A pilot study in kidney transplant recipients suggested that pravastatin may reduce the incidence of acute rejection.[87] A larger study found no effects of a statin on acute rejection after kidney transplantation, however.[82] These negative results were confirmed by

Table 28–4 Advantages and Disadvantages of Antihypertensive Agents in Transplant Recipients

Agent Class	Possible Advantages	Possible Disadvantages
Thiazide diuretics[a]	↓ Edema (mild effect) Low cost Long acting ↓ Osteopenia	↓ Potassium ↑ Serum creatinine ↑ Blood glucose ↑ Cholesterol
Loop diuretics[b]	↓ Edema ↓ HF symptoms	Short acting ↓ Potassium ↑ Serum creatinine
Potassium-sparing diuretics[c]	↑ Potassium	↑ Potassium
Aldosterone receptor blockers[d]	↑ Potassium May improve outcomes in HF	↑ Potassium
β-blockers[e]	May improve outcomes in HF	↓ HDL and triglycerides ↓ Hypoglycemia awareness
Combined α/β blockers[f]	More effective than either alone	
ACE inhibitors[g]	↓ Hemoglobin (↓ polycythemia) May improve outcomes in HF ↓ Proteinuria May preserve kidney function	↓ Hemoglobin (↑ anemia)
Angiotensin II antagonists[h]	↓ Hemoglobin (↓ polycythemia) May improve outcomes in HF ↓ Proteinuria May preserve kidney function	↓ Hemoglobin (↑ anemia)
Calcium channel blockers (NDP)[i]	↑ Cyclosporine levels (↓ cost) ↓ Cyclosporine-induced vasoconstriction	↑ Cyclosporine levels (↑ toxicity) ↓ Heart rate
Calcium channel blockers (DP)[j]	↓ Cyclosporine-induced vasoconstriction	↑ Gingival hypertrophy
α_1 Blockers[k]	↓ Prostatic hypertrophy	
Centrally acting agents[l]	Well-tolerated	
Direct vasodilators[m]	Very effective	↑ Edema ↑ Heart rate

[a]Chlorothiazide, chlorthalidone, hydrochlorothiazide, polythiazide, indapamide, metolazone.
[b]Bumetanide, furosemide, torsemide.
[c]Amiloride, triamterene.
[d]Eplerenone, spironolactone.
[e]Acebutolol, atenolol, betaxolol, bisoprolol, metoprolol, nadolol, pindolol, propranolol.
[f]Carvedilol, labetalol.
[g]Benazepril, captopril, enalapril, fosinopril, moexipril, perindopril, quinapril, ramipril, trandolapril.
[h]Candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, valsartan.
[i]Diltiazem, verapamil.
[j]Amlodipine, felodipine, isradipine, nicardipine, nifedipine, nisoldipine.
[k]Doxazosin, prazosin, terazosin.
[l]Clonidine, methyldopa, reserpine, guanfacine.
[m]Hydralazine, minoxidil.
ACE, angiotensin-converting enzyme; DP, dihydropyridine; HDL, high-density lipoprotein; HF, heart failure; NDP, nondihydropyridine.

two other randomized controlled trials.[66,145] Based on the results of these trials, it seems that statins do not reduce the incidence of acute rejection in kidney transplant recipients. It remains to be seen whether statins may reduce the incidence of chronic allograft nephropathy.

Treatment

Guidelines for the management of dyslipidemia in kidney transplantation have been developed by the National Kidney Foundation.[76] These guidelines closely follow the guidelines developed for the general population by the Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program.[48] A few simple rules can be followed for effective dyslipidemia management after kidney transplantation (Table 28-5).

Elevated triglycerides generally are treated only to prevent the rare occurrence of pancreatitis. Triglycerides that are persistently elevated (≥500 mg/dL [≥5.65 mmol/L]) can cause pancreatitis. The incidence of pancreatitis resulting from hypertriglyceridemia in transplant patients is unknown, but it is probably very low. Nevertheless, the ATP III and National Kidney Foundation guidelines recommend that elevated triglycerides be treated with diet, weight reduction, increased physical activity, abstinence from alcohol, and treatment of hyperglycemia (if present). For patients with elevated fasting triglycerides (≥1000 mg/dL [≥11.29 mmol/L]), the ATP III diet recommendations include a very-low-fat diet (<15% total calories) and medium-chain triglycerides and fish oils to replace some long-chain triglycerides. If these therapeutic lifestyle changes are insufficient to reduce triglycerides to less than 500 mg/dL (<5.65 mmol/L), treatment with a fibrate or nicotinic acid should be considered. Studies from the general population suggest that fibrates and nicotinic acid reduce triglycerides by 20% to 50%. Statins cause less triglyceride lowering, and bile acid sequestrants may increase triglyceride levels. If severe

Table 28–5 **Some "Do's" and "Don't's" of Dyslipidemia Management in Kidney Transplant Recipients**

Do
Treat all patients with LDL >100 mg/dL
Treat non-HDL cholesterol >130 mg/dL if triglycerides are >200 mg/dL
Use diet and a statin as initial therapy
Use additional measures if LDL >130 mg/dL and the patient is at high risk
Don't
Stop the statin if the goal is not achieved
Use a stain and a fibrate
Use a high-dose statin and cyclosporine
Use a statin and cyclosporine and an azole antifungal agent
Use a statin and cyclosporine and a macrolide antibiotic
Use a statin and cyclosporine and a high-dose nondihydropyridine calcium antagonist

Note: To convert LDL cholesterol and HDL cholesterol in mg/dL to mmol/L, multiply by 0.0259. To convert triglycerides in mg/dL to mmol/L, multiply by 0.0113.

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

hypertriglyceridemia is associated with the use of sirolimus, consideration can be given to discontinuing sirolimus, or changing sirolimus to another immunosuppressive agent.

If triglycerides are less than 500 mg/dL (<5.65 mmol/L), but LDL is elevated (≥100 mg/dL [≥2.59 mmol/L]), patients should be treated with dietary modification and, if necessary, a statin. If LDL is less than 100 mg/dL (< 2.59 mmol/L), but triglycerides are greater than 200 mg/dL (>2.26 mmol/L), and non-HDL cholesterol is greater than 130 mg/dL (>3.37 mmol/L), patients also should be treated. Studies in the general population suggest that a lipid-lowering diet can reduce LDL.[48,93,129,177] For transplant patients with LDL 100 to 129 mg/dL (2.59 to 3.34 mmol/L), it is reasonable to attempt diet for 2 to 3 months before starting a statin. The diet should include less than 7% of calories as saturated fat, 10% of calories as polyunsaturated fat, 20% of calories as monounsaturated fat, and total fat of 25% to 35% of total calories. The diet also should contain complex carbohydrates (50% to 60% of total calories) and fiber (20 to 30 g/day), and cholesterol should be less than 200 mg/day. The reduction in LDL that can be achieved with therapeutic diet and lifestyle changes is usually modest. In patients who cannot be expected to reduce LDL to less than 100 mg/dL (< 2.59 mmol/L) by diet, a statin should be started along with diet, if there is no evidence of liver disease.

The dose of statins generally should be reduced in patients treated with cyclosporine because blood levels of most statins are increased by cyclosporine. The addition of other agents that increase cyclosporine and statin blood levels (e.g., azole antifungal agents, macrolide antibiotics, and nondihydropyridine calcium antagonists) should prompt a temporary dosage reduction or discontinuation of the statin. If diet and a statin are insufficient to achieve a target LDL less than 100 mg/dL (<2.59 mmol/L), adding a second agent can be considered. Fibrates generally should not be used in combination with a statin, owing to the risk of myopathy. A bile acid sequestrant can be used in low doses, if taken between doses of cyclosporine or tacrolimus. Perhaps the best choice of a second agent is the new cholesterol uptake inhibitor ezetimibe. Preliminary data suggest that ezetimibe can be used safely in combination with a statin after kidney transplantation.[19,92,94] In some patients at very high risk of IHD, it may be appropriate to consider withdrawing or changing prednisone or cyclosporine, or both, to an immunosuppressive agent that does not increase LDL.

Nephrotic-range proteinuria can increase total and LDL cholesterol and triglycerides.[38,73,74,88,148,171] In some patients, it may be possible to reduce the level of proteinuria and improve the lipid profile with angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists. Few randomized controlled trials have documented the antiproteinuric and lipid-lowering effects of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists in kidney transplant recipients, however. Whether measures to reduce urine protein excretion in kidney transplant recipients are effective, and whether they also reduce plasma lipids, is unclear.

Summary

There is strong evidence from studies in the general population that treating dyslipidemia, particularly with statins, safely reduces the risk for IHD. There are few compelling reasons to believe that this would not also be the case for kidney transplant recipients. Although a well-designed, randomized, controlled trial failed to show a significant reduction in the primary end point with a statin, there was nevertheless a reduction in IHD events and mortality. It seems warranted to use a statin in kidney transplant patients with elevated LDL cholesterol.

Diabetes

Incidence

As the incidence of diabetes increases worldwide, the incidence of ESRD caused by diabetes also is growing. The number of patients with ESRD caused by diabetes who receive a kidney transplantation also is growing. In addition, patients who do not have diabetes at the time of transplantation often develop new-onset diabetes after transplantation (NODAT). The reported incidence of NODAT varies because of differences in the definition of diabetes that have been used, the patient populations that have been studied, the immunosuppressive medication regimen used, and the duration of follow-up.

In clinical trials, NODAT is often diagnosed when insulin therapy is required for at least 1 month. A meta-analysis of observational studies and clinical trials reported that the incidence of NODAT (variously defined) in the first year after transplantation varied from 2% to 50%.[122] In a similar meta-analysis, the incidence of NODAT was approximately 15.4% for patients receiving tacrolimus and about 9.8% for patients receiving cyclosporine.[62] In a study of USRDS patients who had Medicare as their primary beneficiary, NODAT was detected using Medicare claims. Using data from the USRDS, 11,659 Medicare beneficiaries who received a first kidney transplant between 1996 and 2000 were identified.[86] The cumulative incidence of NODAT was 9.1% at 3 months after transplantation, 16% at 12 months after transplantation, and 24% at 36 months after transplantation.[86]

The best evidence-based definition of diabetes is probably that of the American Diabetes Association (ADA) and World

Health Organization (WHO).[9] According to this definition, a patient has diabetes if the following criteria are present:

1. Symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dL (≥11.1 mmol/L). Casual is defined as any time of the day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

or

2. Fasting blood glucose ≥126 mg/dL (≥7 mmol/L). Fasting is defined as no caloric intake for at least 8 hours.

or

3. 2-hour postload glucose ≥200 mg/dL (≥11.1 mmol/L) during an oral glucose tolerance test. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

In the absence of unequivocal hyperglycemia, one of these criteria should be confirmed by repeat testing on a different day.[9]

In addition, because the risk for CVD is present even at blood glucose levels less than those used in the definition of diabetes, the ADA/WHO define impaired fasting glucose to be greater than or equal to 100 mg/dL (≥5.6 mmol/L) but less than 126 mg/dL (<7 mmol/L). Similarly, impaired glucose tolerance is a 2-hour value on an oral glucose tolerance test of greater than or equal to 140 mg/dL (≥7.8 mmol/L) but less than 200 mg/dL (<11.1 mmol/L).[9] Few studies have examined the incidence of NODAT using these ADA/WHO definitions.

When 114 clinically stable Korean transplant recipients underwent oral glucose tolerance tests 9 to 12 months after transplantation, 78 (68%) had glucose intolerance, with 51 (45%) having impaired glucose tolerance and 27 (24%) having NODAT.[124] Similarly, 156 transplant recipients from the Indian subcontinent were given oral glucose tolerance tests 2 weeks, 6 weeks, 3 months, and 6 months after transplantation. Of these 156 patients, 80 (51%) were glucose intolerant, with 42 (27%) having impaired glucose tolerance and 38 (24%) having NODAT.[114] Finally, 173 white transplant recipients underwent oral glucose tolerance tests 10 weeks after transplantation, and 90 (52%) were found to have impaired glucose tolerance[64]; 5 (3%) had impaired fasting glucose, 50 (29%) had impaired glucose tolerance, and 35 (20%) had NODAT.[64] The results of these studies are remarkably consistent, suggesting that during the first post-transplant year, 20% to 24% of transplant recipients develop NODAT, and another 24% to 29% have impaired glucose tolerance. These studies suggest that the incidence of NODAT is much higher than reported in clinical trials and observational studies.

Pathogenesis

The pathogenesis of NODAT is poorly defined. It is likely a combination of increased insulin resistance and decreased secretion. Numerous clinical correlates to NODAT have been identified in observational studies.*

Risk factors for NODAT that are potentially modifiable include corticosteroids,[10,63,71,108,114,137,164] cyclosporine,[18,114,144,150] tacrolimus,[8,10,17,57,86,108,150] obesity,† donor source (deceased versus living),[18,86,151] acute rejection,[32,61,63,138,144,151,164,166] and hepatitis C infection.[17,57,86,176] Risk factors that are not modifiable include family history,[61,63,151,176] pretransplant hyperglycemia,[29,57] age,‡ African-American ethnicity,[8,18,32,53,86,118,137,150,151] and Hispanic ethnicity.[86,118,151]

Association with Cardiovascular Disease and Other Outcomes

Diabetes is an important risk factor for IHD in the general population. Diabetes at the time of transplantation, particularly if it has caused ESRD, is also a major risk factor for post-transplant CVD (see Tables 28-2 and 28-3). In addition, some observational studies have linked NODAT to the subsequent development of IHD.[29,45] NODAT also has been associated with infection,[18,118,151] acute rejection,[8,63,108,164] graft failure,[86,118,144] death-censored graft failure,[86] and all-cause mortality.[31,53,86,139] None of these associations with NODAT prove cause and effect. It is likely that one or more risk factors poorly accounted for in multivariate models, but associated with NODAT, also could increase the risk for poor outcomes and explain the association with NODAT. It is equally likely, however, that NODAT would cause or contribute to post-transplant CVD and other poor outcomes if exposure to this risk were of sufficient duration.

Treatment and Prevention

Observational studies have suggested that the better the blood glucose control in diabetes, the lower the risk of CVD.[37,95,132] It is possible in these observational studies, however, that patients with easier to control diabetes are at lower risk for CVD, and that controlling blood glucose with exogenous insulin or oral hypoglycemic agents would have little effect on the incidence of CVD. In the end, only randomized trials can determine whether diabetes treatment strategies can reduce the incidence of CVD. Although some randomized controlled trials have shown that intensive blood glucose control reduces microvascular disease complications,[41,159] it has been less certain whether intensive blood glucose control also reduces the risk for macrovascular disease complications such as IHD.[42,159] An extended follow-up of the original study patients from the Diabetes Control and Complications Trial showed a reduction in CVD events among patients who had been treated with intensive blood glucose control in the original study.[125] The balance of evidence from the general population suggests that intensive blood glucose control reduces macrovascular disease events. Intensive blood glucose control comes at a price, however, of increased hypoglycemia, and achieving adequate blood glucose control may not always be possible.

Whether the results of intervention trials in the general population can be extrapolated to kidney transplant recipients is unknown. Patients with ESRD caused by diabetes typically have very brittle, difficult-to-control diabetes, with autonomic neuropathy and frequent, severe hypoglycemic reactions. Whether the risk-to-benefit ratio of intensive glucose control is the same in kidney transplant recipients as in patients who

*References 8, 10, 17, 18, 29, 32, 53, 57, 61, 63, 71, 86, 108, 114, 118, 137, 138, 144, 150, 151, 164, 176.

†References 10, 17, 18, 29, 32, 71, 86, 108, 114, 137, 150.

‡References 10, 18, 29, 32, 57, 61, 63, 86, 114, 118, 137, 138, 144, 151, 164, 176.

were carefully selected for randomized trials in the general population is questionable. Pancreas transplantation may be an answer for some patients with type 1 diabetes and ESRD. Whether the additional risk of pancreas transplantation outweighs the benefits of better blood glucose control is unclear, however. Islet transplantation holds great promise, but it is still experimental, and long-term islet function is unusual.

Treatment of diabetes after kidney transplantation is similar to treatment of diabetes in the general population. Oral hypoglycemic agents are effective. The insulin-sensitizing thiazolidinediones can be used after transplantation but may be associated with edema and even CHF. Metformin is an effective agent for improving blood glucose control in the general population and has been shown in clinical trials to reduce the incidence of complications from diabetes. Metformin can cause severe lactic acidosis, however, in patients with reduced kidney function. Because kidney transplant recipients are prone to develop acute kidney dysfunction, most consider metformin to be contraindicated in kidney transplant recipients. In the end, clinicians and patients often are left with managing diabetes with various strategies of administering short-acting and long-acting exogenous insulin.

It is better to prevent than to treat diabetes. Preventing NODAT can start with lifestyle modification, including diet, weight reduction, and exercise. Lifestyle modification has been shown to reduce the risk of type 2 diabetes in nontransplant patients with elevated fasting or postload plasma glucose.[91] Few data on the effectiveness of lifestyle modification in kidney transplant recipients are available. At present, the best strategy for reducing the risk of NODAT is probably to minimize the use of calcineurin inhibitors (cyclosporine and especially tacrolimus) and corticosteroids in individuals who are at increased risk of developing NODAT (Table 28-6). These goals must be balanced, however, against the risk of acute rejection and graft failure. It is hoped that future immunosuppressive agents will effectively prevent acute rejection without increasing the risk of NODAT.

Summary

There is good evidence from studies in the general population that diabetes causes IHD. There also is growing evidence in nontransplant patients that intensive blood glucose control in diabetic patients prevents IHD. Diabetes also is a risk factor for IHD after kidney transplantation, and diabetes is likely important in the pathogenesis of IHD in this population. Control of blood glucose in kidney transplant recipients is more difficult, however, given the propensity to severe hypoglycemia in patients with long-standing diabetes and kidney disease. Likewise, the prevention of NODAT by avoiding immunosuppressive agents that seem to contribute to the risk of new-onset diabetes after transplantation must be weighed against the risk of acute rejection and graft failure.

Acute Rejection and Allograft Function

Acute rejection episodes and their treatment have been shown to be an independent risk factor for IHD after kidney transplantation.[70,80] Proteinuria also has been reported to be associated with the risk of IHD.[80] Similarly, reduced kidney function, assessed by serum creatinine, has been found to be an independent risk factor for major adverse cardiac events.[49]

Many of the risk factors for CVD after kidney transplantation are exacerbated by immunosuppressive medications (see Table 28-6). The use of adequate immunosuppressive medication is crucial to preventing acute rejection and maintaining good allograft function. The current challenge to reducing the risk for CVD after kidney transplantation is to select the immunosuppressive medication regimen that minimizes CVD risk factors, while minimizing the risk for rejection and maximizing long-term allograft function (see Table 28-6). Currently, there is no ideal regimen to accomplish these often conflicting goals, so the relative risks for rejection and CVD must be weighed in each individual patient. In addition, efforts to use adequate immunosuppressive medication in the early post-transplant period (when the risk of rejection is high) can be followed by a strategy to reduce or withdraw agents that may no longer be needed in the late post-transplant period (when the risk for rejection declines, but the risk for CVD continues to increase).

Lifestyle Modifications That May Favorably Affect Multiple Risk Factors

Studies from the general population suggest that exercise and treatment of obesity have beneficial effects on dyslipidemias, blood pressure, and glucose intolerance. There are few randomized controlled trials, however, showing that these lifestyle modifications lead to a reduction in CVD events. There are even fewer studies of the effects of lifestyle modifications in kidney transplant recipients. Painter and coworkers[128] randomly allocated kidney transplant recipients

Table 28–6 **Effects of Immunosuppressive Agents on Cardiovascular Disease Risk**

Drug	Dyslipidemia	Diabetes	Hypertension	Renal Dysfunction
Corticosteroids	↑↑	↑↑	↑↑	–
Cyclosporine	↑↑	↑	↑↑	↑↑
Tacrolimus	↑	↑↑	↑	↑↑
Sirolimus	↑↑	–	–	↑(?)
Mycophenolate mofetil	–	–	–	–
Azathioprine	–	–	–	–

↑↑ = Can increase the incidence or severity or both of the risk factor markedly.
↑ = Can increase the incidence or severity or both of the risk factor somewhat.
– = Has no known effect on the incidence or severity of the risk factor.

to exercise ($n = 51$) versus usual care ($n = 45$). At 12 months after transplantation, there were no differences between the two groups in total cholesterol or coronary heart disease risk estimated by the Framingham risk prediction equation. There was a trend toward higher HDL in the exercise group ($P = .07$), and there was an inverse relationship between maximal exercise capacity and coronary heart disease risk.[128] Additional studies are needed. Meanwhile, it seems prudent to recommend diet and exercise to kidney transplant recipients to reduce CVD risk.

The results of observational studies in the general population also suggest that moderate beer or wine consumption reduces the risk of CVD.[40] There have been no large, randomized trials of interventions with moderate alcohol consumption in the general population, however. It is unknown whether moderate beer or wine consumption reduces CVD risk or is instead a marker of other characteristics that reduce CVD risk. Similarly, there are no studies in kidney transplant recipients examining whether recommending moderate alcohol consumption reduces the risk of CVD. The risk of adverse effects from moderate alcohol consumption could be higher in transplant recipients compared with the general population. Recommending moderate alcohol consumption is probably not a strategy to be adopted in kidney transplant recipients without further study.

Homocysteine

Epidemiological data suggest that homocysteine may contribute to CVD in the general population. Genetic epidemiological studies of "mendelian randomization" have substantiated further that homocysteine may be a risk factor for CVD.[120] Randomized controlled trials have failed to show a benefit of folic acid regimens, which reduce homocysteine levels, on CVD outcomes. The Folic Acid for Vascular Outcome Reduction In Transplantation (FAVORIT) study is an ongoing trial in kidney transplant recipients to determine whether folic acid would reduce homocysteine and CVD events. Recommendations to use folic acid or other strategies to reduce CVD events in kidney transplant recipients will depend on the results of this important trial.

Antioxidant Vitamins

Many data have implicated oxidative injury in the pathogenesis of systemic atherosclerosis. It was natural to assume that antioxidant vitamins would be protective. Numerous studies in the general population have failed to show, however, that antioxidant vitamins reduce CVD events. There is a suggestion that vitamin E may increase all-cause mortality.[119] Currently, the use of vitamin E and other antioxidant vitamins is not indicated in kidney transplant recipients. The vitamin E story shows the need for large, randomized controlled trials to establish the role of even seemingly innocuous therapies for CVD.

FUTURE DIRECTIONS

More recent observational studies in the general population have focused on nontraditional risk factors for IHD. A 52-country study of 15,152 cases and 14,820 controls examined the population attributable risks of "traditional" risk factors for AMI, including cigarette smoking, dyslipidemias, hypertension, diabetes, abdominal obesity, psychological factors, consumption of fruits and vegetables, regular alcohol consumption, and regular physical exercise. Collectively, these nine risk factors accounted for 90% and 94% of the population attributable risk in men and women.[178] Managing these "traditional" risk factors could have a substantial impact on IHD. There is evidence that more attention is being paid to managing traditional risk factors after kidney transplantation (see Fig. 28-4).

Additional studies are needed to confirm the importance of traditional risk factors in kidney transplant recipients. It is possible that the pathogenesis of IHD in kidney transplant recipients differs in important ways from that in the general population. Ideally, randomized controlled trials targeting traditional risk factors should be done in kidney transplant recipients. It is unlikely, however, that it would be possible to perform randomized trials with most risk factors, and it may be necessary to extrapolate the results of randomized trials in the general population to kidney transplant recipients. If it can be confirmed that traditional risk factors are associated with IHD in kidney transplant recipients, greater effort could and should be directed to reducing these known risk factors.

Studies suggest that there is still a high prevalence of dyslipidemias, hypertension, and cigarette smoking in kidney transplant recipients. Obesity and glucose intolerance are increasing. Finding effective ways to manage these risk factors would likely have an immediate effect on the incidence of IHD. It is especially important to find new immunosuppressive medication regimens that minimize risk, not only rejection but also risk factors for IHD.

Finally, studies are needed to define better the role for screening for IHD before and after transplantation. The high prevalence of IHD may make the predictive value of screening tests more favorable in transplant candidates and recipients than in the general population. Whether revascularization would reduce the morbidity and mortality of IHD remains an important, unanswered question.

SUMMARY

Preventing CVD after kidney transplantation requires a comprehensive clinical strategy. Early referral and pretransplant screening for CVD may help prevent post-transplant CVD events. Perioperative β blockers also may be effective. Management of traditional risk factors before and after transplantation includes aspirin prophylaxis, cigarette abstinence, treatment of hypertension, treatment of dyslipidemias, and intensive blood glucose control. Although the risk for CVD can be reduced by minimizing the use of immunosuppressive agents that adversely affect cardiovascular risk factors, the management of risk factors also must include a strategy of optimal immunosuppression to prevent acute rejection and maximize long-term kidney function. Numerous lifestyle modifications that may favorably affect cardiovascular disease risk factors should be encouraged. A multidisciplinary approach that emphasizes evidence-based management of traditional risk factors is currently the best approach to reducing the risk for CVD after kidney transplantation.

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Chapter 29

Infection in Renal Transplant Recipients

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Successful management of infections in renal transplant recipients is complicated by factors related to immune function in the host and the epidemiology of infection in the immunocompromised host.[18] Transplant recipients are susceptible to a broad spectrum of infectious pathogens, manifest diminished signs and symptoms of invasive infection, and may develop systemic signs (e.g., fever) in response to noninfectious processes (e.g., graft rejection, drug toxicity) with multiple processes often present. Immunocompromised patients tolerate invasive, established infection poorly with high morbidity and mortality, lending urgency to the need for an early, specific diagnosis to guide antimicrobial therapy. Given the T lymphocyte dysfunction inherent to transplantation immunosuppression, viral infections in particular are increased. These viral infections not only contribute to graft dysfunction, graft rejection, and systemic illness but also enhance the risk for other opportunistic infections (e.g., *Pneumocystis* and *Aspergillus*) and virally mediated cancers.

RISK OF INFECTION

The risk of infection in a renal transplant recipient is determined by the interaction of two key factors:

1. The epidemiological exposures of the patient, including the timing, intensity, and virulence of the organisms
2. The patient's "net state of immunosuppression," which reflects a measure of all host factors contributing to the risk for infection

An understanding of these factors for each patient allows the development of differential diagnoses for infectious syndromes for transplant recipients and preventive strategies (prophylaxis, vaccination) appropriate to each individual's risk for infection.

Epidemiological Exposures

Exposures of importance can be divided into four overlapping categories—donor-derived infections, recipient-derived infections, community-derived exposures, and nosocomial exposures (Table 29-1).

Donor-Derived Infections

Infections derived from donor tissues and activated in the recipient are among the least appreciated and most important exposures in transplantation. Some of these infections are latent, whereas others are the result of the occurrence of active infection in the donor at the time of procurement. All known types of infections have been recognized in transplant recipients. Three types of infection merit special attention. First, bacteremic or fungemic infections (staphylococci, *Streptococcus pneumoniae, Candida*, *Salmonella, Escherichia coli*) in donors at the time of donation can selectively adhere to anastomotic sites (vascular, urinary) and may produce leaks or mycotic aneurysms. Second, some viral infections, including cytomegalovirus (CMV) and Epstein-Barr virus (EBV), are associated with particular syndromes and morbidity in the immunocompromised population (see section on selected infections of importance). The greatest risk of these infections is to seronegative (immunologically naive) recipients who receive infected grafts from seropositive donors (latent viral infection). Third, late, latent infections, such as tuberculosis, may activate many years after the initial exposure. Such infections may be difficult to treat when established because of interactions between the antimicrobial agents used to treat them (e.g., rifampin, streptomycin, isoniazid for mycobacteria) and the agents used in immunosuppressive therapy.

Donor screening for transplantation is limited by the available technology and by the time available within which organs from deceased donors must be used. At present, routine evaluation of donors relies on antibody detection (serological) tests for common infections. As a result, some active infections remain undetected because seroconversion may not occur during acute infection. These limitations suggest that to achieve the benefits of transplantation, some organs are implanted carrying unidentified pathogens. This risk is exhibited by clusters of donor-derived *Trypanosoma cruzi* (Chagas' disease), rabies virus, West Nile virus, and lymphocytic choriomeningitis virus infections in organ transplant recipients.

Table 29–1 Significant Epidemiological Exposures Relevant to Transplantation

Donor-Derived
Viral
Herpes group (CMV, EBV, HHV-6, HHV-7, HHV-8, HSV)
Hepatitis viruses (notably B and C)
Retroviruses (HIV, HTLV-I, HTLV-II)
Others
Bacteria
Gram-positive and gram-negative bacteria (*Staphylococcus, Pseudomonas*, Enterobacteriaceae)
Mycobacteria (tuberculous and nontuberculous)
Nocardia asteroides
Fungi
Candida
Aspergillus
Endemic fungi (*Cryptococcus neoformans*)
Geographic fungi (*Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis*)
Parasites
Toxoplasma gondii
Trypanosoma cruzi
Nosocomial Exposures
Methicillin-resistant *Staphylococcus aureus*
Vancomycin-resistant enterococci (also linezolid-resistant and quinupristin/dalfopristin-resistant enterococci)
Aspergillus
Non-*albicans Candida* strains
Community Exposures
Foodborne and water-borne (*Listeria monocytogenes, Salmonella, Cryptosporidium*, hepatitis A, *Campylobacter*)
Respiratory viruses (RSV, influenza, parainfluenza, adenovirus, metapneumovirus)
Common viruses—often with exposure to children (coxsackievirus, parvovirus, polyomavirus, papillomavirus)
Atypical respiratory pathogens (*Legionella, Mycoplasma, Chlamydia*)
Geographic fungi and *Cryptococcus, Pneumocystis carinii (jiroveci)*
Parasites (often distant)
Strongyloides stercoralis
Leishmania
Toxoplasma gondii
Trypanosoma cruzi
Naegleria fowleri

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HTLV, human T cell lymphotropic virus; RSV, respiratory syncytial virus.

Table 29–2 Common Infectious Exclusion Criteria for Organ Donors*

Central Nervous System Infection
Unknown infection of central nervous system (encephalitis, meningitis)
Herpes simplex encephalitis or other encephalitis
History of JC virus infection
West Nile virus infection
Cryptococcal infection of any site
Rabies
Creutzfeldt-Jakob disease
Other fungal or viral encephalitis
Untreated bacterial meningitis (requires proof of cure)
Disseminated Infection
HIV (serological or molecular)
HSV (with active viremia), acute EBV (mononucleosis)
Serological or molecular evidence of HTLV-I/HTLV-II
Active hepatitis A or hepatitis B
Parasitic infections (*Trypanosoma cruzi, Leishmania donovani, Strongyloides stercoralis, Toxoplasma gondii*)
Infections Difficult to Treat on Immunosuppression
Active tuberculosis
SARS
Untreated pneumonia
Untreated bacterial or fungal sepsis (e.g., candidemia)
Untreated syphilis
Multisystem organ failure due to overwhelming sepsis, gangrenous bowel

*These must be considered in the context of the individual donor/recipient.

EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HTLV, human T cell lymphotropic virus; SARS, severe acute respiratory syndrome.

Given the risk of transmission of infection from the organ donor to the recipient, certain infections should be considered relative contraindications to organ donation. Because renal transplantation is typically elective surgery, it is reasonable to avoid donation from individuals with unexplained fever, rash, or infectious syndromes. Common criteria for exclusion of organ donors are listed in Table 29-2.

Recipient-Derived Exposures

Infections in the category of recipient-derived exposures reflect colonization or latent infections that reactivate in the setting of immunosuppression. It is necessary to obtain a careful history of travel and exposures to guide preventive strategies and empirical therapies. Notable among these infections are mycobacterial infection (including tuberculosis), strongyloidiasis, viral infections (herpes simplex virus [HSV] and varicella-zoster virus [VZV] or shingles), histoplasmosis, coccidioidomycosis, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Vaccination status should be evaluated (tetanus, HBV, childhood vaccines, influenza, pneumococcus); if vaccines have not previously been given, they should be considered (Table 29-3). Dietary habits also should be considered, including the use of well water (*Cryptosporidium*), uncooked meats (*Salmonella, Listeria*), and unpasteurized dairy products (*Listeria*).

Table 29–3 Vaccinations to Consider before Transplantation

Measles/mumps/rubella (MMR)
Diphtheria/tetanus/pertussis (DTP)
Poliovirus
Haemophilus influenzae b (Hib)
Hepatitis B
Pneumococcus
Influenza
Varicella

Community Exposures

Common exposures in the community are often related to contaminated food and water ingestion; exposure to infected family members or coworkers; or exposures related to hobbies, travel, or work. Infection caused by common respiratory viruses (influenza, respiratory syncytial virus, and adenovirus) and by more atypical pathogens (HSV, VZV) carries risk for viral pneumonia and increased risk for bacterial or fungal superinfection. Community (contact or transfusion associated) exposure to CMV and EBV may produce severe primary infection in the nonimmune host. Recent and remote exposures to endemic, geographically restricted systemic mycoses (*Blastomyces dermatitidis, Coccidioides immitis*, and *Histoplasma capsulatum*) and *Mycobacterium tuberculosis* can result in localized pulmonary, systemic, or metastatic infection. Asymptomatic *Strongyloides stercoralis* infection may activate more than 30 years after initial exposure owing to the effects of immunosuppressive therapy (Fig. 29-1). Such reactivation can result in either a diarrheal illness and parasite migration with hyperinfestation syndrome (characterized by hemorrhagic enterocolitis, hemorrhagic pneumonia, or both) or disseminated infection with accompanying (usually) gram-negative bacteremia or meningitis. Gastroenteritis secondary to *Salmonella, Campylobacter jejuni*, and a variety of enteric viruses can result in persistent infection, with more severe and prolonged diarrheal disease and an increased risk of primary or secondary bloodstream invasion and metastatic infection.

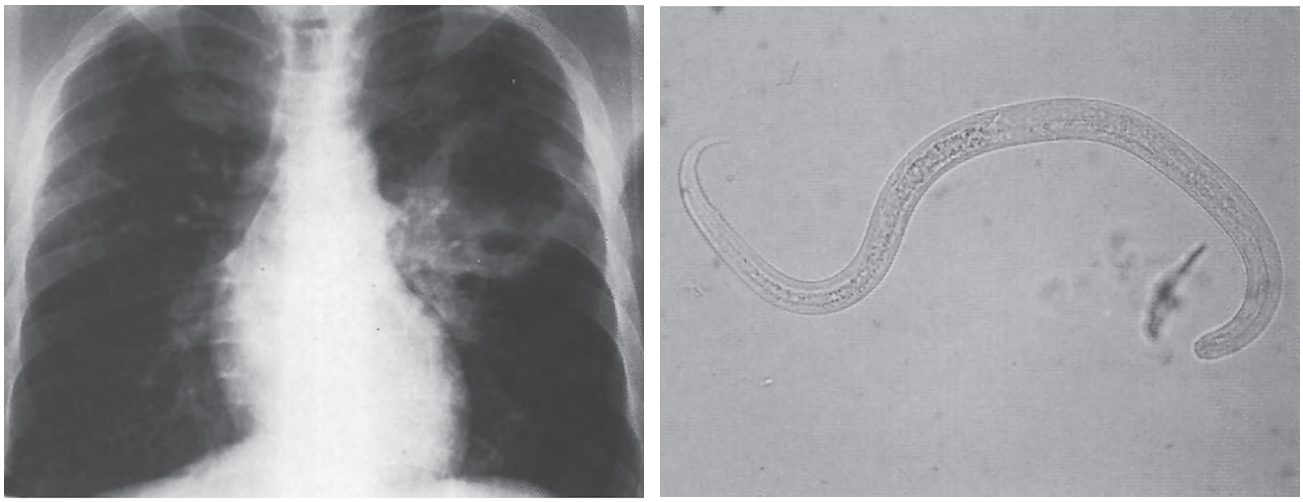
Nosocomial Exposures

Nosocomial infections are of increasing importance. Organisms with significant antimicrobial resistance are present in most medical centers, including vancomycin-resistant, linezolid-resistant, and quinupristin/dalfopristin-resistant enterococci; methicillin-resistant staphylococci, and fluconazole-resistant *Candida*. A single case of nosocomial *Aspergillus* infection in a compromised host should be viewed as a failure of infection control practices. Antimicrobial misuse and inadequate infection control practices have caused increased rates of *Clostridium difficile* colitis. Outbreaks of infections secondary to *Legionella* have been associated with hospital plumbing and contaminated water supplies or ventilation systems. Each nosocomial infection should be investigated to ascertain the source and prevent subsequent infections. Nosocomial spread of *Pneumocystis carinii (jiroveci)* between immunocompromised patients has been suggested by a variety of case series. Respiratory viral infections may be acquired from medical staff and should be considered among the causes of fever and respiratory decompensation in hospitalized or institutionalized, immunocompromised individuals.

Net State of Immunosuppression

The net state of immunosuppression is a qualitative measure of the risk factors for infection in an individual, including immunosuppressive medications and iatrogenic conditions (Table 29-4). Among the most important are the following:

1. The specific immunosuppressive therapy, including number, dose, duration, and sequence of agents
2. Technical difficulties during transplantation, resulting in an increased incidence of leaks (blood, lymph, urine) and fluid collections, devitalized tissue, poor wound healing, and prolonged surgical drainage catheterization
3. Prolonged instrumentation, including airway intubation and use of vascular access devices (e.g., dialysis catheters)
4. Prolonged use of broad-spectrum antibiotics
5. Renal or hepatic dysfunction, or both (in addition to graft dysfunction)



A B

Figure 29–1 Simultaneous *Pneumocystis* pneumonia and bacterial lung abscess secondary to coinfection by *Strongyloides stercoralis* in a Vietnamese kidney transplant recipient. **A,** Chest radiograph shows a lung abscess secondary to *Enterobacter* species. Bronchoscopic examination also revealed simultaneous *Pneumocystis carinii (jiroveci)* and *S. stercoralis* infections. Migration of *Strongyloides* across the wall of the gastrointestinal tract during immunosuppression (hyperinfection) is associated with systemic signs of "sepsis" and central nervous system infection (parasitic and bacterial). **B,** *S. stercoralis* from the lung of the same patient.

Table 29–4 Factors Contributing to the Net State of Immunosuppression

Immunosuppressive therapy—type, temporal sequence, intensity, cumulative dose
Prior therapies (chemotherapy or antimicrobials)
Mucocutaneous barrier integrity (catheters, lines, drains)
Neutropenia, lymphopenia (often drug induced)
Underlying immunodeficiency
Hypogammaglobulinemia from proteinuria
Complement deficiencies
Autoimmune diseases (systemic lupus erythematosus)
Other disease states (HIV, lymphoma/leukemia)
Metabolic conditions (uremia, malnutrition, diabetes, cirrhosis)
Viral infections (CMV, hepatitis B and C, RSV), which lead to immunosuppression
Graft rejection
Cancer/cellular proliferation

CMV, cytomegalovirus; HIV, human immunodeficiency; RSV, respiratory syncytial virus.

6. Presence of infection with an immunomodulating virus, including CMV, EBV, HBV, HCV, or HIV

Specific immunosuppressive agents are associated with increased risk for certain infections (Table 29-5).

TIMETABLE OF INFECTION

With standardized immunosuppressive regimens, specific infections that occur most often will vary in a predictable pattern depending on the time elapsed since transplantation (Fig. 29-2). This is primarily a reflection of the changing risk factors over time (surgery/hospitalization, immunosuppression, acute and chronic rejection, emergence of latent infections, and exposures to novel community infections).[18] The pattern of infections changes with alterations in the immunosuppressive regimen (pulse-dose steroids or intensification for graft rejection), intercurrent viral infection, neutropenia (drug toxicity), graft dysfunction, or significant epidemiological exposures (travel or food). The timeline remains a useful starting point, although altered by the introduction of new immunosuppressive agents and patterns of use, including reduced use of corticosteroids and calcineurin inhibitors, increased use of antibody-based (induction) therapies or sirolimus, routine antimicrobial prophylaxis, improved molecular assays, antimicrobial resistance, transplantation in HIV-infected and HCV-infected individuals, and broader epidemiological exposures (e.g., travel).

Figure 29-2 shows three overlapping periods of risk for infection after transplantation, each associated with differing patterns of common pathogens, as follows:

1. The perioperative period to approximately 4 weeks after transplantation, reflecting surgical and technical complications
2. The period 1 to 6 months after transplantation (depending on the rapidity of taper of immunosuppression and the use of antilymphocyte "induction" therapy), reflecting intensive immunosuppression with viral activation and opportunistic infections
3. The period beyond the first year after transplantation, reflecting community-acquired exposures and some unusual pathogens based on the level of maintenance immunosuppression

The timeline can be used in a variety of ways: (1) to establish a differential diagnosis for a transplant patient suspected to have infection; (2) to provide a clue to the presence of an excessive environmental hazard for the individual, either within the hospital or in the community; and (3) to serve as a guide to the design of preventive antimicrobial strategies. Infections occurring outside the usual period or of unusual severity suggest either excessive epidemiological hazard or excessive immunosuppression.

The prevention of infection must be linked to the risk for infection at various times after transplantation. Table 29-6 outlines routine preventive strategies from the Massachusetts General Hospital. Such strategies serve only to delay the onset of infection in the face of epidemiological pressure. The use of antibiotic prophylaxis, vaccines, and behavioral modifications (e.g., routine hand washing or advice against digging in gardens without masks) may result only in a "shift to the right" of the infection timeline, unless the intensity of immunosuppression is reduced, or immunity develops.

First Phase (0 to 4 Weeks after Transplantation)

During the first month after transplantation, three types of infection occur. The first type is infection present in the recipient before transplantation, which, after inadequate treatment, emerges in the setting of surgery, anesthesia, and immunosuppression. Pretransplantation pneumonia and vascular access infections are common examples of this type

Table 29–5 Immunosuppression and Infection

Antilymphocyte globulins (lytic) and alloimmune response	Activation of latent (herpes)virus, fever, cytokines
Plasmapheresis	Encapsulated bacteria
Costimulatory blockade	Unknown so far
Corticosteroids	Bacteria, *Pneumocystis (carinii) jiroveci*, hepatitis B and C
Azathioprine	Neutropenia, papillomavirus (?)
Mycophenolate mofetil	Early bacterial infection, B cells, late CMV (?)
Calcineurin inhibitors (cyclosporine/tacrolimus)	Enhanced viral replication (absence of immunity), gingival infection, intracellular pathogens
Rapamycin	Excess infections in combination with current agents, idiosyncratic pneumonitis syndrome

CMV, cytomegalovirus.

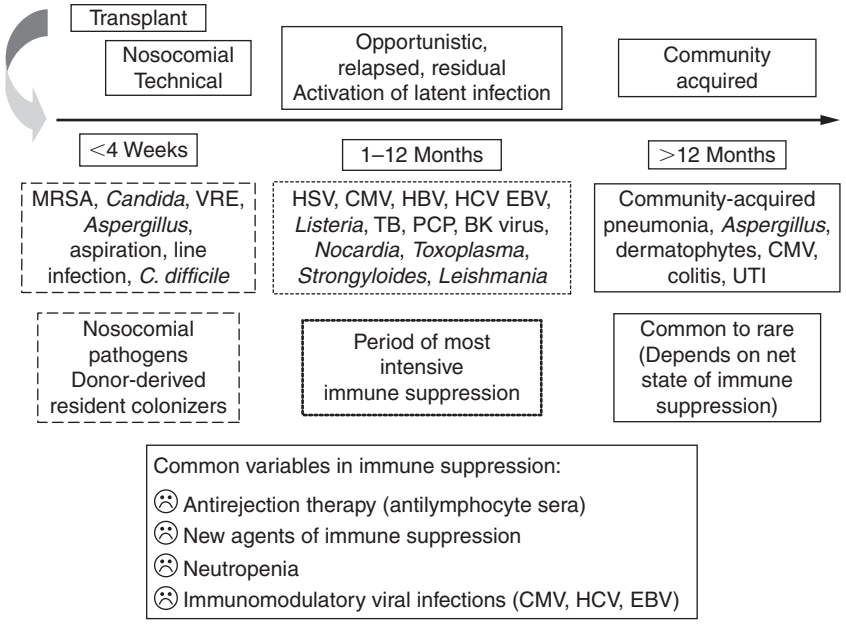


Figure 29–2 The timeline of infection after transplantation. CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; MRSA, methicillin-resistant *Staphylococcus aureus*; PCP, *Pneumocystis carinii (jiroveci)* pneumonia; TB, tuberculosis; UTI, urinary tract infection; VRE, vancomycin-resistant enterococcus.

of infection. Colonization of the recipient with resistant organisms that infect intravenous catheters or surgical drains also is common (e.g., methicillin-resistant *Staphylococcus aureus*). All infection should be controlled or eradicated before transplantation.

The second type of early infection is donor derived. This type may be nosocomially derived (resistant gram-negative bacilli and *S. aureus* or *Candida*) secondary to systemic infection in the donor (e.g., line infection) or contamination during the organ procurement process. The end result is a high risk of infection of vascular suture lines with mycotic aneurysm. Rarely, infections transmitted from donor to recipient may emerge earlier than predicted (e.g., tuberculosis, histoplasmosis).

The third and most common source of infection in the early period is related to the complex surgical procedure of transplantation. These infections include surgical wound infections, pneumonia (aspiration), bacteremia secondary to vascular access or surgical drainage catheters, urinary tract infections, and infections of fluid collections—leaks of vascular or urinary anastomoses or of lymphoceles. These are nosocomial infections and, as such, are due to the same antimicrobial-resistant bacteria and *Candida* infections observed in nonimmunosuppressed patients undergoing comparable surgery. Given the immunosuppression, the signs of infection may be subtle, however, and the severity or duration usually is greater. The technical skill of the surgeons and meticulous postoperative care (i.e., wound care and proper maintenance and timely removal of endotracheal tubes, vascular access devices, and drainage catheters) are the determinants of risk for these infections. Another common infection is *C. difficile* colitis.

Limited perioperative antibiotic prophylaxis (i.e., from a single dose to 24 hours of an antibiotic such as cefazolin) is usually adequate with additional coverage only for known risk factors (e.g., prior colonization with methicillin-resistant *S. aureus*). For pancreas transplantation, perioperative prophylaxis against yeasts is common using fluconazole, mindful of potential increases in sirolimus and calcineurin inhibitor levels when used with azole antifungal agents.

Opportunistic infections are notable for their absence in the first month after transplantation, even though the daily doses of immunosuppressive drugs are at their highest during this time. The implications of this observation are important: It suggests that it is not the daily dose of immunosuppressive drugs that is important but rather the cumulative dose of these drugs—the "area under the curve"—in determining the true state of immunosuppression. The net state of immunosuppression is not great enough to support the occurrence of opportunistic infections, unless an exposure has been excessive. The occurrence of a single case of opportunistic infection in this period should trigger an epidemiological investigation for an environmental hazard.

Second Phase (1 to 6 Months after Transplantation)

Infection in the transplant recipient 1 to 6 months after transplantation has one of three causes:

1. Infection from the perisurgical period including relapsed *C. difficile* colitis, inadequately treated pneumonia, or infection related to a technical problem (e.g., a urine leak, lymphocele, hematoma). Fluid collections in this setting generally require drainage.
2. Viral infections including CMV, HSV, shingles (VZV), human herpesvirus (HHV)-6 or HHV-7, EBV, hepatitis (HBV, HCV), and HIV. This group of viruses is unique. These infections are lifelong and tissue-associated (often transmitted with the allograft

Table 29–6 **Renal Transplantation Routine Antimicrobial Protocols at Massachusetts General Hospital**

***Pneumocystis carinii (jiroveci)* Pneumonia and General Antibacterial Prophylaxis**

Regimen

One single-strength TMP-SMX tablet (containing 80 mg trimethoprim, 400 mg sulfamethoxazole) orally daily for a minimum of 4-6 mo post-transplantation. Patients infected with CMV, with chronic rejection, or with recurrent infections are maintained on lifelong prophylaxis. A thrice-weekly regimen of TMP-SMX prevents *P. jiroveci* pneumonia, but does not prevent other infections (e.g., urinary tract infection, *Nocardia, Listeria, Toxoplasma*, and other gastrointestinal and pulmonary infections)

Alternative Regimen

For patients proven not to tolerate TMP-SMX, alternative regimens include (1) a combination of atovaquone, 1500 mg orally daily with meals, plus levofloxacin, 250 mg orally daily (or equivalent fluoroquinolone without anaerobic activity); (2) pentamidine, 300 mg intravenously or inhaled every 3-4 wk; or (3) dapsone, 100 mg orally daily twice weekly, with or without pyrimethamine. Each of these agents has toxicities that must be considered (e.g., hemolysis in G6PD-deficient hosts with dapsone). None of these alternative programs offers the same broad protection of TMP-SMX

CMV Prophylaxis

CMV Serological Status with or without ALT	Therapy*	Screening (Antigenemia)
$D^+/R^{-\dagger}$	Ganciclovir, 5 mg/kg intravenously for loading dose, then per renal function to discharge; then valganciclovir (in general, 450 mg/day for renal transplants) × 3 mo	Monthly for 6 mo after discontinuation of therapy‡
D^+ or R^+ with ALT	Ganciclovir, 5 mg/kg intravenously for first dose, then per renal function to discharge; valganciclovir daily × 6 mo	Monthly for 6 mo after discontinuation of therapy†
D^-/R^+ (no ALT)	Valganciclovir, 450 mg/day for renal transplants × 3 mo	Symptoms only
D^-/R^-	Famciclovir, 500 mg orally daily × 3-4 mo (or valacyclovir, 500 twice a day, or acyclovir, 400 three times a day); use of CMV-negative or leukocyte-reduced blood	Symptoms, fever/neutropenia
Status unknown with ALS	Ganciclovir, 5 mg/kg intravenously for first dose and daily (corrected for renal function) until serological status determined	

Fungal Prophylaxis

Mucocutaneous candidiasis can be prevented with oral clotrimazole or nystatin 2-3 times per day at times of steroid therapy or in the face of broad-spectrum antibacterial therapy and in diabetic transplant patients. Fluconazole, 200-400 mg/day for 10-14 days, is used to treat prophylaxis failures. Routine prophylaxis with fluconazole is used for pancreas transplants. Other prophylaxis must be determined based on risk for each institution and the presence or absence of colonization or other risk factors for fungal infection

*Drugs are not approved by the Food and Drug Administration at these doses. The doses of antiviral and antibacterial therapies generally *are not* reduced for neutropenia. Consider other options first.

†D^+/R^- = Donor seropositive, recipient seronegative.

‡ALT includes any of the lytic, lymphocyte-depleting antisera.

ALT, antilymphocyte therapy; CMV, cytomegalovirus; G6PD, glucose-6-phosphate dehydrogenase; TMP-SMX, trimethoprim/sulfamethoxazole.

from seropositive donors). More importantly, these viruses are systemically immunosuppressive and predispose to graft rejection. The herpesviruses are prominent given the importance of T cell function in antiviral control and the disproportionate degree of T cell inhibition by most immunosuppressive regimens. Other viral pathogens of this period include BK polyomavirus (in association with allograft dysfunction) and community-acquired respiratory viruses (adenovirus, influenza, parainfluenza, respiratory syncytial virus, metapneumovirus).

3. Opportunistic infection secondary to *P. carinii (jiroveci)*, *Listeria monocytogenes, Toxoplasma gondii, Nocardia, Aspergillus*, and other agents.

In this period, the stage also is set for the emergence of a subgroup of patients—the "chronic ne'er do well"—the patient who requires higher than average immunosuppression to maintain graft function or who has prolonged, untreated viral infections and other opportunistic infections, which predicts long-term susceptibility to many other infections (third phase, discussed later). Such patients may require prolonged (lifelong) prophylaxis (antibacterial, antifungal, antiviral, or a combination) to prevent life-threatening infection.

The specific opportunistic infections that occur reflect the specific immunosuppressive regimen used and the presence or absence of immunomodulating viral infection. Viral pathogens (and rejection) are responsible for most febrile episodes that occur in this period. During this period, anti-CMV strategies and trimethoprim/sulfamethoxazole prophylaxis are effective in decreasing the risk of infection. Trimethoprim/sulfamethoxazole prophylaxis effectively prevents *P. carinii (jiroveci)* pneumonia and reduces the incidence of urinary tract infection and urosepsis, *L. monocytogenes* meningitis, *Nocardia* infection, and *T. gondii*.

Third Phase (>6 to 12 Months after Transplantation)

Recipients who underwent tranplantation more than 6 months previously can be divided into three groups in

terms of infection risk. Most transplant recipients (70% to 80%) have a technically good procedure with satisfactory allograft function, reduced immunosuppression, and absence of chronic viral infection. These patients resemble the general community in terms of infection risk, with community-acquired respiratory viruses constituting their major risk. Occasionally, such patients develop primary CMV infection (socially acquired) or infections related to underlying diseases (e.g., skin infections in diabetes). A second group of patients has chronic viral infection, which in the absence of effective antiviral therapy (often reduction in immunosuppression) produces end-organ damage (e.g., BK polyomavirus leading to nephropathy, HCV leading to cryoglobulinemia or cirrhosis, CMV with chronic graft rejection) or malignancy (e.g., post-transplantation lymphoproliferative disease [PTLD] secondary to EBV, skin or anogenital cancer secondary to papillomaviruses).

A third group of patients has unsatisfactory allograft function and requires more intensive immunosuppressive therapy to preserve graft function. As a result, these patients appear overimmunosuppressed. These patients may have chronic viral infections and represent the "chronic ne'er-do-wells," who are at greatest risk for opportunistic infection. We give these patients lifetime maintenance trimethoprim/sulfamethoxazole prophylaxis and often fluconazole prophylaxis. In this group, one also should consider organisms more often associated with immune dysfunction of acquired immunodeficiency syndrome (AIDS) (*Bartonella, Rhodococcus, Cryptosporidium*, and *Microsporida*) and invasive fungal pathogens (*Aspergillus, Zygomycetes*, and *Dematiaceae* or pigmented molds). Even minimal signs or symptoms warrant careful evaluation in this group of "high-risk" patients.

ASSESSMENT OF INFECTIOUS DISEASE IN RECIPIENT AND POTENTIAL DONOR BEFORE TRANSPLANTATION

Guidelines for pretransplant screening have been the subject of several more recent publications, including a consensus conference of the Immunocompromised Host Society, the American Society for Transplantation Clinical Practice Guidelines for the evaluation of renal transplant candidates, and the American Society of Transplant Surgeons (ASTS) Clinical Practice Guidelines for the evaluation of living renal transplant donors.[5,6,15,16,35,36,61,64,71]

Transplant Donor

Deceased Donor Evaluation

The crucial feature in screening of deceased donors is time limitation. A useful organ must be procured and implanted before some microbiologic assessments have been completed. Major infections must be excluded, and appropriate cultures and samples must be obtained for future reference. As a result, bacteremia or fungemia may not be detected until after the transplantation has been performed. Such infections generally have not resulted in transmission of infection as long as the infection has been adequately treated in terms of use of antimicrobial agents to which the organism is susceptible and time. In recipients of tissues from 95 bacteremic donors, a mean of 3.8 days of effective therapy after transplantation seemed adequate to prevent transmission of susceptible pathogens. Longer courses of therapy in the recipient are preferred targeting known donor-derived pathogens.[22] Bacterial meningitis must be treated with antibiotics that penetrate the cerebrospinal fluid before organ procurement.

Certain acute infections (CMV, HSV, EBV, HIV, and HCV) may be undetected in the period before antibody formation. Viral DNA detection is preferred. Likewise, the donor's clinical, social, and medical histories are essential to reducing the risk of such infections. In the presence of known infection, such infections must be treated before procurement if possible. Several more recent clusters of donor-derived infection have shown the risk for infection secondary to previously unrecognized pathogens, including lymphocytic choriomeningitis virus, Chagas' disease, and HSV, in addition to other, more common pathogens. Major exclusion criteria are outlined in Table 29-2.

Living Donor Evaluation

In contrast to the above-described scenario, the living donor procedure should be considered elective, and the evaluation should be completed and infections should be treated before such procedures. An interim history must be taken at the time of surgery to assess the presence of new infections since the initial donor evaluation. Intercurrent infections (flu-like illness, headache, confusion, myalgias, cough) might be the harbinger of important infection (West Nile virus, severe acute respiratory syndrome [SARS], *T. cruzi*). Live donors undergo a battery of serological tests (Table 29-7), purified protein derivative (PPD) skin test, and, if indicated, chest radiograph. The testing must be individualized based on unique risk factors (e.g., travel). Of particular importance to the renal transplant recipient is the exclusion of urinary tract infections (including yeasts) and bacteremia at the time of donation.

Special Considerations in Procurement

Mycobacterium tuberculosis from the donor represented approximately 4% of reported post-transplant tuberculosis cases in a review of 511 patients by Singh and Paterson.[66] Active disease should be excluded in PPD-positive donors with chest radiograph, sputum cultures, and chest computed tomography (CT) if the chest radiograph is abnormal. Urine acid-fast bacillus cultures may be useful in a PPD-positive kidney donor. Isoniazid prophylaxis of the recipient should be considered for untreated, PPD-positive donors.[4] Factors favoring prophylaxis include a donor from an endemic region, use of a high-dose steroid regimen, or high-risk social environment.

Chagas' disease (*T. cruzi*) has been transmitted by transplantation in endemic areas and more recently in the United States. Schistosomiasis and infection by *S. stercoralis* are generally recipient-derived problems.

Viral Infections Other than Cytomegalovirus

EBV infection is a major risk factor for development of PTLD. The risk is greatest in the EBV-seronegative recipient of an EBV-seropositive allograft (i.e., donor seropositive, recipient seronegative [D^+/R^-]). This situation is most common in pediatric transplant recipients and in adults coinfected with CMV or on higher levels of immunosuppression. Monitoring should be considered for at-risk individuals using a quantitative, molecular assay (e.g., polymerase chain reaction) for EBV.[26,53] EBV also is a cofactor for other lymphoid malignancies.

Table 29–7 Pretransplant Evaluation of Living Donors

Laboratory Test	All Patients	Patients with Exposure to Endemic Area	Quantitative Viral Studies Available (PCR)
Serologies			
CMV	√		√
HSV	√		√
VZV	√		
EBV	√		√
HIV	√		√
HBV: HBsAg	√		√
HBV: anti-HBs	√		
HCV	√		√
Treponema pallidum	√		
Toxoplasma gondii	√		
Strongyloides stercoralis		√	
Leishmania		√	
Trypanosoma cruzi		√	Blood smear
Histoplasma capsulatum		√	
Cryptococcus neoformans		√	Cryptococcal antigen
Coccidioides immitis		√	
Other Studies			
Urinalysis and culture	√		
Skin test: PPD	√		
Chest x-ray (routine)	√		
Stool ova and parasites (*Strongyloides*)		√	
Urine ova and parasites with or without cystoscopy		√ (for kidneys)	√ (schistosomiasis-endemic areas)

anti-HBs, antibody to hepatitis B surface antigen; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; PCR, polymerase chain reaction; PPD, purified protein derivative; VZV, varicella-zoster virus.

VZV screening should be used to identify seronegative individuals (no history of chickenpox or shingles) for vaccination before transplantation. HSV screening is performed by most centers despite the use of antiviral prophylaxis during the post-transplant period. VZV serological status is particularly important in children who may be exposed at school (for antiviral or VZV immunoglobulin prophylaxis) and in adults with atypical presentations of infection (pneumonia or gastrointestinal disease). Other herpesviruses also may reactivate, with HHV-6 and HHV-7 serving as cofactors for CMV and fungal infections and, in endemic regions, Kaposi's sarcoma–associated herpesvirus (HHV-8) causing malignancies.

HBV surface antigen (HBsAg) and HBV core antibody (HBcAb) are used for screening purposes (see Chapter 30 for detailed discussion). A positive HBV surface antibody titer indicates either vaccination or prior infection. HBcAb-IgM positivity suggests active HBV infection, whereas IgG positivity suggests a more remote or persistent infection. The HBsAg-negative, HBcAb-IgG–positive donor may have viral DNA in the liver but may be appropriate as a donor for HBV-infected renal recipients; quantitative assays for HBV should be obtained to guide further therapy. The presence of HBsAg-negative, HBcAb-IgG–positive assays may be a false-positive result or reflect true, latent HBV infection.

HCV infection generally progresses more rapidly with immunosuppression and with CMV coinfection (see Chapter 30 for detailed discussion). HCV-seropositive renal transplant candidates are more likely to develop cirrhosis and complications of liver failure. Therapies for HCV infection are currently limited, particularly in the transplant population; management is often conservative and involves monitoring disease progression by quantitative molecular viral assays with intermittent liver biopsy. Management is likely to change as newer HCV antiviral agents become available (see Chapter 30).

HIV-infected donors have rarely been used. The progression of recipient infection is rapid, and so far outweighs the benefits of transplantation. Based on current criteria, donors may be excluded based on historical evidence of risk factors significant for HIV infection and confirmatory testing.

Human T cell lymphotropic virus I (HTLV-I) is endemic in the Caribbean and parts of Asia (Japan) and can progress to HTLV-I-associated myelopathy/tropical spastic paraparesis or to adult T cell leukemia/lymphoma. HTLV-II is similar to HTLV-I serologically, but it is less clearly associated with disease. Use of organs from such donors is generally avoided.[27,68]

West Nile virus is a flavivirus associated with viral syndromes and meningoencephalitis and may be transmitted by blood transfusion and organ transplantation.[69,70] Routine screening of donors is not advocated other than in areas with endemic infection. Donors with unexplained changes in mental status or recent viral illness with neurologic signs should be avoided.

SARS is a more recently described coronavirus, thought to be associated with exposure to civets or other animals common to the diet of certain regions of China. Tissue persistence is prolonged, and infection of transplant recipients

seems to be severe and often symptomatic. Organ procurement should exclude patients with recent acute illnesses meeting SARS criteria.

Transplant Recipient

The pretransplant period is useful for obtaining travel, animal, environmental, and exposure histories; updating immunizations; and counseling of the recipient regarding travel, food, and other infection risks. Ongoing infection must be eradicated before transplantation. Two forms of infection pose a special risk—bloodstream infection related to vascular access (including that for dialysis), and pneumonia, which puts the patient at high risk for subsequent lung infection with nosocomial organisms. Several other infections are commonly encountered and should be treated and cleared before transplantation. Infected ascites or peritoneal dialysis fluid also must be cleared before surgery. Urinary tract infection must be eliminated with antibiotics with or without nephrectomy. Similarly, skin disease threatens the integrity of one's primary defense against infection and should be corrected even if doing so requires the initiation of immunosuppression before transplantation (e.g., the initiation of immunosuppression to treat psoriasis or eczema). Finally, the history of more than one episode of diverticulitis should initiate an evaluation to determine whether sigmoid colectomy should be done before transplantation.

Among important considerations in transplant recipients are strongyloidiasis, tuberculosis, and AIDS. *Strongyloides* hyperinfestation syndrome (hemorrhagic enterocolitis, pneumonia, gram-negative or mixed bacteremia, or meningitis) may emerge more than 30 years after transplantation. Empirical pretransplantation therapy of *Strongyloides*-seropositive recipients (ivermectin) prevents such infections.

The incidence of active tuberculous disease and the occurrence of disseminated infection secondary to *M. tuberculosis* are higher in the transplant recipient than in the general population. Active tuberculous disease must be eradicated before transplantation. The major antituberculous drugs are potentially hepatotoxic, and significant drug interactions are common between antituberculosis agents and immunosuppressive agents. In patients with active infection, from endemic regions or with high-risk exposures, tuberculosis therapy should be initiated in all PPD-positive individuals before transplantation. Some judgment may be used as to the optimal timing of treatment in individuals without evidence of active or pleuropulmonary disease. Patients at greater risk of tuberculosis infection or exposure include individuals with prior history of active tuberculosis or significant signs of old tuberculosis on chest radiograph, recent tuberculin reaction conversion, known exposure to active disease, protein-calorie malnutrition, cirrhosis, other immunodeficiency, or living exposures (e.g., in a shelter or other group housing).

For many patients receiving antiretroviral therapy, HIV infection has been converted from a progressively fatal disease to a chronic infection controlled by complex regimens of antiviral agents or highly active antiretroviral therapy (HAART). HAART has been associated with reduced viral loads, improved $CD4^+$ lymphocyte counts, and reduced susceptibility to opportunistic infections. In the pre-HAART era, organ transplantation generally was associated with a rapid progression to AIDS, and transplantation was avoided in such individuals. Prolonged disease-free survival with HAART has led, however, to a reconsideration of this policy. Renal transplantation in HIV has been associated with good outcomes in individuals with controlled HIV infection and in the absence of HCV coinfection.[1,67a] Management requires experience with immunosuppressive agents and various HAART regimens.

SELECTED INFECTIONS OF IMPORTANCE

General Considerations

The spectrum of infection in the immunocompromised host is quite broad. Given the toxicity of antimicrobial agents and the need for rapid interruption of infection, early, specific diagnosis is essential in this population. Advances in diagnostic modalities (e.g., CT or magnetic resonance imaging, molecular microbiologic techniques) may greatly assist in this process. The need for invasive diagnostic tools cannot be overemphasized, however. Given the diminished immune responses of the host, and the frequency of multiple simultaneous processes, invasive diagnosis is often the only method for optimal care. The initial therapy is broad by necessity, with a rapid narrowing of the antimicrobial spectrum as data become available.

The first choice of therapy is to reduce the intensity of immunosuppression, with the understanding that the risk of such an approach is graft rejection. For latent viral infections or tuberculosis, activation should be seen as evidence of excessive immunosuppression. In contrast, for intercurrent bacterial or fungal infections, reductions in immunosuppression might be reconsidered when evidence of resolution of infection is established. The selection of the specific reduction may depend on the organisms isolated. Similarly, reversal of some immune deficits (e.g., neutropenia, hypogammaglobulinemia) may be possible with adjunctive therapies (e.g., colony-stimulating factors or antibody). Coinfection with virus (CMV) is common and requires additional therapy.

Viral Pathogens

Cytomegalovirus

CMV is the most important pathogen in transplant recipients. It has a variety of direct and indirect effects.[18,60] The direct effects include the following:

- Fever and neutropenia syndrome with features of infectious mononucleosis, including hepatitis, nephritis, leukopenia, or thrombocytopenia
- Pneumonia
- Gastrointestinal invasion with colitis, gastritis, ulcers, bleeding, or perforation
- Hepatitis, pancreatitis
- Chorioretinitis

With the exception of chorioretinitis, the direct clinical manifestations of CMV infection usually occur 1 to 4 months after transplantation; chorioretinitis usually does not occur until later in the transplant course.

Although CMV is a common cause of clinical infectious disease syndromes, the *indirect effects* of viral infection are equally important. CMV infection produces a profound

suppression of a variety of host defenses, predisposing to secondary invasion by such pathogens as *P. carinii (jiroveci), Candida*, *Aspergillus*, and some bacteria. CMV also contributes to the risk for graft rejection, PTLD, HHV-6 and HHV-7 infections, and acceleration of HCV infection. The mechanisms for these effects are complex, including alteration of T cell number and function and major histocompatibility complex (MHC) synthesis, and the elaboration of an array of proinflammatory cytokines, chemokines, and growth factors.

PATTERNS OF TRANSMISSION

Transmission of CMV in the transplant recipient occurs in one of three patterns—primary infection, reactivation, and superinfection.[18]

Primary Cytomegalovirus Infection. Primary infection occurs most often when seronegative individuals receive grafts from latently infected, seropositive donors (D^+/R^-), with subsequent reactivation of the virus and systemic dissemination after transplantation. Forty percent to 50% of these patients experience direct infectious disease manifestations of CMV, whereas most are viremic, often without symptoms. Primary CMV infection also may occur in seronegative individuals after transfusion or exposure in the community. This disease may be severe.

Reactivation Cytomegalovirus Infection. In reactivation infection, seropositive individuals reactivate endogenous virus after transplantation ($D^{+/-}/R^+$). When conventional immunosuppressive therapy is used (e.g., no antilymphocyte antibody treatment), approximately 10% to 15% experience direct infectious disease syndromes, with a higher rate with the use of induction antilymphocyte therapy. Fifty percent of these individuals are viremic, often without symptoms.

Cytomegalovirus Superinfection. Virus may be reactivated in the setting of an allograft from a seropositive donor transplanted into a seropositive recipient (D^+/R^+).

PATHOGENESIS

Control of CMV infection is via MHC-restricted, virus-specific, cytotoxic T lymphocyte response ($CD8^+$ cells) controlled by $CD4^+$ lymphocytes. Seroconversion is a marker for the development of host immunity. The major effector for (re)activation of virus is the nature of the immunosuppressive therapy administered. Depleting–antithymocyte polyclonal and monoclonal antibodies are direct activators of viral infection (mimicking the alloimmune response) and provoke the elaboration of tumor necrosis factor-α and the other proinflammatory cytokines that enhance viral replication. Cyclosporine, tacrolimus, rapamycin, and prednisone (other than pulse doses) have limited ability to reactivate latent CMV, whereas azathioprine, mycophenolate mofetil, and cyclophosphamide are moderately potent in terms of promoting viral reactivation. These agents also perpetuate infection after it is established.

Allograft rejection is a major stimulus for CMV activation and vice versa. The CMV infection has been linked to a diminished outcome of renal and other allografts. Reinke and colleagues[60] showed that 17 of 21 patients for whom biopsy specimens revealed evidence of "late acute rejection" showed a response to antiviral therapy. Multiple studies have shown that the prevention of CMV infection also resulted in a lower incidence of graft rejection.[41]

DIAGNOSIS

Clinical management of CMV, including prevention and treatment, is important for the transplant recipient. It is based on an understanding of the causes of CMV activation and the available diagnostic techniques. CMV cultures generally are too slow and insensitive for clinical utility. A positive CMV culture (or shell vial culture) derived from respiratory secretions or urine is of little diagnostic value—many patients secrete CMV in the absence of invasive disease. Serological tests are useful before transplantation to predict risk but are of little value after transplantation in defining clinical disease (this statement includes measurements of anti-CMV IgM levels). Should a patient seroconvert to CMV, this is evidence that the patient has been exposed to CMV and has developed some degree of immunity. Seroconversion in transplantation is generally delayed, however, and not useful for clinical diagnosis. The demonstration of CMV inclusions in tissues in the setting of a compatible clinical presentation is the "gold standard" for diagnosis.

Quantitation of the intensity of CMV infection has been linked to the risk for infection in transplant recipients.[7,33,42,50,65] Two types of quantitative assays have been developed—molecular and antigen detection assays. The antigenemia assay is a semiquantitative fluorescent assay in which circulating neutrophils are stained for CMV early antigen (pp65) that is taken up nonspecifically as a measure of the total viral burden in the body. The molecular assays (direct DNA polymerase chain reaction, hybrid capture, amplification assays) are highly specific and sensitive for the detection of viremia. The most commonly used assays include plasma-based polymerase chain reaction testing and the whole-blood hybrid capture assay. Whole-blood and plasma-based assays cannot be directly compared. The highest viral loads often are associated with tissue-invasive disease, with the lowest in asymptomatic CMV infection. Viral loads in the CMV syndrome vary. Either assay can be used in management.

The advent of quantitative assays for the diagnosis and management of CMV infection has allowed noninvasive diagnosis in many patients with two important exceptions:

- Neurological disease, including chorioretinitis
- Gastrointestinal disease, including invasive colitis and gastritis

In these syndromes, the CMV assays are often negative, and invasive diagnosis (biopsy) may be needed.

The central role of assays is illustrated by the approach to management of CMV risk (see Table 29-6). The schedule for screening is linked to the risk for infection. In the high-risk patient (D^+/R^- or R^+ with antilymphocyte globulin) after the completion of prophylaxis, monthly screening is performed to ensure the absence of infection for 3 to 6 months. In the patient being treated for CMV infection, the assays provide an end point for therapy and the initiation of prophylaxis.

CYTOMEGALOVIRUS PREVENTION

Prevention of CMV infection must be individualized for immunosuppressive regimens and the patient. Two strategies are commonly used for CMV prevention—universal prophylaxis and preemptive therapy. Universal prophylaxis involves giving antiviral therapy to all at-risk patients beginning at or immediately after transplantation for a defined period. In preemptive therapy, quantitative assays are used to

monitor patients at predefined intervals to detect early disease. Positive assays result in therapy. Preemptive therapy incurs extra costs for monitoring and coordination of outpatient care, while reducing the cost of drugs and the inherent toxicities. Prophylaxis has the possible advantage of preventing not only CMV infection during the period of greatest risk but also diminishing infections secondary to HHV-6, HHV-7, and EBV. The indirect effects of CMV (i.e., graft rejection, opportunistic infection) also may be reduced by routine prophylaxis. In practice, neither universal prophylaxis nor preemptive therapy is perfect. Infrequently, breakthrough disease and ganciclovir resistance have been observed with both approaches.[34]

Given the risk for invasive infection, patients at risk for primary infection (CMV D^+/R^-) are generally given prophylaxis for 3 to 6 months after transplantation. We use 6 months of prophylaxis in patients receiving depleting anti–T lymphocyte antibodies. Other groups are candidates for preemptive therapy *if* an appropriate monitoring system is in place, and patient compliance is good. Current data support the use of universal prophylaxis (not preemptive therapy), however, in the prevention of indirect effects of CMV infection, including PTLD, opportunistic infections, allograft rejection, and mortality.[34]

TREATMENT

The standard of care for treating invasive CMV disease is at least 2 to 3 weeks of intravenous ganciclovir (5 mg/kg twice daily, with dosage adjustments for renal dysfunction) until a quantitative assay for CMV is negative. In patients slow to respond to therapy and who are seronegative, the addition of 3 months of CMV hyperimmune globulin (150 mg/kg/dose intravenously given every 3 to 4 weeks) may be useful. Relapses occur, primarily in patients not treated beyond the achievement of a negative quantitative assay. The use of completely oral regimens for treatment appears to be effective with the exception of invasive gastrointestinal disease. We treat intravenously until there is evidence of a good response and then switch to oral treatment or oral treatment with close monitoring of quantitative viral load assays, and follow with prophylaxis with 3 months of oral ganciclovir or valganciclovir prophylaxis (based on creatinine clearance). This approach has resulted in rare symptomatic relapses and generally prevents emergence of antiviral resistance.

Numerous issues remain. As noted, the role of oral valganciclovir in treatment remains under investigation. This agent provides good bioavailability but is not approved for this indication. Some relapses occur in gastrointestinal disease because the assays used to follow disease are unreliable in this setting. Repeat endoscopy should be considered to ensure the clearance of infection. The optimal dosing of valganciclovir for *prophylaxis* in renal transplant recipients is also unclear. It is often worth measuring a formal creatinine clearance to ensure adequate dosing.

Alternative therapies are available in intravenous form only, including foscarnet and cidofovir. Foscarnet has been used extensively for therapy of CMV in AIDS patients. Although it is active against most ganciclovir-resistant strains of CMV, we prefer combination therapy (ganciclovir and foscarnet) for organ transplant recipients given the toxicities of high-dose, single-agent therapy, and given the antiviral synergy that has been reported.[45] Cidofovir has been used in renal transplant recipients, often with nephrotoxicity. Foscarnet and cidofovir may exhibit synergistic nephrotoxicity with calcineurin inhibitors. A newer class of agents (dihydroorotate dehydrogenase inhibitors [leflunamide]) that has been approved for immunosuppression and treatment of rheumatological diseases also seems to have useful activity against CMV (and possibly BK polyomavirus). Mirabavir is in clinical trials for CMV prophylaxis and therapy.

Epstein-Barr Virus

EBV is a ubiquitous herpesvirus that infects B lymphocytes. In immunosuppressed transplant recipients, primary EBV infection (and relapses in the absence of antiviral immunity) causes a mononucleosis-type syndrome, generally manifesting as a lymphocytosis (B cell) with or without lymphadenopathy or pharyngitis. Meningitis, hepatitis, and pancreatitis also are observed. Remitting-relapsing EBV infection is common in children and may reflect the interplay between evolving antiviral immunity and immunosuppression. Regardless of its mode of expression, this syndrome should suggest relative overimmunosuppression.

EBV also plays a central role in the pathogenesis of PTLD.[46,49,51,53] The most clearly defined risk factor for PTLD is primary EBV infection, which increases the risk for PTLD by 10-fold to 76-fold. PTLD may occur, however, in the absence of EBV infection or in seropositive patients. Posttransplant non-Hodgkin's lymphoma is a common complication of solid organ transplantation. Lymphomas constitute 15% of tumors among adult transplant recipients (51% in children) with mortality of 40% to 60%. Many deaths are associated with allograft failure after withdrawal of immunosuppression during treatment of malignancy. Compared with the general population, PTLD has increased extranodal involvement, poor response to conventional therapies, and poor outcomes. The spectrum of disease is broad and ranges from benign polyclonal, B cell, infectious mononucleosis–like disease to malignant, monoclonal lymphoma.[30] Most disease is of B cell origin although T cell, natural killer cell, and null cell tumors are described. EBV-negative PTLD has been described, and T cell PTLD has been shown in allografts thought to have rejection or other viral infection. PTLD late (>1 to 2 years) after transplantation is more often EBV-negative in adults. (See Chapter 33.)

The clinical presentations of EBV-associated PTLD vary and include the following:

- Unexplained fever (fever of unknown origin)
- A mononucleosis-type syndrome, with fever and malaise, with or without pharyngitis or tonsillitis (often diagnosed incidentally in tonsillectomy specimens); often no lymphadenopathy is observed
- Gastrointestinal bleeding, obstruction, or perforation
- Abdominal mass lesions
- Infiltrative disease of the allograft
- Hepatocellular or pancreatic dysfunction
- Central nervous system disease

DIAGNOSIS

Serological testing is not useful for the diagnosis of acute EBV infection or PTLD in transplantation. Quantitative EBV viral load testing is required for the diagnosis and management of PTLD.[24,25,43,62] Serial assays are more useful in an individual patient than specific viral load measurements. These assays are not standardized and cannot be directly

compared between centers. Some data suggest that assays using unfractionated whole blood are preferable to plasma samples for EBV viral load surveillance.

MANAGEMENT

Clinical management depends on the stage of disease. In the polyclonal form, particularly in children, re-establishment of immune function may suffice to cause PTLD to regress. At this stage, it is possible that antiviral therapy might have some utility given the viremia and role of EBV as an immunosuppressive agent. With the progression of disease to extranodal and monoclonal malignant forms, reduction in immunosuppression may be useful, but alternative therapies are often required. In renal transplantation, the failure to regress with significant reductions in immunosuppression may suggest the need to sacrifice the allograft for patient survival. Combinations of anti–B cell therapy (anti-CD20, rituximab), chemotherapy (CHOP: cyclophosphamide, hydroxydaunomycin, vincristine [Oncovin], prednisone), or adoptive immunotherapy with stimulated T cells have been used.[11,17,28,67]

Polyomaviruses

Polyomaviruses have been identified in transplant recipients in association with nephropathy and ureteral obstruction (BK virus), and in association with demyelinating disease of the brain (JC virus) similar to that in AIDS. Polyomaviruses are small nonenveloped viruses with covalently closed, circular double-stranded DNA genomes. Adult levels of seroprevalence are 65% to 90%. There seems to be a decrement of antibody positivity in adulthood. BK virus seems to achieve latency in renal tubular epithelial cells. JC virus also has been isolated from renal tissues but seems to have preferred tropism for neural tissues. Reactivation occurs with immunodeficiency and immunosuppression and tissue injury (e.g., ischemia-reperfusion).

BK POLYOMAVIRUS INFECTION

BK virus is associated with a range of clinical syndromes in immunocompromised hosts, including viruria and viremia, ureteral ulceration and stenosis, and hemorrhagic cystitis.[19,31,32,44,47,48,58,59] Active infection of renal allografts has been associated with progressive loss of graft function ("BK nephropathy") in approximately 4% of renal transplant recipients; this is referred to as polyomavirus-associated nephropathy (PVAN). BK nephropathy is rarely recognized in recipients of extrarenal organs. The clinical presentation of disease is usually as sterile pyuria, reflecting shedding of infected tubular and ureteric epithelial cells. These cells contain sheets of virus and are detected by urine cytology as "decoy cells." In some cases, the patient presents with diminished renal allograft function or with ureteric stenosis and obstruction. In such patients, the etiologies of decreased renal function must be carefully evaluated (e.g., mechanical obstruction, drug toxicity, pyelonephritis, rejection, thrombosis, recurrent disease), and choices must be made between increasing immunosuppression to treat suspected graft rejection or reducing immunosuppression to allow the immune system to control infection. Patients with BK nephropathy treated with increased immunosuppression have a high incidence of graft loss. Reduced immunosuppression may stabilize renal allograft function but risks graft rejection. Polyoma-associated nephropathy manifested by characteristic histological features and renal dysfunction is found in about 1% to 8% of renal transplant patients.

Risk factors for nephropathy are poorly defined. Several risk factors have been implicated, although there is no consensus. Nickeleit and colleagues[48] found cellular rejection occurred more commonly in patients with BK nephropathy than controls. Other studies have implicated high-dose immunosuppression (particularly tacrolimus and mycophenolate mofetil), pulse-dose steroids, severe ischemia-reperfusion injury, exposure to antilymphocyte therapy, increased number of HLA mismatches between donor and recipient, deceased donor renal transplants, and presence and degree of viremia in the pathogenesis of disease. The role of specific immunosuppressive agents has not been confirmed. The greatest incidence of BK nephropathy is at centers with the most intensive immunosuppressive regimens.

Diagnosis. The use of urine cytology to detect the presence of infected decoy cells in the urine has approximately 100% sensitivity for BK virus infection but a low (29%) predictive value.[19,32] It is a useful screening tool but cannot establish a firm diagnosis. The use of molecular techniques to screen blood or urine also has been advocated but is more useful in the management of established cases (viral clearance with therapy) than in specific diagnosis.[12,23,29,54,56,57] Hirsch and colleagues[32] showed that patients with BK nephropathy have a plasma viral load statistically significantly higher (>7700 BK virus copies per mL of plasma [P <.001; 50% positive predictive value, 100% negative predictive value]) than patients without such disease.

Given the presence of viremia in renal allograft recipients, it is crucial to reduce immunosuppression whenever possible. The possible coexistence of rejection and BK infection makes renal biopsy essential, however, for the management of such patients. Renal biopsy specimens initially show cytopathic changes in renal epithelial cells with the gradual evolution of cellular infiltration consistent with the diagnosis of interstitial nephritis. Fibrosis is often prominent occasionally with calcification. Immunostaining for cross-reacting SV40 virus shows patchy staining of viral particles within tubular cells.

Treatment. There is no accepted treatment for polyomavirus-associated nephropathy other than a reduction in the intensity of immunosuppression. It is possible to monitor the response to such maneuvers using urine cytology (decoy cells) and viral load measures in blood or urine or both. It is unclear whether reduction of calcineurin inhibitors or antimetabolites should be considered first. Given the toxicity of calcineurin inhibitors for tubular cells, and the role of injury in the activation of BK virus and the need for anti–BK T cell activity, we have generally reduced these agents first. Other centers have selected reduction of the antimetabolite first. Regardless of the approach, renal function, drug levels, and viral loads must be monitored carefully.

Some centers advocate the use of cidofovir for BK nephropathy in low doses (0.25 to 1 mg/kg every 2 weeks).[3,8,10,72] Significant renal toxicity may be observed with this agent, and may add little to reduction in immunosuppression alone. Retransplantation has been achieved in such patients with failed allografts—possibly reflecting immunity developing subsequent to discontinuation of immunosuppression.[52]

JC VIRUS

Infection of the central nervous system by JC polyomavirus has been observed uncommonly in renal allograft recipients as progressive multifocal leukoencephalopathy. This infection generally manifests with focal neurologic deficits or seizures and may progress to death after extensive demyelination. Progressive multifocal leukoencephalopathy may be confused with calcineurin neurotoxicity; both may respond to a reduction in drug levels. These are believed to be distinct entities, but further studies are under way.

Fungal Infections

In addition to the endemic mycoses, transplant recipients are at risk for opportunistic infection with a variety of fungal agents, the most important of which are *Candida, Aspergillus*, and *Cryptococcus neoformans*.

Candida

The most common fungal pathogen in transplant patients is *Candida*, with more than 50% being of non-*albicans* strains. Mucocutaneous candidal infection (e.g., oral thrush, esophageal infection, cutaneous infection at intertriginous sites, candidal vaginitis) is most common in diabetics, with high-dose steroid therapy, and during broad-spectrum antibacterial therapy. These infections are usually treatable through correction of the underlying metabolic abnormality and topical therapy with clotrimazole or nystatin. Thrush also may complicate viral (HSV, CMV) or toxic (drugs including mycophenolate mofetil) esophagitis. Optimal management of candidal infection occurring in association with the presence of vascular access catheters, surgical drains, and bladder catheters requires removal of the foreign body and systemic antifungal therapy with fluconazole or echinocandin.

A special problem in renal transplant recipients is candiduria, even if the patient is asymptomatic. Particularly in individuals with poor bladder function, obstructing fungal balls can develop at the ureteropelvic junction, resulting in obstructive uropathy, ascending pyelonephritis, and the possibility of systemic dissemination. A single positive culture result for *Candida* species from a blood specimen necessitates systemic antifungal therapy; this finding carries a risk of visceral invasion of greater than 50% in this population.

Aspergillus

Invasive aspergillosis is a medical emergency in the transplant recipient, with the portal of entry being the lungs and sinuses in more than 90% of patients and the skin in most of those remaining. Two species, *Aspergillus fumigatus* and *Aspergillus flavum*, account for most of these infections, although amphotericin-resistant isolates (*Aspergillus terreus*) occasionally are recognized. The pathological hallmark of invasive aspergillosis is blood vessel invasion, which accounts for the three clinical characteristics of this infection—tissue infarction, hemorrhage, and systemic dissemination with metastatic invasion. Early in the course of transplantation, central nervous system involvement with fungal infection is most often due to *Aspergillus*; 1 year or later after transplantation, other fungi (*Zygomycetes*, dematiaceous fungi) become more prominent.

The drug of choice for documented *Aspergillus* infection is voriconazole, despite its significant interactions with calcineurin inhibitors and rapamycin. Liposomal amphotericin is an equally effective alternative, and combination therapies are under study. Surgical débridement is usually essential for successful clearance of such invasive infections.

Cryptococcus neoformans *and Central Nervous System Infections*

Central nervous system infection in the transplant recipient may result from a broad spectrum of organisms. Infections are often metastatic to the central nervous system from the bloodstream and lungs. Viral etiologies include CMV (nodular angiitis), HSV meningoencephalitis, JC virus (progressive multifocal leukoencephalopathy), and VZV. Local epidemiology (West Nile virus, Eastern equine encephalitis) also must be considered. Common bacterial infections in addition to the pneumococcus include Lyme disease, *Listeria monocytogenes*, tuberculosis, *Nocardia*, and occasionally *Salmonella*. Brain abscess and epidural abscess have been observed and may be particularly problematic when secondary to methicillin-resistant *S. aureus*, penicillin-resistant *Pneumococcus*, and quinolone-resistant streptococci. As noted earlier, fungi may be metastatic from lungs (*Aspergillus* and *Cryptococcus*) but also may spread from sinuses (*Mucoraceae*), skin (*Dematiaceae*), and the bloodstream (*Histoplasma* and *Pseudallescheria/Scedosporium, Fusarium*). Parasites include *T. gondii* and *Strongyloides*.

Given the spectrum of etiologies, precise diagnosis is essential. A reasonable empirical regimen would treat pneumococcus (ceftriaxone and vancomycin), *Listeria* (ampicillin), *Cryptococcus* (fluconazole or amphotericin), and herpes simplex virus (acyclovir) while awaiting data (lumbar puncture, blood cultures, and radiographic studies). Noninfectious etiologies, including calcineurin inhibitor toxicity, lymphoma, and metastatic cancer, should be included in the differential diagnosis. Molecular assays (HSV) and biopsy (for noninfectious etiologies) may be needed for diagnosis.

Cryptococcal infection is rarely seen in the transplant recipient until more than 6 months after transplantation. In the relatively intact transplant recipient, the most common presentation of cryptococcal infection is that of an asymptomatic pulmonary nodule, often with active organisms present. In the "chronic ne'er-do-well" patient, pneumonia and meningitis are common, with skin involvement at sites of tissue injury (catheters) and in prostate or bone also reported.

DIAGNOSIS AND TREATMENT

Cryptococcosis should be suspected in transplant recipients who present with unexplained headaches (especially when accompanied by fevers), decreased state of consciousness, failure to thrive, or unexplained focal skin disease (which requires biopsy for culture and pathological evaluation) more than 6 months after transplantation. Diagnosis is often achieved by serum cryptococcal antigen detection, but all such patients should have lumbar puncture for cell counts and cryptococcal antigen studies. Initial treatment is probably best with liposomal amphotericin and flucytosine (after obtaining serum levels) followed by high-dose fluconazole until the cryptococcal antigen is cleared from blood and cerebrospinal fluid. Scarring and hydrocephalus may be observed.

Pneumocystis *and Fever with Pneumonitis*

The spectrum of potential pathogens of the lungs in the transplant recipient is too broad for this discussion. Some general concepts are worth mentioning, however. As for all infections in transplantation, invasive diagnostic techniques are often necessary in these hosts. The depressed inflammatory response of the immunocompromised transplant patient may greatly modify or delay the appearance of a pulmonary lesion on radiograph. Focal or multifocal consolidation of acute onset is likely to be caused by bacterial infection. Similar multifocal lesions with subacute to chronic progression are more likely secondary to fungi, tuberculosis, or nocardial infections. Large nodules are usually a sign of fungal or nocardial infection, particularly if they are subacute to chronic in onset. Subacute disease with diffuse abnormalities, either of the peribronchovascular type or miliary micronodules, are usually caused by viruses (especially CMV) or *Pneumocystis*.[20,21]

Additional clues can be found by examining pulmonary lesions for cavitation, which suggests necrotizing infection as may be caused by fungi (*Aspergillus* or *Mucoraceae*), *Nocardia, Staphylococcus*, and certain gram-negative bacilli, most commonly *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.[37,38] CT of the chest is useful when the chest radiograph is negative or when the radiographic findings are subtle or nonspecific. CT also is essential to the definition of the extent of the disease process, to the discernment of the possibility of simultaneous processes (superinfection), and to the selection of the optimal invasive technique to achieve pathological diagnosis.

The risk of infection with *Pneumocystis* is greatest in the first 6 months after transplantation and during periods of increased immunosuppression.[18,20,21] In patients not receiving trimethoprim/sulfamethoxazole (or alternative drugs) as prophylaxis, most transplant centers report an incidence of *Pneumocystis* pneumonia of approximately 10% in the first 6 months after transplantation. There is a continued risk of infection in three overlapping groups of transplant recipients: (1) recipients who require higher than normal levels of immunosuppression for prolonged periods because of poor allograft function or chronic rejection; (2) recipients with chronic CMV infection; and (3) recipients undergoing treatments that increase the level of immunodeficiency, such as cancer chemotherapy or neutropenia secondary to drug toxicity. The expected mortality secondary to *Pneumocystis* pneumonia is increased in patients on cyclosporine compared with other immunocompromised hosts.

The hallmark of infection resulting from *P. carinii (jiroveci)* is the presence of marked hypoxemia, dyspnea, and cough with a paucity of physical or radiological findings. In the transplant recipient, *Pneumocystis* pneumonia is generally acute to subacute in development. Atypical *Pneumocystis* infection (radiographically or clinically) may be seen in patients who have coexisting pulmonary infections or who develop disease while receiving prophylaxis with second-choice agents (e.g., pentamidine or atovaquone). Patients outside the usual period of greatest risk for *P. carinii (jiroveci)* pneumonia may present with indolent disease, which may be radiographically confused with heart failure. In such patients, diagnosis often has to be made by invasive procedures. The role of rapamycin therapy in the clinical presentation is unknown. Numerous patients have been identified with interstitial pneumonitis while receiving rapamycin.[9] This syndrome may occur in the presence or absence of concomitant infections (adenovirus, respiratory syncytial virus, *Pneumocystis*).

DIAGNOSIS, THERAPY, AND PROPHYLAXIS

The characteristic hypoxemia of *Pneumocystis* pneumonia produces a broad alveolar-arterial partial pressure of oxygen gradient. The level of serum lactate dehydrogenase is elevated in most patients with *Pneumocystis* pneumonia (>300 IU/mL). Many other diffuse pulmonary processes also increase serum lactate dehydrogenase levels, however. No diagnostic pattern exists for *Pneumocystis* pneumonia on routine chest radiograph. The chest radiograph may be entirely normal or develop the classic pattern of perihilar and interstitial ground-glass infiltrates. Chest CT scans are more sensitive to the diffuse interstitial and nodular pattern than routine radiographs. The clinical and radiological manifestations of *P. carinii (jiroveci)* pneumonia are virtually identical to the manifestations of CMV. The clinical challenge is to determine whether both pathogens are present. Significant extrapulmonary disease is uncommon in the transplant recipient. Bronchoalveolar lavage may be helpful.

Early therapy with trimethoprim/sulfamethoxazole is preferred; few renal transplant patients tolerate full-dose trimethoprim/sulfamethoxazole for prolonged periods. This reflects the elevation of creatinine owing to trimethoprim (competing for secretion in the kidney), and the toxicity of sulfa agents for the renal allograft. Hydration and the gradual initiation of therapy may help. Alternative therapies are less desirable but have been used with success, including intravenous pentamidine, atovaquone, clindamycin with primaquine or pyrimethamine, and trimetrexate. Although a reduction in the intensity of immunosuppression is generally considered a part of anti-infective therapy in transplantation, the use of short courses of adjunctive steroids with a gradual taper is generally useful.

The importance of preventing *Pneumocystis* infection cannot be overemphasized. Low-dose trimethoprim/sulfamethoxazole is well tolerated and should be used in the absence of concrete data showing true allergy or interstitial nephritis. Alternative prophylactic strategies, including dapsone, atovaquone, and inhaled or intravenous pentamidine, are less effective than trimethoprim/sulfamethoxazole but are useful in patients with significant allergy to sulfa drugs. Trimethoprim/sulfamethoxazole is the most effective agent for prevention of infection caused by *P.carinii (jiroveci)*. The advantages of trimethoprim/sulfamethoxazole include increased efficacy; lower cost; availability of oral preparations; and possible protection against other organisms, including *T. gondii, Isospora belli, Cyclospora cayetanensis, Nocardia asteroides*, and common urinary, respiratory, and gastrointestinal bacterial pathogens. Alternative agents lack this spectrum of activity.

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Chapter 30

Liver Disease in Renal Transplant Recipients

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OVERVIEW OF INCIDENCE AND CLINICOPATHOLOGICAL ASSOCIATIONS OF LIVER DISEASE IN RENAL TRANSPLANT RECIPIENTS

Theoretically, the spectrum of liver disease in renal transplant recipients should mimic the spectrum of disease seen in society. It is axiomatic that renal transplant recipients are at risk for all the acute and chronic liver disorders seen in the nontransplant population. Surveys of the prevalence of chronic liver injury in otherwise healthy subjects suggest that the burden of unrecognized liver disease in the apparently healthy community is high. Among 6917 individuals 12 to 65 years old,[20] 21% had elevated liver biochemistries, and 17.5% displayed convincing features of chronic liver disease after more extensive investigation. Alcohol abuse was the etiological agent in 23%; chronic viral hepatitis in 5%; cirrhosis in 1%; and hepatocellular cancer in 0.07%. This evaluation should be interpreted in relation to time (March 1991 through March 1993) and place (two towns in northern Italy).

A more recent study by Ioannou and colleagues[134] used the National Health and Nutrition Examination Survey (NHANES) conducted between 1999 and 2002 to assess the prevalence of elevated serum transaminase activities in a cohort of 6823 American adults. The prevalence of elevated alanine aminotransferase (ALT) was 8.9%, a result that is more than double that of previously available estimates in similar populations. The prevalence of elevated ALT among individuals without viral hepatitis C or excessive alcohol consumption was 7.3% and was strongly associated with risk factors for nonalcoholic fatty liver disease.

The two above-mentioned studies indicate the potential hazards in estimating the likely prevalence of liver disease in a special population, such as recipients of renal transplantation, in the absence of good data. The increase in nonalcoholic steatohepatitis, the recognition of chronic hepatitis C virus (HCV), and possible changing use of alcohol means that a contemporary assessment of the spectrum of liver disease might be quite different from previous reports, and in one country compared with another.[161,162] Consequently, there have been no comprehensive attempts to characterize liver disease in renal transplant recipients since Allison and associates[6] examined the prevalence and nature of chronic liver disease among 538 patients with functioning renal allografts managed in Scotland between 1980 and 1989. They reported that biochemical evidence of liver dysfunction was observed in 37 patients (7%), 19 (4%) of whom were seropositive for HCV. In addition, histological evidence of hemosiderosis or nodular regenerative hyperplasia was found in a few patients. The work of Allison and associates is most likely an underestimate, given that it was undertaken just as HCV infection was discovered, and, as discussed subsequently, HCV prevalence in renal transplant cohorts has been reported to be 30%.

This chapter discusses some liver disorders that seem to occur in greater frequency in renal transplant recipients compared with the background population. In some circumstances, such as autosomal dominant polycystic disease, the liver and kidney disorders are part of the same underlying disease. In other patients in whom renal failure coexists with liver disease, the two conditions are acquired separately. Chronic infections with hepatotropic viruses (hepatitis B virus [HBV] and HCV) fall into this category.

Liver diatheses that are consequences of the inherent risks of the transplant process are addressed. These particularly relate to the consequences of immunosuppressant medications, either directly, such as focal nodular hyperplasia resulting from azathioprine, or secondary to the effects of immunosuppression, such as infection by cytomegalovirus (CMV),

herpes simplex virus (HSV), and Epstein-Barr virus (EBV) and its related post-transplant lymphoproliferative disorder (PTLD). Liver disease and renal transplantation are linked when renal transplantation is undertaken for renal failure arising in a liver transplant recipient; this is usually due to calcineurin inhibitor renal toxicity (see Chapters 16 and 17).

COMBINED LIVER AND KIDNEY DISEASES

Polycystic Disease

Autosomal dominant polycystic disease is a condition arising from mutations in two distinct genes that result in the development of the renal and liver cysts. Mutations in *AD-PKD1* account for 90% of adult-onset combined kidney and liver polycystic disease, and mutations in *AD-PKD2* account for the remainder.[82] A variant form that is manifested by cysts confined to the liver is due to mutations in an unrelated gene. Patients with mutations in *PKD2* tend to have later onset of disease and approximately 16 years of increased life expectancy compared with patients who have mutations in *PKD1*, but otherwise the natural history is identical regardless of whether *PKD1* or *PKD2* is the mutated gene. Renal cystic disease associated with autosomal dominant polycystic disease may develop into renal failure that requires hemodialysis or renal transplantation. The severity of hepatic cystic disease correlates with the severity of renal cystic disease and the degree of renal dysfunction.

Hepatic cysts are lined with secretory biliary epithelium. These cysts are first noted after puberty. The lifetime risk for expression of hepatic cysts is equal in male and female holders of the genetic defect, but hepatic cysts tend to be larger and more numerous in women. Rapid growth of hepatic cysts under the influence of exogenous estrogens or during pregnancy is well described. Sherstha and coworkers[233] reported that this influence of estrogens was confined to hepatic cyst growth, while sparing kidney cysts.

Symptoms caused by hepatic cysts in adult-onset autosomal dominant polycystic disease are the result of a compartment disorder in which the abdominal cavity is unable to accommodate the cystic mass. Patients with small cysts are asymptomatic. Patients with massive hepatic cysts may experience abdominal pain, early satiety, or dyspnea (Fig. 30-1).

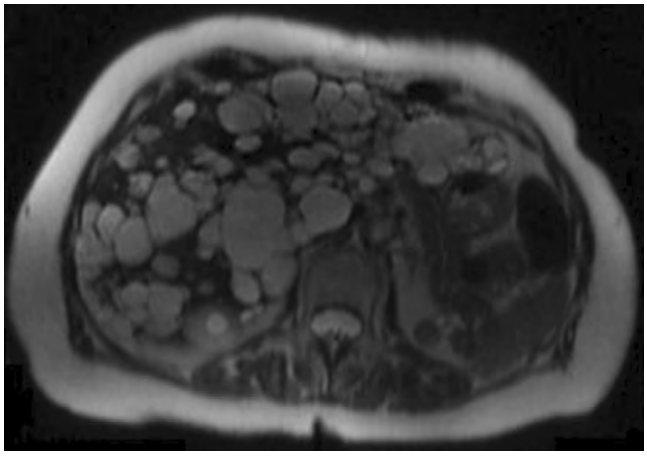

Figure 30–1 The liver has innumerable cysts ranging from small to large in a patient with autosomal dominant polycystic liver/kidney disease.

These "bulk" symptoms may be so troubling as to warrant liver transplantation. Hepatic function and portal hemodynamics are usually normal even in patients with large symptomatic hepatic cysts. Biliary obstruction, portal hypertension, ascites, variceal hemorrhage, and encephalopathy are rare features of autosomal dominant polycystic disease.

There is no good medical therapy for the abdominal symptoms associated with autosomal dominant polycystic disease. Exogenous somatostatin, with or without cyst drainage, is ineffective. Advice for women with symptomatic cysts regarding continuing or stopping oral contraceptive or hormone replacement therapy is largely anecdotal, but it is reasonable to suggest that such patients should give consideration to stopping these agents. Many procedures are described to ameliorate the discomfort associated with liver cysts. Cyst aspiration under sonographic guidance provides temporary relief, but the cysts inevitably recur. Continuous or intermittent drainage through a permanent percutaneous catheter should be strongly discouraged because it runs the risk of converting a sterile cyst into a pyogenic abscess. Surgical approaches include open or laparoscopic cyst fenestration, hepatic resection, and liver transplantation.

Drug-Induced Hepatotoxicity

Drug-induced liver disease can have a wide spectrum ranging from asymptomatic elevations of liver enzymes to acute liver failure with rapid clinical deterioration. With rare exceptions, the histological patterns of liver injury are not diagnostic of drug-related injury. Rather, drug-induced injury is often diagnosed based on a combination of a temporal relationship to a particular drug use, exclusion of other concurrent of preexisting causes of liver dysfunction (e.g., viral hepatitis), and knowledge of common patterns of liver test abnormalities associated with particular drugs.[12,147,172] Improvement of liver tests with discontinuation of the offending medications is further evidence of drug-induced hepatotoxicity, but in certain cases the injury may take weeks to improve after cessation of the medication. Return of the liver injury on rechallenge with the medication confirms the suspicion of drug-induced hepatotoxicity, but this is rarely done in clinical practice.

The severity of drug-related injury is predicted by the degree of impairment of hepatic function. In particular, the presence of jaundice in association with elevated aminotransferases is often an ominous sign of significant hepatocellular injury.[163,194] The severity and specific type of histological injury also can be ascertained by findings on a liver biopsy specimen.

The most common pattern of liver function test abnormalities is acute hepatocellular injury with elevations of aminotransferases greater than twofold normal with lesser elevations of alkaline phosphatase.[266] This pattern is seen with a multitude of medications used in the transplant setting, including immunosuppressive medications,[81] antibiotics,[123] antihyperlipidemics,[200] and drugs for hypertension and diabetes.[29,254]

The mechanisms of drug injury are multiple as well. Toxic metabolites produced by detoxification of medications through the liver, most commonly via cytochrome P-450 mechanisms, may contribute to dose-related hepatotoxicity, such as seen with acetaminophen.[145,266] Other medications may have immunological mechanisms of injury that are

non–dose-related and idiosyncratic.[147] The patient may present with nonspecific systemic symptoms, such as fever, lymphadenopathy, and leukocytosis, and often eosinophilia such as can be seen with phenytoin or nitrofurantoin.[57,148] Other medication toxicities may manifest with a cholestatic pattern of injury that can be acute but may in some cases progress to chronicity and progressive intrahepatic duct loss despite withdrawal of the offending agent. Amoxicillin/clavulanic acid (Augmentin) may produce this kind of injury.[271] Still other drugs may predispose to progressive bland fibrosis ultimately leading to fibrosis, such as seen with methotrexate[173] and high doses of vitamin A.

In transplant patients, the opportunities for drug-related hepatotoxicity abound owing to the use of multiple medications, many of which are metabolized via the same pathways in the liver, increasing the risk of accumulation of hepatotoxic metabolites. Table 30-1 lists common medications that stimulate or block the cytochrome P-450 system within the liver and may influence the serum concentrations of other drugs and their metabolites. Other risk factors for drug-induced liver disease include older age and concomitant alcohol use.[146,163,266]

SPECIFIC IMMUNOSUPPRESSIVE AGENTS IN RENAL TRANSPLANTATION AND HEPATOTOXICITY

Azathioprine (See Chapter 15)

Azathioprine is an antimetabolite agent that is a purine synthesis inhibitor. It is the prodrug of mercaptopurine and inhibits DNA and RNA synthesis. It has been used in solid organ transplantation since the 1960s.[187] A broad range of hepatotoxicity has been associated with the use of azathioprine in renal transplant recipients ranging from vascular lesions (peliosis hepatis, veno-occlusive disease, nodular regenerative hyperplasia) to intrahepatic cholestasis and hepatitis.[31,67,69,81,174,179,188,231] The pathogenesis of azathioprine hepatotoxicity is multifactorial, resulting from endothelial damage,[114] direct hepatotoxicity,[14] and interlobular bile duct injury.[128] Most of these complications are rare.

One of the most lethal complications is the rare occurrence of veno-occlusive disease with obliteration and fibrosis of the central hepatic venule and sinusoidal congestion.[179] Patients present with jaundice, ascites, hepatomegaly, and elevated liver enzymes (typically alkaline phosphatase with minimal increases in aminotransferases). In the first few months after kidney transplantation, veno-occlusive disease can manifest with asymptomatic hyperbilirubinemia and elevated liver enzymes; progression to jaundice, hepatomegaly, and ascites occurs after the first year, often by 3 to 6 years after transplant.[196] The diagnosis is based on the histological appearance.[164] Veno-occlusive disease is associated with a high mortality because of complications of portal hypertension and associated liver failure.[164] With cessation of azathioprine, it rarely has been reported to regress.[152] Other authors have reported successful use of portosystemic shunts (e.g., transjugular intrahepatic portosystemic shunts) for management of portal hypertension with normalization of liver function over the subsequent year.[17]

Other vascular diseases of the liver also have been attributed to azathioprine, including peliosis hepatis (dilated blood-filled cavities within the liver) presumably secondary to endothelial injury within the liver leading to sinusoidal dilation. Nodular regenerative hyperplasia, which can be associated with peliosis and veno-occlusive disease, is rarely seen, and by the time it occurs, portal hypertension with complications of ascites and variceal hemorrhage is often present.[35]

Azathioprine-induced hepatitis has been reported more frequently in kidney transplant recipients with chronic viral hepatitis. In one study of 1035 transplant recipients, 21 fulfilled the criteria for azathioprine hepatitis with jaundice at presentation. Viral hepatitis markers (HCV, HBV, or both) were present in all 20 recipients who were tested. The jaundice disappeared and liver enzymes normalized in all within 4 to 12 weeks of azathioprine discontinuation or dosage reduction. After rechallenge with azathioprine in four patients, jaundice recurred in all four with reappearance of the histological changes.[204] In some of these patients, histological findings were more consistent with azathioprine toxicity than viral hepatitis with intrahepatic cholestasis, centrilobular hepatocellular necrosis, and vascular lesions. Most patients had chronic liver disease secondary to viral hepatitis on histology (18 of 21).

In two patients who underwent repeat liver biopsy 2 and 4 months after withdrawal of azathioprine, histology revealed disappearance of intrahepatic cholestasis and centrilobular hepatocellular necrosis. Rechallenge with azathioprine led to relapse of jaundice and recurrence of azathioprine-associated lesions on liver biopsy specimens. It is difficult to ascertain if the increase in liver enzymes after rechallenge was due to accelerated viral hepatitis in the setting of increased immunosuppression or due to azathioprine toxicity.

In renal transplant recipients with chronic viral hepatitis (HCV, HBV), a policy of azathioprine withdrawal (versus dosage reduction) was associated with greater reductions in elevated bilirubin and aminotransferases compared with baseline, and cirrhosis was seen more frequently in the group with azathioprine dosage reductions only compared with complete withdrawal. The study is limited, however, by

Table 30–1 **Medications That Stimulate or Inhibit the Cytochrome P-450 System and Can Influence the Level of Other Medications Such as Cyclosporine**

Medications that stimulate cytochrome P-450 and can decrease the level of cyclosporine
Trimethoprim/sulfamethoxazole
Isoniazid
Nafcillin
Phenytoin
Carbamazepine
Omeprazole
Medications that inhibit cytochrome P-450 and can increase the level of cyclosporine
Diltiazem
Fluconazole
Tetracycline
Tacrolimus
Sex hormones
Metoclopramide

lack of staging of underlying liver disease at baseline.[65] It has been suggested that in patients with viral hepatitis–associated chronic inflammation, there is reduced catabolism and higher levels of toxic azathioprine metabolites in the liver with resultant increases in rates of fibrosis and cirrhosis and hepatotoxicity.[204,205]

Other potential mechanisms include accelerated course of viral hepatitis owing to the use of more potent immunosuppressive regimens (prednisone/azathioprine/cyclosporine) with improvements occurring as a result of withdrawal of immunosuppression. These theories are difficult to prove. Nevertheless, in solid organ transplantation of viral hepatitis recipients, it is a good policy to use minimal immunosuppression (single or dual regimens rather than triple regimens) to minimize acceleration of viral hepatitis–associated liver disease.

Calcineurin Inhibitor–Induced Hepatotoxicity (See Chapters 16 and 17)

Cyclosporine and tacrolimus are immunosuppressive medications that belong to the class of calcineurin inhibitors.[41,193] They both bind to immunophilins within the cell, and the drug-immunophilin complex binds to calcineurin, which is a serine-threonine phosphatase important in the lymphocyte-activated generation of cytokines that are important in further stimulation of lymphocytes. By binding calcineurin and preventing its phosphatase activity, the calcineurin inhibitors prevent activation of lymphocytes through the cytokine pathway.[245]

Cyclosporine-induced hepatotoxicity is uncommon. Cyclosporine is metabolized via the cytochrome P-450 system, and interactions with medications that inhibit or stimulate this pathway can result in increased or decreased cyclosporine levels, increasing the risk for hepatotoxicity.[104] Commonly used medications that inhibit the cytochrome P-450 system include ketoconazole, fluconazole, erythromycin, and diltiazem; trimethoprim/sulfamethoxazole, isoniazid, phenytoin, and phenobarbital can induce the cytochrome P-450 system and decrease cyclosporine levels.[16] Cyclosporine-induced decrease in bile flow can result from reduced bile acid secretion and is associated with risk of bile duct stones and sludge formation in 2% to 5% of transplant recipients.[169] Rarely, increases in aminotransferases have occurred, mostly in the first 90 days, which respond to dosage reduction. Persistent elevations in aminotransferases are rare, occurring in less than 5% to 10% of renal transplant recipients.[111,203] Transient elevations of bilirubin or aminotransferases are more common, occur early (within the first 3 months after transplantation), and are reversible with dosage reductions or discontinuation.[169]

Among renal transplant recipients without preexisting liver disease, azathioprine-treated patients had a higher incidence of post-transplant chronic liver disease compared with cyclosporine-treated patients.[185] In patients with chronic viral hepatitis and liver transplantation, there is some evidence that cyclosporine may be associated with less progression of viral-induced liver disease than non–cyclosporine-based immunosuppression,[23] although the data are mixed with no difference in patient and graft outcomes in cyclosporine-based versus tacrolimus-based immunosuppression in several large trials.[98,247] In vitro data have suggested direct antiviral activity of cyclosporine on hepatitis C that is distinct from its immunosuppressive action and is not seen with tacrolimus[265] and may be related to blocking of the peptidyl-prolyl isomerase activities of cytochrome P.[192] No evidence exists in renal transplant recipients that examines the outcome of HCV-related liver disease in patients treated with cyclosporine versus tacrolimus.

The mechanisms of cyclosporine toxicity are incompletely understood. Cyclosporine-induced hepatotoxicity may be related to the increase in total intracellular calcium concentration.[190] In the isolated perfused rat liver model, cyclosporine administration was associated with a dose-dependent decrease of the bile flow, more precisely of the bile acid–dependent fraction, as a result of inhibition of bile acid secretion. Cyclosporine had no effect on alkaline phosphatase concentrations, and there were no significant differences in the transaminase levels between the cyclosporine and the control groups. Light microscopy did not reveal any histological evidence of cholestasis or hepatocellular damage.[218]

Tacrolimus has an immunosuppressive mechanism of action similar to cyclosporine.[41] In renal transplant recipients, tacrolimus is associated with fewer episodes of acute rejection, need for salvage immunosuppressive therapy, or ductopenic rejection than cyclosporine. The overall patient and graft survival rates are similar to rates seen with cyclosporine.[203] In HCV-positive liver transplant recipients, liver fibrosis seems to be less accelerated in cyclosporine-treated patients compared with tacrolimus-treated patients, possibly related to some antiviral activity of cyclosporine against HCV.[132] Patient and graft survivals are similar, however, in cyclosporine-treated versus tacrolimus-treated HCV-positive organ recipients.[22] Similar to cyclosporine, tacrolimus levels were higher in HCV-positive renal transplant recipients presumably secondary to impaired cytochrome P-450–related metabolism of tacrolimus.[175] In contrast to cyclosporine, tacrolimus is not associated with reduction in bile flow nor with choledocholithiasis. Also, tacrolimus was associated with less hyperbilirubinemia (0.3%) compared with cyclosporine (3.3%) in renal transplant recipients in a large comparative trial.[178] Elevations in aminotransferases are generally mild, even with supratherapeutic levels,[119] and reversible with dose reduction.

Sirolimus (See Chapter 19)

Sirolimus-induced hepatotoxicity is rare. Elevations of aminotransferases with nonspecific histological changes have been reported. These have resolved with discontinuation of sirolimus and changing to another agent, such as mycophenolate mofetil.[191] Sirolimus hepatotoxicity has been better described in liver transplant recipients. Of 10 patients treated with sirolimus, two had sinusoidal congestion, and one had eosinophilia consistent with a drug-related allergic reaction. Increase in aminotransferases that occurred was less than fivefold, occurred within 21 days of sirolimus initiation and resolved within 27 days of discontinuation of the drug.[189] Cyclosporine can interfere with sirolimus pharmacokinetics and increase its serum concentration, an interaction not seen with tacrolimus.[269] Caution must be exercised, and sirolimus levels must be monitored carefully when calcineurin inhibitors are switched because it may predispose to sirolimus toxicity.

Mycophenolate Mofetil (See Chapter 18)

Mycophenolate mofetil is an ester of mycophenolic acid that is readily absorbed. It inhibits purine synthesis by noncompetitively inhibiting a key enzyme in the de novo purine pathway, inosine monophosphate dehydrogenase. Hepatotoxicity is rare and has been noted in case reports.[170]

Monoclonal Antibodies (See Chapter 20)

Monoclonal antibodies have been used as induction immunosuppression in solid organ transplantation. Use of alemtuzumab (Campath), anti-CD52 humanized antibody, has been shown to accelerate hepatic fibrosis in HCV-infected liver transplant recipients and generally should be avoided in solid organ recipients with chronic viral hepatitis.[177] Anti-CD3 antibodies, which are used less often now for salvage of refractory rejection, have rarely been associated with severe hepatitis with 20-fold elevation of aminotransferases.[107] Cytokine-mediated reactions presumably can cause the occasional hepatotoxicity seen with anti-CD3 antibodies.

HEPATITIS VIRUSES ASSOCIATED WITH RENAL TRANSPLANTATION

Hepatitis B Virus

Viral Structure and Proteins

HBV is a hepatotropic, enveloped, partially double-stranded DNA virus that is a member of the hepadnavirus family.[226] The core of the virus comprises of an RNA-dependent DNA polymerase plus a partially double-stranded DNA. After entry into the hepatocyte, HBV enters the nucleus and forms what is known as covalently closed circular DNA. This DNA is produced by repair of the gapped virion DNA and is the likely source of the transcripts used to produce the viral proteins. The genome of HBV encodes four different genes. The C gene encodes the hepatitis B core antibody (HBcAb), the P gene encodes the hepatitis B polymerase, the S gene encodes three different polypeptides of the surface antigen or protein (pre S1, pre S2, and S), and the X gene encodes proteins potentially involved in transactivation of viral replication.

The hepatitis B viral antigens consist of the hepatitis B core antigen (HBcAg) and a subunit of the core called the hepatitis B early antigen (HBeAg). The HBeAg is released in high concentrations in the plasma during viral replication and is an indirect marker of active viral replication. The envelope protein is referred to as the hepatitis B surface antigen (HBsAg) and is likely responsible for viral binding to the hepatocyte. HBsAg is released in excess in the serum in individuals with chronic HBV infection. Its presence in individuals 6 months after exposure to HBV defines the presence of chronic HBV infection.

Tests for Detection of Hepatitis B Virus

HBV can cause acute and chronic infections. Acute infection is associated with acute hepatitis characterized by acute inflammation and hepatocellular necrosis. The diagnosis rests on detecting HBsAg in the serum of a patient with clinical and laboratory evidence of acute hepatitis (Table 30-2). Patients with a silent, self-limiting infection are able to produce protective antibody (hepatitis B surface antibody [HBsAb]) and ultimately clear the virus. These patients are negative for HBsAg but are positive for HBsAb and HBcAb.

Table 30–2 **Commonly Used Tests for Detection of Hepatitis B Infection and Their Interpretation**

HBsAg	anti-HBs	anti-HBc	Interpretation
+	–	–	Early acute infection
+	–	+	Acute or chronic infection
–	+	+	Cleared HBV infection—immune
–	+	–	Vaccine response—immune

anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen; HBsAg, hepatitis B surface antigen.

Chronic HBV infection is accompanied by evidence of hepatocellular injury and inflammation and is associated with chronic hepatitis. The diagnosis is made by showing persistently elevated serum transaminases and HBsAg in the serum at least 6 months after exposure to HBV infection. Active viral replication is manifested by the presence of HBeAg and high levels of circulating HBV DNA. Eventually, years after initial infection, viral replication may diminish; HBeAg is replaced by antibody to hepatitis B early antigen (anti-HBe), whereas HBsAg and antibody to hepatitis B core antigen (anti-HBc) persist, and HBV viral load may be low or undetectable.

Not all patients with chronic HBV infection develop chronic hepatitis, and some ultimately enter a phase of remission with improvement in liver enzymes despite persistence of HBsAg. These individuals usually are referred to as healthy chronic HBsAg carriers. This terminology is misleading because these patients are at risk for reactivation of infection, and if cirrhosis has already developed, they also are at risk for developing hepatocellular carcinoma (HCC). Some individuals with long-standing infection who are negative for HBeAg and positive for anti-HBe have high serum HBV DNA levels. These individuals have a mutation in HBeAg that prevents its release from the hepatocyte (precore mutant). They continue to have a high risk for cirrhosis and HCC development. Vaccinated individuals are positive for antibody to hepatitis B surface antigen (anti-HBs) only.

Epidemiology of Hepatitis B Virus

ROUTES OF TRANSMISSION

HBV is widespread worldwide with more than 1 billion individuals estimated to be exposed to the virus. Areas of high incidence include China, Southeast Asia, and sub-Saharan Africa.[268] In the United States, more than 1 million individuals are estimated to have chronic infection.[225] HBV is transmitted through the parenteral or sexual routes; transmission via the fecal-oral route does not occur. In countries with a high prevalence of HBV infection, the route of transmission is mainly vertical, at childbirth, or to a lesser degree horizontally among household contacts in the first decade of life. In countries with a lower of prevalence of HBV infection, most infections occur in adulthood and are transmitted sexually and to a lesser extent by intravenous drug use.[5]

NATURAL HISTORY OF HEPATITIS B VIRUS INFECTION

HBV can result in a self-limited acute infection or can progress to chronic liver disease. Progression to chronic

HBV infection after acute infection depends on the age at exposure to the virus. Vertical transmission of HBV is clinically silent but often becomes chronic (in >90% of neonates). Transmission in adulthood is associated with clinically apparent hepatitis in greater than 30% of individuals, but most immunocompetent adults clear the virus (85% to 90%).[5,225] Acute infection in adults when clinically apparent is often associated with jaundice and elevated aminotransferases, with liver histology revealing portal inflammation, interface hepatitis, and lobular inflammation. Chronic infection is associated with varying degrees of portal and lobular inflammation and fibrosis. The jaundice resolves, and aminotransferases are more modestly elevated. In immunosuppressed individuals, including dialysis patients, serum aminotransferases are often normal and are not reliable markers of histological activity of the virus. Liver biopsy is recommended in the pretransplant evaluation of these patients to stage the degree of fibrosis accurately.[83]

The natural history of chronic HBV infection depends on the age at which infection occurs. After perinatally transmitted infection, there is an immune tolerant phase in which high levels of viral replication (with high serum HBV DNA levels) are accompanied by only minimal damage on liver biopsy specimen and normal serum liver enzymes. The immune tolerant phase can last from the first to the third decade of life, after which transition occurs to the immune clearance phase. In this phase, elevated levels of liver enzymes and high serum HBV DNA levels occur. Immune clearances can fail and lead to recurrent phases of HBV replication accompanied by surges of serum HBV DNA and aminotransferases, which increase the risk of cirrhosis and HCC. Some patients can enter further into a nonreplicative phase with disappearance of HBeAg from serum and development of anti-HBe. These patients have detectable HBsAg and may have low levels of HBV viremia. The prognosis in these patients depends on the underlying fibrosis and stage of liver disease (cirrhosis versus not) at the time viral replication ceases. They remain at risk for HCC.[274]

The outcomes of chronic HBV infection vary from an inactive carrier state to cirrhosis and its attendant complications, such as variceal hemorrhage, ascites, and encephalopathy. In addition, the risk of HCC is elevated in chronic HBV even in the absence of cirrhosis and correlates with the serum viral load. The risk of cirrhosis and HCC in chronic HBV also is elevated with concomitant alcohol use and infection with other hepatotropic viruses, such as hepatitis D virus or human immunodeficiency virus (HIV). Increasing duration of infection, male gender, and recurrent phases of viral replication all increase the risk for development of HCC and cirrhosis.[274]

HEPATITIS B VIRUS INFECTION IN PATIENTS ON DIALYSIS AWAITING RENAL TRANSPLANTATION

The incidence and prevalence of HBV infection among patients awaiting renal transplantation have declined in recent years largely as a result of vaccination of patients on dialysis and improved infection control measures during dialysis. Before HBV vaccination, 3% to 10% of patients on dialysis developed this disease,[276] with even higher incidences reported in countries with a high prevalence of HBV infection. In one European study, the prevalence of renal transplant recipients with HBV infection decreased from 3% in 1990 to 2% in 1998.[184] About 1% of patients now on dialysis in the United States are infected with HBV.[97] The incidence and prevalence of HBV in end-stage renal disease patients in developing countries remain high.[54]

Universal vaccination of dialysis patients, although recommended, is not undertaken, with one survey of 12 centers from 11 countries showing routine vaccination of nonimmune subjects in only 66.7% (8 of 12) centers. Vaccination has a lower "take rate" in immunosuppressed end-stage renal disease patients with 50% to 60% of dialysis patients developing adequate titers of anti-HBs antibodies[8,230] and is best undertaken early in the course of dialysis.[8] Despite lower rates of anti-HBs development, there is some evidence that vaccination confers protective T cell responses, and there are reduced rates of HBV infection even if anti-HBs antibodies are not detected in vaccinated dialysis patients.[4]

Higher doses of vaccine may be required, and annual testing of anti-HBs titers should be undertaken with boosters given whenever the anti-HBs titer is less than 10 IU/L. Infection control practices including dedicated dialysis machines and staff for HBV-positive patients are implemented in some units, but not universally, and remain controversial. With these measures, the incidence of HBV infection in dialysis patients has decreased considerably in recent years to approximately 1%.

In dialysis patients, acute exposure to HBV results in chronic infection in most nonvaccinated individuals (80%), likely owing to their immunocompromised state and inability to mount protective antibody and T cell responses.[105] Clinical and histological outcomes in dialysis patients with HBV infection are generally similar to the outcomes seen in immunocompetent individuals. Most of these individuals do not die from liver disease. In one study of dialysis patients in which 30% were infected with HBV, less than 5% died from liver disease. This low percentage may be due to the presence of other comorbidities (competing causes of mortality), such as cardiovascular disease or infections, or due to insufficient length of follow-up.[138]

Pretransplant Management of Hepatitis B Virus–Positive Dialysis Patients

Liver enzymes (aminotransferases) do not accurately reflect the stage of liver disease in patients with chronic viral hepatitis and end-stage renal disease. Patients with chronic HBV on dialysis should undergo liver biopsy for accurate assessment of liver fibrosis (staging) before renal transplantation. Patients with cirrhosis on the biopsy specimen should not be offered renal transplantation alone. Options include remaining on dialysis until there is evidence of portal hypertension and listing for simultaneous liver-kidney transplantation.

Criteria for antiviral therapy in nontransplant patients have included a serum HBV viral load greater than 100,000 copies/mL with evidence of elevated aminotransferases (aspartate aminotransferase [AST], ALT). In patients undergoing renal transplantation, there is increased risk, however, of reactivation of viral replication and increased viral replication after transplantation with exposure to immunosuppressive agents. Because there is worsening of liver disease and worse outcomes of liver disease and renal allograft function after renal transplantation in patients infected with HBV (discussed subsequently), it is prudent to start antiviral therapy before renal transplantation for patients with evidence of active viral replication; this includes patients with positivity for the HBsAg and any detectable viral load.

Lamivudine monotherapy is associated with viral suppression in most patients with end-stage renal disease. The problems with lamivudine include development of resistance with prolonged antiviral therapy, which can result in virological and clinical breakthrough. In one study, Fontaine and colleagues[101] treated five hemodialysis patients with lamivudine for 12 months. HBV DNA became undetectable during treatment in all patients, and one patient developed anti-HBe. Viral breakthrough with re-emergence of serum HBV DNA was seen in two of the five patients at month 7 and 18 of lamivudine therapy.

Post-transplant Prognosis in Hepatitis B Virus–Positive Recipients

After renal transplantation, HBV-infected recipients have decreased survival compared with noninfected recipients. In one study of 1250 renal allograft recipients, with a median follow-up of 125 months, cirrhosis occurred in 30%, and renal allograft survival was reduced compared with recipients without chronic HBV.[105] Overall mortality was similar between HBV-positive and HBV-negative recipients in this study. A study of 51 renal transplant recipients with chronic HBV infection found reduced patient survival and a higher incidence of death resulting from liver failure in the HBV group (44%) compared with non–hepatitis-infected controls (0.6%). In multivariable analysis in the HBV group, the presence of hepatitis B antigen was not an independent predictor of death; patient age, serum creatinine, and proteinuria at 3 months after transplantation were independent predictors of reduced patient survival.[184]

Other large studies have found significant reductions in long-term patient and graft survivals in HBsAg-positive kidney transplant recipients compared with noninfected renal transplant recipients. In a cohort of 128 renal transplant recipients infected with HBV, the 10-year survival was 55% compared with 80% in non–HBV-infected renal transplant recipients.[180] Age at transplant and presence of cirrhosis were independent prognostic factors for survival in this study. Liver disease and sepsis were the major causes of death in the HBV-infected cohort in this study, each accounting for 29% of the deaths in this study. Another study found a significant difference in long-term survival between HBV-positive recipients compared with recipients without chronic viral hepatitis,[15] with a relative risk of mortality of 2.36 for 42 HBsAg-positive recipients. In another study, chronic HBV infection was found to increase the risk of infection in renal transplant recipients.[211] A meta-analysis that included 6050 renal transplant recipients found increased mortality (relative risk of death with HBsAg positivity 2.49) associated with chronic HBV infection and reduced graft survival (relative risk of graft loss 2.49).[87]

Differences in outcomes between studies may result from small numbers in some studies; length of follow-up; heterogeneity of patient characteristics, such as age at transplant, replicative state of HBV, and presence or absence of cirrhosis at time of transplant; and the confounding effect of antiviral therapy for hepatitis B. Studies with larger numbers, longer follow-up, and matched case-control design and multivariate analysis have tended to show a reduction in patient and graft survivals associated with chronic HBV infection in renal transplant recipients.

Risk Factors for Progression of Liver Disease in Renal Transplant Recipients with Hepatitis B Virus Infection

The risk of fatal liver disease after renal transplantation is related to the replicative state of the virus in the recipient. A much higher risk of mortality from liver disease is present in recipients who are HBV DNA–positive or HBeAg-positive compared with recipients who are HBV DNA–negative and HBsAg-positive only.[89] Development of de novo HBV after renal transplantation is associated with rapid viral replication and progression of liver disease.[89] The HBV serological and virological status of the donor and recipient are important risk factors that predict development of de novo HBV infection after renal transplantation. The highest risk of de novo hepatitis exists in recipients who are nonimmune for HBV (HBsAb-negative) and receive an organ from an HBsAg-positive donor.

The risk of transmission from an HBcAb-positive donor (HBsAg-negative, HBcAb-positive, negative serum HBV DNA donor) to an HBV-negative recipient also exists, although it is reduced compared with that seen in liver transplant recipients.[100,171,261] The risk is considerably reduced if the recipient is HBsAb-positive, although not completely eliminated. These patients are less likely to get clinically evident HBV. In one series where HBcAb-positive donors were used for recipients with a prior history of HBV or HBV vaccination, no recipients developed clinically evident HBV, although 27% did develop HBcAb positivity or HBsAb positivity or both after transplant.[171] Prevention of de novo HBV in renal transplant recipients is best achieved by universal vaccination of all dialysis patients. Alternatively, organs from HBsAg-positive recipients can be offered only to recipients with preexisting HBV infection or individuals who have been successfully vaccinated for HBV. Use of HBcAb-positive donors may be restricted to the same individuals.

The risk of clinically evident liver disease and decreased survival also is related to the stage of liver disease at the time of renal transplantation. The presence of cirrhosis was associated with reduced survival in renal transplant recipients.[180] The presence of cirrhosis, if not clinically evident, should be sought on liver biopsy in HBV-positive patients undergoing evaluation for renal transplantation. Cirrhosis is a contraindication for isolated kidney transplantation and should lead to consideration of combined liver-kidney transplantation.

In rare cases, viral replication may become uncontrolled in the setting of immunosuppression after renal transplantation. In this state, the virus may become directly cytopathic and lead to a state of hepatocellular failure with profound cholestasis. The liver biopsy specimen is characteristic with hepatocyte ballooning, cholestasis, and perisinusoidal fibrosis. This condition is called fibrosing cholestatic hepatitis and was first described in liver transplant recipients infected with HBV.[66] When established, the prognosis is poor even with antiviral therapy. Preemptive suppressive antiviral therapy is the judicious strategy to prevent this feared outcome. In rare cases, suppression of viral replication with long-term antiviral therapy has resulted in salvage of liver and graft function (discussed later).

Antiviral Therapy of Chronic Hepatitis B Virus in Renal Transplant Recipients

Renal transplant recipients with active HBV (HBsAg-positive) should be started on antiviral therapy at the time of

transplantation or even during dialysis to prevent worsening of liver disease after transplantation (Table 30-3). In one trial, the efficacy of lamivudine in preventing viral replication after renal transplantation was compared in HBsAg-positive recipients using three strategies: (1) preemptive lamivudine therapy (HBV DNA–positive recipient received lamivudine therapy 0 to 9 months before renal transplantation, $n = 7$), (2) prophylactic lamivudine therapy (HBV DNA–negative recipient received lamivudine therapy before transplantation, $n = 3$), and (3) salvage therapy (HBV DNA–positive recipient, with advanced hepatic dysfunction after transplantation, received lamivudine after transplant, $n = 6$).[117] HBV DNA disappeared in all recipients in all groups on therapy. The recurrence rate of HBV viremia was 10% (1 of 10) in the preemptive and prophylactic group compared with 42% (11 of 25) in a non–lamivudine-treated group. In the group treated for hepatic dysfunction, HBV DNA disappeared in all six cases but recurred in 50% (three of six) while on lamivudine.

In another trial of lamivudine therapy, HBV DNA levels were measured, and lamivudine was started before renal transplantation if the HBV DNA increased to more than 2.83×10^8 copies/mL alone or to more than 2.83×10^7 copies/mL with elevated AST/ALT from 1996 to 2000 (so called de novo group).[51] This strategy was compared with preemptive use of lamivudine for patients who had undergone transplantation before 1996 (when lamivudine became commercially available) and received therapy later after transplantation than the de novo group. Although suppression of HBV DNA and normalization of aminotransferases was achieved in all patients, the survival of the de novo–treated group was comparable to that of HBsAg-negative controls, whereas HBsAg-positive patients who were transplanted before 1996 and received preemptive therapy with increasing HBV DNA after renal transplantation had a higher risk of overall mortality (relative risk 9.7) and liver-related mortality (relative risk 68).

Antiviral therapy should be offered to all HBV-positive individuals (HBsAg-positive) starting ideally before renal transplantation even if HBV serum levels are undetectable. The optimal duration of therapy is yet to be determined and in an immunocompromised host may need to be indefinite. Cessation of antiviral therapy in the immunocompromised host is associated with an increased risk of flare of liver disease and rarely decompensated liver disease in transplant recipients and patients without organ transplantation.[51,165] Durable responses are occasionally seen after seroconversion to HBeAg (development of anti-HBe), but this seroconversion is rare even in immunocompetent individuals.

Treatment may be stopped 6 months after this seroconversion occurs with careful follow-up. In one study, discontinuation of lamivudine was attempted in 12 low-risk patients. These patients had been on lamivudine for at least 9 months, were negative for HBV DNA, had normal liver enzymes and stable immunosuppressive regimens, and had no resistance to lamivudine. HBV DNA levels were measured monthly for 1 year after stopping lamivudine. Withdrawal of lamivudine was successful in 5 of 12 (42%) patients, whereas 7 required retreatment because of resurgence of HBV DNA levels. Of the five patients in whom lamivudine was withdrawn, DNA remained negative in two after 18 months of follow-up and was detectable again in three patients with normal liver enzymes.[51]

Indefinite therapy carries its own risks, including that of antiviral toxicity (rare) and of resistance (high with lamivudine and increasing with newer agents such as adefovir). In nontransplant populations, the risk of resistance is higher with prolonged therapy[165,166] with resistance rates of 40% after 2 years of treatment. The rate of resistance also increases with incomplete viral suppression, where viral loads decrease but are still fairly high. Use of more potent antiviral agents or even combination therapy may be advocated in these individuals to reduce the HBV viral load and reduce the risk of resistance to a single agent. These paradigms also may be applicable to the immunocompromised host but have not yet been tested formally.

Specific Antiviral Agents for Hepatitis B Virus Used in Renal Transplant Recipients

LAMIVUDINE

The most data for antiviral agents for HBV used in renal transplant recipients exist for lamivudine. A dose of 100 mg/day has been shown to be highly effective in suppression of HBV replication and normalization of aminotransferases in greater than 80% of patients.[84,150,216] Cessation of antiviral therapy has been associated with virological and clinical relapse.[216] The risk of resistance also increases with duration of lamivudine therapy. In one study of 29 renal transplant recipients who underwent 60 months of therapy, 14 (48%) developed resistance with flares seen in 11 (79%) of these patients (persistently in 6 of 11). In another study of 14 renal transplant recipients with chronic HBV who received long-term lamivudine therapy (median duration of treatment 64.5 months), resistance to lamivudine appeared in 8 (57%) after a median duration of 15 months. During a 51-month follow-up after viral breakthrough, three of the eight recipients had a clinical breakthrough (ALT >5 × upper limits of normal), and there were no episodes of decompensation.[248]

In a meta-analysis of 14 clinical trials (184 recipients) of lamivudine after renal transplantation, most recipients had HBV DNA clearance (91%) and biochemical normalization (81%), and the risk of lamivudine resistance was 18%.[84] Although HBeAg loss was higher with prolonged therapy, the resistance also was higher, limiting its efficacy. Consideration of newer antiviral agents for prolonged therapy is advisable.

ADEFOVIR

Adefovir dipivoxil, an oral prodrug of adefovir diphosphate, which is a nucleotide analogue of adenosine monophosphate, has shown treatment efficacy in treatment-naive and lamivudine-resistant patients with HBV.[116,176,202] In patients with renal transplants, it has been used in small studies mostly in lamivudine-resistant recipients. In one study of 11 renal transplant recipients, there was significant reduction in HBV DNA after initiation of adefovir with a median decline of 5.5 log in HBV DNA after 12 months of therapy. No virological breakthrough was observed, and no significant changes in creatinine occurred.[102] The favorable resistance profile of adefovir compared with lamivudine even after prolonged therapy—5.9% after 144 weeks of adefovir therapy in immunocompetent individuals[115]—can result in long-term response and could be advantageous in renal transplant recipients. No long-term studies of adefovir have been published in renal transplant recipients.

Table 30–3 **Selected Pretransplant and Post-transplant (Nonliver) Studies of Antiviral Therapy in Hepatitis B Virus Patients**

Study	Patient Population	No.	HBV Antiviral Therapy	Duration of Therapy	HBV DNA Suppression	HBeAg Seroconversion to Anti-HBe	Virological Breakthrough
Pretransplant							
Fontaine et al, 2000[101]	Dialysis patients	5	Lamivudine 10 mg daily in 3, 50 mg 3×/wk in 2	12 mo (7-28 mo)	5/5	1/5	2/5 (at mo 7, 18 of lamivudine)
Duarte et al, 1995[76]	Dialysis patients	2	Interferon alfa 3 million units 3×/wk	3 mo	2/2	2/2	None
Post-Transplant							
Fontaine et al, 2000[101]	Post–renal transplant patients with HBV infection	26	Lamivudine 100 mg/day	16.5 mo (4 to 31mo)	26/26 undetectable	6/26	8/26
Fontaine et al, 2005[102]	Post–kidney transplantation with lamivudine-resistant HBV	11	Adefovir 10 mg/day	15 mo (3-19 mo)	Median change –5.6 log copies/mL (–2.2 to –7.7)	0/6 that were initially $HBeAg^+$	Not detected
Han et al, 2001[117]	Post–kidney transplantation with HBV ($HBsAg^+$)	Group 1—after developing recurrent hepatic dysfunction after renal transplant (6); group 2—preemptive or prophylactic treatment for $HBsAg^+$ recipients beginning before renal transplantation (10)	Lamivudine 100 mg/day	Group 1—follow-up 15-60 mo; group 2—follow-up 9-30 mo	On treatment group 1—6/6; on treatment group 2—11/11	Group 1—0/6; group 2—0/11	Group 1—3/6; group 2—1/10

Chan et al, 2002[51]	Post–kidney transplantation with HBV (HBsAg$^+$)	Period II—post-1996, de novo preemptive therapy before renal transplantation and continued after transplantation (11); period I—pre-1996, preemptive therapy after renal transplantation	Lamivudine 100 mg/day	Period I—36.3 ± 11.4 mo; period II—27.6 ± 14.5 mo	26/26 undetectable	Not mentioned. 3/14 HBeAg$^+$ patients became undetectable	11 (40.7%) became lamivudine-resistant at 9.5-24 mo after starting treatment
Puchhammer-Stockl et al, 2000[209]	Post–kidney transplantation with HBV (HBsAg$^+$)	11	Lamivudine 100 mg/day in 7, reduced dose in 4 per renal function	>12 mo	HBV undetectable in 10/11 by PCR	Not reported	Lamivudine resistance in 5/11 9-15 mo after starting lamivudine
Thabut et al, 2004[248]	Post–kidney transplantation with HBV (HBsAg$^+$)	14	Lamivudine 100 mg/day	Median duration 64.5 mo (6-93 mo)	11/11 undetectable on treatment	None of 4 HBeAg$^+$ patients	Lamivudine resistance with virological breakthrough in 8/14 patients 9-24 mo after starting lamivudine
Chan et al, 2004[52]	Post–kidney transplantation with HBV (HBsAg$^+$)	29	Lamivudine 100 mg/day	56.7 ± 12.5 mo	29/29 undetectable initially	5/15 who were HbeAg$^+$ initially developed anti-Hbe	14/29 (48%) developed lamivudine resistance (10-35 mo after starting treatment) with virological breakthrough occurring in all 14

anti-HBe, antibody to hepatitis B early antigen; HBeAg, hepatitis B early antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

OTHER NEWER AGENTS

Other nucleotide and nucleoside analogues are now available for use in HBV-infected individuals, including entecavir and tenofovir.[55,258] Advantages include potency, low rates of resistance allowing prolonged therapy without breakthrough, and efficacy in lamivudine-resistant patients. No data exist in renal transplant recipients.

INTERFERON

Use of interferon is associated with an unacceptably high risk of precipitating renal allograft rejection, sometimes irreversible despite salvage immunosuppressive therapy. With the availability of other antiviral agents for HBV, use of interferon in renal transplant recipients should be avoided.[78,186]

Treatment of Fibrosing Cholestatic Hepatitis B in Renal Transplant Recipients

Fibrosing cholestatic hepatitis B is a histological and clinical variant of hepatitis B characterized by hepatocyte ballooning, cholestasis, minimal inflammation, periportal fibrosis, and massive viral replication (Fig. 30-2). This condition was first described in HBV-infected recipients of liver allografts but has been subsequently described in other immunosuppressed states, such as after renal transplant and bone marrow transplantation.[140] Patients often develop rapidly progressive liver failure, and spontaneous recovery is rare. Lamivudine has been reported to be useful in case reports resulting in successful resolution of the severe acute hepatitis and hepatic failure associated with this condition.[53]

Hepatitis C Virus

Viral Structure

For many years, patients with elevated liver enzymes and evidence of chronic liver disease were an enigma. The discovery of hepatitis A virus and HBV between 1967 and 1973[93] was a medical breakthrough but left many unanswered questions. For the next 16 years, patients with non-A, non-B hepatitis virus became increasingly recognized as having a form of chronic liver disease. In 1989, Choo and colleagues[59] published the first account of HCV, which was described further as a single-stranded, enveloped, positive-sense RNA virus. HCV is classified in the Flaviviridae family. The genome of 9400 nucleotides contains two noncoding regions in 5′ and 3′ flanking a large reading frame, which codes for a polyprotein of 3000 amino acids; this polyprotein is cleaved further into structural (C, E1, E2) and nonstructural (NS1, NS2, NS3, NS4, NS5) proteins. The positive RNA acts as a cap-independent messenger; the transcription is mediated by the NS5 RNA polymerase. After the maturation step, the virion is liberated by exocytosis leaving a relatively intact cell. As with other RNA viruses, the HCV genome displays a high degree of variability, especially in the E2/NS1, E1, NS3, and NS5b regions.[207] The 5′ noncoding region is highly conserved between HCV isolates and is instrumental in the reverse transcription and amplification of HCV RNA by polymerase chain reaction (PCR).[36]

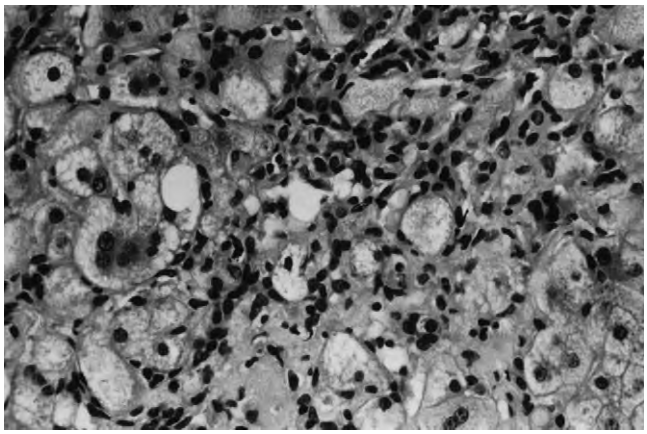

Figure 30–2 Perisinusoidal fibrosis and hepatocyte ballooning without inflammatory infiltration. Characteristic histological appearance of fibrosing cholestatic hepatitis B.

Hepatitis C Virus Species

HCV can be thought of as a spectrum of similar viruses. Six HCV genotypes with several distinct subtypes have been identified throughout the world[236] with an additional six proposed by HCV researchers.[37] Within a genotype or subtype, the genome of HCV is highly mutable owing to the lack of efficient proofreading capabilities. As the virus replicates over time, selective pressures from the immune system or antiviral treatments or both cause the viral populations to evolve. These mutant versions of genotypes are called quasi-species. The heterogeneity of this virus is what allows it to evade immunological detection and elimination, thus far preventing the development of a vaccine.

Epidemiological studies done on the HCV genotypes have shown significant regional variation. Genotype 1 is found worldwide and is the most common (60% to 70% of isolates) in the United States, Europe,[79] Japan, and Taiwan. Although less common, genotypes 2 and 3 also are found in these areas, with genotypes 4, 5, and 6 being rarely encountered. Genotype 3 is predominant in India, the Far East, and Australia.[126,139] Genotype 4 is present in North Africa and the Middle East,[181] with a particularly high incidence in Egypt. Genotype 5 has been detected most frequently in South Africa, whereas genotype 6 has been isolated to Hong Kong.[277] HCV infection does not confer immunity, and infection with multiple genotypes is common, especially in intravenous drug users and in individuals who required multiple blood transfusions.[156]

The clinical significance of viral genotypes is unclear, but important differences have been shown. Amoroso and coworkers[11] followed patients with acute viral hepatitis and found that patients infected with genotype 1 developed chronic infection at a significantly higher rate compared with patients infected with genotypes 2 or 3. Regarding the genotypic sensitivities to treatment, there is compelling evidence that genotypes 2, 3, or 5[160] are more responsive to interferon-based treatments than genotypes 1 and 4.[71] Current recommendations for treatment durations take these findings into consideration.

Clinical Manifestations of Hepatitis C Virus Infection in Immunocompetent Hosts

HCV generally is a chronic infection, and its acute form often goes unrecognized. Twenty percent to 30% of patients with acute HCV have symptoms 2 to 12 weeks after the exposure.[251,264] The symptoms are generally mild and

include lethargy, nausea, vomiting, jaundice, and anorexia. Serum aminotransferases can range from twofold to tenfold above normal. Rarely, acute HCV can lead to fulminant hepatic failure,[90] although this is more likely to occur when there is already significant underlying liver disease. Acute HCV is detected by testing for HCV RNA, which is the earliest marker identifiable.[91] HCV antibodies may not be detected for weeks to months after the exposure and may not develop in immunocompromised individuals.[208]

Chronic HCV develops in 85% of individuals who are exposed. The clinical course is remarkably nonspecific in most with varying degrees of fatigue and arthralgias. Studies have estimated 20% to 35% of patients have progression of liver disease to cirrhosis over 20 to 30 years.[206] A study by Cacoub and associates[43] found that 38% of HCV patients presented with at least one clinical extrahepatic manifestation. Associated findings include hematological disorders, such as cryoglobulinemia and lymphomas, and porphyria cutanea tarda and other rashes. Commonly, dry eyes and mouth, pruritus, renal disease including membranoproliferative glomerulonephritis, and diabetes are present.

Incidence, Prevalence, and Transmission of Hepatitis C Virus in Renal Transplant Patients

It is estimated that 4 million people in the United States are HCV antibody carriers, of whom 2.7 million are viremic.[7] The United Network for Organ Sharing database has 67,226 potential recipients on its renal transplant waiting list.[255] A significant number of these patients on the renal transplant waiting list, especially patients on renal replacement therapy, are infected with HCV. Obtaining accurate data regarding infection rates in this transplant-associated population is complicated by several factors, including the insidious and indolent nature of the disease in the setting of uremia[88]; regional variations of the HCV genome; the use of nonstandardized diagnostic methods[38,125]; and the absence of good, prospective, well-powered studies.

The history of patients with chronic kidney disease is important to include in a discussion of renal transplant patients and concomitant HCV infection. Seventy percent to 80% of patients who are transplanted have been on renal replacement therapy for a period of time.[227] HCV prevalence in hemodialysis units across seven countries was reported in the Dialysis Outcomes and Practice Patterns Study (DOPPS) and showed a mean HCV prevalence of 13.5% with a range between the countries of 2.6% to 22.9%. HCV prevalence is higher in Japan, Italy, and Spain and lower in Germany and the United Kingdom. The United States had a 14% HCV prevalence and a hemodialysis seroconversion rate of 2.5%/100 patient-years.[99] Historically, blood products were the major contributor to infection in these patients. This method of transmission has been virtually eliminated in recent years with extremely reliable screening methods[74,151] and decreased transfusion requirements directly related to the increased use of hematopoietic growth factors.[74,125] Despite these improvements, studies show de novo infections do occur in dialysis units, although clearly identifiable risk factors have not been reproducibly shown.[68]

As transplant waiting lists soar to record levels, programs of all organ types are faced with decisions regarding the use of extended criteria (previously called marginal) donor organs, including those positive for HCV antibody. Historically, allocation of HCV-positive organs has been restricted to HCV-positive recipients. A study by Abbott and associates[2] published data showing that the practice of transplanting organs from HCV-positive donors into HCV-negative recipients is more common than previously thought, especially when the recipient is older or African American or both. Although transplantation of HCV-positive organs into HCV-negative recipients is a risk factor for poorer outcomes in the renal transplant patients,[2,96,112,122] the practice continues in dire circumstances and is another contributing factor to the overall incidence of HCV in this patient population.

Impact of Pretransplant Hepatitis C Virus on Post-transplant Outcomes

PATIENT AND GRAFT SURVIVAL

Controversy exists regarding the impact of pretransplant HCV infection on the outcome of renal transplantation. Initially, studies of short follow-up periods suggested that neither patient nor graft survival was altered after transplantation despite a logarithmic increase in HCV RNA levels.[157,158,197,240] Several of these studies found no significant difference in the rate of liver complications in the HCV-infected patients. This information regarding degree of liver disease was frequently ascertained by measuring biochemical markers. Studies since these publications have shown this method of detecting liver disease is unreliable. Orloff and colleagues[197] reported the liver biopsy findings at 3 to 7 years after kidney transplantation in HCV-positive subjects; 12% had chronic active hepatitis, 50% had mild hepatitis, and 38% had normal histology. HCV conferred no adverse effect on patient or graft survival. Lee and coworkers[158] agreed that HCV infection did not reduce renal allograft or patient survival; however, they identified more liver disease and a greater prevalence of life-threatening sepsis in the HCV-infected recipients.

In contrast, studies with more lengthy follow-up after transplantation have found decreased patient or graft survival in HCV-positive renal transplant recipients.[118,159,201,229] Periera and coworkers[201] compared the prevalence of post-transplantation liver disease and graft and patient survivals in HCV-positive and HCV-negative kidney transplant recipients. Among recipients who were HCV-positive before transplantation, the relative risk of post-transplantation liver disease was 5, of graft loss was 1.3, and death was 3.3. There was a significant increase in death resulting from sepsis with a relative risk of 9.9. Similarly, Hanafusa and associates[118] found clinically significant hepatitis in 55% of HCV-positive kidney transplant recipients. These investigators also found a significant decline in the 20-year survival in the HCV-positive patients compared with the HCV-negative cohort (64% versus 88%).

In a meta-analysis of observational studies after renal transplantation that included eight studies, the presence of HCV antibody was an independent risk factor for death and graft failure after renal transplantation (relative risk for death 1.79 [95% confidence interval 1.57 to 2.03]) and for renal graft failure (relative risk 1.56 [95% confidence interval 1.35 to 1.80]). HCC and liver cirrhosis were more frequent causes of mortality in HCV-positive than HCV-negative recipients.[87] Whether HCV infection results in increased rates of progression of hepatic fibrosis in the setting of renal transplantation compared with immunocompetent hosts is controversial.[229]

Most studies regarding post-transplant HCV outcomes comprise chronically infected recipients, usually subjects who acquired HCV during hemodialysis. The subsets of solid organ transplant recipients who become infected with HCV in the perioperative period have a markedly different course, however. Delladetsima and colleagues[70] followed 17 such patients by biochemical and histological markers for a mean of 7 years. Six (35%) patients died a median of 6 years after transplantation as a result of fibrosing cholestatic hepatitis, vanishing bile duct syndrome, cirrhosis, miliary tuberculosis, and myocardial infarction. Overall, the yearly fibrosis progression rate was five times that of age-matched immunocompetent HCV-infected patients.[242] These studies suggest that HCV acquired at the time of transplantation may have a particularly aggressive course.

HEPATITIS C VIRUS AND POST-TRANSPLANT DIABETES IN RENAL TRANSPLANT RECIPIENTS

The association of diabetes mellitus and HCV has become increasingly apparent more recently in the immunocompetent HCV population and particularly after solid organ transplantation in HCV-infected patients. The overall incidence of post-transplant diabetes mellitus has been reported to vary from 10% to 54%; post-transplant diabetes mellitus has shown similar long-term effects as diabetes mellitus types 1 and 2 with cardiac and renal dysfunction in a significant proportion.[86] Yildiz and coworkers[273] reported a case-controlled study of 43 renal transplant recipients with post-transplant diabetes mellitus in which 72% were HCV-infected compared with a prevalence of 37% in the recipients without post-transplant diabetes mellitus ($P = .002$). This association also was observed by Bloom and associates,[26] who reported post-transplant diabetes mellitus occurred more frequently in HCV-positive than HCV-negative patients (39.4% versus 9.8%; $P = .0005$). Bloom and associates[26] found that among the HCV-positive patients, there was an eight times increased incidence of post-transplant diabetes mellitus in patients treated with tacrolimus (58%) compared with cyclosporine (7.7%).

HEPATITIS C VIRUS AND POST-TRANSPLANT NEPHROPATHY

Post-transplant renal disease is common among HCV-positive recipients of any organ. Although the causes of renal injury after transplantation are multifactorial in nature, chronic allograft nephropathy among renal transplant recipients and nephrotoxicity owing to calcineurin are the most common etiologies. Of the additional etiologies likely to arise in patients infected with HCV, membranoproliferative glomerulonephritis is the most common followed by membranous nephropathy, minimal change disease, and renal thrombotic microangiopathy. These may be recurrent or manifest de novo.[183]

Membranoproliferative glomerulonephritis has been reported in 45% of HCV-positive renal transplant recipients who underwent renal biopsy for worsened renal function. In the HCV-negative group, the incidence was only 5.9%.[183] De novo disease was found in 18% of the membranoproliferative glomerulonephritis patients, and chronic renal allograft nephropathy was similar in HCV-positive and HCV-negative recipients.[62] Initially, membranoproliferative glomerulonephritis and chronic allograft nephropathy appear similarly with proteinuria and can be a diagnostic dilemma requiring electron microscopy to differentiate the two. Membranoproliferative glomerulonephritis is associated with subendothelial electron-dense deposits compared with only thickening and duplication of glomerular basement membranes in transplant nephropathy.[64]

Immunosuppressive Strategies in Renal Transplant Patients Infected with Hepatitis C Virus

No studies have been done to determine optimal immunosuppressive regimens in renal transplant recipients infected with HCV. As mentioned in a previous section, studies have shown tacrolimus as an additive risk in HCV patients for the development of post-transplant diabetes mellitus.[259]

Hepatitis C Virus Antiviral Therapy

PRETRANSPLANT ANTIVIRAL THERAPY

Eradication of HCV before transplantation has several theoretical and real benefits. HCV is associated with worse patient and graft survivals and increased risk of post-transplant diabetes mellitus and de novo glomerulopathy. Eradication of HCV before transplantation might mitigate some of these adverse outcomes.[63,130,143] Interferon therapy after transplantation is associated with reduced treatment response rates, a greater incidence of organ rejection, and impairment of renal function.[217] It would be best if treatment could be undertaken before embarking on the solid organ transplant (Table 30-4).

Results of treatment of HCV in dialysis patients varies, with sustained virological rates ranging from 16% to 68%.[85] These rates are not significantly different from those seen in the non–end-stage renal disease population, and in many reports the rates are higher than in patients with normal renal function. The higher sustained virological rates in patients with normal renal function may be due to higher circulating levels of interferon in patients on dialysis[215] or lower viral loads in patients on hemodialysis.[85] Histological improvements have been reported by studies in which the hepatic activity index was compared on sequential liver biopsy specimens before and after interferon therapy. Similarly post-transplantation improvements in hepatic activity index were seen to persist in patients treated with interferon while on the waiting list compared with patients who were not given interferon before renal transplantation.[130] Post-transplant glomerulopathy also is reduced by pretransplant interferon therapy. Of 78 renal transplant recipients, 15 received interferon before transplantation, and 10 of 15 were HCV RNA–negative at transplant. Only 1 of the 15 (6.7%) developed de novo glomerulonephritis after transplantation compared with 19% of nontreated HCV-positive renal transplant recipients (12 of 63).[63]

Most studies report treatment regimens including interferon monotherapy administered for 6 to 12 months. Interferon side effects in the dialysis population vary but seem to be more frequent than in non–end-stage renal disease patients. Discontinuation rates are 51% compared with studies of non–end-stage renal disease patients, where dropout rates are approximately 20%. The higher discontinuation rates may be secondary to a longer half-life of interferon in dialysis patients.

Ribavirin is renally excreted, and its use has been avoided in dialysis patients, not least because of the fear of hemolysis.

Table 30–4 **Selected Pretransplant and Post-transplant (Nonliver) Studies of Antiviral Therapy in Hepatitis C Virus Patients**

Study	Patient Population	No.	Antiviral Therapy	Follow-up after Therapy	ETVR	SVR	Biochemical Response	Histological Response	Side Effects/Discontinuation	Outcome after Transplant
Pretransplant										
Casanovas-Taltavull et al, 1995[49]	Dialysis patients	10	IFN 3 million units 3×/wk, tapering to 1.5 μ 3×/wk for 1 yr	6 mo	1/10	2/10	9/10	—	IFN stopped in 3	4 of 5 maintained normal renal function, 1 had acute vascular rejection
Huraib et al, 2001[130]	Renal transplant candidates	30	15 patients—IFN 3 million units 3×/wk for 1 yr, 11 had renal transplant (group A); 15 patients—no antiviral therapy, 10 had transplant (group B)	12 mo	—	4/11	—	—	Minimal or no dosage adjustment	HAI at 1 yr after transplant lower in group A (1.19) than group B (5.5)
Benci et al, 1998[21]	Dialysis patients	10	IFN 1 million units 3×/wk, for relapsers 3 million units 3×/wk, 1 yr total therapy	6 mo	—	3/10	—	—	IFN stopped in 1	—
Campistol et al, 1999[47]	Dialysis patients	19 received IFN, 17 controls	IFN 3 million units 3×/wk for 6 mo	3-33 mo	14/19	8/19	—	—	IFN stopped in 10/19	3 remained HCV RNA$^-$ and 1 relapsed
Casanovas-Taltavull et al, 2001[48]	Dialysis patients	29	IFN 3 million units 3×/wk for 6 mo and 1.5 million units 3×/wk for 6 mo	41 ± 28 mo	23/28	18/28	18/28	—	IFN stopped in 7/29	8 remained HCV RNA$^-$ and 1 relapsed
Post-transplant										
Tang et al, 2003[244]	Acute de novo HCV infection after renal transplant	4	IFN 3 million units 3×/wk + ribavirin 1000-1200 mg/day for 48 wk	15-42 mo	3/4	3/4	3/4	—	Dose-dependent hemolysis, no renal dysfunction	—
Rostaing et al, 1995[217]	Renal transplant recipients with HCV	15 treated (group A) and 15 controls (group B)	IFN 3 million units 3×/wk for 6 mo	12 mo	4/14	0/14	10/14	—	Renal failure in 5/14, renal function recovered in only 2 despite steroid pulse	—

Table continued

Table 30–4 **Selected Pretransplant and Post-transplant (Nonliver) Studies of Antiviral Therapy in Hepatitis C Virus Patients—cont'd**

Study	Patient Population	No.	Antiviral Therapy	Follow-up after Therapy	ETVR	SVR	Biochemical Response	Histological Response	Side Effects/Discontinuation	Outcome after Transplant
Shu et al, 2004[234]	Renal transplant recipients with HCV	11	IFN 1 million units 3×/wk + ribavirin 600 mg/day for 48 wk	NR	5/11	3/11	10/11	—	IFN stopped in 3, 1 with acute graft failure	—
Fontaine et al, 2004[103]	Renal transplant recipients with HCV	13	Ribavirin 724 ± 224 mg/day for 22.6 ± 13.3 mo	22.6 ± 13 mo	0/13	0/13	Decrease in mean AST from 128 to 53	Metavir activity score decreased from 2.46 ± 0.78 to 1.23 ±1.01	1 patient required erythropoietin	—
Kamar et al, 2003[142]	Renal transplant recipients with HCV	16 received ribavirin (group A) and 32 controls (group B)	Ribavirin starting at 1000 mg/day adjusted to hemoglobin, 1 yr therapy	12 mo	0/16	0/16	Decrease in mean ALT from 85 to 48	No improvement in inflammation and fibrosis; improvement in proteinuria	3 cases ribavirin stopped despite erythropoietin therapy	—
Durlik et al, 1995[77]	Renal transplant recipients (7 with HCV alone, 6 HBV alone and 2 HBV, HCV, HDV)	15	IFN 3 million units 3×/wk for 6 mo	3-53 mo	—	—	ALT improved in all (normal in 50%)	—	Rejection in 5/15, 4 lost grafts (3 from irreversible rejection)	—
Therret et al, 1994[250]	Renal transplant recipients with HBV, HCV, or both	13	IFN 3 million units 3×/wk for 6 mo	3-26 mo	—	—	AST, ALT improved significantly on therapy, returned to previous levels after end of treatment	—	IFN stopped in 7, acute renal failure in 2 patients (1 chronic rejection)	—
Tokumoto et al, 1996[253]	Renal transplant recipients with HCV	6	IFN 10 million units daily for 2 wk, then 5-10 million units 3×/wk for 22 wk	17-27 mo	3/6	3/6	6/6 normalized AST, ALT	—	1 with renal failure (acute vascular rejection)	—

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ETVR, end of treatment virological response, defined as undetectable HCV RNA at end of therapy; HAI, hepatic activity index; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; IFN, interferon; SVR, sustained virological response, defined as undetectable HCV RNA 6 mo after the end of therapy.

Discontinuation because of severe hemolytic anemia may occur despite low doses of 200 mg three times a week in dialysis patients.[243] Some pilot studies have reported ribavirin use in addition to interferon in patients on dialysis.[34] In the study by Bruchfeld and coworkers,[34] lower doses of ribavirin were used (170 to 300 mg/day) along with erythropoietin and iron, and monitoring of ribavirin levels was done. A sustained virological response was seen in one of the six patients treated, and there was no evidence that adding ribavirin in dialysis patients provided any added therapeutic benefit. Longer term and larger studies with preemptive hematopoietic growth factors are needed to improve tolerance and measure virological response.

There is considerable clinical experience, although few studies, using pegylated interferon monotherapy in dialysis patients with chronic HCV. In one study, 16 patients were randomly assigned to 0.5 μg/kg/wk or 1 μg/kg/wk of pegylated interferon alfa-2b for 48 weeks. Sustained viral response was 40% in the 1 μg/kg group and 22% in the 0.5 μg/kg group. Adverse effects, primarily hypertension and infection, led to discontinuation of therapy in 56% (five of nine patients) in the 1 μg/kg group and in 28% (two of seven patients) in the 0.5 μg/kg group.[219]

The data have repeatedly shown an increase in side effects in this population. Response rates using pegylated interferon may not be better than with standard interferon in dialysis patients because the half-life of regular interferon is increased in patients on dialysis. The combination of pegylated interferon with ribavirin has been used in limited numbers, usually with reduced doses of ribavirin (170 to 300 mg/day in one study) in patients on dialysis. Interferon-related side effects were common. Sustained virological response in one study of six patients was 50%.[33] More data on safety, tolerability, efficacy, and pharmacokinetics of combination therapy are needed in dialysis patients before routine use and doses can be recommended. Monitoring of ribavirin levels may be useful to maintain ribavirin plasma concentration of 10 to 15 μmol/L.

Long-term maintenance of response is generally good after a successful virological response pretransplantation and after renal transplantation. Casanovas-Taltavull and associates[48] reported that of 14 dialysis patients who received interferon, 9 were HCV RNA–negative at the time of transplant, and 8 of the 9 remained HCV RNA–negative at long-term follow-up of 41 ± 28 months. Persistent biochemical normalization after renal transplantation is seen in most patients treated with interferon.

Interferon therapy is associated with reasonable response rates in dialysis patients with frequent maintenance of response after renal transplantation. Given the lower patient and graft survival rates after renal transplantation in HCV-positive compared with HCV-negative patients, interferon should be considered for renal transplant candidates infected with HCV and showing active viral replication. A liver biopsy should be performed to assess underlying activity and stage of HCV-related liver disease. This information can help guide expected response rates and aggressiveness of therapy. Patients with advanced fibrosis or cirrhosis or both need to be considered for a dual-organ transplant.

POST-TRANSPLANT ANTIVIRAL THERAPY FOR HEPATITIS C VIRUS

Post-transplantation interferon therapy generally is contraindicated in organ transplant recipients other than recipients of liver allografts; this is due to multiple reports of precipitation of renal failure and organ rejection owing to interferon therapy (see Table 30-3). Sustained virological responses are rare,[217] and there is a significant incidence of renal allograft dysfunction in a third of interferon-treated patients. In the report by Rostaing and colleagues,[217] renal function was recoverable in only two of the four patients receiving methylprednisolone therapy. Rejection may be due to enhanced HLA-DR expression stimulated by interferon, inhibition of prostaglandin synthesis leading to immunologically mediated nephropathy, or stimulation of antibody production by B cells. In a few patients, interferon and ribavirin combination therapy has been associated with sustained virological rates without renal dysfunction.[244] Interferon alfa therapy should be limited to patients with severe recurrence of HCV, such as advanced fibrosis/cirrhosis or fibrosing cholestatic HCV, or in the setting of well-constructed, appropriately powered clinical trials.

Ribavirin monotherapy has been associated with reduction in aminotransferases and necroinflammation in renal transplant recipients but no virological response. Another study in these patients showed biochemical improvement without histological or virological improvement.[142] Although ribavirin does not decrease viral replication, the histological efficacy shown in some studies and decrease in AST/ALT may be due to decreased lymphocytic proliferation, decreased synthesis of proinflammatory cytokines, and a decrease of T helper type 2 cytokine production favoring a T helper type 1 profile. This area remains speculative.

HEPATOCELLULAR CARCINOMA AFTER RENAL TRANSPLANTATION

In the setting of immunosuppression, loss of tumor surveillance can lead to higher risk for various malignancies. HCC is more common after renal transplantation (incidence 1.4% to 4%) than in the general population (incidence 0.005% to 0.015%).[135,168,195,214] In areas endemic for HBV, the most common tumor after renal transplantation is HCC (20% to 45%).[56,58]

Most of these cases are related to chronic viral hepatitis (HBV and HCV), which has a high incidence in the renal transplant population. In one study of 534 renal transplant recipients between 1980 and 1998 with follow-up to 2003, 6 recipients were diagnosed with HCC (incidence 1.1%). In this cohort, the incidence of HCC was 2.29% among renal transplant recipients with chronic viral hepatitis.[214] HCC was diagnosed 45 to 244 months after transplantation and was larger than 5 cm in all. Four recipients had multiple lesions. Three of the six had α-fetoprotein values greater than 400 ng/mL.

Estimated survival was worse than that expected for similar stage tumors in nontransplanted populations. In one study, median survival was 6 months.[214] Other studies have shown high mortality rates (69%) for HCC cases after renal transplantation.[56] Because outcomes after HCC are poor, preventive measures are important, including vaccination of renal transplant waitlist patients for HBV, antiviral therapy for HCV and HBV in the dialysis population, continued antiviral treatment for HBV in renal transplant recipients, and exclusion of patients with end-stage renal disease and cirrhosis from isolated kidney transplantation and, in select cases, consideration of these patients for combined liver-kidney transplantation.

In renal transplant recipients infected with HBV or HCV with uncontrolled viral replication or advanced fibrosis or cirrhosis, surveillance for HCC should be undertaken with ultrasonography or computed tomography (CT) every 6 months along with α-fetoprotein levels. α-Fetoprotein is useful in the post-transplant setting and in immunocompetent patients for the diagnosis of HCC.[58]

SYSTEMIC INFECTIONS RESULTING IN HEPATITIS AND LIVER DISEASE

Numerous systemic infections have hepatitis as part of the clinical manifestation. Foremost among these are infections caused by herpesviruses, which are major pathogens in organ transplantation. Other infections primarily involving the liver also are reviewed.

Liver Abscess

Pyogenic liver abscess does not represent a specific liver disease but is a final common pathway of many pathological processes. The incidence of pyogenic liver abscess ranges from 8 to 20 cases per 100,000 hospital admissions[137]; a population-based study reported 2.3 cases per 100,000 individuals per year.[144] A population-based study found no increased risk of pyogenic liver abscess in renal transplant recipients.[144]

Abscesses may be classified by presumed route of hepatic invasion: (1) biliary tree, (2) portal vein, (3) hepatic artery, (4) direct extension from contiguous focus of infection, and (5) penetrating trauma.[137] Major causes of pyogenic liver abscess are suppurative cholangitis and pyelophlebitis from diverticulitis, pancreatitis, or appendicitis. Any systemic bacteremia may spread to the liver to cause an abscess. Direct extension may occur with cholecystitis, perinephric abscess, or a subphrenic abscess. Approximately 50% of pyogenic liver abscesses are cryptogenic.[210] Comorbid illnesses, such as diabetes, malignancy, and cirrhosis, are common in patients with liver abscesses and may be predisposing factors.

The microbiology of pyogenic liver abscess varies with the route of infection. Most are polymicrobial, however. *Escherichia coli* and *Klebsiella pneumoniae* are the most common pathogens.[210] Viridans streptococci and enterococci also frequently are found.

Although fever and constitutional symptoms are frequent, only 1 in 10 patients presents with the classic triad of fever, jaundice, and right upper quadrant tenderness. Right upper quadrant tenderness and hepatomegaly are found in half of patients.[279] Although liver function tests are abnormal in most patients, elevation is usually modest. Alkaline phosphatase elevation is present in two thirds of patients; small elevations may be seen in liver transaminases and bilirubin. Radiographic imaging using ultrasonography or CT is essential to making the diagnosis. Microbiological diagnosis rests on obtaining purulent material from the abscess cavity, which should be sent for Gram stain and culture. Treatment consists of antimicrobial therapy for 3 to 4 weeks and drainage of the abscess. Drainage may be accomplished by percutaneous aspiration with or without placement of a drainage catheter.[27] In recent years, investigators have reported success with treatment of small abscesses with antibiotic therapy alone.[45]

Amebiasis is a far less common cause of liver abscess in the United States but one that must be considered in patients living in or traveling to countries where the prevalence of amebiasis is high, such as Mexico, India, East and South Africa, and portions of Central and South America.[129] There is a marked male predominance, and amebic liver abscesses are usually solitary.[239] Clinical signs and symptoms and liver test abnormalities do not help to distinguish amebic from pyogenic liver abscesses. Serology for antibodies to *Entamoeba histolytica* is useful to determine current or past infection. After confirmation of an abscess on imaging, if amebic rather than pyogenic liver abscess is suspected, treatment with metronidazole for 10 days is necessary. Renal transplant recipients traveling to areas endemic for amebiasis should be counseled to avoid ingestion of potentially contaminated food and water, such as fresh produce that cannot be adequately cooked.[154] Boiling water before use is essential to destroy the cysts of *E. histolytica*, which are not killed by low-dose iodine or chlorine tablets.

Mycobacterial Infection

Tuberculosis is an important cause of morbidity and mortality among renal transplant recipients. The risk of active tuberculosis is approximately 50-fold higher in renal transplant recipients compared with nontransplant patients; most reactivation disease has been reported to occur in the first year after transplantation.[3,223] In a series of 520 renal transplant patients in Turkey, 22 (4.2%) developed tuberculosis.[272] Pleuropulmonary involvement accounted for more than half the cases; two patients had granulomatous hepatitis. Other series in renal transplant recipients also have found that the most frequent primary site of infection is the lung.[13] Liver involvement with tuberculosis is rare; when present, it is usually associated with pulmonary or gastrointestinal involvement with tuberculosis. Three patterns of tuberculous liver involvement have been reported[9]: (1) diffuse involvement of the liver in association with tuberculosis at other body sites; (2) miliary involvement of the liver with no other known organ involvement (granulomatous hepatitis); and (3) focal lesion in the liver, either an abscess or a tuberculoma.[113,241]

Constitutional symptoms and fever are common but nonspecific. A modest degree of transaminase and alkaline phosphatase elevation is common. Imaging and tissue staining for acid-fast bacilli and culture for mycobacteria are required to confirm the diagnosis. Isolated hepatic tuberculous abscess in renal transplant patients has been rarely described in case reports[44] and should be considered in the differential diagnosis of solitary masses in the liver, especially in patients from countries with high endemicity rates for tuberculosis.

Given the high risk of reactivation, potential kidney transplant recipients should be screened for latent tuberculosis with a tuberculin skin test. Chemoprophylaxis with isoniazid should be offered if the induration is greater than or equal to 5 mm.

Viral Infections (See Chapter 29)

Herpesviruses

The herpesviruses include CMV, EBV, HSV, human herpes virus (HHV)-6, HHV-7, HHV-8, and varicella-zoster virus (VZV). The herpesvirus family is responsible for considerable morbidity and mortality in transplant recipients. In particular,

CMV remains a major health threat after solid organ transplantation. All the herpesviruses can remain latent in tissues after acute infection. Liver involvement frequently is a part of the clinical presentation of herpesvirus-related diseases.

Cytomegalovirus

CMV is the most important pathogen in transplant recipients.[153] In contrast to the other herpesviruses, such as HSV and VZV, which remain latent in highly restricted areas of the body, once acquired, latent CMV can be found in multiple body sites. CMV infects humans of all ages, although the peak period of viral acquisition in the general population occurs early in life.[43,238] Infection in children is usually asymptomatic. Depending on the population surveyed, the prevalence of CMV antibody seropositivity in various regions ranges from 40% to 100%.[43,238]

Approximately 50% of transplant patients excrete CMV in body secretions (e.g., saliva and urine) at some stage after organ transplantation[235]; this usually begins in the first month after transplant surgery. Viral shedding reaches peak levels during the second and third months after transplantation, at which time it may be associated with disease.[110] The incidence of symptoms related to CMV infection varies among different types of allograft recipients. In general, liver, pancreas, lung, intestinal, and heart transplant recipients have a greater incidence of CMV disease than do kidney transplant recipients. Symptomatic infections occur in approximately 39% to 41% of heart-lung, 9% to 35% of heart, 22% to 29% of liver and pancreas, and 8% to 32% of renal transplant recipients not receiving antiviral prophylaxis.[235]

CMV is an active inducer of some members of the herpesvirus family.[1] Increases in EBV antibody titers are seen in transplant recipients with symptomatic CMV infection.[1] After renal transplantation, HHV-6 reactivation and the simultaneous detection of HHV-6 and CMV DNA in either urine or serum is a strong predictor of CMV disease.[124] CMV is an immunomodulating virus, and CMV infection has been shown to increase susceptibility to infection with other opportunistic agents, including *Pneumocystis carinii* and *Aspergillus fumigatus*.[28,213] Other indirect adverse effects linked to CMV are allograft rejection[220] and atherosclerosis.[256]

CMV infection is defined as isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen.[167] CMV disease (pneumonia, colitis, hepatitis) is diagnosed by the presence of signs or symptoms of tissue injury combined with virus isolation or histopathological or immunohistochemical evidence of CMV in tissue samples.[167]

In solid organ transplant patients, three patterns of CMV infection are observed, each with a different propensity for causing clinical disease, as follows:

1. Primary infection develops in a CMV-seronegative individual who receives an organ from a CMV-seropositive donor.
2. Superinfection or reinfection occurs when a seropositive transplant recipient receives an allograft from a seropositive donor, and reactivation of the latent virus of donor origin occurs.
3. Reactivation occurs when latent CMV reactivates after transplantation in a CMV-seropositive recipient. It is impossible to distinguish superinfection from reactivation infection, unless viral genetic studies are used.

A major factor influencing CMV reactivation after transplantation is the type and intensity of immunosuppressive therapy.[131] A higher incidence of tissue-invasive CMV disease has been found in mycophenolate mofetil–treated patients receiving more than 2 g of mycophenolate mofetil per day compared with lower doses of the drug or azathioprine.[222,249] The use of antithymocyte or antilymphocyte globulin and muromonab-CD3 (OKT3) monoclonal antibodies, either as induction therapy or for allograft rejection treatment, increases the risk of symptomatic CMV infection, especially in CMV-seropositive individuals, with CMV disease being diagnosed three to four times more frequently than in patients not receiving antilymphocyte therapy.

Regardless of the pattern and type of CMV transmission, most patients who develop symptomatic disease do so 1 to 4 months after transplantation. Primary disease is usually more severe than reactivation disease. CMV disease occurring later in the post-transplantation period may be noted in association with community-acquired primary infection, relapsing disease, or the use of antilymphocyte antibody therapy to treat rejection.

Hepatitis is a major clinical manifestation of CMV disease. In an immunocompetent patient, the disease is usually mild and self-limiting, although rare cases of fulminant CMV hepatitis have been described.[228] In the transplant recipient, CMV hepatitis is a more severe illness, usually with other organ involvement or disseminated disease, and is common. In a series of 97 renal transplant recipients with CMV disease, half had evidence of CMV hepatitis; the severity of hepatitis was greater in primary disease than in cases of reactivation.[221]

In an autopsy series of four immunocompromised patients with overwhelming CMV infection, Ten Napel and colleagues[246] showed that liver cell damage was extensive, but inflammatory infiltration was less prominent than in immunocompetent patients with CMV infection. Intracellular CMV inclusion bodies were found in the hepatocytes, vascular endothelium, and bile epithelium.

Serology for CMV antibodies is most useful to determine past exposure to the virus but is less helpful for diagnosis of acute disease in transplant patients. Detection of CMV DNA in the serum or tissue is usually accomplished using a variety of commercially available tests, such as PCR, shell vial, or DNA capture. Treatment of CMV hepatitis should be undertaken promptly with intravenous ganciclovir in addition to supportive care and reduction of immunosuppression whenever feasible. Resistance to ganciclovir, although well described in case reports, is infrequent in renal transplantation.[24,257]

Prophylaxis of transplant patients at risk for CMV disease should be started immediately after transplantation with acyclovir or ganciclovir. Ongoing debate surrounds the issue of whether prophylaxis against CMV should be administered universally to all transplant patients at risk for CMV, or whether serial monitoring of CMV viremia should be employed for patients with low to intermediate risk and preemptive therapy started only if viremia occurs. A systematic review that included 1980 solid organ transplant recipients found that either strategy reduced the risk of CMV disease, but only the universal prophylaxis approach reduced bacterial and fungal infections and death.[141]

Epstein-Barr Virus

EBV, a member of the human Gammaherpesvirinae family, is a ubiquitous pathogen. More than 90% of the world's

population is infected.[25] Virus is shed intermittently into saliva[270] and is believed to be transmitted through close contact with oral secretions. EBV infection may occur as primary or secondary infection (reactivation). Primary infection occurs in individuals with no previous exposure to EBV and usually is defined by the appearance of antibodies to EBV viral-capsid antigen. Childhood disease is usually asymptomatic. Infection acquired in adolescence or young adulthood frequently causes the clinical syndrome of acute infectious mononucleosis, characterized by fever, pharyngitis, and lymphadenopathy in 75% of patients.[80] The liver frequently is involved in acute infectious mononucleosis, but frank hepatomegaly is uncommon. Jaundice is apparent in 5% to 9% of patients. Liver function test abnormalities include serum transaminases two to three times above baseline, whereas alkaline phosphatase and bilirubin increases are less frequent (60% and 45%). Liver function test abnormalities peak with acute illness and return to normal over 1 to 2 months. In instances where liver biopsy specimens have been obtained, minimal swelling and vacuolization of hepatocytes can be seen accompanied by a lymphocytic or monocytic infiltrate in portal regions.[75]

EBV establishes latency[18] and may reactivate later. The risk of reactivation is especially high in immunosuppressed patients. Primary infection with EBV after transplantation may manifest as a febrile illness with constitutional signs and symptoms.

EBV has a central role in the pathogenesis of PTLD,[245] although not all PTLD is caused by EBV. EBV-driven PTLD occurs in 15% of solid organ transplant recipients, but its incidence depends on the organ transplanted, type and intensity of immunosuppression, and the EBV immune status of the donor and recipient, with an EBV-naive recipient and EBV-seropositive donor conferring greater risk.[109] PTLD is the most common form of post-transplant malignancy in pediatric transplant recipients and is an important cause of morbidity and mortality in adult transplant recipients. One percent to 3% of renal transplant recipients develop EBV-related PTLD, which has a spectrum of presentations ranging from benign polyclonal lymphoproliferation to extranodal solid tumors at any site, including the liver.

PTLD has a bimodal distribution with an early peak occurring within 2 years of transplantation and a later peak after 2 years. Early-onset PTLD is associated with primary EBV infection, as might occur in an EBV-naive recipient receiving an EBV-seropositive allograft. Serial monitoring for EBV viremia in transplant patients has been used to predict the development of EBV-associated PTLD.[182,252,262] Wide variation in the types of assays used and the frequency and timing of monitoring make it difficult to compare and generalize the results of these studies, but generally, although asymptomatic fluctuations in EBV viral load are common after transplantation, patients with PTLD are more likely to have higher viral loads for a more sustained period.

The mainstay of treatment of EBV disease after transplantation is reduction in immunosuppression. This approach is most effective in hyperplastic or polymorphic forms of PTLD. Late-onset PTLD is much less likely to respond to immunosuppression. Antiviral therapy with acyclovir or ganciclovir is controversial but is employed in many transplant centers in patients with detectable EBV viremia. Most EBV-infected cells within PTLD lesions are transformed B cells that are not in the lytic phase. Antiviral therapy has no effect on latently infected B cells and would not be expected to affect the natural history of PTLD. More recent data suggest that chemotherapy may be used to induce lytic EBV infection in EBV-transformed cells, and antiviral therapy with ganciclovir or acyclovir may be used to treat the lytic form.[94,95] Other available treatment options include interferon, intravenous immunoglobulin, anti–B cell monoclonal antibodies such as rituximab, conventional cytotoxic chemotherapy, radiation, and surgery. A detailed discussion of these strategies is beyond the scope of this chapter but may be found elsewhere.[109]

Herpes Simplex Virus

HSV is an alpha herpesvirus with a genome consisting of a linear, double-stranded DNA molecule.[267] The two types of HSV, HSV-1 and HSV-2, have 50% sequence homology. HSV have a worldwide distribution, and humans seem to be the only natural reservoir.[267] The development of type-specific serological assays has allowed accurate determination of seroprevalence rates of the two types of HSV, which range from 56% to 60% for HSV-1 and from 15% to 18% for HSV-2 in the U.S. general population.[232] Transmission of HSV infection occurs through close contact with an individual who is shedding virus at a peripheral site, at a mucosal surface, or in genital or oral secretions.[267] On entry of the virus into mucosal surfaces or abraded skin, viral replication is initiated with subsequent infection of autonomic or sensory nerve endings. The virus is transported to the nerve cell bodies in ganglia—most frequently the trigeminal ganglia with HSV-1 and sacral nerve root ganglia with HSV-2.

First episodes of HSV, or primary infection, are frequently accompanied by systemic signs and symptoms and have a longer duration of symptoms.[10,127] The virus establishes latency in ganglia and may reactivate. Immunocompromised patients have been found to have more severe and more frequent reactivation.[263] In renal transplant recipients, the incidence of HSV infection has been reported to be 30% to 50% in the absence of prophylaxis.[136,224]

Hepatitis with HSV has been well described in the renal transplant population. Kusne and colleagues[155] reported a series of 12 cases of HSV hepatitis, which developed a median of 18 days after solid organ transplantation. The clinical features included fever, herpetic stomatitis, and abdominal pain, usually in association with disseminated disease. Clinical features associated with mortality included bacteremia, hypotension, disseminated intravascular coagulation, and gastrointestinal bleeding. HSV hepatitis was associated with 67% mortality in this patient population.

Conclusive diagnosis of HSV hepatitis rests on demonstration of viral involvement of liver tissue. HSV has been associated with diffuse and focal liver involvement. Histologically, hepatocytes have enlarged ground-glass nuclei with chromatin margination (Fig. 30-3). Transplant recipients who present with fever, progressive transaminase elevation, and abdominal symptoms with or without evidence of cutaneous herpes simplex infection should prompt consideration of HSV hepatitis and treatment with intravenous acyclovir at 5 mg/kg/day. Oral acyclovir for prophylaxis against HSV has greatly reduced the incidence of HSV infection.[136,224]

Varicella-Zoster Virus

VZV is another herpesvirus that causes two distinct diseases—varicella and herpes zoster. Primary infection with VZV

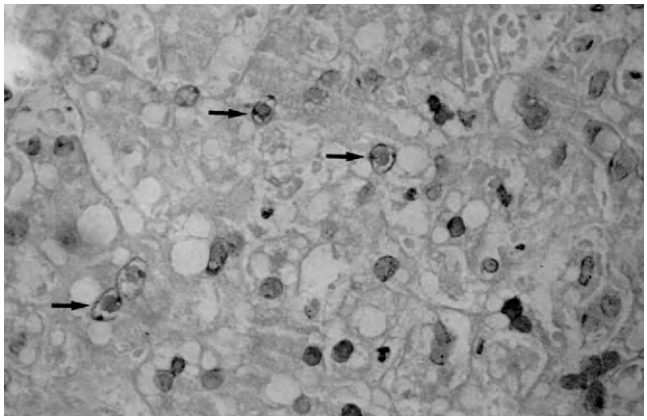

Figure 30–3 Hepatocyte necrosis caused by herpes simplex infection. These cells contain eosinophilic nuclear inclusions surrounded by clear halos, with clumped chromatin along the nuclear membrane, showing Cowdry type A inclusions (*arrows*). (From Velasco M, Llamas E, Guijarro-Rojas M, et al: Fulminant herpes hepatitis in a healthy adult: a treatable disorder? J Clin Gastroenterol 28:386, 1999.)

causes varicella in susceptible hosts, with a peak incidence in March through May in the United States. VZV is transmitted through aerosolized droplets from nasopharyngeal secretions or contact with vesicular fluid from skin lesions. Children generally develop mild disease compared with adults or immunocompromised patients, such as patients with underlying malignancy, steroid use, or immunosuppressive therapy, HIV infection, or solid organ transplantation. Although only 0.1% of varicella infections develop in this population, 25% of varicella-related deaths occur in this patient population.

The clinical manifestations of varicella include the characteristic generalized vesicular rash with pruritic lesions at various stages of development after a prodrome of fever and malaise. Other organ involvement, such as pneumonia and encephalitis, is infrequent but is associated with considerable morbidity and mortality.[106] Adults have a 25-fold higher incidence of complications compared with children. Hepatic involvement with varicella is uncommon but has been described in transplant recipients (Fig. 30-4). A study

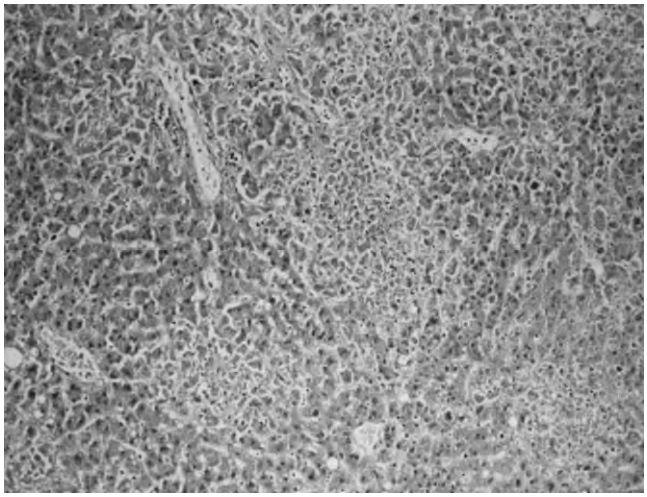

Figure 30–4 Liver with scattered necrotic areas from varicella infection (hematoxylin and eosin 300×.) (From Os I, Strom EH, Stenehjem A, et al: Varicella infection in a renal transplant recipient associated with abdominal pain, hepatitis, and glomerulonephritis. Scand J Urol Nephrol 35:330, 2001.)

assessing clinical features of liver transplant patients with varicella hepatitis showed that the most common presenting features were cutaneous vesicular lesions, fever, and acute abdominal or back pain. The rash may not be apparent at the time of hepatic involvement, however, and the diagnosis of varicella hepatitis may be delayed. In case reports, high-dose acyclovir (10 mg/kg every 8 hours) has been shown to treat varicella hepatitis successfully.

The currently available live varicella vaccine is not recommended after transplantation.[19] Pretransplant vaccination with the live varicella vaccine has been found to be safe and immunogenic in children with chronic kidney failure, children on dialysis, and children with chronic liver disease, who were varicella-naive. Broyer and coworkers[32] showed a reduction in the incidence of varicella infection in pediatric kidney transplant recipients after the pretransplant administration of varicella vaccine. Prevention of varicella primary infection in susceptible transplant recipients after exposure includes use of varicella immunoglobulin given within 72 hours of exposure.[50]

Similar to all herpesviruses, VZV establishes latency and may subsequently reactivate.[61] Reactivation of latent VZV typically results in a localized skin infection known as herpes zoster or shingles, a syndrome characterized by a painful, unilateral vesicular eruption in a restricted dermatomal distribution. Analysis of a large administrative database found the overall incidence of herpes zoster to be 3 cases per 1000 person-years.[133] The incidence of herpes zoster in patients receiving care for HIV, transplantation, or cancer was considerably higher at 10 cases per 1000 person-years. In a retrospective study of 869 solid organ transplant recipients at the University of Alberta, Gourishankar and associates[108] found that the incidence of herpes zoster was 7.4% in renal transplant recipients, with a median time of onset of 9 months. The only independent risk factor for herpes zoster in renal transplant patients was antiviral therapy other than CMV prophylaxis, usually intravenous ganciclovir or low-dose acyclovir. The authors postulated that this variable was a marker for identifying patients at high risk for reactivation of herpesviruses.

Disseminated zoster in transplant patients can be a severe, prolonged illness. In a case series of four renal transplant recipients who developed primary (one recipient) or reactivation (three recipients) VZV infection, all four had multiorgan involvement, and three of the four developed hepatitis.[92] Primary varicella infection generally is a more severe illness than reactivation disease. Fehr and colleagues[92] reviewed all cases of herpes zoster in renal transplant recipients and found 34 reported cases, most of which were primary infections. Analysis of these cases showed that disseminated intravascular coagulation and hepatitis occurred in half of the cases, and pneumonitis occurred in 29% of patients. The overall mortality was 34%, although it seems to have decreased over time from 53% to 22%. Although these data are based on compilation of cases from the literature, and firm conclusions cannot be drawn regarding the impact of VZV infection in the renal transplant population, they highlight the severity of this infection in this patient population.

Treatment of disseminated zoster in transplant patients should be undertaken promptly with high-dose acyclovir. Patients with disseminated zoster should be hospitalized and placed in airborne and contact isolation to minimize nosocomial transmission. A new vaccine against herpes zoster

(Zostavax) has been approved by the Food and Drug Administration for individuals 60 years old or older, based on a large randomized trial of 38,000 adults, in whom the vaccine reduced the burden of herpes zoster infection by 61% ($P < .001$).[199] Immunocompromised patients were excluded from this trial, and because this vaccine is a live vaccine, it is not recommended for use in transplant recipients.

Human Herpesvirus-6 and Human Herpesvirus-7

HHV-6 and HHV-7 are ubiquitous lymphotropic herpesviruses and were initially isolated from patients with lymphoproliferative disorders.[46] The cellular host range of HHV-6 and HHV-7 includes $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, natural killer cells, macrophages, megakaryocytes, glial cells, and epithelial cells. Besides directly infecting cells, HHV-6 is a powerful inducer of cytokines (e.g., tumor necrosis factor-α and interferon-γ).[237]

HHV-6 has two subtypes (A and B) that differ from each other by 4% to 6% at the nucleotide level.[73] Seroprevalence surveys have found that HHV-6 infection occurs in most children by age 3 years, and the prevalence in adults is greater than 90%. HHV-6 DNA can be detected in saliva and peripheral blood mononuclear cells of 90% of healthy individuals. HHV-6 also can be recovered in vivo from a broad range of tissues, such as lymph nodes, peripheral blood mononuclear cells, renal tubular cells, salivary glands, and the central nervous system. HHV-6B is the predominant variant detected in healthy adults; much less is known about the epidemiology of HHV-6A.

The major childhood clinical syndrome caused by HHV-6 primary infection is exanthema subitum. Infection in immunocompetent adults is usually benign, manifesting as fever with lymphadenopathy or an infectious mononucleosis–like syndrome. HHV-6 is increasingly recognized as a pathogen in transplant patients[60]; cases of interstitial pneumonitis, bone marrow suppression, hepatitis, and encephalitis have been reported in solid organ transplant recipients. HHV-6 also has been proposed as a possible cause of acute liver failure in nontransplant patients who eventually required liver transplantation,[121] and pretransplant HHV hepatitis has been shown to be a risk factor for post–liver transplant HHV-6 hepatic involvement.[120]

Given the high rate of HHV-6 seropositivity in the general population, most infections in transplant patients are proposed to result from reactivation of the latent virus. HHV-6 reactivation has been shown to predispose to primary CMV infection and disease in renal transplant recipients at risk for CMV.[72] The clinical significance of HHV-7 infection in solid organ transplantation has not been fully defined, although it has been shown to increase the risk for CMV disease in renal transplant recipients.[149] Infection with HHV-6 and HHV-7 usually occurs 2 to 4 weeks after transplantation; this characteristic timing of onset distinguishes HHV-6 from CMV, which usually occurs later, 6 to 12 weeks after transplantation. Donor transmission of HHV-6 also has been documented.

The usefulness of virus isolation, serology, and qualitative PCR for diagnosis of HHV-6 and HHV-7 is limited because most patients have positive tests for these even in the absence of clinical disease.[278] Qualitative PCR often cannot distinguish between latent and active infection. During the past few years, virus load measurements through quantitative PCR have been explored with promising results.[278] Quantifying virus load should allow better definition of the contribution of HHV-6 to post-transplant complications.[212]

No controlled study has been performed for prevention or treatment of HHV-6 infection in transplant recipients. The first step is reduction of immunosuppression. Ganciclovir, foscarnet, and cidofovir all have in vitro activity against HHV-6,[40] and reports of the effects of antiviral therapy in HHV-6 hepatitis have been published.[42] In contrast, HHV-7 is much less susceptible to ganciclovir,[275] and in studies in transplant patients, the prevalence of HHV-7 viremia did not seem to be reduced by oral or intravenous ganciclovir.[30,39]

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Chapter 31

Neurological Complications after Renal Transplantation

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Neurological disease commonly arises as a complication of kidney transplantation. Benign to life-threatening neurological disease may be encountered hours to years after transplantation. Neurological consultation may be obtained for a variety of reasons, including altered mental status, new-onset seizures, sudden hemiplegia, or slowly progressive numbness and tingling. Diagnosis and treatment are best undertaken in conjunction with a neurologist acquainted with transplantation. Diagnostic confusion can be caused by the residue of prior neurological disease, the coexistence of multiple diagnoses, and the suppression of normal inflammatory responses by immunosuppression.

Over the years, as surgical techniques have been refined, and immunosuppressants have been improved, transplant complications have declined. An early, large retrospective study found the neurological complication rate to be 30% over an 18-year period.[1] Two more recent studies have found lower rates of 8% and 10% over a 26-year period and a 19-year period.[56,101] Neurological complications may be underdiagnosed, however. In a prospective brain magnetic resonance imaging (MRI) study, 30% of 187 renal transplant patients had abnormal neuroradiological findings.[3]

Neurological disease can result from the disease process underlying renal failure. This possibility is important to realize so that symptoms are not ascribed to the transplant when they may have been extant before the procedure. This chapter discusses the most commonly encountered preexisting neurological syndromes. When one suspects de novo neurological disease in a renal transplant patient, it is helpful to localize the area of neurological dysfunction broadly into central nervous system (CNS) or peripheral nervous system (PNS) dysfunction and to assess the timing of complication onset (acute, subacute, or chronic), to aid in differential diagnosis.

NEUROLOGICAL DISEASE PRECEDING RENAL TRANSPLANTATION

Diseases that underlie kidney failure often cause coincidental injury to the nervous system, which may not be discovered until long after transplantation. Patients with long-standing uremia frequently have signs of chronic PNS toxicity. Additionally, dialysis has been associated with at least two forms of neurological disturbance—dialysis dysequilibrium syndrome and dialysis dementia.[18]

Systemic Disease

Disease processes that cause renal insufficiency commonly cause progressive injury to the nervous system. These underlying disease processes include diabetes mellitus, hypertension, autoimmune diseases such as systemic lupus erythematosus, and human immunodeficiency virus (HIV). Diabetes and hypertension predispose patients to small vessel disease. Ischemic strokes may manifest with acute neurological deficits or may occur subclinically, with gradual accumulation of cognitive deficits. Diabetes is known for its effects on the peripheral nerves as well, primarily causing a painful sensory neuropathy. Systemic lupus erythematosus is associated with cognitive dysfunction, headache, seizures, chorea, cerebrovascular events, myelopathy, polyneuropathy, and mononeuropathy.[83] Other autoimmune disorders may disturb the nervous system similarly.[18] HIV is capable of innumerable syndromes affecting the nervous system; the most common are dementia, vacuolar myelopathy, and sensory neuropathies.[73]

Uremia

Acute and chronic uremia produce characteristic neurological syndromes. Acutely, an increase in blood urea nitrogen (BUN)

produces an encephalopathy characterized by fluctuating level of consciousness, seizures, and prominent asterixis accompanied by diffuse weakness.[28] Chronic uremia may cause milder symptoms and signs, such as anorexia, insomnia, restlessness, and mild asterixis.[89] Uremic encephalopathy correlates less with levels of BUN and more with rate of increase, with rapid BUN accumulation causing a more severe alteration in consciousness.[98] The mechanism underlying the encephalopathy is not well established but may be secondary to abnormalities in brain energy usage, accumulation of toxic organic acids in the CNS, or direct toxic effects of parathyroid hormone in the CNS.[18,69]

Chronic uremia as seen in end-stage renal disease is a well-known cause of a length-dependent, axonal, symmetrical, sensorimotor polyneuropathy that is partially reversible with correction of renal function.[15] Autonomic neuropathy leads to postural hypotension, sudomotor abnormalities, impotence, and gastrointestinal disturbances. The autonomic impairment may be partially responsible for significant blood pressure lability seen frequently during dialysis.[61]

Dialysis Dysequilibrium Syndrome and Dialysis Dementia

Dialysis dysequilibrium syndrome was first recognized in the 1960s when patients were rapidly dialyzed over short periods. Today, dialysis is performed slowly and intermittently, and the syndrome is seen in a milder form when a patient initiates dialysis. Dialysis dysequilibrium syndrome is characterized by headache, irritability, restless legs, agitation, somnolence, seizures, muscle cramps, and nausea. These symptoms may stabilize or improve with long-term dialysis. The syndrome is thought to be caused by increased intracranial pressure and cerebral edema from the osmotic gradient that develops between the plasma and brain during rapid dialysis.[18]

Dialysis dementia is a progressive encephalopathy thought to be related to aluminum intoxication; this is less commonly seen because aluminum-rich dialysate is not widely used, and because dietary aluminum intake is restricted.[28,67] Dialysis dementia affects a subset of patients who are on dialysis longer than 1 year and manifests initially with hesitancy of speech, leading to speech arrest, followed by decline in intellect, delusions, hallucinations, seizures, myoclonus, gait disturbance, and death within 6 months to 1 year. When diagnosed, the patient is treated with deferoxamine.[18]

APPROACH TO THE RENAL TRANSPLANT PATIENT WITH NEUROLOGICAL DISEASE

Although a few neurological illnesses may occur at any time after transplantation, most problems are likely to occur as immediate, subacute, or chronic complications of transplantation. Within each time period, neurological syndromes may be divided into central and peripheral etiologies. CNS dysfunction localizes to any abnormality of the brain or spinal cord. PNS dysfunction localizes to the nerve roots, peripheral nerves, or muscle.

Central Nervous System Dysfunction

Encephalopathy

CNS illness often manifests as altered mental status, also known as encephalopathy. The hallmark of encephalopathy is reduced attention span with a decreased or fluctuating level of consciousness. Patients typically are disoriented to varying degrees, with poor awareness of their environment and circumstances surrounding their illness, although it is rare for a patient to lose orientation to self. Encephalopathy may or may not be accompanied by seizures. The etiologies are numerous, ranging from infection to metabolic derangement to multiple embolic strokes. CNS dysfunction may occur in the absence of encephalopathy; this is seen with focal seizures or neurological deficits from a stroke or mass lesion.

Seizures

A seizure is a symptom of CNS dysfunction, and an underlying etiology should be sought. Seizures are common after transplantation, estimated to occur in 6% to 36% of post-transplant patients.[35,39,74] In a review of 119 renal transplants in children, 17% of the children had seizures over a period of 10 years. Most occurred less than 55 days after transplant.[6] The etiologies included hypertensive encephalopathy, fever with infection, and acute allograft rejection. Of the patients with post-transplant seizures, 25% had a history of seizures before transplantation.

Seizures are classified as being either partial in origin—electrical focus in one region of the brain—or generalized—electrical abnormality coming from the entire brain. An electroencephalogram may help define the patient's seizure type. Routine electrolytes, magnesium, and drug levels of cyclosporine and tacrolimus should be obtained. If brain imaging by MRI is unrevealing for a mass lesion, spinal fluid should be examined for signs of increased intracranial pressure, infection, inflammation, abnormal cytology, and, with complaints of severe headache, subarachnoid hemorrhage.

Treatment of seizures is best directed toward correction of the underlying abnormality. While awaiting these treatments to take effect, benzodiazepines can be used on a short-term basis; however, these can cause sedation, which may compromise the neurological examination of an already encephalopathic patient. Multiple antiepileptic medications can be tried if a patient is at risk to develop more seizures. The cytochrome P-450–inducing anticonvulsants (phenytoin, carbamazepine, and phenobarbital) may affect immunosuppressive agents metabolized by the liver. The clearance of cyclosporine and corticosteroids is increased in the presence of these anticonvulsants.[64] Levetiracetam may be preferable because of its minimal effects on the liver. Isolated seizures in the setting of organ transplantation rarely lead to epilepsy, and long-term anticonvulsant therapy is seldom needed.[6,66]

Peripheral Nervous System Dysfunction

PNS illness encompasses any neurological abnormality affecting (1) the nerve roots exiting the spinal cord, known as radiculopathy; (2) the peripheral nerves, known as neuropathy; and (3) the muscle, termed myopathy. Disease affecting the nerve roots may cause weakness, numbness, and pain, as in the case of Guillain-Barré syndrome. The peripheral nerves typically are affected in a length-dependent fashion causing slowly progressive numbness and tingling. A focal nerve may be compressed during surgery, however, causing an asymmetric weakness and numbness in the distribution of that nerve. Myopathy may manifest with cramps, myalgias, and weakness of proximal muscles and is typically symmetrical. Difficulty with standing

from a seated position and walking up stairs are common complaints.

IMMEDIATE NEUROLOGICAL COMPLICATIONS

Neurological complications that occur within days of renal transplantation have characteristic etiologies, which help with the differential diagnostic possibilities. We have categorized these complications into disorders involving the CNS and disorders involving the PNS (as described earlier).

Central Nervous System Dysfunction

Hypoxic-Ischemic Insult and Perioperative Sedation

In the immediate postoperative period, transplant patients may exhibit behavioral changes ranging from a mild confusional state to severe encephalopathy. Acute confusional states often are related to a global hypoxic-ischemic insult. Neuroimaging with computed tomography (CT) or MRI may aid with this diagnosis. In the absence of evidence of ischemia, other causes (see later) should be explored. In patients with renal or hepatic failure, poor metabolism and excretion of anesthetics and other sedating medications should be considered. Altered mental status occurring 2 to 5 days after surgery may be the result of intensive care unit (ICU) psychosis, which may resolve with neuroleptics or environmental reorientation.[66]

Electrolyte Imbalance

Electrolyte abnormalities are common after transplantation. Postoperative polyuria is often treated with fluid replacement in the form of hypotonic saline. If sodium decreases to less than approximately 120 mEq/L, generalized tonic-clonic seizures and worsening mental status from cerebral edema may occur.[5,78] Hypomagnesemia also is known to cause seizures. Although anticonvulsants may help, treatment of the seizures is best achieved by correcting the electrolyte imbalance. Sodium is corrected using normal or slightly hypertonic saline. The sodium should be corrected slowly (≤10 mEq/L over 24 hours) because rapid correction can lead to central pontine myelinolysis, as discussed subsequently.

Rejection Encephalopathy

The term rejection encephalopathy has been used to describe an episode of acute graft rejection particularly in young patients that is accompanied by altered mental status.[6] The entity was initially proposed based on a case series of 13 patients who exhibited a reversible acute neurological syndrome that coincided with severe acute rejection of the transplanted kidney.[41] Young transplant patients may be particularly susceptible because 11 of these patients were younger than 20 years of age.

The patients developed various combinations of seizures, headache, confusion, disorientation, and irritability, and one had papilledema. Acute rejection was defined by presence of graft swelling and tenderness, fever, weight gain, and hypertension. Patients with encephalopathy had a greater increase in serum creatinine compared with patients without encephalopathy. No differences were noted between the groups when comparing blood pressure or rate of increase of blood pressure. There were no differences in serum electrolytes, weight gain or fluid retention, or type of immunosuppressant in the two groups.

The patients in this cohort had an excellent prognosis with no residual sequelae.[40,41] It is unclear whether rejection encephalopathy should be regarded as a direct consequence of graft rejection or a reflection of the accumulation of metabolic and physiological insults occurring during severe graft rejection and its treatment.

Hypertensive Encephalopathy

Hypertensive encephalopathy has been reported after transplantation. The diagnosis should be considered when other causes of altered mental status have been excluded. Sometimes, the entity, also called malignant hypertension, is accompanied by papilledema and seizures.[6,25] It is thought to be the cause of death in some patients, especially in children after renal transplantation.[101,119] The diagnosis of hypertensive encephalopathy can be aided by MRI, which reveals a characteristic posterior leukoencephalopathy, which is reversible after blood pressure is controlled.

Infection

Despite immunosuppressant doses being high during this period, CNS infection within 1 month of transplantation is uncommon. When infections are present, it often suggests that the infection was present before transplantation, was acquired from the donated organ, or is related to surgical complications such as the presence of an indwelling catheter.[90] These infections are usually due to common pathogens found in the general, nonimmunosuppressed population.[22,49]

Central Pontine Myelinolysis

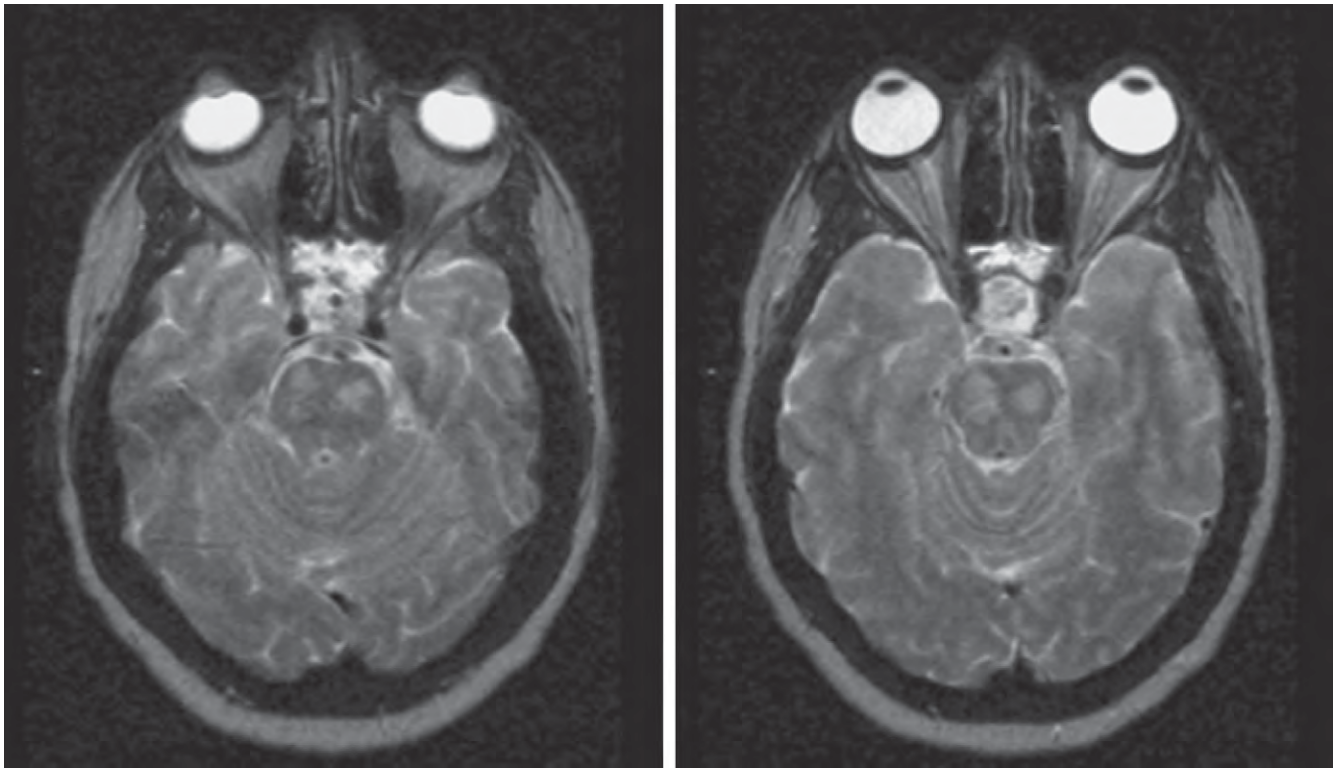
Central pontine myelinolysis is rare in recipients of renal transplants and occurs more frequently after liver transplantation (Fig. 31-1).[59,84,131] It usually occurs within 10 days of transplantation and is seen after rapid correction of chronic hyponatremia.[77,108] Patients develop symmetrical limb weakness with extensor plantar responses over hours to days. Facial and bulbar musculature may be paralyzed. In severe cases, a locked-in state develops, in which the patient remains fully conscious but no voluntary movements are possible apart from vertical eye movements, a state that may be misinterpreted as coma. Death and chronic disability are common, and full recovery is rare. Because many cases of this disastrous neurological disorder seem to be iatrogenic, it is recommended that the serum sodium correction should not exceed 10 mEq/L in 24 hours.[84,114,130,131]

Peripheral Nervous System Dysfunction

Peripheral nerve injuries during renal transplantation are uncommon, with estimates of 2% to 5%.[2,18,104] The most common sites involved are the femoral nerve, lateral femoral cutaneous nerve, the lumbosacral plexus, and the ulnar nerve. Nerve damage is thought to occur by several mechanisms, including ischemia, compression from malpositioning a pharmacologically paralyzed patient, compression by local hematoma formation, or stretching of the nerve owing to prolonged retraction.

Femoral Neuropathy

A prospective study found that 4 of 184 patients (2.2%) developed acute femoral neuropathy that was ipsilateral to



A B

Figure 31–1 Central pontine myelinolysis. A 52-year-old woman with end-stage renal disease on hemodialysis presents with 2 days of progressive lethargy and tetraparesis. **A** and **B**, Axial T2-weighted MR images of the brain show bilateral pontine signal hyperintensity consistent with the diagnosis of central pontine myelinolysis. The patient had gradual and complete improvement of her weakness.

the side of the renal transplant. It developed 24 to 48 hours postoperatively, and all patients had excellent motor recovery in 4 to 9 months.[104] Femoral neuropathy is typically noticed early after surgery but may not be apparent until the patient attempts to walk. Nerve damage may occur from stretching of the nerve secondary to self-retaining retractors.[120] Another mechanism is ischemia to the femoral nerve during anastomosis of the graft renal artery to the internal iliac artery by a "steal phenomenon."[55] On neurological examination, patients exhibit unilateral weakness of knee extension; loss of the patellar reflex; and decreased sensation on the anterior-medial aspect of the thigh, knee, and calf. Neuropathic changes on nerve conduction studies and electromyography typically are seen 1 week after injury. Compressive femoral neuropathies usually resolve entirely, but this takes several months and can be incomplete.[75,96,97,109,120,132] The lateral femoral cutaneous nerve is often exposed and retracted during transplantation and was injured in 2.4% of patients in one series.[109] Injury to this nerve causes numbness over the lateral aspect of the thigh.

Lumbosacral Plexopathy

Lumbosacral plexopathy is seen when the internal iliac artery is used for revascularization of the graft, particularly in diabetic patients.[44] This lesion occurs postoperatively with buttock pain and weakness of ankle dorsiflexion and eversion and sometimes proximal leg weakness. Recovery occurs but may be incomplete.

Ulnar Neuropathy

Ulnar neuropathy may occur from mechanical trauma at the elbow, from the weight of the patient and physician on the adducted arm, and from the blood pressure cuff compressing the cubital fossa. Arms with and without an arteriovenous fistula seem to be affected equally.[133] Patients with diabetes seem to be more susceptible.[102] Patients may have sensory complaints in the medial aspect of the hand, including the ring and little fingers.

SUBACUTE NEUROLOGICAL COMPLICATIONS

Within weeks of renal transplantation, many of the neurological complications are related to immunosuppression directed at the transplanted kidney. Central dysfunction from calcineurin inhibitors is often manifested by altered mental status that may be accompanied by seizures. The severe manifestations of calcineurin inhibitor toxicity usually develop within the first 3 months of therapy and have been reduced with the use of a microemulsion preparation that allows for steadier absorption.[126]

PNS dysfunction from immunosuppressants manifests as symmetrical paresthesias or as myopathy. Another category of a PNS dysfunction that occurs weeks after transplantation is Guillain-Barré syndrome, which can be life-threatening if not diagnosed quickly and appropriately managed.

Central Nervous System Dysfunction

Cyclosporine

Cyclosporine-related neurological side effects are more common in liver transplant recipients, possibly as a result of associated hypocholesterolemia and hyponatremia.[1,27] In renal transplant patients, cyclosporine is estimated to be responsible for approximately 20% of neurological complications.[12,57,85] These side effects range from tremor and paresthesias to a serious leukoencephalopathy. Limb tremor is the most common side effect of cyclosporine. It is a fine tremor in the upper extremities that is most prominent while holding hands in posture, typically seen within the first 3 months.[57,126] Many instances of tremor and paresthesias are not sufficiently troublesome to warrant reducing effective immunosuppressive therapy. A lower extremity pain syndrome has been associated with cyclosporine and renal transplant patients, termed calcineurin inhibitor pain syndrome. Nine patients on cyclosporine developed severe pain in their feet in one study. MRI showed bone marrow edema in the painful bones.[42]

Confusion, coma, cortical blindness, cerebellar syndromes, hemiplegias, and flaccid quadriparesis all have been described in cyclosporine recipients. The multifocal disorder including various combinations of these features has been termed reversible posterior leukoencephalopathy; this is a clinical-radiological syndrome with other etiologies such as malignant hypertension and preeclampsia.[53,54,113] A prospective brain MRI study was performed in 187 kidney transplant recipients and 29 liver transplant recipients. In the patients who received a kidney transplant, 1.6% had findings consistent with reversible posterior leukoencephalopathy (two with cyclosporine toxicity and one with tacrolimus toxicity), whereas 20.1% of liver transplant recipients met criteria for the diagnosis.[3] Classically, the posterior white matter is involved; however, it is now known to affect the frontal lobes and gray matter as well (Fig. 31-2).

The neurological syndrome and brain imaging abnormalities usually resolve within 2 weeks after stopping cyclosporine or after dosage reduction if blood levels were particularly high. Although the syndrome is usually reversible, a small percentage of patients progress to death or have incomplete recovery.[103] Cortical blindness is a rare complication and is usually completely reversible with reduction or withdrawal of cyclosporine.[34] The mechanism of reversible posterior leukoencephalopathy is thought to be related to disruption of the blood-brain barrier, possibly mediated by astroglial cellular effects on endothelial permeability.[31]

Tacrolimus

Tacrolimus is frequently the alternative immunosuppressant to cyclosporine and usually is a satisfactory replacement in cases of severe cyclosporine-associated neurological side effects. Studies of liver transplant recipients have shown neurotoxic side effects, however, in 20% to 30% of patients.[79,128] Tacrolimus and cyclosporine were compared in a prospective unblinded randomized trial of 400 patients after renal transplantation. The tacrolimus group reported higher rates of all neurological side effects; tremor was significantly greater than in the cyclosporine group at 54% versus 34%, as were paresthesias at 23% versus 15%.[94] Side effects usually occur within the first months of therapy and are more common at higher doses. Generalized seizures, tremor, ataxia, encephalopathy, nightmares, and agitation have occurred, most resolving with dose reduction.

A leukoencephalopathy similar to that caused by cyclosporine can be seen on MRI.[110,117] This syndrome usually manifests with occipital headache, nausea, and vomiting, followed by seizures and visual disturbances. Tacrolimus blood levels may be high, although not invariably, and the disorder resolves with dosage reduction.

OKT3 Monoclonal Antibody

OKT3 therapy for acute rejection is associated with multiple neurological side effects. In one series of 21 patients with acute renal allograft rejection treated with OKT3, 29% had headache associated with nausea and vomiting, and 14.2% presented with severe neurological alterations.[36] OKT3 also is associated with aseptic meningitis, which typically occurs 2 to 7 days after treatment.[88] Such patients develop various degrees of fever, photophobia, and headache. The syndrome may resolve spontaneously even if OKT3 therapy is continued.[71]

Steroids

High-dose steroid therapy may cause mood alteration in the form of mania and depression and occasionally causes psychosis requiring anxiolytics and antipsychotics if the steroid dosage cannot be reduced safely. Epidural spinal lipomatosis is a well-described but uncommon complication in the post-transplant population, related to the use of steroids for immunosuppression.[116]

Peripheral Nervous System Dysfunction

Cyclosporine

Limb paresthesias are common in patients taking cyclosporine. Many patients report burning sensation of the limbs, but clinical and electrophysiological evaluation usually does not reveal evidence of peripheral neuropathy. If neuropathy is present in such patients, it is usually attributable to prolonged uremia before transplantation or other predisposing conditions. Whether cyclosporine alone causes neuropathy is debatable.[14,123]

Tacrolimus

A severe demyelinating sensorimotor peripheral neuropathy has been associated with tacrolimus use in liver transplant patients. Whether this neuropathy also occurs in renal transplant recipients receiving tacrolimus is unknown.[16]

Steroids

Steroids have long been associated with myopathy, but the prevalence has not been well established. Steroid myopathy does not seem to be dose-dependent, occurring with acute and long-term use.[4,58] Current study of steroid-induced myopathy is in the context of ICU patients who are receiving steroids and neuromuscular blocking paralytic agents. It does not seem to be related to length of ICU stay.[13] One prospective study followed 281 liver transplants and identified four patients who developed acute quadriplegic myopathy postoperatively. These four patients were receiving typical steroid doses. All had significantly higher intraoperative complications and required longer ICU and hospital stays than the average transplant patient. Muscle pathology

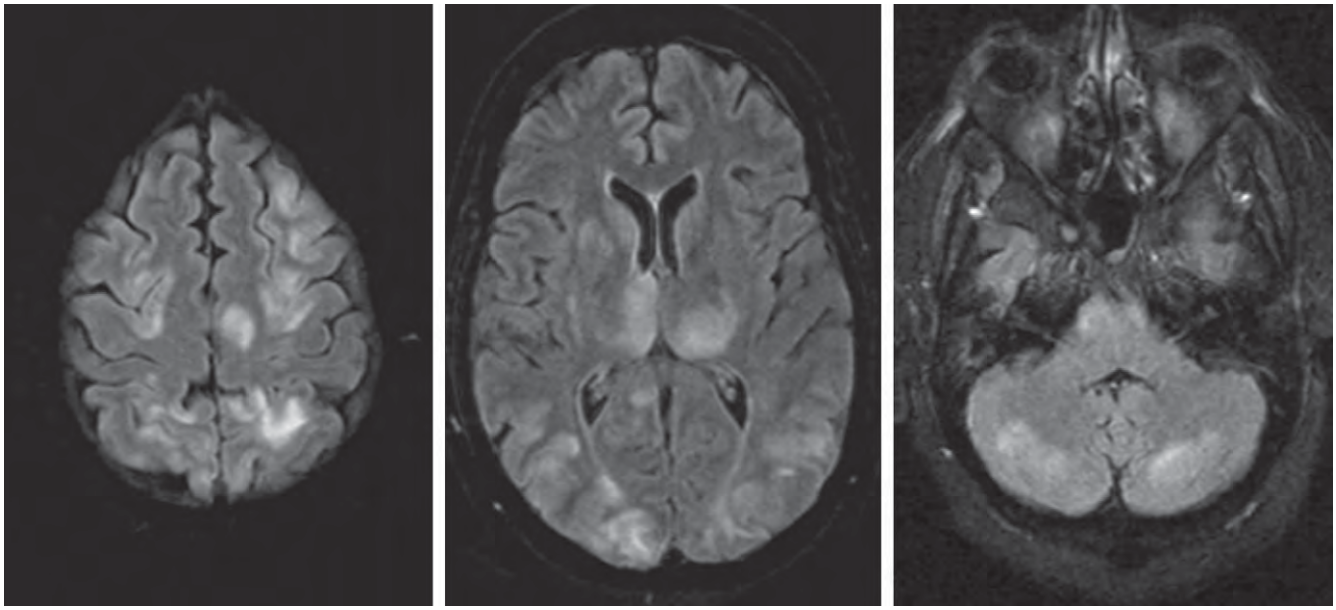


Figure 31–2 Reversible posterior leukoencephalopathy secondary to cyclosporine toxicity. A 45-year-old woman with a history of non-Hodgkin's lymphoma had undergone a matched unrelated donor bone marrow transplant 1 month previously. Over 24 hours, she developed altered mental status and generalized tonic-clonic seizures. Cyclosporine was found to be at a toxic blood level of 806 ng/mL. **A-C**, Axial fluid-attenuated inversion recovery MR images of the brain show abnormal hyperintense signal in the cortex and subcortical white matter of the occipital and parietal lobes and cerebellum bilaterally. There is extension into the frontal lobes (**A**), deep gray matter of the basal ganglia and thalami (**B**), and pons (**C**). Cyclosporine was discontinued, and the patient was given tacrolimus. She had no further seizures, and her mental status returned to normal.

showed loss of myosin in the thick muscle fibers. All patients had improvement and were able to walk but had mild persistent proximal weakness at long-term follow-up.[76]

Guillain-Barré Syndrome

Subacute tetraparesis caused by Guillain-Barré syndrome has followed renal transplantation and is associated with transmitted cytomegalovirus (CMV) infection or reactivation of latent CMV infection.[8,32] In some cases, the patient is CMV negative.[17] One renal transplant patient with Guillain-Barré syndrome was found to have bacteremia with *Campylobacter jejuni*, a common prodromal infection in nontransplant Guillain-Barré syndrome patients.[68] Guillain-Barré syndrome typically manifests as an ascending paralysis over 2 to 3 days with areflexia, often accompanied by a mild ascending sensory loss. It may progress quickly to involve the respiratory muscles, requiring intubation. Nerve conduction studies show a proximal demyelinating polyneuropathy. Treatment is with total plasma exchange or intravenous immunoglobulin.[72] Although many patients have full recovery within months, others may have permanent neurological deficits in the form of weakness and sensory loss.[33]

CHRONIC NEUROLOGICAL COMPLICATIONS

There are few PNS complications that begin months after renal transplantation. The most common chronic CNS complications are infection and stroke. Infection can occur at any time after transplantation, but risk increases significantly at 1 month after the transplant operation.[38] Ischemic and hemorrhagic strokes may occur at anytime but are typically seen many months after transplantation. Primary CNS lymphoma can occur during this time, often manifesting several months after transplantation but in most cases within 1 year.

Infection (see Chapter 29)

At some point after transplantation, 5% to 10% of transplant patients develop a CNS infection, with 44% to 77% of the infections resulting in death.[22] An Indian cohort of 792 renal allograft recipients found that CNS infections constituted most neurological complications, accounting for 39% of brain dysfunction.[101] CNS infection can be divided into four categories based on clinical presentation: (1) meningitis, including acute bacterial and insidious fungal infections; (2) encephalitis or meningoencephalitis; (3) focal brain abscess; and (4) progressive dementia.

Meningitis

A nonimmunosuppressed patient with acute meningitis presents with fever, nuchal rigidity, headache, and confusion; meningitis progresses quickly to death if untreated over 24 to 48 hours. CNS infection in transplant patients may be difficult to diagnose because immunosuppressant therapy minimizes the symptoms and signs that would normally develop from meningeal inflammation. Transplant patients with advanced CNS infections may present with few clinical signs of infection.

Listeria monocytogenes is the most common cause of acute and subacute bacterial meningitis in transplant patients. Other common pathogens include *Haemophilus influenzae, Neisseria meningitidis*, and *Streptococcus pneumoniae*.

Fever and headache most commonly develop over 1 to several days. Focal neurological deficits, impaired consciousness, and meningismus are encountered in less than half of cases.[95] *Listeria* infection may occur at any time after transplantation but rarely within the first month.[49] Analysis of cerebrospinal fluid (CSF) shows pleocytosis, increased protein, and normal or reduced glucose concentration. Gram stain may be positive in less than one third of the cases.[81] CSF cultures positive for *Listeria* may develop late, and blood cultures may reveal the organism first.[30,112] Diagnosis is easiest in patients with purely meningitic syndromes in whom there is a high chance of positive cultures from blood or CSF. Confirmation of the diagnosis may prove difficult in patients with nonmeningitic *Listeria*. The most common nonmeningitic form of CNS listeriosis is a meningoencephalitis, which manifests with ataxia and multiple cranial nerve abnormalities, such as oculomotor weakness or dysarthria.[30,65] *Listeria* may manifest as a focal brain abscess with a higher mortality rate. Twenty-five percent of these patients also have meningitis, and almost all patients become bacteremic.[26,30]

In a patient with subacute or chronic meningeal symptoms, such as low-grade fevers and mild headache, fungi are the most common etiological agent and are associated with a 70% mortality. In the Indian cohort described earlier, of the 31 renal allograft patients who had CNS infection, cryptococcal meningitis occurred in 12, mucormycosis occurred in 6, and aspergillosis occurred in 1 patient.[101]

Cryptococcus neoformans meningitis usually develops more than 6 months after engraftment with insidious clinical progression.[49] One review showed that clinical presentation of cryptococcal meningitis in organ transplant recipients can vary, including encephalopathy (64%), nausea and vomiting (50%), fever (46%), headache (46%), nuchal rigidity (14%), visual loss (7%), and seizures (4%). The mean length of symptoms before the diagnosis of meningitis was 17 days (range 2 to 30 days).[121] The CSF opening pressure frequently is increased. Culturing the organism from CSF may take weeks, and immunological detection of CSF cryptococcal antigen is recommended as a quick, reliable diagnostic method. Brain imaging in organ transplant patients with cryptococcal meningitis may be normal or reveal nonspecific results.[121] Antifungal treatment with intravenous amphotericin B or fluconazole or both may eradicate the infection in most patients without necessitating a reduction in immunosuppression that might jeopardize graft survival.[49,124] Other chronic meningeal infections are *Mycobacterium tuberculosis, Strongyloides stercoralis, Coccidioides immitis*, and *Histoplasma capsulatum*.

Encephalitis

Patients with viral encephalitis (also called meningoencephalitis) exhibit prominent confusion and difficulty forming new memories. Cranial neuropathies are common when the brainstem is involved. Headache and fever are only variably present. Proven CMV encephalitis is rare in transplant recipients but when seen may be associated with retinitis.[9] Brain MRI may show white matter abnormalities or meningeal enhancement, or may be normal. The CSF should be sent for CMV polymerase chain reaction, which reliably indicates CMV infection in the CNS.[21] Making the diagnosis is important because of the prospect for treatment with ganciclovir or foscarnet and the need to reduce the immunosuppressant drug regimen.[48,124]

Varicella-zoster virus is a common post-transplant infection that affects many organs and causes a brainstem encephalitis. Other offending agents that produce encephalitis include *Toxoplasma gondii*, human herpesvirus-6, *S. stercoralis*, and *Cryptococcus neoformans*.[62] West Nile virus has potential to cause a severe meningoencephalitis in transplant recipients. It has been transmitted by the organ donor and acquired naturally in communities where the virus is endemic.[20,29,60]

Focal Brain Infections

Cerebral abscesses in transplant recipients are usually due to aspergillosis or less often due to candidal abscess, cryptococcosis, nocardiosis, toxoplasmosis, mucormycosis, or listeriosis. *Aspergillus fumigatus* usually occurs at 3 months post–renal transplantation with a mean incidence of 0.7% in kidney transplant recipients.[107] Aspergillosis in the CNS usually causes sudden focal neurological deficits or seizures. The stroke-like onset of symptoms reflects invasion of cerebral blood vessels by fungus with distal embolization. There is evidence of disseminated disease in one third of cases, most commonly involving the lung.[11,107] Head CT or MRI may show single or multiple lesions with little mass effect or contrast enhancement. Lung or cerebral biopsy is required for diagnosis. The mortality rate in transplant recipients with invasive aspergillosis ranges from 74% to 92%.[107] Downward deterioration is rapid, and most patients die despite antifungal therapy.[11,49,125]

A brain abscess from *Nocardia asteroides* is frequently disseminated from a pulmonary focus. Clusters of patients with nocardial infection may occur in transplant units.[7] Associated subcutaneous lesions may be palpable, and biopsy specimens can be obtained. *T. gondii* is a rare CNS infection in renal transplant recipients. It commonly occurs as multiple progressive mass lesions but also may cause diffuse encephalopathy or a meningoencephalitis.[82,118] Imaging studies may not always lead to the diagnosis. The presence of multiple ring-enhancing lesions is characteristic, but this also is seen in other focal infections and neoplasms.[3] Mucormycosis is common in transplanted diabetics and nearly always fatal.[101] It starts in the paranasal sinuses, producing periorbital edema and proptosis and subsequently may invade the intracavernous carotid artery, leading to cerebral artery emboli and strokes.[19]

Progressive Dementia

Progressive multifocal leukoencephalopathy (PML) is a rare and fatal condition producing widespread demyelination within the CNS. Initially described in patients with acquired immunodeficiency syndrome, PML was eventually identified in many immunosuppressed individuals. It is caused by polyomavirus infection, usually JC virus but sometimes SV40 or BK virus.[92] The clinical presentation is insidious, with progressive dementia, blindness, or bilateral weakness. More focal presentations cause hemiparesis, hemianopia, and rarely seizures.

The diagnosis is suggested by the history in conjunction with brain MRI, which shows diffuse subcortical white matter T2 hyperintensity. Mass effect and contrast enhancement are unusual. Definitive diagnosis requires tissue showing demyelination and identification of virus particles in enlarged oligodendrocyte nuclei by electron microscopy.

When invasive diagnostic procedures are not performed, a presumptive diagnosis of PML can be made by demonstration of JC virus DNA in the CSF, together with clinical and radiological findings compatible with PML.[63] Although JC virus DNA is detected in the CSF of 90% of patients with PML, a negative polymerase chain reaction result cannot reliably be used to rule out the infection.[37] Patients die within months to 1 year after a relentlessly progressive decline. Occasionally, the decline may be more abrupt after an explosive onset, and there have been rare survivors for years. Currently, there is no evidence that antiviral treatments alter the condition.

Stroke (see Chapter 28)

Stroke competes with infection as the most frequent neurological complication of kidney transplantation, but it is the most frequent cause of neurological illness among chronic complications.[2,70,99,101] A review of 403 patients who received one kidney graft between 1979 and 2000 found a stroke prevalence of 8% at 10 years, one third of which were cerebral hemorrhages. The mean age was 50 years (range 23 to 63 years). The mean time from transplant to stroke was 49.3 months. Three risk factors were identified as predictors of stroke: diabetic neuropathy, peripheral vascular disease, and age older than 40.[86] Another large retrospective review found a post-transplant stroke prevalence of 9.5%, with most occurring more than 6 months after transplantation.[2]

In a single-center study of 1600 kidney transplants between 1983 and 2002, 105 patients died, and 60.3% died with a functioning graft. Stroke was the second greatest cause of death at 17%, preceded only by infection, which accounted for 24% of deaths. After stroke, the most frequent causes were cardiovascular disease at 16%, malignant neoplasm at 15%, and hepatic failure at 11%.[105] A retrospective study looking at causes of death from renal transplantation from 1970 to 1999 found that the percentage of deaths from stroke increased over the years from 2.4% to 8% as the percentage of graft rejection at death decreased.[50]

Ischemic Stroke

Various risk factors contribute to the increase in stroke after transplantation.[2] Age older than 40 years places a transplant recipient at particular risk.[2,51] One study noted an increased stroke risk in patients whose renal failure originally was due to hypertension.[51] This association was not found in another survey, however, which noted a clear association of ischemic stroke with underlying polycystic renal disease, a condition in which hypertension is common.[2] Diabetes mellitus and systemic lupus erythematosus also predispose a patient to stroke after transplantation.[2]

Hyperlipidemia occurs in renal failure and persists to some degree after transplantation, likely contributing to accelerated atherosclerosis.[51] Long-term steroid therapy may accelerate atherosclerosis; this effect might prove to be less in the modern era of cyclosporine and tacrolimus immunosuppression, where overall steroid doses are lower. No studies have yet assessed possible changes in the incidence of thromboembolic disease as a result of the introduction of calcineurin inhibitors.

Ischemic stroke usually manifests with abrupt onset of a focal neurological deficit, such as hemiparesis, speech disturbance, clumsiness, or visual field cut. Headache may be present but is rarely severe. Head CT does not typically show signs of stroke in the first 24 hours after stroke, unless the stroke is particularly large. Brain MRI may show restricted diffusion 30 minutes after the onset of an ischemic event. If the neurological deficit recovers within 24 hours, it is termed a transient ischemic attack, rather than a completed stroke. Transient ischemic attacks are investigated in the same way as completed strokes to modify risk factors and to initiate low-dose aspirin therapy as prophylaxis against future stroke.

Potentially reversible stroke risk factors include hypertension, smoking, and diabetes mellitus. If multiple strokes occur in the presence of fever, prominent headache, or markedly lymphocytic CSF, infection and cerebral vasculitis should be considered. The fungal infections aspergillosis and mucormycosis can present as stroke after hyphal invasion of cerebral arteries with distal embolization. Cerebral vasculitis has been reported in immunosuppressed transplant recipients.[100]

Hemorrhagic Stroke

A retrospective study by the Mayo Clinic identified 10 cases of intracerebral hemorrhage among 1573 patients who received a renal transplant between 1966 and 1998. Six of the 10 patients died. The interval from renal transplantation to intracranial hemorrhage ranged from 12 to 114 months (average 57 months). All patients with intracranial hemorrhage had poorly controlled hypertension. Patients with polycystic kidney disease had a tenfold increased risk of developing a hemorrhage, and patients with diabetes mellitus had a fourfold increased risk. Most cerebral hemorrhages were catastrophic and fatal but overall were responsible for only 1% of the deaths after renal transplantation.[127]

Primary Central Nervous System Lymphoma

Non-Hodgkin's lymphoma is the second most common neoplasia occurring after solid organ transplantation.[91] A study comprising 145,000 deceased donor kidney transplants between 1985 and 2001 found 1094 cases of non-Hodgkin's lymphoma. Of the 0.7% patients who developed non-Hodgkin's lymphoma, 11.7% of those were diagnosed as primary CNS lymphoma with a 38% 5-year survival.[87] Histologically, non-Hodgkin's monoclonal B lymphocyte proliferation accounts for most primary CNS lymphomas. It typically occurs within 1 year of transplantation, with a median interval of 9 months (range 5.5 to 46 months).[46] In a study of 25 patients who developed primary CNS lymphoma after renal transplantation, the mean age at diagnosis was 46 years. The diagnosis was made 4 to 264 months after transplant (median of 18 months).[111]

Patients may present with a single lesion or multifocal lesions; the latter are seen 33% to 72% of the time.[46,80,111] The lesions are often supratentorial and periventricular in location. Cerebral lymphoma can invade the meninges, but malignant meningitis more often reflects spread from a systemic primary. Two risk factors have been identified in the development of primary CNS lymphoma: (1) the intensity of immunosuppressive regimen and (2) Epstein-Barr virus seropositivity.[52,115,122,129] Epstein-Barr virus is suspected to play a causative role in cerebral lymphoma, based on serum antibody responses, immunostaining, and DNA hybridization studies of biopsy specimens.[10,43,45,47]

Patients usually present with neurological deficits that worsen over several weeks. In a French study of 25 patients with primary CNS lymphoma after renal transplantation, the most common presenting symptom was a focal neurological deficit in 84%, either an isolated deficit or associated with seizures or increased intracranial pressure.[111] Headache usually is a late symptom, often reflecting increased intracranial pressure or meningeal involvement.[46] Less frequent neurological presentations include malignant meningitis, spinal cord lesions, and visual disturbance from ocular deposits.[46]

In immunocompetent patients, brain MRI shows primary CNS lymphoma lesions as homogeneously enhancing with gadolinium. In transplant patients, the lesions may show homogeneous, heterogeneous, or no enhancement (Fig. 31-3). Ring enhancement may be easily mistaken for glioblastoma multiforme or abscess. In primary CNS lymphoma, the CSF may have modestly elevated protein levels and low glucose but often does not show the presence of lymphomatous cells.[111] With diffuse lymphomatous involvement of the meninges, multiple cytological specimens may be required before histological confirmation is forthcoming.

A suspected diagnosis of primary CNS lymphoma should be confirmed by prompt neurosurgical biopsy. High-dose steroid therapy before obtaining the biopsy specimen may interfere with the reliability of histological diagnosis.[23,106] Biopsy is associated with significant morbidity and mortality secondary to hemorrhage.[93] Resection of the tumor does not seem to enhance long-term survival, and there is substantial morbidity after attempts to resect a deep-seated tumor.[23]

The outcome of post-transplant primary CNS lymphoma is poor.[93] In a large study of non-Hodgkin's lymphoma in

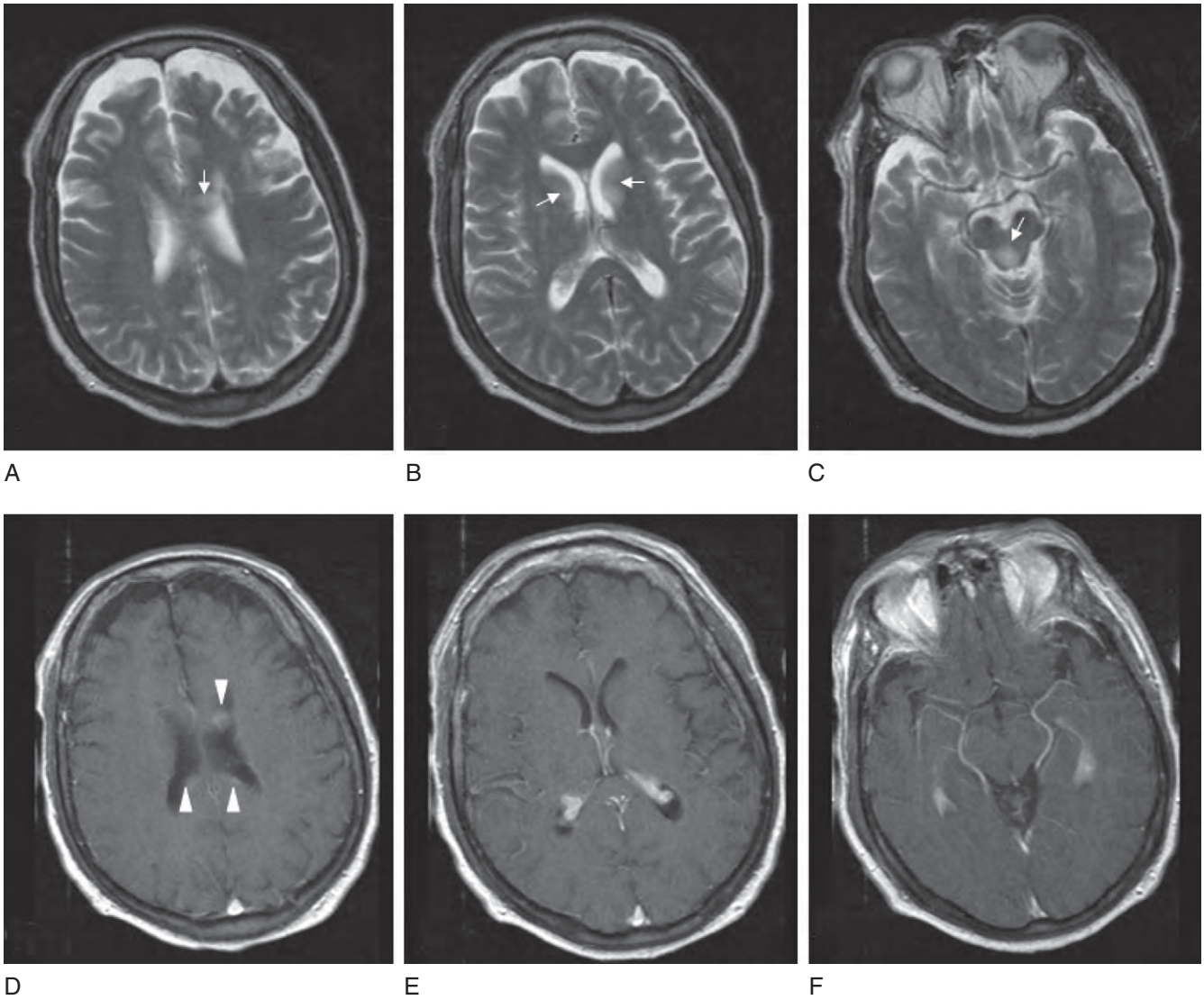


Figure 31–3 Primary central nervous system lymphoma. A 56-year-old man with a history of deceased donor renal transplant developed lethargy and altered mental status 1 year after transplantation. Cerebrospinal fluid cytology showed monomorphic large B cells consistent with primary central nervous system lymphoma. **A-C**, Axial T2-weighted MR images of the brain show regions of hyperintensity (*arrows*) in the corpus callosum (**A**), bilateral caudate (**B**), and periaqueductal region of the midbrain (**C**). **D-F**, Contrast-enhanced axial T1-weighted MR images show subtle enhancement of the lesions of the corpus callosum (*arrowheads* in **D**) with no enhancement of the lesions in the caudate and midbrain. The patient was treated with intrathecal methotrexate and later died as a result of sepsis.

145,000 deceased donor kidney transplants, of patients diagnosed with primary CNS lymphoma, 38% had a 5-year survival.[87] Most commonly, initial treatment is with reduction of immunosuppressive therapy; however, this rarely results in clinical remission alone. There are many treatment options, including intraventricular infusion of monoclonal antibodies, chemotherapy, and radiotherapy, each of which yields only 50% clinical remission. In the French cohort of 25 patients, the median survival across all treatment regimens was 26 months. An improved median survival of 42 months was reported when high-dose cytosine arabinoside and intrathecal methotrexate were combined with radiotherapy.[23] Intravenous methotrexate before radiotherapy produces tumor response in 85% of patients, but this combined therapy carries a high risk of leukoencephalopathy in a few years, causing dementia, ataxia, and incontinence, especially in older patients.[24] Optimal treatment regimens for primary CNS lymphoma are currently being sought, and patients should be managed by an oncologist experienced in this area.

SUMMARY

Neurological problems are major contributors to morbidity and mortality in transplant recipients. Many problems occur months or years after engraftment and may never come to the attention of the transplant surgeon. It is helpful to approach a patient with neurological disease by broadly localizing disease to the CNS or PNS. In the immediate postoperative period, encephalopathy with or without seizures may occur secondary to a variety of conditions. Compressive femoral neuropathy may occur as a perioperative neurological complication. Weeks after the transplantation, the most common neurological problems are related to immunosuppressant drugs, which may induce encephalopathy, tremor, neuropathy, or myopathy. Guillain-Barré syndrome is seen rarely. Chronic neurological complications tend to be caused by CNS infection, stroke, or primary CNS lymphoma.

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Chapter 32

Nonmalignant and Malignant Skin Lesions in Renal Transplant Patients

Aoife Lally • Cristina Bordea • Vanessa Venning • Fenella Wojnarowska

Skin problems are a common and important consequence of renal transplantation and a cause of concern to patients and physicians. Patients present with skin manifestations of their drug regimens. Established immunosuppressive agents, such as steroids and cyclosporine, and newer agents, such as sirolimus, are associated with dermatological side effects, which may cause functional or esthetic problems. More significantly, the state of nonspecific immunosuppression renders the transplant recipient susceptible to many bacterial, viral, and fungal infections and predisposes the patient to the development of premalignant and malignant skin lesions, which may cause significant morbidity or mortality. There is a similar profile of drug cutaneous side effects in renal transplant recipients of all racial groups, but the consequences of immunosuppression differ markedly with racial group, skin type, and geographical location. In patients of Northern European ancestry, the dominant long-term problem is nonmelanoma skin cancer. In tropical and subtropical areas, infections predominate, and Kaposi's sarcoma is seen. We reviewed cutaneous disease among patients attending the Oxford Renal Transplant Unit and found the most common skin problems are malignant and premalignant lesions, specific drug-induced cutaneous changes, warts, fungal infections, acne, folliculitis, and seborrheic dermatitis (Fig. 32-1).

DRUG SIDE EFFECTS

Many iatrogenic cutaneous effects are dose related, occurring early after transplantation and decreasing in severity as immunosuppression doses are reduced to maintenance levels. Other effects are more persistent and occur later after transplantation. Because transplant recipients are often taking many medications in addition to their immunosuppressive drugs, it must be taken into consideration that nonimmunosuppressive medications may play a role in the etiology of some cutaneous signs seen in this population. There is a high degree of variation in quality of reports of post-transplantation iatrogenic cutaneous effects in the literature. Often, assumptions are made regarding the exact role played by individual immunosuppressive agents in the etiology of skin disease. We summarize here the major cutaneous findings generally attributed to individual immunosuppressive agents. Controversies regarding the independent roles played by individual immunosuppressive agents in the pathogenesis of cutaneous malignancy are discussed later.

Corticosteroids

Cushingoid effects of corticosteroids have been reported in most studies that have looked at the cutaneous effects of steroids in renal transplant recipients.[11,36,89,144] Purpura and some redistribution of body fat are reported in greater than

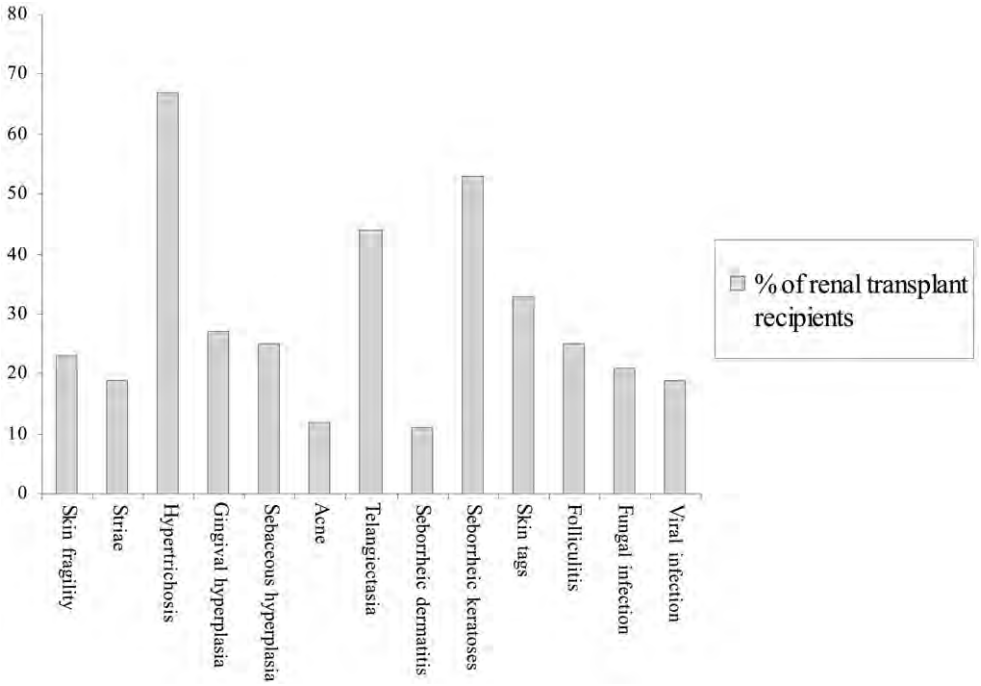

Figure 32–1 Graph showing the frequency of different nonmalignant cutaneous findings in a survey of renal transplant recipients attending the Oxford Renal Transplant Unit.

90% of patients, and more than half developed atrophic friable skin with poor wound healing. Striae, facial erythema, telangiectasia, generalized skin dryness, and rough skin over the upper arms and thighs (keratosis pilaris) caused by blockage of the hair follicle orifices by keratin plugs also are observed. There is marked variation in individual susceptibility, and some of the latter changes mentioned may occur commonly in healthy individuals.

Corticosteroids stimulate the pilosebaceous unit, possibly through an androgen-mediated mechanism, and this is responsible for the appearance of hirsutism and steroid acne (Fig. 32-2). Steroid acne may develop within 2 to 3 weeks of the start of treatment, and although it generally remits as the prednisolone dosage is lowered, it can be persistent on maintenance doses. The condition resembles acne vulgaris, affecting only androgen-dependent areas of skin bearing sebaceous glands (i.e., face, chest, back, and upper arms). Steroid acne is distinguished from acne vulgaris by the scarcity of open comedones (blackheads); the predominant lesions are discrete superficial monomorphic papulopustules, which may be present on the face, back, and chest.[154] Severe forms of acne also may occur, with deep-seated inflammatory nodulocystic lesions capable of scarring. In addition, perioral dermatitis, characterized by redness and papulopustules around the mouth and nose, is observed in transplant recipients receiving systemic steroids.[2]

Cyclosporine

The skin is one of the principal sites of accumulation of cyclosporine,[118] and mucocutaneous side effects of cyclosporine have been recognized since the introduction of this drug, the most common being gum hypertrophy (Fig. 32-3)[168] and hypertrichosis (Fig. 32-4).[33] Gum hyperplasia has a reported frequency of 8% to 70%.[42,168] Nifedipine produces similar gum hyperplasia and is synergistic.[155] The onset may be within the first month of cyclosporine treatment, but there is a sharp increase in incidence around 3 to 6 months.[17] The changes may be more severe in patients with poor oral hygiene,[168] although they also occur in otherwise healthy mouths.[17] Hypertrichosis of some degree probably develops in 100% of cyclosporine-treated patients.[98] Keratosis pilaris may precede the appearance of thick pigmented hair over the trunk, back, shoulders, arms, neck, forehead, and cheeks. Severe hypertrichosis seems to be more common in dark-skinned individuals, a finding that suggests that some individuals may be genetically predisposed to the development of side effects.[17] It does not seem to be an androgen-mediated side effect because cyclosporine-induced hypertrichosis is not confined to androgen-dependent areas of skin[117] and is independent of sex hormone levels.[98]

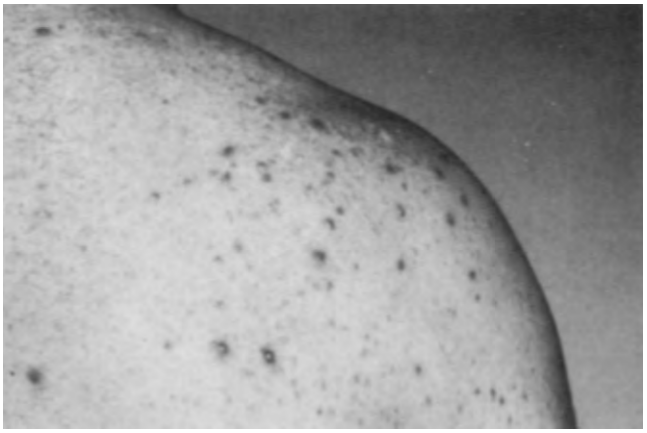

Figure 32–2 Steroid acne with monomorphic inflamed lesions with few comedones. (See color plate.)

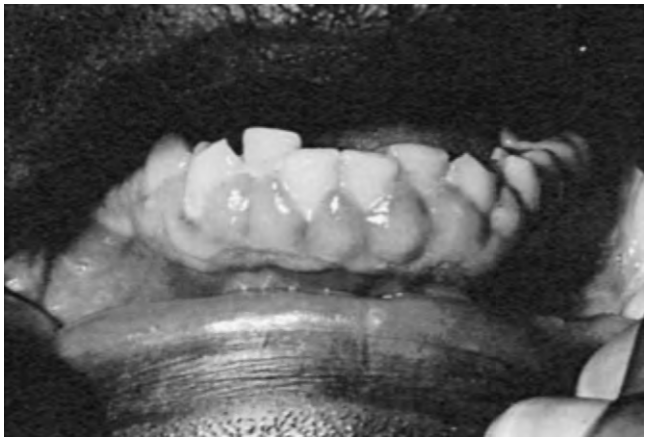

Figure 32–3 Gingival hypertrophy in a patient on cyclosporine alone.

Bencini and colleagues[17] described many other skin lesions, all of pilosebaceous origin, occurring in cyclosporine-treated renal transplant recipients—epidermal (pilar) cysts in 28% of patients, sebaceous hyperplasia in 10%, and acne in 15%. The pilosebaceous unit is a structure also modified by corticosteroids, making differentiation between the effects of the two drugs difficult; in many cases, they seem to be acting synergistically. Reduced clearance of prednisolone during cyclosporine treatment may account for some of this synergy.[123,124] There have been a few case reports of acne keloidalis nuchae[9,34] and hypertrophic pseudofolliculitis barbae[94] (other disorders of the pilosebaceous unit) occurring in cyclosporine-treated patients. Of a cohort of 197 white male patients, we observed hypertrophic pseudofolliculitis barbae in 5 patients.[178a]

Tacrolimus

Mucocutaneous findings, such as gingival hypertrophy and hirsutism, are less commonly observed than with cyclosporine.[30,51,159] Alopecia is recognized in association with tacrolimus therapy and in one series occurred in 28.9% of renal transplant recipients when other potential causes for alopecia were ruled out.[165]

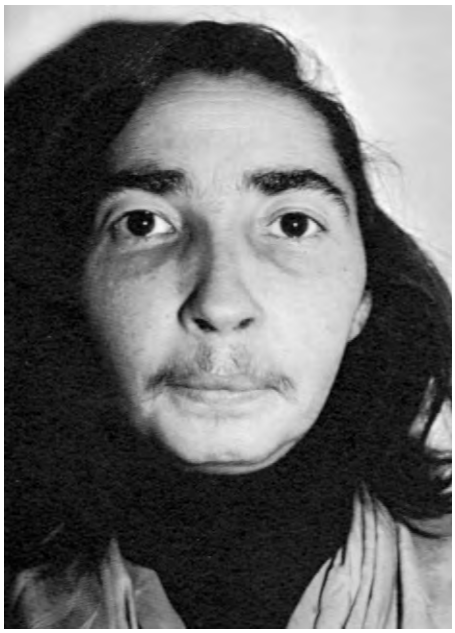

Figure 32–4 Hypertrichosis in a 35-year-old woman on cyclosporine alone 3 months after transplantation. (See color plate.)

Mycophenolate Mofetil

Mycophenolate mofetil seems to have a low incidence of skin side effects with fewer side effects documented compared with azathioprine.[153] There is increased susceptibility to herpes simplex and zoster[166] and cytomegalovirus infections.[176]

Sirolimus

The first study to quantify and characterize in detail the cutaneous effects in renal transplant recipients receiving sirolimus was undertaken in France.[104] This study examined 80 patients who had been taking sirolimus for a mean of 18 months. Disorders of the pilosebaceous unit were frequently observed with acneiform eruptions being the most common—observed in 46%. Scalp folliculitis was often seen in combination with acne, and males were affected more commonly than females. Chronic edema was seen in 55% of patients. Mucous membrane pathologies also were very common. Aphthous ulceration was significantly associated with sirolimus therapy and was observed in 60% of the population studied. Nail disorders were seen in 24% of the patients taking sirolimus. During the 3-month period after completion of the study, 12% of patients had to stop sirolimus secondary to cutaneous effects, including hidradenitis suppurativa, severe acne, severe limb edema, and aphthous ulceration.

A short-term study such as this one cannot provide sufficient information regarding the long-term effects of sirolimus on the skin. It is postulated that sirolimus may reduce risk of cutaneous malignancy, given its antiangiogenic and antiproliferative effects. This effect already has been shown in cases of Kaposi's sarcoma in renal transplant recipients,[161,182] where switching to a sirolimus-based immunosuppressive regimen resulted in resolution of Kaposi's sarcoma. Early studies suggest that risk of skin cancer may be reduced in patients switched to a sirolimus-based immunosuppressive regimen.[180] Investigators should await the outcome of ongoing studies before drawing definitive conclusions regarding the influence of sirolimus on post-transplantation nonmelanoma skin cancer rates.

Management of Drug Side Effects

Many drug side effects require no specific treatment and tend to improve as doses are reduced to maintenance levels. Most cutaneous effects of immunosuppressive medication result in esthetic problems. Compliance is often an issue for this reason, particularly in young renal transplant recipients, so it is important to address cosmetic side effects appropriately. First-line treatment for drug-induced acne is the use of topical agents. More severe cases require oral antibiotics, such as minocycline, doxycycline or oxytetracycline, given as a 3- to a 12-month course. In severe cases, isotretinoin is given at a dose of 0.5 mg/kg or 1 mg/kg for a minimum of 4 months, although cheilitis, paronychia, and effects on lipids are sometimes troublesome.

There is no specific treatment for gingival hyperplasia. Some investigators stress the importance of good oral hygiene and antiseptic mouthwashes in primary prevention. In severe cases, gingivectomy may be indicated.[168]

A few patients develop hypertrichosis of such severity as to be a major cosmetic problem. When treatment is required, the hair may be removed by any method acceptable to the patient. Electrolysis and laser have longer lasting effects, although cost can be an issue.

INFECTIONS

Skin infections are a common sequela to renal transplantation and may be caused by bacteria, fungi, viruses, or parasites. The incidence depends on duration and intensity of immunosuppression and geographical location.

Bacterial Infections

Pyogenic Bacteria

Bacterial infections of the skin are common in renal transplant recipients, and patients are at continuing risk of these infections. Prevalence seems to depend greatly on geographical location, with bacterial infections being most common in tropical and subtropical areas.[15,100,101] Apart from wound infections, the range of clinical lesions encountered in transplant patients includes folliculitis, impetigo, furuncles, abscesses, cellulitis, and erysipelas, and the lesions tend to run a more severe and protracted course than usual.[68] As in normal subjects, group A streptococci and *Staphylococcus aureus* are the most common causative organisms, although the possibility of unusual pathogens should be borne in mind, particularly in cases of cellulitis. The resident skin flora of transplant recipients is similar to that of normal individuals with no increased carriage of potential pathogens.[119] In view of the risk of serious infections, antibiotic treatment should be started promptly on clinical grounds but only after obtaining appropriate specimens for bacteriological confirmation.

Mycobacteria

Atypical mycobacterial infections occasionally occur in renal transplant patients and produce exceptionally disseminated nodular skin lesions. Several species have been reported in renal transplant recipients, including *Mycobacterium marinum*[64] and *Mycobacterium chelonae*.[132] These organisms vary in their resistance to standard antituberculous drugs and may prove extremely persistent.

Fungal Infections

Fungal and yeast infections are common and affect most renal transplant recipients in tropical and subtropical countries. Although many fungal infections are minor, some are severe and life-threatening.[101] Superficial infections by fungi and yeasts are extremely common in the immunocompromised host, and treatment is more difficult than in immunocompetent patients.

Pityriasis Versicolor

The *Malassezia* group of yeast-like fungi (previously known as *Pityrosporum*) produces a distinctive eruption with multiple, minimally scaly macular lesions widely scattered over the trunk and upper arms known as pityriasis versicolor (Fig. 32-5). The macules may be hyperpigmented or hypopigmented and usually are asymptomatic apart from their appearance. The prevalence of pityriasis versicolor in renal transplant patients is reported to be 18% to 25%.[36,89,101] The diagnosis is made clinically. Topical preparations (shampoo or cream) or, less frequently, oral itraconazole are used. Relapses are common. Persistence of hypopigmentation for many months is common and does not imply failure of treatment, although lesions with scaling usually harbor fungus.

Dermatophyte Infections

The skin of patients who are chronically immunosuppressed is more frequently colonized with potentially pathogenic fungi than that of healthy control subjects.[89,151] The rate of dermatophyte carriage on clinically normal skin was estimated as 12% in renal transplant patients compared with 6.8% in a control population.[89] There are more fungal infections in male patients and in warm climates.[101] The common sites for fungal infections in renal transplant patients are body, feet, scalp, and nails. Skin infections may be clinically typical (i.e., annular lesions with scaling at the margins), although extensive skin involvement, Majocchi's granuloma, or atypical nodular lesions have been reported (Fig. 32-6).[19,46,145] Nails infected with dermatophyte fungi typically are yellowish, crumbly, and distorted, with heaped-up debris under the free edge. Fingernail infections and involvement of multiple nails are seen more commonly in immunocompromised patients than in other patients. Whenever fungal infection is a possibility, skin scrapings or nail clippings or both should be sent for microscopy and fungal culture.

Topical imidazoles and terbinafine are used to treat dermatophyte infections of the skin. Extensive, nodular, and granulomatous infections all require systemic treatment. Nail infections respond only to systemic treatment, but topical nail preparations may suppress the infection. Most renal transplant recipients are not offered therapy, however, because risks usually outweigh benefits.

Candida

Infections by *Candida albicans* usually are superficial and localized, although skin lesions also may accompany systemic candidiasis. The yeast thrives in moist intertriginous sites, such as the inframammary folds, groin, vulva, and digital web spaces, producing the familiar well-demarcated glazed erythema, satellite lesions, and curdy plaques. Vesicles and

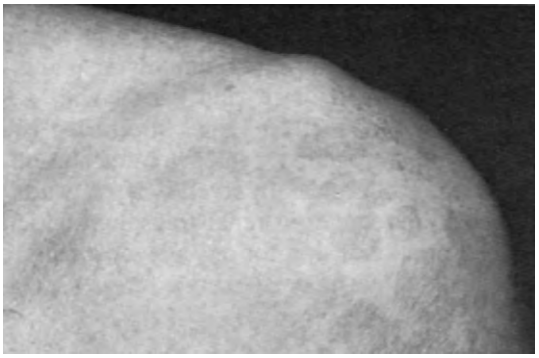

Figure 32–5 Pityriasis versicolor. Pigmented macular lesions with superficial scaling over the shoulder region. (See color plate.)

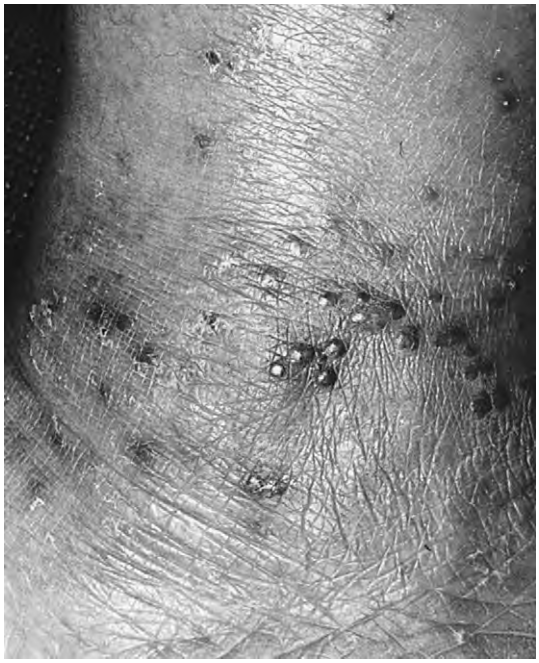

Figure 32–6 Dermatophyte infection shows grouped papules on the lower leg; biopsy and culture were needed to confirm the diagnosis.

superficial pustules occasionally may be present. Obesity, diabetes, and occlusion (e.g., under rings) are additional predisposing factors. Angular cheilitis and stomatitis are other common presentations.[68] Chronic paronychia, with a tender heaped-up nail fold, usually is associated with *C. albicans* infection, although other *Candida* species (e.g., *Candida parapsilosis*) may be found. Frequent hand wetting and loss of the protective cuticle are important predisposing factors. Culture of *Candida* from skin swabs and nail clippings helps confirm the clinical diagnosis.

Candida intertrigo responds to topical imidazole preparations. Oral candidiasis may be treated with local nystatin or imidazole. Treatment with systemic fluconazole may require double the usual dose, and there are interactions with cyclosporine (see Chapter 16). Chronic paronychia is managed by frequent, liberal application of an imidazole cream around the nail fold, which serves a barrier function in addition to therapy for *Candida*.

Cryptococcal Infections

Impaired cellular immunity predisposes to infection by *Cryptococcus neoformans*. Cutaneous involvement by *Cryptococcus* usually accompanies disseminated systemic infection (see Chapter 29), although in a series from India only half of the patients infected had skin nodules.[36] Primary cutaneous cryptococcosis occurs rarely and usually is the result of inoculation of the pathogen because of injury.[152] The skin lesions accompanying systemic cryptococcosis are described variously as papulonodular, acneiform, and ulcerative and more rarely as an acute cellulitis resembling bacterial infection.[81] Biopsy and aspiration of subcutaneously injected sterile saline provides material for histological examination and culture. The management of cryptococcosis is discussed in Chapter 29. In areas of the world where such organisms are endemic, *Histoplasma capsulatum* and other species must be included in the differential diagnosis of acute cellulitis.[49] Other rare deep cutaneous mycoses encountered in renal transplant recipients include mycetomas,[87] chromoblastomycosis,[174] and *Cladophialophora bantiana*.[80]

Viral Infections

The herpesviruses and the human papillomaviruses (HPV) are the two groups of viruses affecting skin that are important in renal transplant patients.

Herpesviruses

Immunosuppression can lead to reactivation of latent infection by various members of the herpesvirus group. Herpes simplex and varicella-zoster viruses produce severe infections in the immunocompromised host (see Chapter 29). Human herpesvirus-8 is believed to be associated with Kaposi's sarcoma (see Chapter 33).

Herpes simplex may manifest with persistent small single or grouped erosions in renal transplant recipients and require systemic treatment with acyclovir or valaciclovir. If the infection is minor, topical acyclovir may suffice. Herpes zoster manifests with blisters, which may be purpuric in a localized dermatomal distribution but also may be generalized. The blisters usually are preceded and accompanied by pain and itch. Treatment requires prompt systemic antiviral therapy.

Human herpesvirus-8 is believed to be associated with Kaposi's sarcoma. Kaposi's sarcoma manifests in the skin with purple plaques, which sometimes may resemble bruises, and nodules. This infection is most common in patients from the Mediterranean, Middle East, and parts of Africa. Kaposi's sarcoma is discussed in detail in Chapter 33.

CYTOMEGALOVIRUS

Cytomegalovirus infection involving the skin is unusual, and no specific lesion exists. Plaques, nodules, vesicobullous lesions, cutaneous vasculitis, oral lesions, and perineal ulceration have been described.[76,96,113]

EPSTEIN-BARR VIRUS

Oral hairy leukoplakia, a persistent hypertrophic white plaque on the border of the tongue, is associated with opportunistic Epstein-Barr virus infection. Originally described in patients infected with human immunodeficiency virus (HIV), this lesion is no longer regarded as specific for HIV and seems to be associated with immunosuppression in general, including transplant recipients.[66,77,78,83]

Human Papillomaviruses

HPV is a small, nonenveloped DNA virus of the Papovaviridae family.[147] The heterogeneous group of HPV includes the causative organisms for common warts, plantar warts, flat warts, and genital warts. Interest has been focused on HPV because of evidence pointing to the oncogenic potential of certain types (discussed subsequently).

EPIDEMIOLOGY AND CLINICAL FEATURES

The prevalence of warts in renal transplant recipients varies in different series. In early studies, 31% to 87% of renal transplant recipients were affected.[26,60,139,160] In more recent studies, there is geographical variation, with the highest prevalence recorded in New Zealand.[74] Warts are less common in children than in adults[11,112]; the incidence of warts in children remains stable for at least 7 years after transplantation.[48]

The number of patients with warts and the number of warts per patient correlate with the duration of immunosuppression.[11,23,36,74,100,144] Warts also occur more commonly in transplant patients with a history of high sun exposure,[26,111,150] which is in accordance with the high rates in New Zealand.[74] In one series, warts were more common in individuals with a history of burning and failure to tan on sun exposure.[111] Sun exposure also plays a role in determining the distribution of warts, with sun-exposed areas having the highest numbers of warts[11,36,74,100] and sun protection reducing the numbers.[11] Although warts predominate on sun-exposed skin, they are not confined to these sites, and in children 50% of warts are plantar.[48]

Warts are almost always multiple and sometimes quite numerous.[11,23,36,74,100,112,144] Common warts are the most frequent clinical type and usually are multiple and may number many dozens of lesions (Fig. 32-7). Other clinical types observed in transplant recipients include flat warts, unusual wart lesions with a pityriasis versicolor–like appearance, plantar warts,[103] and genital warts.[11,29,139] In our experience, warts occurring on severely sun-damaged skin may be difficult to distinguish clinically from other keratotic lesions, including solar keratoses, keratoacanthomas, and squamous cell carcinomas (SCCs). All of these lesions may coexist. Warts in transplant recipients show little tendency to remit and seem more resistant to treatment than usual.

VIROLOGY

To date, 86 distinct HPV types have been identified and fully sequenced, and more than 130 putative novel sequences have been partly characterized.[71] In broad terms, HPV types 2 and 4 (less commonly HPV types 1, 3, 27, 29, and 57) are found in common warts; types 1, 2, and 4, in plantar warts; types 3, 10, and 28, in flat (plane) warts; types 5, 8, and others, in epidermodysplasia verruciformis; and types 6, 11, 16, 18, and others, in genital warts. Many HPV types have been identified within warts from renal transplant recipients. The most common types are HPV 2, 3 and 4, with HPV types 1, 5, 6, 8, 10, 11, 16, and 18 occurring less frequently.* More than one HPV type can occur in a single patient,[74,169] and infections can occur at sites not normally associated with certain HPV types. HPV types 1 and 4, usually associated with plantar warts, and HPV types 6, 11, 16, and 18, usually confined to mucosal lesions, all have been identified in skin warts from transplant recipients. The rare type, HPV 5, has been associated in transplant recipients with warts and with plaques of warts resembling those seen in patients with epidermodysplasia verruciformis (discussed later).[74,102]

*References 29, 47, 60, 74, 102, 130, 139, 140, 169, 178.

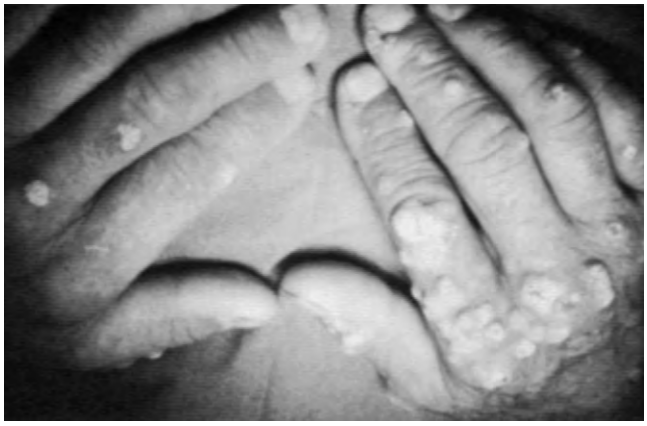

Figure 32–7 Extensive common warts on the hands of a renal transplant recipient.

MANAGEMENT

In many transplant patients, the lesions are typical clinically and present no problem with diagnosis. On severely sun-damaged skin, however, multiple keratotic lesions, including warts, solar keratoses, keratoacanthomas, and SCCs, may coexist and be difficult to distinguish clinically from one another. In such doubtful cases, biopsy may be helpful, although some lesions seem to be mixed histologically, with dysplasia coexisting with viral changes in a single lesion.[22] Treating the warts rarely results in cure. Over-the-counter wart paints or gels may be of variable benefit, and duct tape has been used with some reported improvement.[53] Hypnosis, hyperthermia, and raw garlic cloves all have been reported in the literature as potential treatments for cutaneous warts. Cryosurgery using liquid nitrogen is rarely effective, and repeated treatments are required because recurrence is common. Curettage may be undertaken for bulky lesions and filiform lesions. Topical treatments, such as 5-fluorouracil and imiquimod (discussed later), also may be used. Lasers may be used in the treatment of warts (carbon dioxide and Nd:YAG), but pain, scarring, and cost are limiting factors with this therapy.

Parasitic Infestations

Scabies is a common infestation affecting 3% of Turkish and 12% of Indian patients,[144,173] although it is reported rarely. Scabies may present the typical clinical picture of intense generalized pruritus with burrows and other lesions that characteristically favor the hands, feet, and genitals but spare the head and neck. There may be papular lesions. The intense itching sometimes can be masked in patients taking prednisolone, however, and the clinical picture may be atypical in other respects. The distribution of lesions can be unusual, with face and scalp involvement[171] or a flexural predilection,[7] and exceptionally heavy mite infections are possible, producing widespread scaling mimicking chronic eczema (Norwegian or crusted scabies).[46,179] The epidermal scales harbor numerous mites, seen readily on light microscopy; such patients are highly infectious, and in a hospital they may become the focus of a local epidemic. More than one application of a scabicide (e.g., permethrin 5%) to the whole body including the head is likely to be required to achieve cure. All contacts must be treated simultaneously to prevent reinfection.

INFLAMMATORY AND NONINFLAMMATORY CUTANEOUS FINDINGS

The expression of preexisting or new inflammatory diseases may be influenced by immunosuppression in renal transplant recipients. There is a paucity of information in the literature, however, regarding prevalence of inflammatory or noninflammatory benign cutaneous findings in this population.

Seborrheic Dermatitis

Seborrheic dermatitis (seborrheic eczema) manifests with erythema, pruritus, and scaling, and affects 1% to 3% of the immunocompetent population.[67] The face—particularly the eyebrows and nasolabial folds—ears, scalp, and trunk all may be involved. Involvement of the groin, other body folds, and genitals can be troublesome and complicated by secondary infection with *S. aureus*. The etiology of this condition is not fully understood, but *Malassezia* (previously *Pityrosporum*) yeasts are thought to play a role. Seborrheic dermatitis is a well-recognized manifestation of immunosuppression in HIV infection, occurring in 30% to 83% of this immunosuppressed population.[50,156] The reported incidence in renal transplant recipients is lower at 4% to 14%.[11,100] In the Oxford renal transplant population, we found point prevalence of seborrheic dermatitis to be 11% (see Fig. 32-1). Treatment is with topical steroids in combination with antiyeast preparations—hydrocortisone and clotrimazole or miconazole for the face and skin folds, and these or more potent steroids for other regions (e.g., clobetasone and tetracycline and nystatin; betamethasone and clotrimazole or fusidic acid if there is bacterial infection).

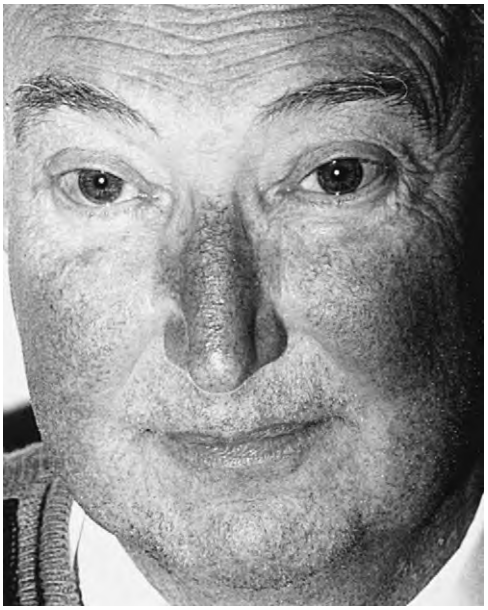

Figure 32–8 Extensive telangiectasia in a transplant recipient who had taken nifedipine for many years.

Psoriasis

Preexisting psoriasis often ceases to be a problem after transplantation because of the immunosuppressive medication. Cyclosporine is a recognized (second-line) treatment for psoriasis. If psoriasis is persistent and does not respond to simple topical measures, increasing the dose of cyclosporine should be considered. Phototherapy must be used with great caution because of the photocarcinogenesis, and psoralens and ultraviolet A (PUVA) therapy should be avoided.

Eczemas

Endogenous eczemas, such as atopic eczema, lichen simplex, pompholyx, and discoid eczema, seem to be rare in renal transplant recipients, presumably because of immunosuppression, but some cases are reported.[100] Similarly, the exogenous eczema, contact dermatitis, is rare.

Urticaria and Type I Allergy

Preexisting idiopathic urticaria and angioedema often are less troublesome during periods of high immunosuppression, but as the doses are lowered, these conditions may require treatment. Cetirizine is the antihistamine of choice in renal transplant recipients because this has the least potential for interaction with cyclosporine. Type I allergy to foods (e.g., nuts, fruits, shellfish, wasps, and bees) persists, and the need for availability of emergency epinephrine is not abolished by immunosuppression.

Telangiectasia and Poikiloderma of Civatte

Marked telangiectasias are seen in some renal transplant recipients and are partially due to systemic steroids (see earlier) and in some cases to nifedipine (Fig. 32-8).[167] The two drugs may be synergistic. A review of 82 Northern European renal transplant recipients found that 90% had clinical evidence of photodamage.[40] This study revealed that the grade of photodamage, including the presence of telangiectasia, was strongly associated with calcium channel blockers. Marked photodamage-induced changes, known as poikiloderma of Civatte (telangiectasia on the side of the neck with sparing of the "V" under the chin), has been reported from New Zealand in almost 10% of patients.[74] Treatment is difficult, but laser therapy may be helpful.

Seborrheic Keratoses

Seborrheic keratoses (seborrheic warts) are benign warty growths with a variety of clinical appearances, which are common in the immunocompetent population, particularly with increasing age. They have been observed in renal transplant recipients,[11,74,144] but it is unclear whether they are more common in this population. Their importance lies in their frequent confusion with dysplastic lesions, and we have observed a possible association with nonmelanoma skin cancer risk.[91] Seborrheic keratoses vary in color from skin-colored to deep brown or black. They are raised plaques with an irregular warty surface and may have a greasy appearance. These warts are usually multiple and vary in size from a few millimeters to a few centimeters. They do not require any treatment but are removed easily by curettage, which also allows histological confirmation of the diagnosis, or they may be treated with cryosurgery.

Skin Tags

Skin tags are pedunculated benign lesions that vary in size. They are frequently seen together with seborrheic keratoses. They are commonly seen in patients with diabetes mellitus and patients with an increased body mass index. There is little mention of them in literature pertaining to studies on renal transplant recipients. Euvrard and colleagues[48] found multiple minute skin tags on the neck and axillary folds of 5.5% of a pediatric transplant population. In adults

attending the Oxford Renal Transplant Unit, we found skin tags in 32%; in some patients, these were a major cosmetic problem (Fig. 32-9).

Nail Changes

Nail disorders of many kinds are common. A comprehensive review of 205 renal transplant recipients found nail pathology in 56.6%.[143] Leukonychia, absence of lunula, onychomycosis and longitudinal ridging were the most common nail findings in this study. Transverse white banding of the fingernails has been described in two renal transplant recipients,[73,97] in both cases in association with acute rejection. Nail brittleness and splitting (onychoschizia) have been described in childhood renal transplant recipients.[112]

PREMALIGNANT AND MALIGNANT SKIN CONDITIONS

In the long term after kidney transplantation, skin cancers are the most common malignancy in patients of European descent (Fig. 32-10) (see Chapter 33).[61,99,115,126,135] The first report of an increased risk of skin cancer in transplant patients was published by Walder and colleagues in 1971.[175] The clinical features described in the article summarize well the main characteristics of skin cancers developing in transplant patients. These characteristics include reversal of the usual SCC-to-basal cell carcinoma (BCC) ratio reported in the immunocompetent population, tendency for the lesions to be multiple, increased age at transplantation of the patients who subsequently developed skin cancer, and increased prevalence of keratoses on sun-exposed sites with rapid evolution of some of them into SCCs.[175]

The premalignant and malignant skin conditions reported to occur most frequently in transplant recipients include solar keratosis, keratoacanthoma, Bowen's disease, SCC, BCC, malignant melanoma, and Kaposi's sarcoma. Cases of angiosarcoma, Merkel's cell carcinoma,[65,126] sebaceous carcinoma,[23,126] trichilemmal carcinoma,[59] and a pure cutaneous plasma cell tumor also have been reported. SCC has been the most common reported condition in long-term retrospective studies[24,99,148] and was the most common presenting tumor.[24] In prospective studies of Hispanic renal transplant recipients[52,56] and retrospective studies of Italian[54,164] and Hungarian renal transplant recipients,[92] BCC occurred more often than SCC.

Figure 32–9 Extensive skin tags on the neck of a renal transplant recipient. (See color plate.)

The SCC/BCC ratio reverses compared with the general population. The SCC/BCC ratio changes from 1:4 to 1.5:1 in Australia and Slovakia[122,135] and from 1:8 to 3.6:1 in the Netherlands.[70] Many patients have multiple tumors at the time of diagnosis. Over time, in our experience, almost two thirds of the patients developed more than one skin cancer. In the Oxford group, one patient had more than 70 skin tumors removed, with lesions occurring as frequently as once a month. Cases have been reported of patients with more than 100 cancers each.[126] Hyperkeratoses develop frequently on sun-exposed sites, some of which undergo malignant change, and in some patients multiple SCCs develop in the hyperkeratotic areas.[107,175] The dorsum of the hands and forearms sometimes take on a characteristic appearance described as transplant hand with what is referred to by some authors as field cancerization. This appearance is a "dry and somewhat scaly skin with increasing numbers of either verrucae planae or actinic keratoses, or both" (Fig. 32-11).[23]

The latent period between transplantation and presentation with skin cancer varies from a few months to more than 20 years. The cumulative incidence increases with the time after transplantation, yet varies with the level of sun exposure and skin type. In Queensland, Australia (latitude 27 degrees south), the cumulative incidence of nonmelanoma skin cancer increased with the duration of immunosuppression: 29.1%, 52.2%, 72.4%, and 82.1% of patients given immunosuppression for less than 5 years, 5 to 10 years, 10 to 20 years, and more than 20 years, respectively.[135] The cumulative incidence of skin cancer in a white renal transplant population in England (latitude 52 degrees north), was 9%, 27%, 43%, and 61% after 5 years, 10 years, 15 years, and 20 years of immunosuppression, respectively.[24]

Renal transplant patients present with skin cancers approximately 20 to 30 years earlier than their nonimmunosuppressed counterparts. In the immunocompetent population in the United Kingdom, the mean age at presentation is 70 years for BCC and 73 years for SCC.[88] In our transplant population, the mean age at presentation with skin cancer was 56 years.[24] Similar results have been reported from the United States.[38] The relative risk of developing nonmelanoma skin cancer after transplantation has been reported from a few centers and ranges from 3.5 in Sweden[23] to 20 in Australia.[69] The risk of skin cancer is higher in men than in women.[24,25,61] It is unclear whether the increased risk in men is due to differences in the levels of sun exposure, or whether other factors might be involved; one study that specifically investigated the history of sun exposure in male and female transplant patients found similar levels of exposure in the two groups. The incidence of malignant melanoma also is increased in transplant recipients: 3.2-fold in Ohio,[28] 4.4-fold in Australia,[177] and 8-fold in the United Kingdom.[93]

Most skin cancers occur on sun-exposed areas, pointing to the effect of ultraviolet (UV) exposure in the pathogenesis (Fig. 32-12).[24,135] An increased frequency of SCC also

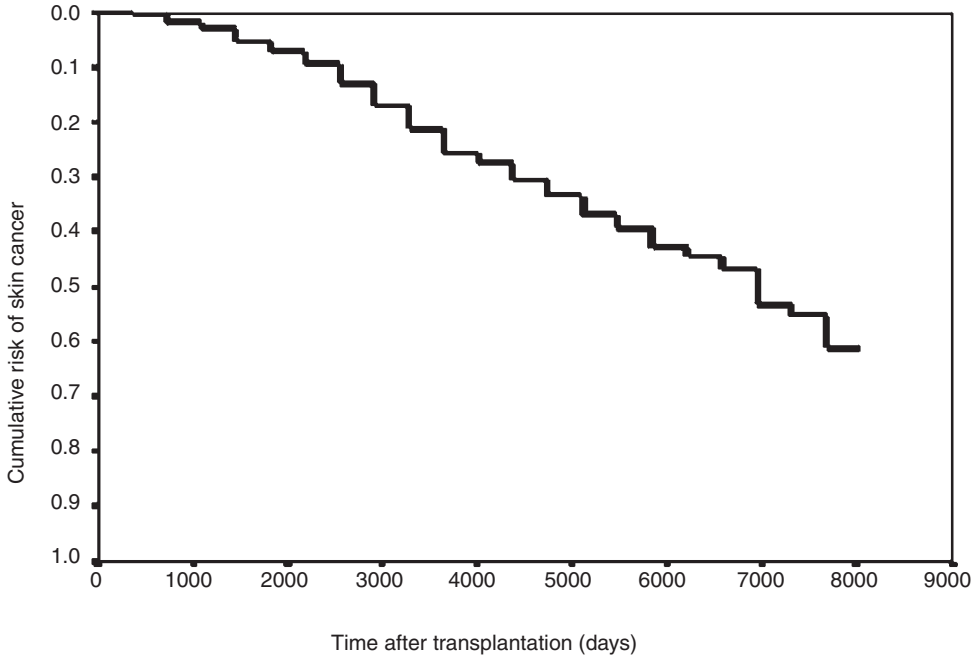

Figure 32–10 Graph illustrating the cumulative risk of cutaneous malignancy after transplantation in white renal transplant recipients attending the Oxford Renal Transplant Unit.

was noticed in renal transplant patients in areas not usually exposed to UV.[24,135] Invasive SCC and SCC in situ are located preferentially on the head and neck and dorsum of hands, whereas BCC develops frequently on the head and neck and trunk.[70]

SCC is more of a problem than BCC in transplant patients, with multiple lesions developing in the same patient, and perhaps there is an increased tendency to recur and metastasize.[149] Of the 3087 transplant patients with cancer reported from around the world to the Cincinnati Tumor Transplant Registry, 179 (5.8%) developed lymph node metastases.[126] Of these, 75% were from SCC; 17%, melanoma; 7%, Merkel's cell tumor and 1%, BCC. Of the patients, 5% died of their skin cancers, with 61% of deaths caused by SCC; 34%, melanomas; 4%, Merkel's cell tumors; and 1% (one patient), BCC.[126] Most cases of aggressive SCC occurred in Australia.[149]

In pediatric (<18 years old) renal transplant recipients, skin cancers are the most common post-transplant malignancy.[128] Almost 20% of cases occurred in childhood, and half of them were malignant melanomas.[127] In a Dutch pediatric renal transplant population, the standardized risk for nonmelanoma skin cancer was 222-fold higher compared with the general population.[41]

PREMALIGNANT SKIN TUMORS

Solar Keratosis

Solar keratoses manifest as localized areas of adherent hyperkeratosis on sun-exposed skin and are associated histologically with dysplastic changes in the basal epidermis, together with evidence of solar damage. The reported incidence of solar keratosis after transplantation in the

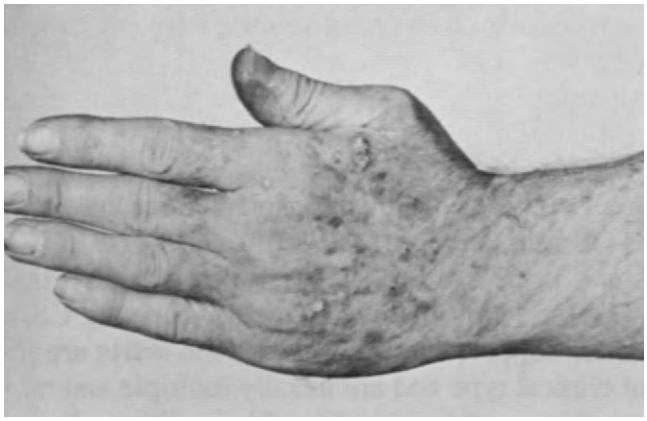

Figure 32–11 "Transplant hand." Sun-damaged skin on the hand of a renal transplant recipient shows solar keratosis. (See color plate.)

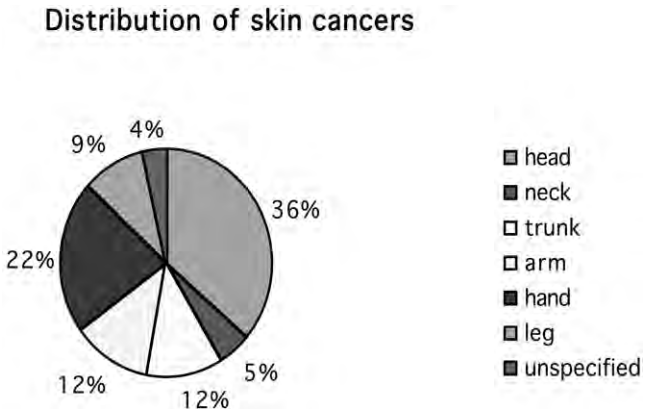

Figure 32–12 Anatomical distribution of cutaneous malignancies in renal transplant recipients attending the Oxford Renal Transplant Unit. (See color plate.)

United Kingdom ranges from 7.4%[26] to 22.3%.[150] In a New Zealand renal transplant population, with an average 9.5 years of continuous immunosuppression, the prevalence of solar keratoses was found to be 42.3%.[74]

The lesions may appear 2 to 6 months after transplantation. In immunocompetent patients, the malignant potential of solar keratoses is regarded as low, although a slow-growing SCC may develop after a prolonged latency. In transplant recipients, keratoses tend to be multiple, tend to recur after conservative treatment, and may evolve rapidly into SCC.[175]

Bowen's Disease

Typically manifesting as a persistent scaly erythematous plaque on exposed or covered skin, Bowen's disease represents true carcinoma in situ and has malignant potential. McLelland and coworkers[111] reported a prevalence of Bowen's disease of 5.8%. In our series, the prevalence reached 9%.[24] In our experience, the lesions may be atypical clinically and manifest as banal keratotic lesions for which the differential diagnosis must include solar keratosis, keratoacanthoma, warts, and SCC.

Porokeratosis

Porokeratosis is an unusual condition characterized by annular lesions with a distinctive raised keratotic edge (Fig. 32-13). The variant repeatedly described in transplant patients[16] and in other immunosuppressed patients[95] consists of multiple small (1- to 2-cm) lesions distributed widely on the limbs (disseminated superficial actinic porokeratosis). Rarely, a variant called porokeratosis of Mibelli has transformed into SCC, although this complication never has been described in a renal transplant recipient. Treatment is unsatisfactory; emollients, mild keratolytics, and cryosurgery all have been tried.

MALIGNANT SKIN TUMORS

Keratoacanthoma

Common in the immunocompetent population, keratoacanthoma manifests as a firm, rapidly growing, dome-shaped tumor of 1 to 2.5 cm in diameter with a central keratin-filled crater (Fig. 32-14). Keratoacanthomas occur mainly on sun-exposed areas but can develop on any hairy cutaneous site. These lesions are normally self-limiting and regress spontaneously in immunocompetent individuals. Any rapidly growing skin lesion occurring in a transplant patient is an indication for surgical excision, however, and if histologically suggestive of keratoacanthoma, it is still best managed as an SCC because differentiating between the two is very difficult.

Squamous Cell Carcinoma

In renal transplant recipients, SCC usually manifests as a rapidly growing, raised, keratotic lesion with or without central ulceration, often sore and with an indurated base (Fig. 32-15). If a central ulcer is present, the border does not always resemble the classic description. Some patients may present with multiple lesions within the same area (Fig. 32-16). SCC is a true, invasive carcinoma of the surface epidermis, which can spread to the lymph nodes and in some cases cause death. After surgery, patients need to be followed up to check for local and regional recurrence.

Basal Cell Carcinoma

BCC is a slowly developing tumor with a tendency toward local invasion and tissue destruction, although the metastatic potential is extremely low. In contrast to SCC, the clinical appearance of BCC (Fig. 32-17) in transplant patients is similar to that in immunocompetent patients. Follow-up is required after surgical excision because patients are at risk of local recurrence and further lesions.

Malignant Melanoma

Four main clinicopathological variants of malignant melanoma are described: superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, and acral lentiginous or palmoplantar melanoma. All variants, apart from the rare amelanotic melanoma, manifest as a changing pigmented lesion, which is not typical of other benign nevi found on the patient's skin. A history of a changing

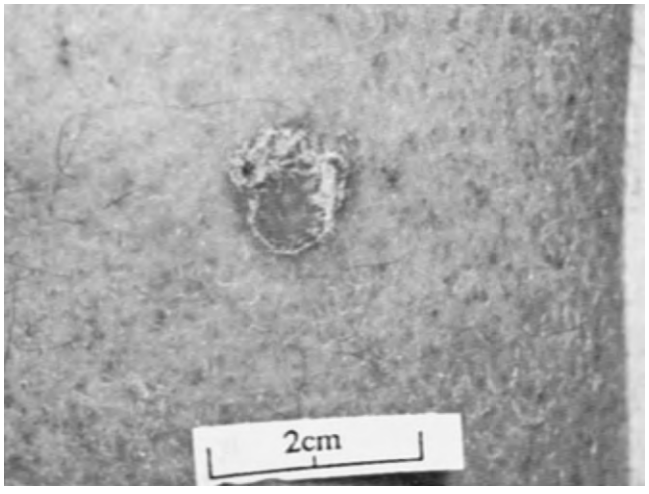

Figure 32–13 Typical annular lesion of porokeratosis showing the distinctive keratotic edge and slightly atrophic center. (See color plate.)

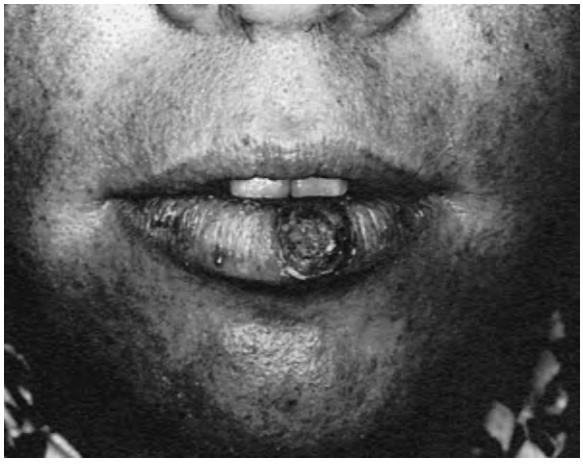

Figure 32–14 A keratoacanthoma on the lip of a young man 5 years after renal transplantation. The lesion had first appeared 6 weeks previously, rapidly reaching the appearance shown.

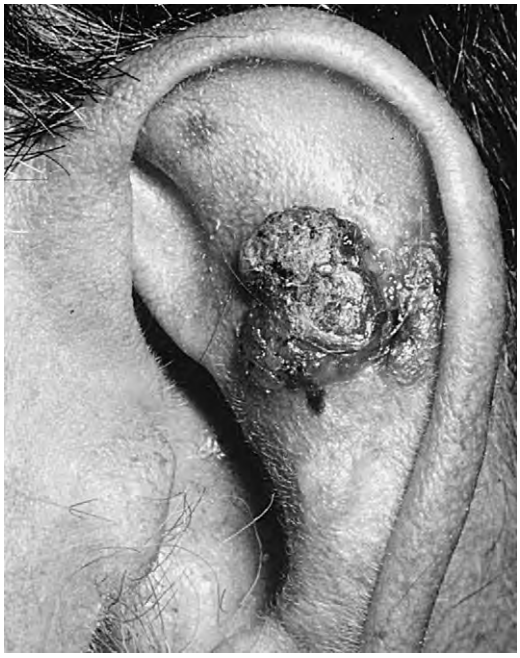

Figure 32–15 Squamous cell carcinoma on the ear.

lesion is significant, and changes can affect size, shape, and color. There can be signs of inflammation, oozing or bleeding, itch, or altered sensation. Particular attention must be paid to examining pigmented lesions in transplant patients to detect any early changes, and in our experience numerous thin melanomas are detected in this way. Any suspicious lesion must be excised and examined histologically. Subsequent management depends on the pathology

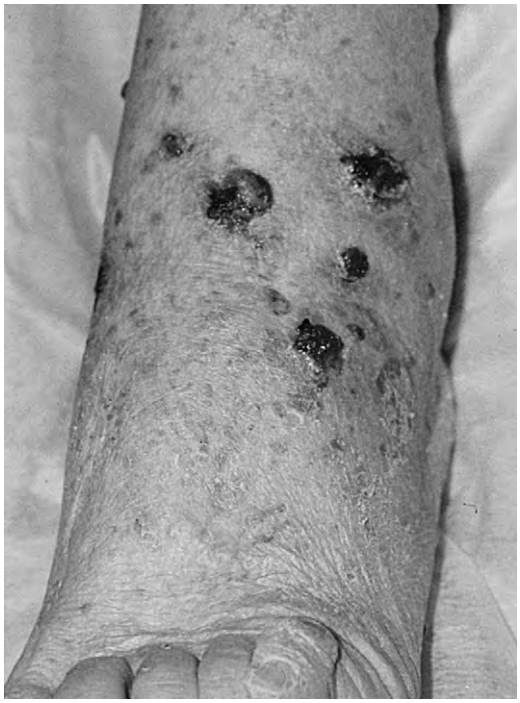

Figure 32–16 Multiple squamous cell carcinomas on the leg of a woman with signs of lymphedema.

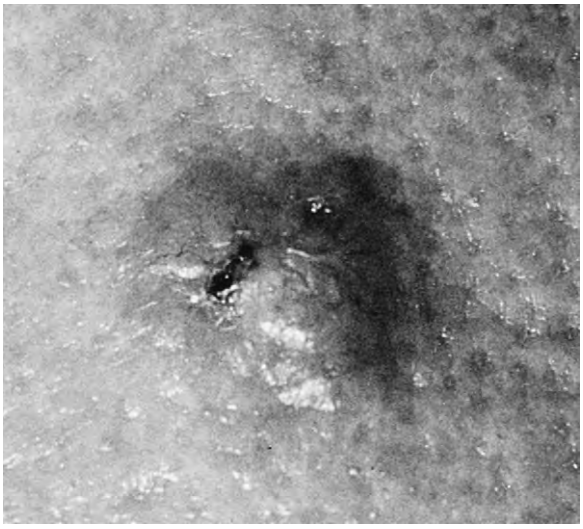

Figure 32–17 Nodular basal cell carcinoma with some early ulceration on the face.

(e.g., Breslow thickness) and presence or absence of lymph nodes.

Risk Factors and Pathogenesis

Skin phototype, UV exposure, duration and type of immunosuppressive therapy, genetic factors, and possibly infection with HPV are thought to contribute to the pathogenesis of skin cancers in renal transplant recipients.

Skin Phototype and Ultraviolet Exposure

In immunocompetent and immunosuppressed patients, most skin cancers develop on sun-exposed areas. The incidence of skin cancer is highest in white patients living in tropical and subtropical climates. Epidemiological studies show a relationship between skin cancer and sun exposure. Nonmelanoma skin cancers tend to be related to cumulative exposure, whereas melanomas (with the exception of lentigo maligna melanoma) seem to be related to exposure in childhood or intermittent high-dose exposure.[181]

It is believed that UV acts by initiating a cascade of events in the skin, starting with absorption by a chromophore or chromophores locally and ending in immunomodulation. The proapoptotic protein p53 is a major target for UV-induced damage. In addition to direct damage, UV radiation can cause DNA damage indirectly through oxidative stress.[5] UV radiation can reduce the number of Langerhans' cells and impair their ability to stimulate proliferative T cell responses in vitro.[10] It is possible that antigens encountered through UV-exposed skin are presented differently, or not at all, from those encountered through normal skin. It is reasonable to speculate that these defects might contribute further to the breakdown of immunosurveillance already impaired by immunosuppressive drugs and enhance the development of premalignant and malignant lesions.

Immunosuppressive Drugs

Azathioprine increases the speed of UV-induced skin cancer development in animal models.[85] Other effects of azathioprine include chromosome breaks[82] and inhibition of repair of UV-induced damage.[84] Findings from a more recent study

showed that normal exposure to sunlight may induce chronic oxidative stress and increase the levels of oxidative DNA lesions in the skin of patients taking azathioprine.[121]

In vitro studies have shown that cyclosporine has the ability to inhibit antigen-processing and accessory functions of epidermal Langerhans' cells.[45,57] Some investigators have found a decrease in the number of Langerhans' cells in the skin of transplant recipients,[20,129,146,158] detectable 3 days after starting the treatment.[58] New data suggest that cyclosporine can promote cancer progression by a direct cellular effect that is independent of its effect on the host's immune cells and may involve the production of transforming growth factor-β.[75] Topical and systemic corticosteroids are known to deplete epidermal Langerhans' cells[8,14,21] and impair their antigen-presenting capacity.[1,8,14]

There has been much discussion about the risk associated with different immunosuppressive regimens. Disney and coworkers[44] found that skin cancer occurred significantly more frequently in patients treated with both cyclosporine and azathioprine than with either of these drugs alone. In the group of patients treated with cyclosporine only, the incidence was slightly higher for the first 6 years after transplantation, after which it reached levels similar to the group treated with azathioprine alone. In a randomized comparison of two cyclosporine regimens, patients who received the low-dose cyclosporine had significantly fewer warts and premalignant and malignant skin lesions but had more rejection episodes.[43]

Human Papillomavirus

As mentioned previously, HPV is well recognized as the causative agent of common warts and condyloma acuminatum. The first evidence that HPV infection is associated with SCC of the skin was found in patients with the rare, genetically determined condition epidermodysplasia verruciformis. Epidermodysplasia verruciformis is characterized by the development of numerous flat, wart-like lesions, which in 30% to 50% of patients progress to SCCs 20 to 30 years later. A specific group of closely related HPV types, the epidermodysplasia verruciformis group, especially HPV 5 and occasionally HPV 8, 14, 17, 20, or 47, has been isolated from greater than 90% of SCCs from these patients.[105] After transplantation, there is an increase in viral skin infections, and the tumors with the highest incidence are those thought to arise from oncogenic viruses. A role for HPV in the etiology of nonmelanoma skin cancer in renal transplant recipients analogous to that of epidermodysplasia verruciformis–related skin cancer seems plausible.[71,131,162] Clinically, squamous dysplasia and SCC develop in close proximity and are usually preceded by viral wart lesions.[70] In some patients, the skin changes resemble those of patients with epidermodysplasia verruciformis. Histologically, viral warts and keratotic skin lesions often display varying degrees of epidermal dysplasia, and some SCCs retain HPV-associated features on microscopic examination.[18]

Virological data confirmed a high prevalence of HPV: 69% to 88% in transplant SCCs with epidermodysplasia verruciformis HPV types predominating. No specific HPV type was associated with malignancy, however, and mixed infections with one or two HPV types were common. A high prevalence of HPV DNA has been reported in transplant premalignant lesions. The prevalence of HPV in BCCs was lower than in SCCs.[71] HPV also has been identified in normal skin and in benign hyperproliferative lesions. The ubiquity of HPV and the low viral load of nonmelanoma skin cancer questioned the potential role of HPV in the etiology of skin cancer.

Researchers have investigated the molecular mechanism of HPV-induced carcinogenesis. Purdie and colleagues[133] identified in the upstream regulatory region of HPV 77 (a cutaneous HPV identified in transplant patients) a sequence that is responsive to activation of p53 by UV radiation, leading to stimulation of HPV 77 promoter activity. A similar p53 recognition site has been identified in the noncoding region of HPV 8.[4] In vitro, UV radiation–induced release of proinflammatory cytokines by keratinocytes was enhanced in the presence of HPV 20 and HPV 27, and the cytokines seemed to control the promoter activity of the virus.[141] The two main viral oncoproteins are E6 and E7. Jackson and Storey[79] showed that E6 of HPV 5, HPV 10, and HPV 77 could inhibit UV-induced apoptosis by inactivation of the proapoptotic protein Bak. In addition to inhibition of apoptosis, HPV 5 and HPV 18 cells expressing the E6 protein were shown to have reduced ability to repair UV-induced damage.[62] In an in vitro skin equivalent, keratinocytes expressing the E7 gene of HPV 8 acquire an invasive phenotype and migrate through and invade the underlying dermis. Additionally, HPV E7 alters the normal differentiation program of the cells, resulting in hyperkeratosis and horn pearl formation.[3]

Genetic Factors

Mutations in p53 are common in skin cancers. Polymorphism of the p53 gene at codon 72 was found to be linked to the risk of skin cancer in transplant patients in two studies,[32,108] but was not confirmed in a further two studies.[12,120] The glutathione S-transferase GSTP1C allele was associated with an increased risk of SCC.[106] GSTM1 null genotype was associated with an increased risk of SCC. The interval from transplantation to development of tumors was shorter in GSTM1 null patients with high levels of sun exposure and in smokers. GSTP1*Ile homozygotes developed more SCCs.[134] GSTM1 AB and GSTM3 AA alleles were associated with fewer SCCs. GSTM1 AB and GSTP1 Val/Val also were associated with fewer BCCs in patients on high-dose prednisolone therapy.[55]

MANAGEMENT

Management of premalignant and malignant skin conditions should begin before transplantation with education programs. Potential renal transplant recipients must be informed about the increased risk of cutaneous malignancy after transplantation. Information may be found on the British Association of Dermatologists website (www.bad.org.uk/public/leaflets/awaiting_transplant.asp).

Patients with a history of skin cancer before transplantation have an increased risk of developing skin cancer after transplantation. Patients with a prior history of malignant melanoma also have reduced survival rates.[35] Although UV exposure is just one factor important in the etiology of skin cancer, it is the sole factor that can be avoided. Efforts must be made to reduce sun exposure, and patients need to be educated about the dangers of UV exposure so that they understand the rationale and avoid further damage. To be of maximal benefit, sun protection measures should start as early as possible (as soon as the patients are accepted

for transplantation). Advice should be centered on sun avoidance, including appropriate clothing (wide-brimmed hats, long-sleeved shirts, long pants), avoidance of sunbathing and sunbed use, and when feasible a change of outdoor activities so that midday sun is avoided. Sunscreen creams providing broad-spectrum protection against UVA and UVB are appropriate, but they should not be regarded as a substitute for sun avoidance.[171] Despite an awareness by most renal transplant recipients of the dangers of sun exposure, several studies have revealed that UV avoidance strategies used by most patients are inadequate.[31,163] In another published survey, reasons given for not using sunscreens included cost of sunscreens, cosmetic unacceptability, and simple forgetfulness.[114]

The clinical appearance of skin cancers in renal transplant patients does not always resemble that in immunocompetent patients. A study looking at the accuracy of clinical diagnosis of suspected premalignant and malignant skin lesions reiterated the need for biopsy of any suspicious lesion in this population.[39] If clinical diagnosis is in doubt, biopsy or surgical excision with histological examination is preferred to locally destructive therapy. This approach provides histological diagnosis, assessment of aggressiveness, and confirmation of the adequacy of excision. Patients who have had SCCs removed need to be checked for lymph node metastases. In our experience, metastatic spread is uncommon and has occurred only with skin cancers that are known to have a high risk of metastatic spread in immunocompetent patients (e.g., sebaceous carcinoma, Merkel's cell tumor, malignant melanoma, and SCC of the lip, ear, and scalp).

Some renal transplant patients progressively develop widespread and numerous warty skin lesions, particularly on the dorsum of the hands, and it may be difficult to distinguish clinically between benign and malignant lesions. In the past, such patients may have undergone excision of a large area and reconstruction of the defect with a skin graft harvested from a non–sun-exposed site, but with the advent of topical therapies, this approach is used less often. These patients with so-called field cancerization continue to prove challenging from a management point of view, with an ongoing risk of multiple cutaneous malignancies. In practice, therapeutic decisions are made based on size and number of lesions, anatomical site involved, and the general health of the patient.

Surgery

Surgical therapeutic options include cryosurgery, curettage and cautery, traditional excision of the lesion in question, Mohs' micrographic surgery, and photodynamic therapy. Apart from cryosurgery and photodynamic therapy, all methods yield a histopathological sample.

Cryosurgery with liquid nitrogen may be performed on premalignant lesions and warts. Curettage and cautery allows a histological sample to be obtained and tissue destruction to be carried out to the base of the lesion to obtain hemostasis and reduce recurrence. This technique is done under local anesthetic and can be used to treat extensive warty lesions, hyperkeratotic actinic keratoses, Bowen's disease, and superficial BCCs and SCCs. Excision is the treatment of choice for any clinically indeterminate lesion, all suspicious pigmented lesions, and most BCCs and SCCs. Mohs' micrographic surgery is a surgical technique developed in the 1940s with the aim of achieving 100% histologically clear margins with minimal loss of surrounding tissue. It is indicated in particular for tumors at sites where preservation of tissue for optimal cosmetic result is required (e.g., BCC of the nose). Photodynamic therapy uses topical porphyrin precursors in combination with a light source. Light exposure activates the porphyrin resulting in free radical production and cell death. This technique can be used to treat large areas of premalignant lesions or field cancerization, such as the bald scalp of men or the dorsum of the hands. It also may be a therapeutic option for superficial BCCs. Pain is often the major limiting factor of this treatment from the patient's perspective.

Topical Therapy

Topical Retinoic Acid

Retinoic acid was the first topical therapy with demonstrated efficacy in the treatment of premalignant cutaneous lesions. Several studies have shown the efficacy of tretinoin (Retin-A) cream in the treatment of solar keratoses.[137] The clinical effect was associated with an increase in the number of Langerhans' cells.[138] This treatment has been largely superseded by newer agents.

Topical 5-Fluorouracil

5-Fluorouracil is an antimetabolite that inhibits pyrimidine metabolism and DNA synthesis. There are many variations in therapeutic regimens recommended. It is typically used twice daily once or twice a week or every night for several weeks and causes extensive erythema and discomfort of clinical and subclinical disease, while normal skin remains unaffected. This discomfort naturally may lead to problems with compliance if patients are unable to tolerate the treatment. 5-Fluorouracil is used to treat solar keratoses and Bowen's disease, and large areas covered with keratotic and warty lesions may be treated at one time (e.g., the dorsum of the hand or bald scalp). A randomized comparison of photodynamic therapy with 5-fluorouracil in the treatment of Bowen's disease found photodynamic therapy to be superior.[142] Topical 5-fluorouracil, applied by a patient at home, is more convenient, however, than attendance at the hospital for photodynamic therapy, which has limited availability.

Topical Imiquimod

Imiquimod, whose action is mediated by Toll-like receptors on cell surfaces, is an immunomodulator. Imiquimod upregulates production of interferon-α, tumor necrosis factor-α, interleukin (IL)-6, IL-8, and IL-10. It has no effect on T cell proliferation, IL-2 production, or IL-2 receptor expression.

Topical imiquimod 5% is used three to five times a week for 16 weeks for the management of warts, actinic keratoses, Bowen's disease, and superficial BCCs, and has been shown to be effective in immunocompetent individuals. Moderate improvement was observed in a few renal transplant recipients with recalcitrant warts who were treated with imiquimod (reducing frequency for 24 weeks).[72] Another study (randomized double-blind, placebo-control) from the same group showed topical 5% imiquimod cream to be safe on skin areas of 60 cm^2 in renal transplant recipients and

effective in reducing cutaneous dysplasia and the frequency of squamous tumors developing in high-risk patients.[27] In a small study of renal transplant recipients, 5% topical imiquimod applied at night four times weekly for 6 weeks cleared small BCCs (mean tumor area 52 mm^2) clinically and histologically.[172] A combination of topical imiquimod and topical 5-fluorouracil was effective in treatment of a few transplant recipients with Bowen's disease.[157] Graft function was unaffected by the actions of imiquimod on the local innate and possibly adaptive immunity.

Topical Diclofenac

Topical diclofenac 3% in a gel preparation used twice daily for 180 days has been shown to be effective and well tolerated in the management of actinic keratoses,[136] but to date no studies have been published regarding use in the immunosuppressed renal transplant population.

Altering the Immunosuppression Regimen

Reducing a transplant recipient's immunosuppressive medication is thought to play a role in the management of post-transplant cutaneous malignancies. The burden of skin cancer for which this management strategy should be considered is unclear, however. Reduction of immunosuppression for management of Kaposi's sarcoma and post-transplant lymphoproliferative disease is well established (see Chapter 33). The decision to make any alterations to the immunosuppression regimen to lessen the burden of cutaneous malignancy should be weighed against the risk of graft rejection. Because there are no randomized control trials in this area, an expert consensus survey was done to formulate guidelines for the graduated reduction of immunosuppression for increasing skin cancer risks.[125]

Systemic Retinoids

Medical prophylaxis together with treatment of established skin lesions is a useful alternative to surgery in some patients. Synthetic analogues of vitamin A, the retinoids isotretinoin (13-*cis*-retinoic acid) and etretinate, are known to suppress epithelial dysplasia and neoplasms in nontransplant patients.[90,116] Low-dose etretinate (0.3 mg/kg/day) over 3 to 26 months produced a significant reduction in the number of skin cancers during the first 6 months of treatment and a trend toward a longer term reduction at 18 months of treatment.[63] Etretinate has now been replaced by its metabolite acitretin.

Short-term treatment with acitretin, 0.5 mg/kg/day, reduced temporarily the development of new SCCs[170] and the number of keratotic skin lesions,[13] but these recurred after discontinuation of treatment. Long-term prophylactic treatment with acitretin, 0.3 mg/kg/day, reduced significantly the development of new nonmelanoma skin cancers in renal transplant patients during the period of treatment, with well-tolerated side effects.[109]

There are no published reports of the use of isotretinoin in transplant recipients. In contrast to etretinate, isotretinoin does not increase natural killer cell numbers,[6,110] and this has been proposed as a theoretical advantage in terms of safety to the graft.

Side effects are usual and sometimes troublesome. Dry mucous membranes leading to desquamation causes sore, cracked lips in most patients. Skin dryness, pruritus, and hair changes are observed less frequently. Reversible biochemical changes, including hyperlipidemia, chiefly affecting triglyceride levels, and disturbances of liver enzymes are common and require monitoring. Bones and joints are affected in a few patients, causing myalgia, arthralgia, and reduced exercise tolerance. All retinoids are highly teratogenic, and in view of the long half-life, acitretin is contraindicated in female patients who may wish to conceive in the next few years. Active contraception is mandatory (for duration, see manufacturers' recommendations), and pregnancy should be excluded before starting treatment.

The antineoplastic effects of retinoids are reversible. On cessation of treatment new lesions develop. One possible concern has been that the rate of tumor development may be accelerated in the immediate post-treatment period.[86,90]

SUMMARY

Much variation in management approaches exists with regard to cutaneous disease in renal transplant recipients. A survey of U.S. dermatologists revealed that most respondents saw transplant recipients only after development of cutaneous malignancy.[37] Ideally, patients should be seen regularly in a dedicated transplant dermatology clinic for complete cutaneous examination and education on UV avoidance. Patients with no history of skin malignancy should be seen on an annual basis; patients with a history of malignancy should be seen more frequently and should come to the clinic on short notice if new lesions develop.

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Chapter 33

Cancer in Dialysis and Renal Transplant Patients

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For dialysis patients and renal transplant recipients, the risk of malignancy is considerably greater than it is in the general population. In some cases, de novo cancer develops; in others, cancer is transferred with a donor organ, and occasionally there is recurrence of preexisting cancer in a recipient. This chapter discusses all of these aspects of the cancer problem in dialysis and transplant patients, with the exception of skin malignancy, which is one of the greatest cancer risks faced by these patients, but which has been considered separately in Chapter 32.

CANCER IN DIALYSIS PATIENTS

Soon after the appearance of the first reports of cancer arising de novo in renal transplant recipients,[32,94] it was suggested that patients on dialysis programs, many awaiting transplantation, also were at heightened risk of cancer development.[78] The reasons for this were not immediately apparent. There have now been many reports confirming that the incidence of malignancy is considerably greater in patients on dialysis than it is in the population at large. Most of these cancers affect the renal tract, however, directly or indirectly, and there has been ongoing controversy over whether dialysis patients are more susceptible to malignancies that do not affect the renal tract. Although several authors have concluded that there is an overall increase in the incidence of malignancy in patients with chronic renal failure,[49,70,78,83,125,129] others have found no increase,[120] or an increase only in non-Hodgkin lymphoma.[64] Rarely, renal failure may be a consequence of malignancies such as those arising in the lung and colon because they lead to glomerulopathy.[37] It has been suggested that this glomerular disease in cancer patients could be a result of tumor-associated antigens. Nephrotic syndrome is most often associated with Hodgkin's disease. Malignant disease of the kidney or ureter can impair renal function by causing obstruction, and occasionally renal dysfunction results from a treatment-related nephropathy secondary to radiation or drugs.

Magnitude of the Cancer Problem in Dialysis Patients

Some of the most comprehensive long-term data on the development of malignancy in dialysis patients are available from the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry. This registry has collected information on all patients in Australia and New Zealand who have received dialysis since 1963. In the 1997 ANZDATA Report,[118] the incidence of cancer in 21,093 patients who had been on dialysis for a mean of 2.2 years was documented. Of these patients, 3% developed skin malignancies, and a further 2.5% developed malignancies of other organs. The rate of cancer development (excluding renal tract tumors) was approximately 1.5 times that expected in the general population, matched for age. In a subsequent ANZDATA report,[23] the incidence of cancer in 33,822 patients (87,039 person-years) who had undergone dialysis in Australia and New Zealand between 1980 and 2003 was documented. Standard incidence ratios (SIR)—the ratio of observed to expected events—for cancer risk, excluding nonmelanoma skin cancers (NMSC) were calculated, with the results standardized for age, gender, and calendar year with the Australian general population. The data are shown in Table 33-1. Overall, the SIR for cancer risk was 1.70. For individual cancer types, the highest SIR was for Kaposi's sarcoma (10.46). Very high SIRs also were reported for multiple myeloma (8.11); tumors of the kidney, ureter, and urethra (7.96); tumors of the thyroid and other endocrine glands (6.02 and 8.82, respectively); and tumors of the vulva and vagina (4.02 and 6.05, respectively). ANZDATA Registry data also are reported and discussed in an article by Vajdic and colleagues.[129]

Table 33–1 **Standardized Incidence Ratios for Cancer Risk (Excluding Nonmelanocytic Skin Cancer) Experienced by Patients Undergoing Dialysis in Australia and New Zealand, 1980 to 2003***

Site of Cancer	Observed Cancer	Expected Cancer	SIR	95% CI
All registrable cancers	*1469*	*861.91*	*1.70*	*1.62-1.79*
Head, neck, and lip	26	10.73	2.47	1.68-3.63
Esophagus	22	12.16	1.81	1.19-2.75
Stomach	32	23.80	1.34	0.95-1.90
Small intestine	9	2.40	3.76	1.95-7.22
Colorectal	144	134.11	1.07	0.91-1.26
Liver	21	7.43	2.83	1.84-4.34
Gallbladder	7	6.44	1.09	0.52-2.28
Pancreas	21	19.44	1.08	0.70-1.66
Nasal cavity	3	1.44	2.08	0.67-6.46
Larynx	8	8.78	0.91	0.46-1.82
Trachea, bronchus, and lung	186	108.49	1.71	1.49-1.98
Other thoracic organs	3	0.74	4.04	1.30-12.51
Bone and articular cartilage	4	1.01	3.95	1.48-10.53
Melanoma	88	71.18	1.24	1.00-1.52
Mesothelioma	11	5.72	1.92	1.07-3.47
Kaposi's sarcoma	8	0.76	10.46	5.23-20.92
Connective and other soft tissue	6	5.33	1.13	0.51-2.51
Breast	124	84.75	1.46	1.23-1.75
Vulva	6	1.49	4.02	1.81-8.96
Vagina	3	0.50	6.05	1.95-18.76
Cervix uteri	20	6.64	3.01	1.94-4.67
Corpus uteri	15	13.72	1.09	0.66-1.81
Ovary	13	10.16	1.28	0.74-2.20
Other female genital organs	0	0.44	0.00	—
Penis and other male genital organs	2	1.05	1.90	0.48-7.60
Prostate	89	143.77	0.62	0.50-0.76
Testis	0	2.00	0.00	—
Kidney, ureter, and urethra	197	24.74	7.96	6.93-9.16
Bladder	120	35.36	3.39	2.84-4.06
Eye	0	2.13	0.00	—
Brain and central nervous system	22	12.25	1.80	1.18-2.73
Thyroid gland	33	5.48	6.02	4.28-8.47
Other endocrine glands	4	0.45	8.82	3.31-23.50
Unknown primary site	65	34.32	1.89	1.49-2.42
All lymphomas	48	34.18	1.40	1.06-1.86
Immunoproliferative neoplasms	3	0.68	4.44	1.43-13.78
Multiple myeloma	89	10.97	8.11	6.59-9.98
Leukemias	17	21.85	0.78	0.48-1.25

*Analysis of 33,822 patients (87,039 person-years), standardized for age, gender, and calendar year with Australian general population.
CI, confidence interval; SIR, standard incidence ratio.
Data from Chapman JR, Webster A: Cancer Report 2004. In Ross GR (ed): ANZDATA Registry Report 2004. Adelaide, South Australia, Australia and New Zealand Dialysis and Transplant Registry, 2004, pp 101–103.

It is more difficult to obtain comprehensive data on the incidence of malignancy in dialysis patients treated in other countries. In Japan, a nation with a large population of patients on long-term dialysis, an analysis of deaths caused by cancer (including renal tract tumors) revealed that the relative risk (RR) of cancer mortality for dialysis patients was substantially increased compared with the general population (male RR 2.48; female RR 3.99).[55]

To examine the question of malignancy in dialysis patients, a major international study was undertaken by Stewart and coworkers,[123] involving analysis of pooled data for patients who received dialysis for end-stage renal disease (ESRD) during the period 1980 to 1994. A cohort of 834,884 patients treated in the United States, Europe, Australia, and New Zealand was assembled. The observed frequency of cancer among these patients during 2,045,035 person-years of follow-up was compared with the frequency of cancer in the respective background populations. Patients with NMSC were excluded. It was found that the overall risk of cancer was increased in patients with ESRD, and that the distribution of tumor types in dialysis patients resembled the pattern seen after transplantation. The excess risk was largely ascribed to effects on the kidney and bladder of underlying renal or urinary tract disease, or to loss of renal function. Also considered likely to be responsible was an increased susceptibility to viral carcinogenesis.

During the short mean follow-up of 2.5 years in this study, 3% of the study population developed cancer. The expected number of individuals developing cancer in the population at large was lower so that the SIR was 1.18. In younger patients (<35 years old), the risk of cancer was considerably higher (SIR 3.68), and this risk gradually decreased with increasing age. Particularly high risks were observed for cancer of the kidney (SIR 3.60), the bladder (SIR 1.50), and the thyroid and other endocrine organs (SIR 2.28). Excess numbers of cancers occurred in several organs in which viruses have been suspected as causative carcinogenic agents, whereas cancers of the lung, colon and

rectum, prostate, breast, and stomach were not consistently increased.

Reasons for Increased Risk of Cancer in Dialysis Patients

Patients maintained on dialysis are potentially at risk of cancer for several reasons, including the presence of chronic infection, especially in the urinary tract; a depressed immune system; previous treatment with immunosuppressive or cytotoxic drugs; nutritional deficiencies; and altered DNA repair mechanisms.[130] In addition, the underlying disease leading to renal failure, the persistent metabolic changes associated with it, and the development of certain complications such as acquired renal cystic disease may predispose to cancer. Some forms of genitourinary disease are known to predispose to renal, ureteric, or bladder tumors. The risk of renal cancer is increased in patients with inherited or acquired cystic disease of the kidney.[62,76] The main determinant of acquired cystic disease of the kidney and renal cell cancer seems to be the total duration of renal impairment, rather than the duration of dialysis treatment.[91] Other conditions predisposing to cancer include Balkan nephropathy and analgesic nephropathy, both of which are associated with a high risk of developing tumors of the renal pelvis and ureter.[28,72]

Particular Problem of Renal Tract Malignancy in Patients with End-Stage Renal Disease

Pathology studies have shown that renal tumors are more common in the pretransplant ESRD population than had previously been reported on the basis of radiological imaging.[30] A large study undertaken by Maisonneuve and colleagues[75] was important because, as the authors point out, most of the previous studies had been too small to detect potentially important findings on less common types of tumors or small increases in risk, or to study the relationship between cancer and the various causes of renal failure or the method of dialysis treatment (hemodialysis or peritoneal dialysis).[12,28,53,55,58,92,103,107,116] The study showed an overall increased risk of cancer in patients with ESRD, as had several previous studies. Generally, the types of cancer developing in patients with ESRD were similar to the cancer types observed with increased frequency in transplant recipients. Most common were cancers of the urinary tract, but cancers of the tongue, liver, lower genital tract in women, external genitalia in men, and thyroid and lymphomas and multiple myeloma also were observed to have an increased incidence. In seeking to explain their findings, the authors of this study suggested that viral infections were likely to be important as causative agents for some of the tumors.

Viral infections occur in about 10% of patients after transplantation,[38] but data about the frequency of viral infections in dialysis patients are sparse. There is no doubt, however, that ESRD patients have a greater than normal exposure to hepatitis B and hepatitis C viruses,[87] and this probably accounts for the observed excess of liver cancer. Human papillomavirus is thought to play a role in the development of cancers of the tongue, cervix, vagina, vulva, and penis.[6,29] In dialysis and transplant patients, the increased risk of developing lymphomas is thought likely to be due to activation of dormant Epstein-Barr virus (EBV).[135,136] A possible explanation for the observed increase in risk of thyroid and other endocrine tumors is the repeated examination and imaging of the neck in relation to the diagnosis of secondary hyperparathyroidism. In support of this hypothesis is the observation that the frequency of thyroid tumors increases with duration on dialysis.

An important point to emerge from the study by Maisonneuve and colleagues[75] is that the risk of cancer was not related to the type of dialysis. It was concluded that the uremic state, rather than any treatment-related phenomenon, was likely to be the cause of the increased risk. The uremia is thought likely to lead to an impairment of immunity, perhaps by interference with DNA repair mechanisms or by causing a reduction in antioxidant defense. Chronic infections and inflammatory processes, potentially associated with the development of malignancies, are more common in patients with renal failure. A final point to consider is that any degree of renal impairment could lead to the accumulation of carcinogenic compounds.[130]

In the above-mentioned study by Stewart and coworkers,[123] it also was concluded that dialysis itself conferred no additional risk of cancer other than by prolonging exposure to the uremic state. These authors reported that in the dialysis population, the risk of developing cancer of the kidney or bladder was relatively (but not absolutely) greater at younger ages, and in women rather than men. They found that the dialysis population exhibited a risk of cancers of the kidney and urinary tract over and above the heightened risk of cancer seen in many other sites. They reported that there was no excess risk of kidney cancer in patients with ESRD owing to polycystic disease, and noted that primary renal disease accounted for almost all of the excess risk of urothelial cancer, whether in the bladder or elsewhere in the urinary tract. They determined that the carcinogenic potential of acquired renal cystic disease was greater than that of primary (hereditary) polycystic renal disease. Stewart and coworkers[123] reported that the SIR for kidney cancer increased significantly with time on dialysis, whereas the SIR for bladder cancer progressively decreased.

Screening for Cancer in Dialysis Patients

Several authors have suggested that routine cancer screening in patients on long-term dialysis is not cost-effective.[24,50,60] Others have argued, however, that although general cancer screening is not cost-effective in dialysis patients, selective screening in younger patients and for known cancer types is warranted.[75,85] Parathyroid cancer is a good example, and this condition should be suspected in dialysis patients if rapid changes in serum parathyroid hormone levels are observed.[102] Careful and regular screening for premalignant and malignant skin lesions is another good example; this is likely to be of particular value in countries such as Australia, where frequent exposure to intense solar ultraviolet (UV) radiation is almost inevitable. Ishikawa and associates[56] proposed that screening is valuable in the detection of renal cell cancer, and pointed out that survival is best in young patients with a short duration of dialysis, and when the renal cell cancer is detected by screening, rather than by direct reporting of symptoms. Satoh and coworkers[110] likewise suggested that early diagnosis of renal cell cancer by regular imaging of patients with ESRD who are on dialysis would result in an improved outcome.

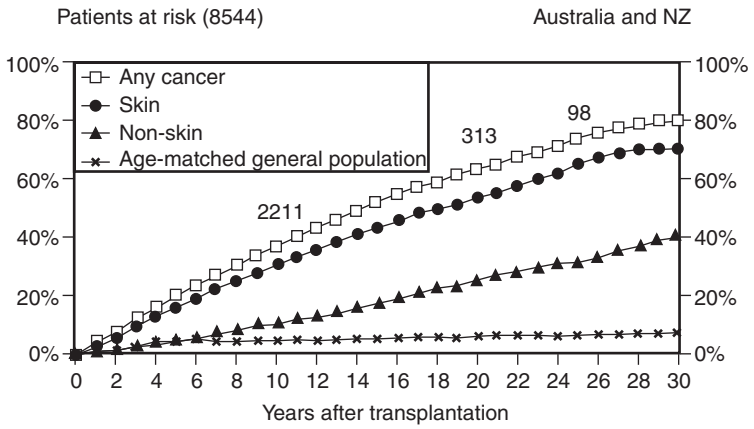

Figure 33–1 Cumulative risk of cancer post-transplant in Australia and New Zealand, 1965 to 2000. Primary cadaver and living unrelated donors. Patient and graft survived 90 days after transplantation. (Data from Sheil AG: Cancer Report 2001. In Ross GR (ed): ANZDATA Registry Report 2004. Adelaide, South Australia, Australia and New Zealand Dialysis and Transplant Registry, 2001, pp 84–90.)

Management of Cancer in Dialysis Patients

If malignancy does develop in a patient on dialysis, the condition should be treated in the conventional way; this usually involves surgical resection. For dialysis patients who have surgical treatment for malignancy, postoperative complications are, however, as expected, much higher than usual.[26] If surgery is not considered appropriate, chemotherapy may be possible, but individual drug dosage adjustments are likely to be required.[13] Treatment with radioactive iodine can be undertaken for thyroid cancer in patients on dialysis, but dosage adjustment is necessary because iodine is cleared mainly by the kidneys or by the dialysis process.[51]

CANCER IN KIDNEY TRANSPLANT RECIPIENTS

The magnitude of the cancer problem in kidney transplant recipients is well illustrated in Figure 33-1. Based on comprehensive, long-term data from the ANZDATA Registry, this figure shows that 10 years after receiving a renal allograft, approximately 10% of all patients will have developed a cancer (excluding NMSC).[117] After 20 years, this figure has increased to approximately 25%, and after 30 years to approximately 40%, by which time 80% of renal transplant recipients in Australia and New Zealand would have developed a cancer of any type.

The risk of cancer development is particularly great in patients who are older when they first undergo transplantation. For men who are younger than 35 years old at the time of first kidney transplantation, the adjusted risk of developing cancer (excluding NMSC) after 10 years is 4.2, whereas for men who are 55 years old or older at the time of transplantation, it is 24.6. For women, the corresponding risk values after 10 years are 5.8 and 20.9. The overall results of this analysis are shown in Table 33-2. From Table 33-2, it is possible to give an estimate of a patient's risk of developing a cancer (excluding NMSC) according to gender and age at transplantation. This information allows clinicians to identify patient groups at higher risk of developing malignancy and may be useful for pretransplant counseling when informed consent is being obtained. Vajdic and colleagues[129] present in detail ANZDATA Registry data relating to the risk of cancer after renal transplantation.

Transmission of Cancer from the Donor

Early in the history of renal transplantation, it became apparent that cancer in the transplanted organ or at other sites occurred frequently in recipients who received apparently normal kidney allografts from cancer-affected donors.[77,82,95] It was soon recognized that organs retrieved from such donors could harbor malignant cells that had the potential to proliferate in the recipient, causing death.[77,82]

Table 33–2 **Absolute Cancer Risk in the Clinical Setting (Excluding Nonmelanocytic Skin Cancer) in Australia and New Zealand by Time after First Kidney Transplant, 1963 to 2003***

	Risk of Nonskin Cancer by Age at Transplantation (%)							
	< 35 yr		35-44 yr		45-54 yr		≥ 55 yr	
Years Since Transplant	Male	Female	Male	Female	Male	Female	Male	Female
5	1.3	2.1	3.1	3.7	5.7	6.8	10.1	9.6
10	4.2	5.8	7.5	9.6	14.4	14.7	24.6	20.9

*Adjusted risk of ≥1.

Data from Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry.

Although most patients with a transplanted cancer ultimately die of the malignancy, early experiences showed that cure occasionally could be achieved by stopping the patient's immunosuppressive therapy with a view to precipitating rejection of the transplanted organ and the cancer, and then removing the allograft.[80,134]

Because of the almost universally disastrous results of transplanting organs from a donor known to have cancer other than a primary brain tumor or a NMSC, such individuals are generally excluded as potential organ donors. This exclusion applies to cadaver and living donors. The situation is not always straightforward, however. The donor may have had a cancer many years earlier and be considered to have been cured. A cancer in the donor may not be recognized at the time of donation, and only discovered subsequently when it becomes clinically apparent (in the case of a living donor) or when an autopsy is performed (in the case of a cadaver donor). If an autopsy is not performed after cadaver donation, the diagnosis of malignancy in the donor may never be documented.

Generally, transplantation of organs from donors with a history of NMSC and donors dying of primary intracerebral tumors has been regarded as safe because such tumors rarely metastasize.[25] The risk of transferring malignancy is considerably greater, however, for primary brain tumors with a high histological grade of malignancy or after previous surgery on them, and under such circumstances organ donation is generally considered inappropriate.[39,47] Even when every reasonable precaution is taken, transfer of malignancy to the recipient from a donor with a brain tumor occasionally occurs.[14,27,31] Great caution is required, and strenuous efforts must be made to exclude the presence of cancer in every potential organ donor.[1] In addition, an early autopsy examination of the cadaver donor should be encouraged and conducted whenever possible.

Similarly, despite all efforts to avoid the situation, kidneys occasionally are transplanted from donors without brain tumors who are subsequently discovered to have primary or metastatic malignancies.[9,47,93] There is general consensus that these renal grafts should be removed as soon as possible, with reinstatement of dialysis. All potential recipients must be made aware of the possibility that malignancy might be transferred with the donor organ, and this risk should be included in informed consent documentation. The matter has medicolegal implications, and there have been instances of litigation by patients who have received organs from a donor with cancer.

Because of the serious shortage of donor organs, the possibility of relaxing the stringent requirement that donors have no history of serious malignancy is sometimes considered if the malignancy seems to have been treated successfully. Instances of tumor transmission from donor to recipient many years after apparently successful treatment of malignancy in the donor have been documented, however. Melanoma is the malignancy most commonly transferred from a donor to a transplant recipient,[85] and it has been suggested that organs from an individual who has ever had an invasive melanoma should not be used for transplantation purposes.[73] In the case reported by MacKie and associates,[73] an individual who had a melanoma treated, apparently successfully, 16 years earlier became a kidney donor after dying from a presumed subarachnoid hemorrhage. Both kidney recipients developed metastatic melanoma. An autopsy of the donor was not performed, but it seems likely that the cause of death of the donor was bleeding from a melanoma metastasis in the brain. There are numerous other reports in the literature of melanoma transferred with donor organs, with an interval of many years after apparently successful treatment of the primary melanoma in many of them.[35,101,124]

A common situation is the potential donor who becomes brain dead as a result of an intracerebral hemorrhage. Even with currently available high-resolution imaging modalities, a small metastasis that has been the cause of the hemorrhage may be undetectable. Whenever possible, urgent postmortem examination of the brain of the donor should be performed in these situations before the organs are transplanted, to exclude the possibility that the intracerebral hemorrhage is due to malignancy. Buell and coworkers[14] have provided compelling evidence in support of the proposal that a limited brain autopsy after donation should be performed whenever the cause of a donor's brain death is unclear. These authors reviewed information submitted to the Israel Penn International Transplant Tumour Registry from multiple individual international registries. They found that the most common diagnostic error was with intracranial hemorrhage, which in some countries, such as Australia, is the most common cause of brain death for cadaver renal donors.[36]

Even the most extensive donor screening cannot provide an absolute guarantee of a cancer-free status. If a transplant recipient develops cancer, the other recipients of organs from the same donor should be investigated as soon as possible and monitored carefully. Occasionally, the development of a post-transplant lymphoproliferative disorder (PTLD) in a recipient may be a direct result of viral transmission from the donor.[18] In these cases, EBV usually has been detected in the tumor cells.

A possible exception to the general rule that precludes the transplantation of organs from a patient with a known malignancy is when a donor kidney is found to have a small, incidental renal cell cancer with a low histological grade. It has been suggested that this cancer can be managed by excision before transplantation with a low risk of tumor occurrence in the recipient.[15] Donor exclusion criteria are considered in detail in Chapters 6 and 7.

Development of De Novo Cancers in Renal Transplant Recipients

Early reports of de novo cancers arising in immunosuppressed transplant recipients undoubtedly underestimated the long-term risk.[74] These estimates were based on single-center and registry reports, which indicated that malignancies (excluding NMSCs) arose in 2% to 8% of transplant recipients. Some more recent estimates of risk also are likely to be serious underestimates because transplant recipient populations on which the incidence of cancer is based are biased by the large numbers of recently transplanted patients compared with the fewer long-surviving patients. A large study in the United States that attempted to determine the true incidence of malignancy in renal transplant recipients reported only the cancer rates in the first, second and third years after transplantation.[59]

When incidence figures are determined on the basis of the number of years since transplantation, a different picture emerges, with reports of 34% to 50% of immunosuppressed transplant recipients developing cancer if they are followed

for 20 years or more after transplantation.[41,84,119] Long-term data from Australia and New Zealand reveal that in recipients of cadaver kidneys transplanted 30 years earlier, the incidence of skin cancer is 75%, and the incidence of nonskin cancer is 33%, with some form of cancer (either skin or nonskin cancer) developing in 80% of patients.[118] Transplant registry data from other countries also show a substantial risk of cancer development in renal transplant recipients, which steadily increases as the time since transplantation lengthens.[1,8,11]

Reasons for the Increased Risk of Cancer in Transplant Patients

Numerous mechanisms are likely to contribute to the increased risk of cancer in immunosuppressed allograft recipients. Some of these are the same mechanisms responsible for the development of malignancy in patients with ESRD and in patients on dialysis. In transplant recipients, however, other mechanisms also are involved, and the relative importance of each of these may vary with the type of cancer. The dominant factors are believed to be impaired immune surveillance for neoplastic cells and depressed antiviral immune activity.

Impaired Immune Surveillance

A century ago, Ehrlich (1909) proposed that abnormal cells arise frequently in normal individuals as a result of somatic mutation, viral infection, or some other mechanism. If these abnormal cells are not eliminated, they have the potential to become autonomous and to develop into a malignant process. Based on the assumption that the immune system is important in eliminating such abnormal cells,[126] it was logical to make the further assumption that any impairment of immune surveillance could result in cancer.[17,61] These concepts have been subjected to close scrutiny, and much work has been done to assess the importance of immune surveillance in cancer development, by undertaking laboratory and clinical studies.

Several pieces of evidence support the importance of immune surveillance in protection against cancer in humans. One is the observed increase in cancer incidence with increasing age. Another is the well-documented increase in cancer incidence that occurs in congenital and acquired immunodeficiency states,[63] particularly in patients with the acquired immunodeficiency syndrome.[42,132] Particularly powerful additional evidence comes from experience with human transplantation, including the transfer of malignancy to immunosuppressed transplant recipients, the increased incidence of de novo cancer in these individuals, and studies showing that the immunosuppression can lead to tumor recurrence.[40] On the basis of all these pieces of evidence, it is now generally accepted that most malignancies arise from abnormal cells that have not been eliminated by the immune system in the usual way.

The mechanisms by which immunosuppressive agents lead to the development of cancer are complex. Their effects seem to be due at least partially to their ability to act as potentiating agents for other oncogenic stimuli, such as oncogenic viruses, chemical carcinogens, and UV light. Immunosuppressive agents with powerful antilymphocyte activity, including cyclosporine, antilymphocyte globulin, antithymocyte globulin (ATG), and anti–T cell antibodies, may potentiate the effects of oncogenic viruses by eliminating T lymphocytes or impairing their normal function. In early studies, a clear potentiating effect of antilymphocyte globulin on cancer development was observed when the agent was used in conjunction with oncogenic viruses[2,68] or chemical carcinogens.[4,20,106]

Oncogenic Viruses

Viruses that have oncogenic properties have long been recognized in experimental studies.[2,114] Organ transplant recipients are particularly susceptible to viral infections, some of which are known to be potentially oncogenic in humans. These include EBV, cytomegalovirus, herpes simplex, herpes zoster, hepatitis B, hepatitis C, and the human papillomaviruses. The fact that the most common types of cancer occurring in transplant recipients are those in which oncogenic viruses are known to be causative is unlikely to be coincidental. Viral oncogenesis is considered to play a role in the development of most post-transplant lymphomas and lymphoproliferative disorders, cancers of the skin and cervix, and hepatomas.[34] The rapidity with which some malignancies occur after transplantation also is consistent with the concept that viral oncogenesis is involved because the start of immunosuppression is likely to produce very rapid viral transformation.

Chronic Antigenic Stimulation and Immune Regulation

It has been suggested that the continuing presence of foreign allograft antigens in a recipient may be important in cancer causation. This possibility is supported by evidence that chronic lymphoid stimulation results in a high incidence of malignant lymphomas.[121] The mechanism may be a direct consequence of protracted antigenic stimulation of the lymphoreticular system, with continued stimulation of lymphoid tissue leading to hyperplasia and ultimately to neoplasia.

Environmental Factors

A range of factors could account for the observed regional variations in the pattern of cancers that occur in patients transplanted at different centers around the world. A striking example of environmental effect is the association between the development of skin cancer in white transplant recipients and solar UV exposure. This association undoubtedly accounts for the high incidence of skin cancer in renal transplant recipients in Australia and New Zealand (see Chapter 32). Exposure to UV light also can cause immunosuppression that may influence the development of other forms of cancer, such as non-Hodgkin's lymphoma.[5,90] Other factors that might predispose to the development of malignancy include viral infections encountered by patients before or after transplantation and local practices in viral infection prevention, detection, and therapy. Such factors operate against a background of general influences, such as age, gender, and genetic diversity, and depend on the length of time after transplantation. The complex interactions of such factors determine the incidence and pattern of post-transplant malignancy for each individual transplant center.

Direct Neoplastic Action of Immunosuppressive Drugs (see Chapters 15 to 21)

The immunosuppressive drugs used to prevent and treat rejection in transplant recipients generally have the effect of increasing the risk of cancer. This is consistent with the

concept that malignancies arise when immune surveillance is impaired. Paradoxically, some of these immunosuppressive drugs also may have antineoplastic properties.[44]

Calcineurin Inhibitors (Cyclosporine and Tacrolimus)

There is now a considerable body of experimental and clinical evidence that the calcineurin inhibitor cyclosporine promotes rather than induces the development of cancer. The calcineurin inhibitor FK506 (tacrolimus) seems to have similar effects, again supported by experimental studies[113] and clinical studies.[57] It seems clear that calcineurin inhibitors as a group are associated with post-transplant malignancy. The effect seems to be due to aberrant production of cytokines that regulate tumor growth, metastasis, and angiogenesis.[44] There also is evidence, however, that cyclosporine inhibits multidrug resistance in cancer cells,[128] and that it can even be combined with cytotoxic drugs, such as paclitaxel, to inhibit tumor growth in some cases.[69]

Inhibitors of the Mammalian Target of Rapamycin

Rapamycin (sirolimus) and its derivatives are immunosuppressive agents that bind with high affinity to mammalian Target of Rapamycin. The basis for the immunosuppressive activity of these agents is their action in blocking interleukin-2 stimulation of lymphocyte proliferation. There is accumulating evidence that rapamycin-based compounds have antineoplastic properties,[67,108] and there have been several reports that the incidence of post-transplant malignancy is markedly lower in patients who receive sirolimus-based immunosuppression or sirolimus in association with calcineurin inhibitors compared with patients receiving calcineurin inhibitor therapy alone.[19,44,79]

Corticosteroids

Corticosteroids have anti-inflammatory and immunosuppressive properties, and their effects on the immune system are complex. Although they have been used clinically for several decades, their exact mechanisms of action are still not clearly understood. Their primary effects seem to be a result of inhibition of the production of T cell lymphokines, which are needed to amplify macrophage and lymphocyte responses. They also cause lymphopenia as a result of redistribution of lymphocytes from the vascular compartment into lymphoid tissues, and they inhibit the migration of monocytes.

Corticosteroids, such as prednisone and prednisolone, have been used as part of most immunosuppressive regimens since human organ transplantation began, but it is difficult to assess their role in the causation of cancer in transplant recipients because almost always they have been used in conjunction with other immunosuppressive therapy. Although there is some experimental evidence that corticosteroids increase the risk of malignancy,[131] and it is known that there is an increased incidence of Kaposi's sarcoma in patients receiving them for long periods,[127] corticosteroids also are used in combination with other drugs to treat certain types of cancer, including lymphomas. The contribution of corticosteroids to the development of cancer in transplant recipients is unclear.

Azathioprine

Azathioprine was one of the earliest agents used to prevent rejection in human transplant recipients. It disrupts the synthesis of DNA and RNA, causing immunosuppression by interfering with lymphocyte proliferation. When used as a single agent to treat autoimmune diseases, azathioprine is associated with an increased risk of lymphomas and an increased risk of a wide range of solid neoplasms, including squamous cell carcinomas,[10] urinary bladder tumors,[111] breast carcinomas,[66] and brain tumors.[112] In a large follow-up study of 1000 renal transplant recipients, it was found that patients who received azathioprine had a lower cumulative incidence of tumors after transplantation than patients who received cyclosporine.[81] It was unclear, however, whether this was due to the drugs themselves or to the overall intensity of the immunosuppression.

Mycophenolate Mofetil

Mycophenolate mofetil, now established as an effective immunosuppressive drug in organ transplantation, was originally developed as an antineoplastic agent.[133] Its main mode of action as an immunosuppressant is through blockage of the de novo purine synthesis pathway.[3] Preliminary analysis of data from large transplant registries suggests that the rate of development of cancer in patients receiving mycophenolate mofetil is lower than the rate in patients receiving other immunosuppressive therapies, but longer follow-ups of patients treated with this agent are required before firm conclusions can be drawn.

Lymphocyte-Depleting Agents

Although a common pathway for many immunosuppressive agents used in organ transplantation seems to be the suppression of lymphocyte proliferation, some agents are known or are thought to act by causing the death of lymphocytes. Examples are antilymphocyte globulin and antithymocyte globulin, both polyclonal antibodies; the monoclonal antibody muromonab (OKT3), which is directed against the CD3 antigen complex found on all mature human T cells; and more recently developed antilymphocyte antibodies, such as the anti-CD25 antibodies basiliximab and daclizumab, which are highly specific interleukin-2 receptor blockers. After administration of these agents, the total lymphocyte count decreases as lymphocytes, especially T cells, are lysed after antibody binding and complement deposition on the cell surface, inactivated by binding to T cell receptors, or cleared from the circulation and deposited in the reticuloendothelial system. Overall, lymphatic depletion is thought to increase the risk of malignancy by reducing the effectiveness of an individual's immune surveillance.

Types of Cancer in Renal Transplant Recipients

Overview

The distribution of malignancies that occur in kidney transplant recipients differs considerably from that in the general population[22]; comprehensive data from the ANZDATA registry, collected since 1963, show this clearly (Table 33-3). In the 2004 ANZDATA report, several analyses of cancer developing in kidney transplant recipients were reported.[23] These were based on cancer rates for the Australian general population

Table 33–3 **Standardized Incidence Ratios for Cancer Risk (Excluding Nonmelanocytic Skin Cancer) in Patients Undergoing at Least One Kidney Transplant in Australia and New Zealand, 1980-2003***

Site of Cancer	Observed Cancer	Expected Cancer	SIR	95% CI
All registrable cancers	*1545*	*495.08*	*3.12*	*2.97-3.28*
Head, neck, and lip	63	22.77	2.77	2.16-3.54
Esophagus	29	6.14	4.73	3.28-6.80
Stomach	15	12.07	1.24	0.75-2.06
Small intestine	3	1.49	2.01	0.65-6.23
Colorectal	141	72.76	1.94	1.64-2.29
Liver	19	3.97	4.78	3.05-7.49
Gallbladder	8	3.21	2.49	1.25-4.98
Pancreas	16	9.30	1.72	1.05-2.81
Nasal cavity	5	0.92	5.41	2.25-13.00
Larynx	11	5.54	1.99	1.10-3.59
Trachea, bronchus, and lung	108	53.85	2.01	1.66-2.42
Other thoracic organs	6	0.57	10.60	4.76-23.60
Bone and articular cartilage	5	1.01	4.94	2.06-11.87
Melanoma	183	57.64	3.18	2.75-3.67
Mesothelioma	4	2.97	1.35	0.51-3.59
Kaposi's sarcoma	28	1.06	26.44	18.26-38.29
Connective and other soft tissue	12	3.80	3.16	1.79-5.56
Breast	87	69.52	1.25	1.01-1.54
Vulva	41	0.90	45.60	33.58-61.93
Vagina	12	0.33	36.02	20.46-63.43
Cervix uteri	46	6.97	6.60	4.94-8.81
Corpus uteri	18	9.75	1.85	1.16-2.93
Ovary	8	7.56	1.06	0.53-2.12
Other female genital organs	0	0.32	0.00	—
Penis and other male genital organs	11	0.62	17.81	9.86-32.16
Prostate	53	54.72	0.97	0.74-1.27
Testis	0	4.36	0.00	—
Kidney, ureter, and urethra	125	14.73	8.49	7.12-10.12
Bladder	82	15.97	5.14	4.14-6.38
Eye	4	1.50	2.67	1.00-7.12
Brain and central nervous system	16	9.59	1.67	1.02-2.72
Thyroid gland	27	5.96	4.53	3.11-6.61
Other endocrine glands	4	0.43	9.37	3.52-24.97
Unknown primary site	70	16.74	4.18	3.31-5.28
All lymphomas	231	22.74	10.16	8.93-11.55
Immunoproliferative neoplasms	3	0.29	10.23	3.30-31.73
Multiple myeloma	15	5.62	2.67	1.61-4.42
Leukemia	32	12.28	2.61	1.84-3.69

*Analysis of 13,077 patients (110,395 person-years), standardized for age, gender, and calendar year with Australian general population.
CI, confidence interval; SIR, standard incidence ratio.
Data from Chapman JR, Webster A: Cancer Report 2004. In Ross GR (ed): ANZDATA Registry Report 2004. Adelaide, South Australia, Australia and New Zealand Dialysis and Transplant Registry, 2004, pp 101–103.

collected and reported by the Australian Institute of Health and Welfare, with adjustments made to standardize the results by age, gender, and calendar year from 1980 to 2003. The expected number of cancers was based on data collected by the National Cancer Statistics Clearing House (NCSCH). The data are likely to be reliable because in all Australian States and Territories it is a legal requirement that all new cases of cancer are reported to the relevant State or Territory cancer registry, and this information is in turn forwarded to the NCSCH.

Using this methodology, the absolute cancer risk (excluding NMSC) was determined for 14,354 patients who received a first renal transplant between 1963 and 2003. Median follow-up was 7 years (interquartile range 2.7 to 13.2 years). Various predictors of post-transplant malignancy were investigated, including age at transplantation, gender, donor source, era of transplantation, and primary kidney disease. Each potential predictor was examined alone (univariate analysis), and the predictors that showed a significant relationship with diagnosis of a post-transplant malignancy were entered into a multivariate Cox proportional hazards model, to show the effect of each predictor after allowing for the effect of other predictors. Results were stratified by predictors showing significant effect modification and reported as hazard ratios with 95% confidence intervals.

Of the 14,354 kidney recipients in the data set, 1412 (9.8%) developed a cancer (other than a NMSC). Univariate analysis revealed that there was a significantly increased risk of cancer with increasing age at transplantation ($P < .0001$), for women ($P < .002$), for cadaver donors ($P < .0001$), for recipients with primary disease other than diabetes ($P = .003$), and for recipients transplanted after 1985 ($P < .001$). When allowing for all effects in the multivariate model, however, gender, age, and primary renal disease remained significant predictors of cancer development but donor source ($P = .25$) and era of transplantation ($P = .87$) did not. The importance of age at transplantation in cancer development in men and women has been mentioned previously and is apparent from the data shown in Table 33-2.

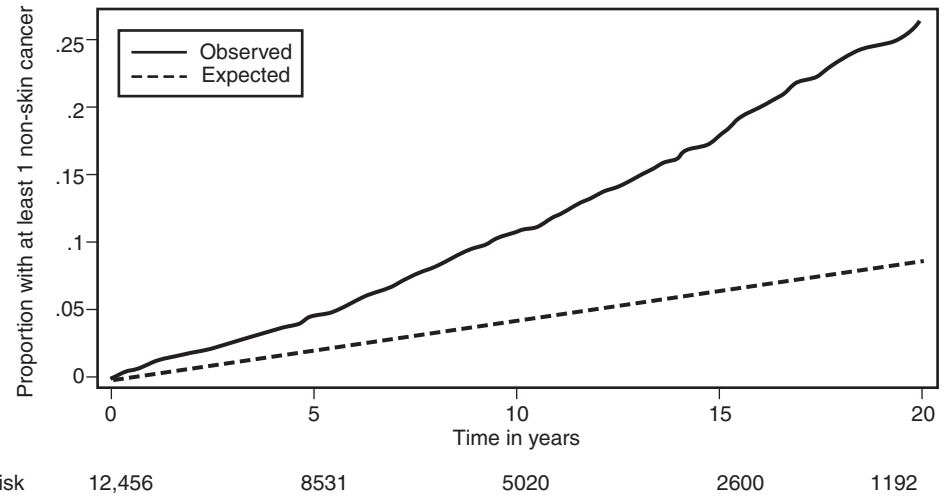

Figure 33–2 Lifetime cumulative risk of at least one cancer (excluding nonmelanocytic skin cancer) after first transplant in Australia and New Zealand, 1980 to 2003. Patients become at risk at the time of the first transplant and cease to be at risk at the time of death or last known follow-up. Expected curve is calculated for a general population of the same age and sex distribution. (Data from Chapman JR, Webster A: Cancer Report 2004. In Ross GR (ed): ANZDATA Registry Report 2004. Adelaide, South Australia, Australia and New Zealand Dialysis and Transplant Registry, 2004, pp 101–103.)

The cumulative risk of developing at least one cancer (excluding NMSC) after renal transplantation is shown in Figure 33-2. The risk after 10 years is approximately twice that expected in the general population, whereas after 20 years, it is approximately three times the expected risk.

Skin Malignancies

Skin malignancies are the most common types of cancer developing in renal transplant recipients and are a particular problem in parts of the world where predominantly white populations are exposed regularly to high-intensity solar UV light. Skin malignancies in transplant recipients are discussed in Chapter 32.

Genitourinary Malignancies

The most frequent malignancies occurring in transplant recipients are those of the genitourinary system, constituting approximately one third of the total. Of these, the female genital tract is particularly at risk, with a greatly increased RR for squamous cell carcinomas of the vulva and vagina and in situ and invasive carcinomas of the cervix. These cancers have a known association with human papillomavirus infection. The relative risk of developing malignancy of the bladder, kidney, and urethra also is greatly increased. Retained native kidneys represent a cancer risk because the cause of the renal failure may have been a condition known to predispose to malignancy, such as analgesic nephropathy,[65] and because the retained kidneys may have developed acquired cystic disease with its own malignant potential. It is for the latter reason that renal cell cancer is the most common malignancy after renal transplantation in Japan.[52]

Post-Transplant Lymphoproliferative Disorders

Thought to be due to primary or reactivated infection with EBV, PTLDs are common in the early post-transplant period.[89] The term EBV-associated PLTD includes all clinical syndromes of EBV-associated lymphoproliferation, ranging from uncomplicated post-transplant infectious mononucleosis to true malignancies that contain clonal chromosomal abnormalities.[104] EBV-associated malignancies affect approximately 1% of renal transplant recipients,[118] the greatest incidence being in the first post-transplant year (0.2%/yr), with a reduced incidence thereafter (0.04%/yr).[88] PTLDs occur most commonly when intense immunosuppression is used to treat resistant episodes of graft rejection. Pediatric graft recipients also are at particular risk. PTLDs regress completely in some patients when immunosuppressive therapy is reduced,[122] with or without concurrent antiviral therapy,[45] sometimes with evolution to non-Hodgkin's lymphoma, or they may progress to a fatal outcome.

A marked increase in the incidence of PTLDs in renal transplant recipients was reported after the introduction of cyclosporine[99] and tacrolimus.[86] These observations raised concern that the drugs themselves might have a specific role in lymphoma causation; however, registry reports from different countries suggest that this is not the case.[88,118] It is now generally believed that PTLDs and malignant lymphomas are an inevitable consequence of effective immunosuppressive therapy regardless of the particular immunosuppressive agents used. The effect of EBV infection, whether as a primary event or as a reactivation of a previous infection, is thought to be mediated by B lymphocyte proliferation secondary to inhibition of the T cell–dominated immune responses produced by powerful immunosuppression.[45] The T cell–suppressive, B cell–stimulatory cytokine interleukin-10 has been implicated.[7]

It has been known for nearly 40 years that EBV is linked to the development of Burkitt's lymphoma[48] and to nasopharyngeal carcinoma. EBV is ubiquitous, with 95% of the adult population in most countries having serological evidence of prior exposure. The possibility of reactivation is high if immunosuppression is excessive. In children who undergo transplantation, approximately 50% are likely to be EBV negative at the time, resulting in susceptibility to primary infection from the environment or directly from a virus-positive graft or blood transfusion.[33]

The widespread lymphoproliferative response to EBV infection has histological features ranging from polymorphic B cell hyperplasia to monomorphic lymphoma. In some of these patients, the lymphoproliferation results in tumor masses in which the lymphoid cells are usually of a polyclonal type. In approximately one third of patients, the lesions are

monoclonal, the hallmark of true malignant lymphomas.[46,122] Although the most common of the malignant lymphomas that occur in transplant recipients are large cell lymphomas, the whole range of malignant lymphomas has been recorded, including lymphoblastic lymphomas, Hodgkin's disease, and a variety of poorly defined malignancies. Hodgkin's disease accounts for only approximately 2% of lymphomas in organ transplant recipients, however, compared with 34% in the general population.[97] Approximately half of the patients with lymphoma have localized disease, and half have disseminated disease. When the disease is localized, the area most commonly affected is the central nervous system. In patients with disseminated disease, the liver, spleen, lymph nodes, bone marrow, and central nervous system are usually involved. Approximately one third of recipients with disseminated disease have involvement of the renal allograft.

The frequency with which lymphomas occurring in transplant recipients involve the central nervous system is notable. In approximately 40% of lymphomas in transplant recipients, the brain or spinal cord is involved compared with 2% of such malignancies in the general population. These lymphomas involving the central nervous system are frequently multicentric.

Kaposi's Sarcoma

A viral etiology also is well established for Kaposi's sarcoma developing in organ transplant recipients. Genetic predisposition has an important role as well, and Kaposi's sarcoma occurs more frequently in immunosuppressed transplant recipients of Italian, Greek, Jewish, Arabic, and African ancestry,[100] no matter where these patients are resident when they receive their transplant. The incidence of Kaposi's sarcoma in any transplant population depends largely on the proportion of patients with Mediterranean heritage in that population. In countries such as the United States and Australia, Kaposi's sarcoma affects approximately 0.25% of renal allograft recipients, contributing 2% to 3% of all cancers. In Japan, Kaposi's sarcoma is extremely rare,[52] whereas it is common in the Middle East, affecting approximately 5% of recipients in Saudi Arabia, contributing 40% to 70% of all cancers.[105] Men are affected three times as frequently as women, and almost 50% of cases occur within the first year after transplantation.[100] The evidence that herpesviruses are involved in the development of Kaposi's sarcoma is compelling,[21] and it has been shown that the specific herpesvirus involved (human herpesvirus type A) can be transmitted by renal allografts.[109] The role of immunosuppression in the development of Kaposi's sarcoma is shown by the fact that withdrawal of immunosuppression sometimes results in complete remission.[100]

Kaposi's sarcoma developing in transplant recipients tends to be multicentric, with the development of tumors composed of spindle-shaped cells with endothelium-lined vascular spaces, red blood cell extravasation, and clusters of inflammatory cells. Of transplant patients with this condition, 60% have involvement of the skin or the oropharyngolaryngeal mucosa or both.[96] In these sites, the lesions appear as circumscribed purplish macules or as granulomas that fail to heal (Fig. 33-3). The remaining patients have visceral disease, particularly involving the gastrointestinal tract or the respiratory system. Approximately 40% of patients with nonvisceral lesions have complete or partial remission after cessation or reduction of immunosuppressive therapy, although with reduced immunosuppression, more than 50% of these patients lose their grafts to rejection. Patients with visceral involvement usually fail to respond to any form of therapy. There is some anecdotal evidence that rapancycin (sirolimus) may be an effective therapy.[9a,41a] (See Chapter 19.)

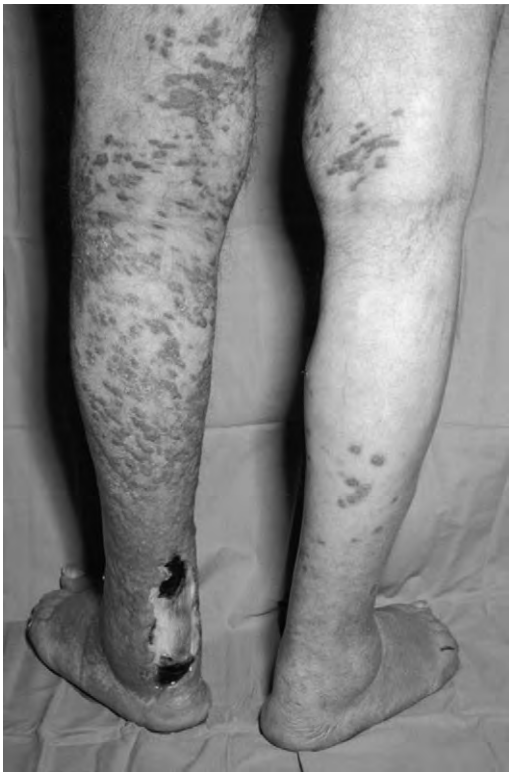

Figure 33–3 Renal transplant patient with Kaposi's sarcoma and an area of post-treatment cutaneous radionecrosis over the Achilles tendon. This area of ulceration was completely healed 1 year later. (See color plate.)

Other Malignancies

In addition to the malignant diseases already discussed, many other malignancies occur in renal transplant recipients with increased frequency relative to the general population. These include hepatoma (RR 7.3), well known to be associated with infection with the hepatitis B virus, and various malignancies of the gastrointestinal tract, particularly the esophagus (RR 5.2) and the large bowel (RR 2.4). There is no significant increase in the incidence of breast cancer in renal transplant recipients (RR 1.0), whereas only two cancers have a significantly reduced incidence—prostate cancer (RR 0.7) and ovarian cancer (RR 0.7).[23] It is thought likely that a diminished hormonal drive in renal transplant recipients is responsible for this reduced incidence of malignancy in the prostate and ovary.

Time of Cancer Presentation

In nonimmunosuppressed individuals, known carcinogens such as tobacco, UV light, and ionizing radiation have long latent periods between exposure and the development of malignancy. In immunosuppressed renal transplant recipients, the process of oncogenesis is greatly accelerated. In Australia, the mean time of appearance for lymphomas, Kaposi's sarcoma, and malignancy of the endocrine glands is approximately 6 years after transplantation; for cancer affecting the

respiratory tract, it is 8 years; for breast cancer, genitourinary system cancers, and leukemia, it is 9 years, and for cancer of the alimentary tract, it is 10 years.[118] Non-Hodgkin's lymphoma has its peak incidence within 1 year of transplantation, however, with a decreased frequency thereafter. Although the increased risk of malignancy continues indefinitely, the average times of appearance of various malignancies is gradually lengthening as the mean follow-up period for transplant recipients is extended.

Management of Cancer in Kidney Transplant Recipients

Because early diagnosis offers the best chance of effective treatment, clinicians caring for renal transplant recipients must be constantly alert to the possibility of cancer development. Regular clinical review by transplant clinicians is essential, with periodic gynecological review of female recipients, and careful dermatological surveillance for all recipients considered to be at risk of developing skin malignancy. Localized nonskin malignancies should be treated by standard surgical excision, with adjuvant radiotherapy or chemotherapy as considered appropriate. If complete surgical excision is possible, it is usually considered reasonable to continue immunosuppressive therapy. If metastatic disease is present or develops, however, most clinicians withdraw immunosuppression, arrange excision of metastases if these appear to be single or localized, institute chemotherapy if surgical removal is impossible, and remove the allograft when rejection occurs. As expected, survival rates are lower in transplant recipients than they are in the general population (e.g., colorectal cancer).[16]

The treatment of PTLDs, particularly if they become apparent in the early post-transplant period, is to cease or reduce immunosuppression in the hope that B cell proliferation would not have progressed to the stage of monoclonal malignancy. Treatment with antiviral agents is usually given concurrently. Other forms of therapy are sometimes employed, but a detailed consideration of the treatment of PTLDs is beyond the scope of this chapter; it has been reviewed elsewhere.[43,71] Generally, localized lymphoma is treated when possible by surgical excision, or, when this is not possible, by radiotherapy. When lymphoma occurs late after transplantation and is multicentric, it is usual to withdraw immunosuppression, treat with chemotherapy or radiotherapy as appropriate for the histological type of malignancy, and deal with rejection of the allograft if it occurs.

TRANSPLANTATION IN PATIENTS WITH A HISTORY OF CANCER

A question that frequently arises is whether it is safe for patients who have a history of malignancy to undergo transplantation. The known predisposition of immunosuppressed patients to develop malignancy means that the risk of reactivating a latent malignancy would be high. In an early review of the problem, Penn[98] reported patients with nonrenal malignancies who had been treated before transplantation. Of 119 patients with tumors involving the breast or a variety of internal organs, 18 (14%) developed recurrence or metastasis, mostly from tumors of the breast, bladder, or large bowel. Although recurrence generally was less likely to occur with greater time from treatment of the cancer to transplantation, 28% of the recurrences occurred in patients who had been treated an average of 7 years before transplantation. There were 22 patients with prior lymphatic malignancies, and the disease persisted or recurred in 50%. Most of the recurrences were in patients who had multiple myeloma. Nine of the 11 patients were not being treated or were not in remission at the time of transplantation, or the existing malignancies had not been recognized at the time of transplantation. Generally, recurrences did not occur in patients treated more than 2 years before transplantation or who were in remission at the time of transplantation.

Previously treated melanoma presents a particular problem because in nonimmunosuppressed patients this disease can recur more than 25 years after apparently successful treatment,[115] indicating that in some cases the disease persists but is controlled by the individual's immune defenses. The risks of recurrence of melanoma after transplantation are considerable, and if transplantation is contemplated, screening with a sensitive test such as a positron emission tomography scan should be performed before proceeding, although microscopic metastatic disease would not be detected using this test or any other investigation presently available.

Currently, most clinicians consider it reasonable to offer transplantation to patients with ESRD after treatment of low-grade cancers such as NMSC and in situ cancer of the cervix. For patients who have had other cancers treated successfully, it is generally considered appropriate to defer transplantation for at least 2 years. Patients who have had a cancer with a particularly poor prognosis, such as melanoma, probably should not be considered for transplantation until several years have passed from the time of successful treatment of that malignancy.

PREVENTION OF CANCER IN RENAL TRANSPLANT RECIPIENTS

As indicated earlier, all reasonable measures should be taken to exclude malignancy in every patient before offering transplantation. Cigarette smoking should be prohibited, and appropriate advice should be given about sun protection in areas where there is a high risk of skin malignancy. Pretransplant dermatological assessment is advisable, and existing skin lesions should be treated. In female patients, pretransplant gynecological assessment should be mandatory, and any abnormality of the uterine cervix should be treated adequately before transplantation. Pretransplant viral studies should be undertaken, including tests for hepatitis B, hepatitis C, cytomegalovirus, HIV, EBV, herpes simplex, and herpes zoster. Donor viral studies also should be routine to avoid or at least document viral transmission. After transplantation, the use of prophylactic antiviral agents may be considered for patients who are judged to be at high risk for viral infection, such as cytomegalovirus-negative or EBV-negative recipients who receive organs from donors positive for these viruses, or for recipients receiving high-dose immunosuppression to treat rejection (these measures are discussed in detail in Chapter 29). By preventing or controlling infections, it is hoped that the risk of post-transplant malignancy will be reduced.

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Chapter 34

Pancreas and Kidney Transplantation for Diabetic Nephropathy

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Type 1 diabetes, which most commonly manifests in childhood, continues to represent a therapeutic challenge. Secondary diabetes complications, observed in 30% to 50% of patients who live more than 20 years after onset of the disease, result in poor quality of life (QOL), premature death, and considerable health care costs.[79] The principal determinant of the risk of devastating diabetes complications is the total lifetime exposure to elevated blood glucose levels.[17] Establishing safe and effective methods of achieving and maintaining normoglycemia would have substantial implications for the health and the QOL of individuals with diabetes.

The Diabetes Control and Complications Trial (DCCT) showed that, given a qualified diabetes care team and intensive insulin treatment control, near-normalization of glycemia could be achieved and sustained for several years. Such a near-perfect level of treatment would increase a patient's burden of day-to-day diabetes management, be difficult to implement for many patients, require more attention and medical services than are routinely available in clinical practice,[10] and be accompanied by an increased frequency of severe hypoglycemia.[17] Currently, the only way to restore sustained normoglycemia without the associated risk of hypoglycemia is to replace the patient's glucose-sensing and insulin-secreting pancreatic islet beta cells either by the transplantation of a vascularized pancreas[120] or by the infusion of isolated pancreatic islets.[112] The tradeoff is the need for immunosuppression to prevent rejection of allogeneic tissue, and for this reason, most pancreas or islet transplant recipients have been adults, but the potential for application earlier in the course of the disease exists, particularly in diabetic children already on immunosuppression for other indications.[4]

By the mid-1990s, more than 1500 pancreas transplants were being done annually worldwide (Fig. 34-1), as reported to the International Pancreas Transplant Registry (IPTR).[36] By 2005, about 25,000 vascularized pancreas transplants had been performed, approximately three fourths in the United States, with very large series at some centers.[125] Most were done to establish insulin independence in patients with de novo type 1 diabetes mellitus, but enteric drainage pancreas transplants have been used to correct endocrine and exocrine deficiency after total pancreatectomy in some patients[40,43] and from diseases such as cystic fibrosis in other patients.[111]

More than 120 institutions in the United States and nearly the same number outside the United States have performed pancreas transplants.[36] The IPTR was founded in 1980 to analyze the cases.[119] In 1987, reporting of U.S. cases became obligatory through the United Network for Organ Sharing (UNOS), and annual reports have been made since then.[35-37]

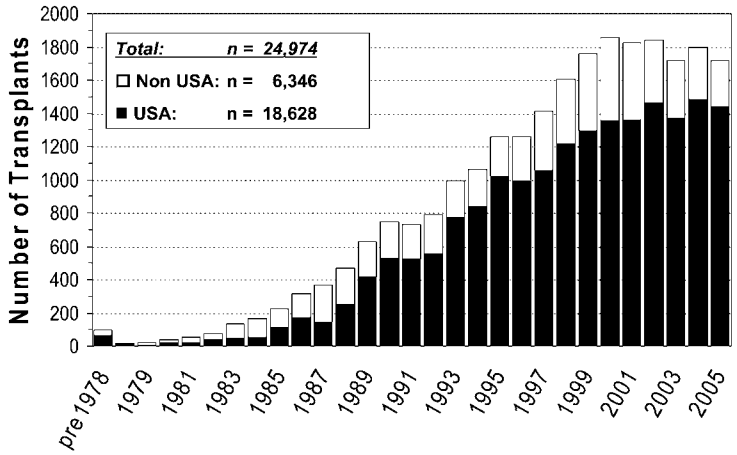

Figure 34–1 Annual number of U.S. and non-U.S. pancreas transplants reported to the International Pancreas Transplant Registry, 1978 to 2005.

HISTORY

The first clinical pancreas transplant was performed in 1966 by Kelly and Lillehei, simultaneous with a kidney transplant, in a uremic diabetic patient at the University of Minnesota.[53] Shortly thereafter, a few institutions around the world began to perform pancreas transplants, as detailed in a comprehensive history in another book.[124]

The success rate (long-term insulin independence) with pancreas transplantation was initially low, but it increased considerably in the 1980s, leading to increased application (see Fig. 34-1). Innovations in surgical techniques and in immunosuppression were responsible for the improved success rates.

The first pancreas transplant was a duct-ligated segmental (body and tail) graft,[53] but this approach was associated with multiple complications. In a series of 13 more pancreas transplants between 1966 and 1973 at the University of Minnesota,[61,62] Lillehei and colleagues devised the whole pancreas–duodenal transplant technique to the iliac vessels with enteric drainage via a duodenoenterostomy to native small bowel, which is now a routine at most centers. The initial results were not as good as today, however, and several surgeons devised alternative techniques during the 1970s and early 1980s.[124] Dubernard and colleagues[22] in Lyon, France, introduced duct injection of a synthetic polymer as a method to block secretions and cause fibrosis in the exocrine pancreas of a segmental graft with sparing of the endocrine component, and many pioneering centers adopted this technique, although it is little used today. Gliedman and associates[30] introduced urinary drainage via a ureteroductostomy for segmental grafts, and Sollinger and coworkers[105] later modified this approach with direct anastomosis of a duodenal patch of a whole-pancreas graft to the recipient bladder. Nghiem and Corry[83] did further modification of urinary drainage, retaining a bubble of duodenum for duodenocystostomy as Lillehei and associates[62] had done for duodenoenterostomy.

From the early 1980s until the mid-1990s, the bladder drainage technique with duodenocystostomy was the predominant technique for pancreas transplants. The bladder drainage technique had a low acute complication rate and was helpful in monitoring for rejection by detection of a decline in urine amylase activity, but chronic complications, such as recurrent urinary tract infections or dehydration from fluid loss via the exocrine secretions, were common. In the mid-1990s, a switch occurred, and enteric drainage, as described by Lillehei and colleagues[62] and never totally out of fashion,[108,125] overtook bladder drainage as the predominant technique. In addition, portal rather than systemic venous drainage began to be used by some groups for enteric drainage whole-pancreas duodenal transplants.[99] Portal venous drainage was originally introduced by Calne in 1984[14] for segmental pancreas grafts as a more physiological technique and was applied by several groups sporadically over the years.[124]

With advances in immunosuppression, including the introduction of cyclosporine by Calne and associates in 1979,[15] tacrolimus by Starzl and coworkers in 1989,[109] and mycophenolate mofetil by Sollinger and coworkers in 1995,[93] bladder drainage had become less important for monitoring for rejection. In recipients of simultaneous pancreas and kidney (SPK) transplants from the same donor, the kidney could be monitored for rejection episodes (elevation of serum creatinine) as a surrogate marker for pancreas rejection before there was sufficient pancreas damage to cause hyperglycemia. In solitary pancreas transplants, serum creatinine could not be used as a marker for rejection, however, and in such cases bladder drainage remained useful and continues to be applied.[124]

INDICATIONS AND CATEGORIES

Indications

A pancreas transplant is performed to treat diabetes mellitus, most commonly in conjunction with a kidney transplant for patients with kidney failure or dysfunction secondary to diabetic nephropathy (see Recipient Categories). For such patients, the decision to undergo a pancreas transplant is not difficult. Because they are already candidates for a kidney transplant, they would require lifelong immunosuppression. The only significant additional risk of a pancreas transplant is the surgical risk associated with the operative procedure. The options available for such patients include undergoing both transplants simultaneously (from a deceased or a living donor or a combination of both) or undergoing the two transplants sequentially (usually the kidney transplant first,

followed weeks or months later by the pancreas transplant). Which option is best depends on the individual patient's medical status, the availability of donors, and personal preference. These options are discussed in more detail later.

For diabetic patients with preserved kidney function, the decision to undergo a pancreas transplant must balance the risks of long-term immunosuppression with risks of long-term insulin therapy. The decision is easiest for patients with brittle diabetes who have rapid fluctuations in blood glucose levels, frequent episodes of diabetic ketoacidosis, or significant hypoglycemic unawareness.[46] For such patients, a successful pancreas transplant becomes a lifesaving procedure. Even for patients with less severe diabetes, a pancreas transplant can improve QOL markedly and, to some extent, halt progression of secondary complications of diabetes.

Recipient Categories

Diabetic pancreas transplant recipients can be divided into two broad classifications: (1) patients with nephropathy to such a degree that they also undergo a kidney transplant, either simultaneously or sequentially, and (2) patients, usually without end-stage renal disease, who undergo only a pancreas transplant. Within these two broad classifications are several recipient categories. The traditional categories are as follows:

1. SPK transplant
2. Pancreas after kidney (PAK) transplant (in the interval between the two transplants, the recipient would be in the kidney transplant alone [KTA] category)
3. Pancreas transplant alone (PTA)
4. Kidney after pancreas (KAP) transplant (in the interval between the two transplants, the recipient would be in the PTA category)

Worldwide, in most SPK transplants, both organs have come from same donor, and the donor has been a deceased donor,[35] but at the University of Minnesota, from 1994 through 2002, 10% were from a living donor.[125] Other centers have done living donor same donor SPK transplants as well.[5] For some SPK transplants, each organ has come from different donors, and transplants have been done with a simultaneous deceased donor pancreas and a living donor kidney.[41,123] A simultaneous deceased donor pancreas and a living donor kidney transplant is conceptually similar to a different-donor PAK transplant (living donor kidney followed by a deceased donor pancreas) but has the advantage of achieving the overall objective (correction of uremia and diabetes) with one operation in the recipient, while preempting dialysis (if the waiting time for the deceased donor pancreas is short). If the waiting time is predicted to be long for a deceased donor pancreas, the simultaneous deceased donor pancreas and a living donor kidney advantage of ultimately having one operation may not offset the disadvantage of having to go on maintenance dialysis while waiting.

Most PAK recipients have had two deceased donor organs, either a living donor kidney followed by a deceased donor pancreas (most common in our series) or a deceased donor kidney followed by a deceased donor pancreas. A few sequential living donor kidney and living donor pancreas transplants from different donors have been done.[125] In most KAP recipients, each organ came from different donors, either a deceased donor pancreas followed by a living donor kidney (most common) or a deceased donor pancreas followed by a deceased donor kidney. If a kidney and pancreas candidate with high plasma renin activity has a negative crossmatch against a potential living donor, we would advise a living donor KTA with subsequent placement on the PAK waiting list or a living donor same donor SPK transplant if a suitable donor is willing to give both organs.

At the University of Minnesota, we offer uremic diabetic candidates all options: a living donor same donor SPK transplant, a simultaneous deceased donor pancreas and a living donor kidney transplant on either a standby or a fortuitous basis, or a living donor KTA transplant (from a donor willing or suitable only to give the kidney) followed by a deceased donor PAK transplant. Nearly all of our primary deceased donor PAK transplants are in recipients of a living donor KTA. Most PAK transplants that follow a deceased donor kidney transplant are pancreas retransplants in recipients of a previous SPK transplant whose pancreas graft failed (usually for technical reasons) while the kidney continued to function. Few PAK recipients have had a preceding primary deceased donor KTA because uremic diabetic patients who are candidates for a pancreas and a kidney transplant and who do not have a kidney living donor are nearly always placed on the waiting list for a deceased donor SPK.

If a PTA candidate with moderately advanced nephropathy (e.g., glomerular filtration rate of 60 mL/min) has an identified living donor for a kidney in case one is needed, a deceased donor pancreas transplant can be done first—knowing that if the native kidneys deteriorate (not all do), a living donor kidney can be added preemptively as uremic symptoms appear. If the candidate does not have a living donor for a kidney, a judgment has to be made about the value of correcting diabetes at the possible expense of accelerating the decline in kidney function by exposure to calcineurin inhibitors. At the University of Minnesota,[125] as has been done elsewhere,[60] we put such patients on calcineurin inhibitors before transplantation. If kidney function deteriorates acutely, we stop the drug and place the patient on the waiting list for a deceased donor SPK transplant; the wait may be long, but the zero–HLA mismatch lottery always gives a chance of a preemptive transplant, even before reaching the glomerular filtration rate level (20 mL/min) that confers eligibility for waiting time points. Not all PTA patients with moderately advanced nephropathy experience progressive deterioration of kidney function while on calcineurin inhibitors. For some patients, their native kidney morphology improved after correction of the diabetic state.[27]

In the University of Minnesota experience with more than 400 PTA recipients, about 4% went on to have a KAP within 1 year, and 10% had a KAP within 5 years of pancreas transplant.[125] Only 6% of the KAP recipients at the University of Minnesota were on dialysis at the time of kidney transplant.[125] About half of the KAP recipients had a functioning pancreas at the time of kidney transplant. About one third of the KAP transplants at the University of Minnesota have been done in conjunction with a pancreas retransplant (SPK); most patients in this subgroup had rejected the PTA graft because calcineurin inhibitor levels were kept low in an attempt to preserve native kidney function. By adding a normal kidney to the pancreas retransplant, adequate calcineurin inhibitor levels can be maintained to prevent rejection.

Our KAP recipients with functioning pancreas grafts underwent kidney transplant to obviate early uremic symptoms

and primary chronic fatigue and to obtain the full benefit of immunosuppression. This strategy has been highly effective. At 1 year, graft survival rates in KAP recipients of a solitary kidney are 96%.[125] Two thirds of the KAP transplants of a solitary kidney were from a living donor, greatly facilitating the process. Even for PTA candidates with moderately advanced nephropathy who tolerate pretransplant calcineurin inhibitors with minimal early deterioration, we encourage early identification of potential living donors for a kidney so that the KAP option can be expedited whenever appropriate.

In contrast to the subgroup of PTA recipients whose native kidney function deteriorated to the point where a KAP was done, there is a subgroup of PTA recipients with moderately advanced nephropathy (per pretransplant native kidney biopsy specimens) whose lesions completely or partially resolve 5 to 10 years after transplantation.[27] These PTA recipients were probably spared from a kidney transplant; based on their original biopsy findings, progressive deterioration would have been predicted had they remained diabetic.

The native kidney function of some PTA candidates is in a gray zone. Diabetic nephropathy may be moderately advanced, but uremic symptoms are absent or minimal. Some such candidates are extremely sensitive to the nephrotoxic effect of calcineurin inhibitors.[60] At the University of Minnesota, we place all PTA candidates on calcineurin inhibitors before transplantation, then measure kidney function and monitor symptoms. If kidney function declines substantially and symptoms appear, the calcineurin inhibitor is stopped, and the patient becomes a candidate for a kidney transplant, ideally from a living donor. If no living donor is available, the candidate is placed on the waiting list for a deceased donor SPK transplant.

The patient would remain on the PTA list if his or her diabetes is extremely labile, recognizing that the interval until dialysis is necessary could be shortened by reintroduction of a calcineurin inhibitor at the time of a PTA. Such an extreme approach is prompted by the fact that, in the United States under the UNOS system, waiting time points for a deceased donor kidney (KTA, SPK, or KAP) do not accumulate until the candidate's creatinine clearance is 20 mL/min or less. Many candidates for a deceased donor SPK transplant have a creatinine clearance greater than 20 mL/min when initially evaluated.

Diabetic patients referred as potential PTA candidates encompass a broad range of kidney function. Patients with a creatinine clearance of 100 mL/min or better are at low risk for calcineurin inhibitor–induced reduction of kidney function to the point where a kidney transplant is indicated. Some patients with a creatinine clearance of 50 to 60 mL/min are sensitive to calcineurin inhibitors, others are resistant, and some have kidneys with the capacity to stabilize functionally and improve morphologically after a PTA.[27] For patients in the gray zone, the findings on native kidney biopsy specimens, kidney function while on calcineurin inhibitors, and availability of living donors are our three main guides to selecting the treatment plan: PTA or simultaneous or sequential kidney and pancreas transplants. A calcineurin inhibitor–free protocol using anti–T cell maintenance also is being developed.

ALLOCATION

The allocation scheme must accommodate candidates for a solitary pancreas transplant and candidates for a deceased donor kidney transplant. In some organ procurement organizations, usually single-center organizations, SPK candidates are given priority over KTA candidates when the pancreas and a kidney from a deceased donor is suitable for transplantation. Some organ procurement organizations have no, or few, solitary PTA candidates listed. In such organizations, the local use of deceased donor pancreata depends on whether SPK candidates are given priority over KTA candidates (diabetic and nondiabetic). If priority is given to SPK candidates, theoretically, half of the kidneys would go to uremic diabetics (even though they comprise less than half of the combined SPK and KTA list). The result would be shorter kidney waiting times for patients with diabetic nephropathy than for patients with other causes of end-stage renal disease. The proportion of uremic diabetic patients who are listed for an SPK transplant (versus KTA) approaches 100% in some organ procurement organizations, so virtually all KTA candidates are nondiabetic.

In practice, not all deceased kidney donors are judged to have a pancreas suitable for transplantation. Even with the extreme policy of full priority of SPK over KTA candidates for a kidney from deceased donors with a suitable pancreas, less than half of the locally procured deceased kidneys are transplanted in SPK recipients. With such a policy, waiting times are shorter for diabetic SPK (versus nondiabetic or diabetic KTA) candidates. About 25% of kidney transplant candidates are diabetic, so in organ procurement organizations with an extreme policy the pancreata from all deceased donors with a suitable pancreas tend to be used.

At the other end of the spectrum are organ procurement organizations (usually multicenter) that give no priority to SPK candidates. In such organizations, the kidneys are allocated to the two highest ranked suitable candidates on the specific list generated for a deceased donor. The donor pancreas is used locally for an SPK candidate but only if that candidate is one of the two highest ranked suitable candidates for the kidney. Other organ procurement organizations have allocation schemes that fall between the extremes.

Compared with nondiabetic kidney transplant candidates, uremic diabetics have a high mortality rate while waiting for transplants (6% per year according to UNOS). This fact provides one rationale for including medical priority in a deceased kidney allocation scheme (as is the case in liver and heart allocation); a pancreas allocation scheme that gives full priority to SPK candidates in effect incorporates medical priority.

Meanwhile, living donors are needed to compensate for the shortage of deceased donors. Rejection rates have declined for deceased and living donor recipients, so the main incentive to use living donors is to eliminate the waiting time and high mortality rate in certain candidates while waiting. As more diabetics are listed for deceased donor pancreas transplant, the waiting time is expected to approach or exceed that for deceased donor kidneys, and the incentive to use living donors for pancreas transplant is expected to increase. Incentives to use living donors for segmental pancreas transplants have included the ability to induce an insulin-independent and dialysis-free state with one operation (SPK), and the elimination or reduction of waiting time for candidates in any category (PAK, PTA, and SPK) who have a high potential for a long wait on the deceased donor transplant list (e.g., because of high plasma renin activity). When diabetic candidates for pancreas transplants with low plasma

renin activity are waiting 2 to 4 years for a deceased donor, the incentive to take the pancreas living donor option increases, as has happened for kidney transplants.

Methods to screen potential pancreas living donors for suitability have been developed. Briefly, volunteers are suitable to be hemipancreas donors if they have a body mass index less than 28 kg/m^2 (to minimize the need for increased insulin secretion to compensate for obesity), no history of gestational diabetes, and normal glucose tolerance with a threefold increase in first phase blood insulin concentration on intravenous arginine and glucose stimulation. In our experience, living donors who meet these criteria retain normal glucose tolerance postdonation; any changes in glucose or insulin levels would be no greater in magnitude than the changes in creatinine clearance that are seen after kidney donation.

Our islet autograft cases show the potential to increase the efficiency of islet preparation and transplantation from deceased donors by duplicating, as nearly as possible, ideal conditions (very short preservation time, elimination of purification process with reduced tissue volume from half of a pancreas). The cases also show the potential to transplant more than one recipient with islets from one pancreas. The precedent for splitting a deceased pancreas for transplantation as immediately vascularized grafts (head and tail) into two diabetic recipients goes back to 1988[126] and preceded the use of split deceased liver transplants.[23]

SPECIFIC RISK FACTORS

The preceding sections outlined algorithms for pancreas transplants in general diabetic and uremic diabetic patients. Some candidates have risk factors that require special consideration, however. Jehovah's Witnesses do not allow blood transfusions. Most pancreas transplants are done without substantial blood loss, but, as is true for any major surgery, some patients may need transfusions. The Jehovah's Witnesses we have transplanted all have survived the operation,[49] but they faced an above-average risk.

Chronic viral infection (e.g., human immunodeficiency virus [HIV] or hepatitis C virus [HCV]) also pose additional risks for allograft candidates. With modern HIV therapeutic agents, infected patients have been successfully transplanted.[59] HIV-positive diabetics should be considered for pancreas transplantation according to clinical indications. HCV can recur in liver allograft recipients, but overall outcomes have been good. In kidney allograft recipients, HCV does not seem to progress more than in renal failure patients on dialysis.[110] HCV-positive uremic diabetic patients have had SPK or PAK transplants in our program; the incidence of progressive liver disease was no different from that of nondiabetic KTA recipients. We see no reason to withhold pancreas transplantation from asymptomatic HCV-positive diabetics.

The age of pancreas transplant recipients theoretically has no limits. Analyses of pancreas transplant outcome by recipient age have shown that the rejection rate is lower for recipients who are older than 45 years old.[35,125] In the PTA category, patient survival rate at 1 year is nearly 100% in the group older than 45 years old, and graft survival rate is significantly higher than in younger recipients. This finding is consistent with studies showing a blunting of primary immune responses as individuals age. In the older group, the main risk factor to address is cardiovascular disease. Candidates should be screened for coronary artery disease; if present, it should be corrected before pancreas transplant, even if asymptomatic.[64]

Pancreas transplants have been done in diabetic children (<18 years old).[4] Pediatric SPK recipients have had less rejection than pediatric PTA recipients.[4] In the early experience, juvenile PTA recipients had more frequent or severe rejection episodes than adults. The immunosuppressive regimen for pediatric patients must be more aggressive than that for adults. Living donors are particularly attractive for pancreas transplants in children because the rejection rates for all types of organ allografts are lower than with deceased donors. Obtaining a sufficient beta cell mass should nearly always be possible with parental donors of pediatric recipients.

Diabetic patients with exocrine deficiency as a result of a total pancreatectomy for benign disease (usually chronic pancreatitis) also are special cases. Ideally, pancreatectomized patients should have had diabetes prevented by an islet autograft (if they were nondiabetic before the total pancreatectomy). Some become diabetic from the chronic pancreatitis before the pancreatectomy, however. Others have an insufficient yield of autologous islets to prevent diabetes. Still others have had the pancreatectomy at institutions not offering islet autotransplants. The combination of diabetes and exocrine deficiency poses a special problem. Erratic food absorption coupled with exogenous insulin predisposes to hypoglycemic events. Such patients would benefit most from an enteric drainage pancreas transplant so that exocrine and endocrine deficiencies are corrected.

Some patients with severe exocrine deficiency from chronic pancreatitis are not diabetic. Some are pain-free, and exocrine deficiency is the sole problem. Oral enzyme therapy usually improves food absorption but not in all. Enteric drainage pancreas transplants have abolished steatorrhea and the need for oral enzyme therapy in some patients with exocrine deficiency.[40,111] There is a rationale to treat exocrine deficiency by enteric drainage pancreas transplant in patients with serious nutritional problems. We have done so by adding a second enteric drainage pancreas transplant in a totally pancreatectomized patient whose initial bladder drainage pancreas transplant corrected only diabetes.[125] For technical reasons, a conversion from bladder drainage to enteric drainage could not be done, so the steatorrhea and malabsorption persisted despite heavy administration of pancreatic enzymes orally. The enzyme deficiency was solved by the enteric drainage pancreas transplant, leaving the functioning bladder drainage graft in place.[125]

PROCEDURE

Surgical Techniques

The pretransplant evaluation does not differ substantially from that which is undertaken for diabetic kidney transplant recipients. Examination of the cardiovascular system is most important because significant coronary artery disease may be present without symptoms. Noninvasive testing may not identify such disease, so coronary angiography is performed routinely. In PTA candidates, detailed neurological, ophthalmological, metabolic, and renal function testing may be needed to assess the degree of progression of secondary complications. When patients are placed on a waiting list, their medical condition should be reassessed yearly or more frequently.

As mentioned in the history section, a variety of techniques have been used for management of the exocrine secretions and venous drainage of pancreas transplants. Most pancreas grafts are procured from multiorgan deceased donors, and because the liver and pancreas share the origins of their arterial blood supply, a whole-organ pancreas graft usually requires a reconstruction.[11,66] The tail of the pancreas is supplied by the splenic artery originating from the celiac axis, and the head of the pancreas is supplied by the pancreaticoduodenal arcades originating from the superior mesenteric artery and the hepatic artery. Because the latter goes with the liver, along with the celiac axis, the usual approach is to attach an arterial Y-graft of the donor iliac vessels, with anastomosis of the hypogastric artery to the graft splenic artery and the external iliac artery to the graft superior mesenteric artery, leaving the common iliac artery of the Y-graft for anastomosis to the recipient arterial system, usually the right common iliac artery. The portal vein of the pancreas graft can be anastomosed to the recipient's common iliac vein (usually after the hypogastric veins have been doubly ligated and divided) or vena cava, or to the recipient's superior mesenteric vein.

When venous drainage is to the recipient's iliac vein, the whole-pancreas graft can be oriented with the head directed into the pelvis or into the upper abdomen. When directed cephalad, enteric drainage is the only option. When directed caudally, the duodenum can be anastomosed to either the bladder (Fig. 34-2) or the bowel (Fig. 34-3). Figure 34-2, showing the bladder drainage technique, also depicts a kidney transplant to the left iliac vessels, but as mentioned, enteric drainage is more common than bladder drainage (Fig. 34-4).

With the bladder drainage technique, the anastomosis may be hand sewn or performed with an end-to-end anastomosis (EEA) stapler brought through the distal duodenum (which is subsequently stapled closed) for connection to the post of the anvil projected through the posterior bladder via an anterior cystostomy (see Fig. 34-2). The inner layer is reinforced with a running absorbable suture for hemostasis and for burying the staples under the mucosa.

With the enteric drainage–systemic venous drainage technique, the anastomosis also may be hand sewn in a side-to-side fashion (see Fig. 34-3); stapled in a side-to-side fashion, including using an EEA stapler inserted into the distal graft duodenum with the post projected through the side for connection to the anvil inserted into recipient bowel through an enterotomy closed around the post with a purse-string; or hand sewn in an end-to-side fashion. The enteric anastomosis can be done directly to the most convenient proximal small bowel loop of the recipient (depicted) or to a Roux-en-Y segment of recipient bowel that is created at the time. Outcome analyses (see later) do not show any statistical advantage to creation of a Roux-en-Y loop.

For portal drainage of the pancreas graft venous effluent (Figs. 34-5 and 34-6), the head and duodenum of the graft is oriented cephalad, and the graft portal vein is anastomosed directly to the recipient superior mesenteric vein. In Figure 34-5, the pancreas graft is ventral to the recipient small bowel mesentery so that the venous anastomosis is to the ventral side of the superior mesenteric vein, and the arterial Y-graft must be brought through a window of mesentery for anastomosis to the recipient's aorta or common iliac artery. The graft duodenum is anastomosed to recipient small bowel by the same techniques described for systemic venous drainage, with or without (depicted) a Roux-en-Y loop of recipient bowel.

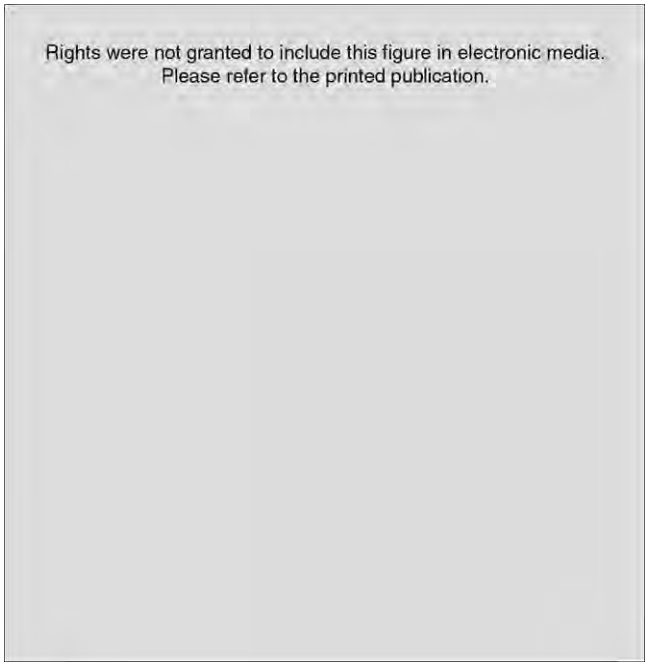

Figure 34–2 Simultaneous pancreas-kidney (SPK) transplantation using a whole pancreas–duodenal graft from a deceased donor with systemic venous drainage to the right iliac vein and bladder drainage of the pancreas exocrine secretions via a duodenocystostomy. The pancreas and the kidney are placed intraperitoneally through a midline incision. The donor splenic artery, supplying the pancreatic tail, and the donor superior mesenteric artery, supplying the pancreatic head, have been joined by a Y-graft constructed from the donor common/external/internal iliac artery complex during a benchwork procedure, and the base of the Y-graft is anastomosed to the recipient common iliac artery. The mid-duodenum is anastomosed to the posterior dome of the bladder, and the duodenal stumps are oversewn. The kidney graft could be from a living donor or the same deceased donor as the pancreas graft, but in either case it is preferentially placed to the left iliac vessels so that the right side, with its more superficial vessels, can be used for the pancreas transplant. In this illustration, the donor ureter was implanted into the bladder using the Politano-Leadbetter technique via an anterior cystotomy, a technique that also allows the duodenocystostomy to be performed with an EEA stapler with internal oversewing of the anastomotic line using an absorbable suture to cover the staples, followed by closure of the cystotomy. When enteric drainage is used for an SPK transplant, however, an external ureterocystoneostomy is usually done. (From Gruessner RWG, Sutherland DER [eds]: Transplantation of the Pancreas, color plate xiv. New York, Springer-Verlag, 2004.)

An alternative approach for portal venous drainage of the pancreas graft effluent is to place the pancreas retroperitoneally by reflecting the right colon to the left and exposing the dorsal surface of the superior mesenteric vein, as described by Boggi and coworkers.[12,13] The arterial Y-graft can be anastomosed directly to the right common iliac artery, but this approach mandates creation of a Roux-en-Y limb of recipient bowel to bring through the small bowel or transverse colon mesentery for a graft duodenoenterostomy.

Other techniques can be used, including duct injection for a segmental graft. Segmental grafts are rarely used except in the few cases of living donor pancreas transplants,[5,39,122] and most of these have the exocrine secretions managed by a ductoenterostomy to a Roux-en-Y limb of recipient

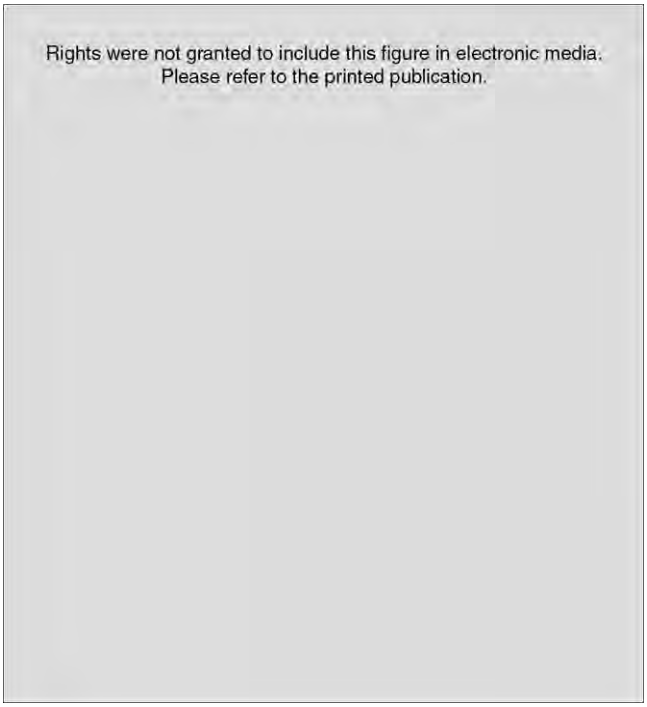

Figure 34–3 Whole pancreas–duodenal transplantation from a deceased donor with systemic venous drainage and enteric drainage of graft exocrine secretions to a proximal loop of recipient jejunum. In this case, a side-to-side stapled or hand-sewn duodenojejunostomy is illustrated. The pancreas with its vascular anastomosis (donor Y-graft to recipient common iliac artery, donor portal vein to recipient common iliac vein) is implanted in the standard fashion on the right side of the pelvis. (From Gruessner RWG, Sutherland DER [eds]: Transplantation of the Pancreas. New York, Springer-Verlag, color plate XVII, 2004. (See color plate.)

bowel or by ductocystostomy (depicted) to the recipient's bladder (Fig. 34-7). Segmental pancreas transplants from living donors, with or without a kidney transplant, are particularly useful in candidates who would otherwise have a long wait for a deceased donor organ, such as candidates with a high level of HLA antibodies but with a negative crossmatch to a living volunteer. More details are given on the variety of surgical techniques in pancreas donors (deceased and living) and recipients in a book dedicated to pancreas transplantation.[8]

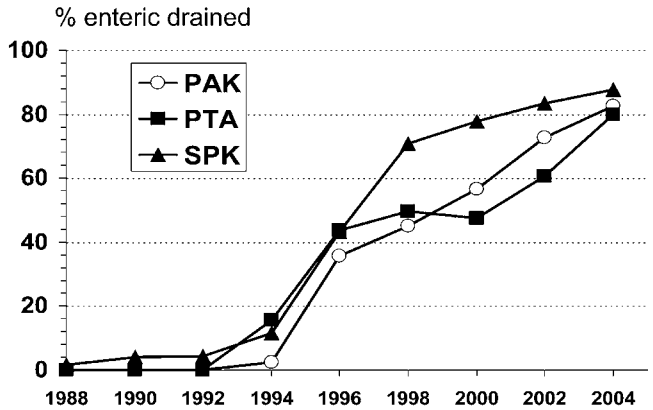

Figure 34–4 Percentage of enteric drainage pancreas transplants performed in the United States from 1988 through 2004 by recipient category. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

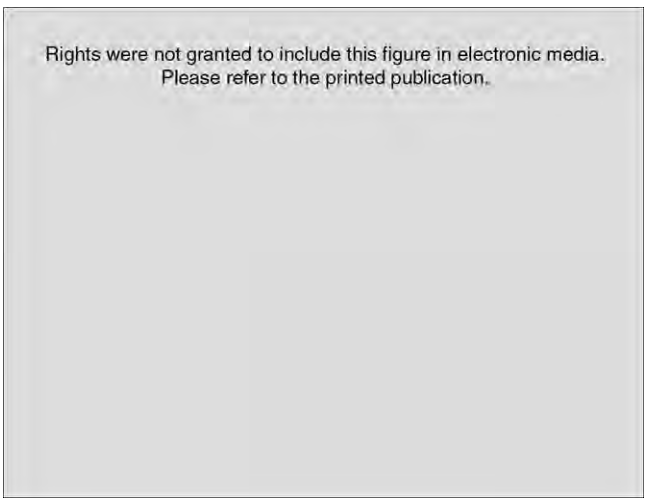

Figure 34–5 Whole pancreas–duodenal transplantation from a deceased donor with portal venous drainage via an end-to-side anastomosis to the recipient superior mesenteric vein accessed below its confluence with the splenic vein. Drainage of exocrine secretions is via a side-to-side duodenojejunostomy, about 40 to 80 cm distal to the ligament of Treitz. Note the cephalad position of the pancreatic head when portal venous drainage is done, as opposed to the caudal orientation possible with systemic venous drainage, no different than that needed when bladder drainage is done. In this illustration, the pancreas graft overlies the root of the small bowel mesentery, with the duodenal segment below the transverse colon, and the arterial Y-graft anastomosed to the recipient common iliac artery through a mesenteric tunnel. A retroperitoneal approach under the right colon also is possible, in which case the arterial Y-graft can be anastomosed directly to the recipient iliac artery, but the enteric anastomosis must be via a Roux-en-Y limb of recipient bowel brought through the mesentery. If a kidney is simultaneously transplanted to the left iliac vessels, the ureter can be implanted into the bladder using the extravesical ureterocystoneostomy (Lich) technique, as illustrated. (From Gruessner RWG, Sutherland DER [eds]: Transplantation of the Pancreas. New York, Springer-Verlag, color plate xx, 2004.) (See color plate.)

Immunosuppression

Immunosuppression management of pancreas transplant recipients is similar to that of recipients of other solid organ transplants, including kidney transplants, which most pancreas recipients also receive. Induction immunosuppression with anti–T cell monoclonal or polyclonal depleting or nondepleting

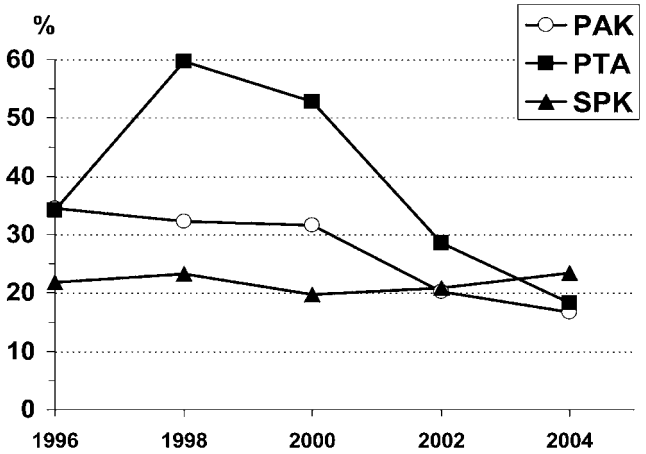

Figure 34–6 Percentage of portal drainage in enteric drainage pancreas transplants performed in the United States from 1996 through 2004 by recipient category. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

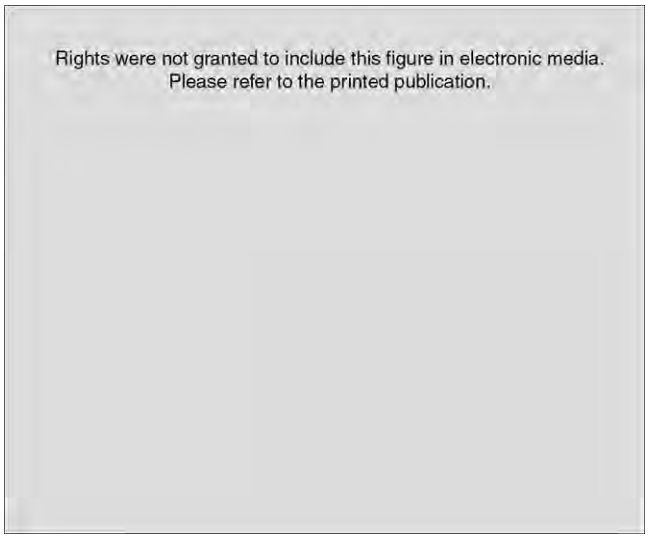

Figure 34–7 Living donor segmental (body and tail) pancreas transplantation to right iliac vessels (systemic venous drainage) and bladder drainage of exocrine secretions through a ductocystostomy via an intraperitoneal approach. The donor splenic artery and splenic vein are anastomosed end-to-side to the recipient external iliac artery and vein, after ligation and division of all hypogastric veins to bring the main vein as superficial as possible. The splenic artery anastomosis is lateral and proximal to the splenic vein anastomosis. A two-layer ductocystostomy is constructed. The pancreatic duct is approximated to the urothelial layer (inner layer) using interrupted 7-0 absorbable sutures over a stent *(inset)*. If a kidney is transplanted simultaneously, the donor ureter is implanted into the bladder using the extravesical ureterocystoneostomy (Lich) technique. (From Gruessner RWG, Sutherland DER [eds]: Transplantation of the Pancreas. New York, Springer-Verlag, color plate xvi, 2004.) (See color plate.)

agents may be used or reserved for rejection episodes. Maintenance immunosuppression usually consists of a combination of a calcineurin inhibitor (cyclosporine or tacrolimus) with the dosage and blood levels adjusted to minimize nephrotoxicity and an antiproliferative agent (mycophenolate mofetil or sirolimus), with or without prednisone. Steroid-free regimens are common for all organ transplants, including the pancreas.[51]

MANAGEMENT

Intraoperative Care

Most centers prefer placement of a central venous line (preferably internal jugular) for volume monitoring (central venous pressure), whereas others are most comfortable with pulmonary artery catheters (Swan-Ganz). Blood glucose is monitored hourly and usually controlled with an insulin drip. At the time of organ reperfusion, adequate volume status and blood pressure are imperative to avoid graft hypoperfusion. Before revascularization, diuretics are frequently given to promote early kidney graft function in SPK recipients (using furosemide) and reduce pancreas graft swelling (using mannitol, 1 g/kg). On completion of the procedure, the abdomen is copiously irrigated with antimicrobial solutions (e.g., containing bacitracin and amphotericin).

Postoperative Care

In the initial postoperative period, serum glucose levels are followed closely, and an intravenous insulin infusion is continued to maintain the serum glucose 80 to 110 mg/dL. Persistent elevation or acute increase in the serum glucose to more than 200 mg/dL requires immediate evaluation with Doppler ultrasonography or radionuclide scanning to assess graft perfusion and function.

The sentinel sign of rejection in SPK recipients is an increase in serum creatinine. After elimination of other possibilities for an abnormal creatinine level (dehydration, calcineurin toxicity, ureteral obstruction, bladder dysfunction, or vascular compromise), a percutaneous renal biopsy with ultrasound guidance is warranted. In some SPK recipients, serum amylase or lipase levels may increase, while creatinine levels remain stable. In such situations, a renal transplant biopsy is still warranted, especially if an enteric portal–drained pancreas is present.[103] Only in rare cases is a pancreas biopsy necessary to determine rejection if the kidney and the pancreas are from the same donor. It has been shown, however, that in an SPK recipient, one organ remains rejection-free.[45,65,128] For PTA and PAK recipients, the ability to follow rejection is more difficult.

Pancreas recipients with bladder drainage exocrine secretions may result in the obligatory loss of at least 1 to 2 L/day of pancreatic exocrine and duodenal mucosal secretions rich in bicarbonate and electrolytes into the urine. Fluid and bicarbonate supplementation is necessary for these recipients. For pancreas recipients with bladder drainage of exocrine secretions, urinary amylase levels can be monitored.[89-91] Studies have shown that urinary amylase levels expressed in units per hour are more consistent compared with measurements in units per liter and lead to more accurate assessment of pancreas graft function. An analysis of a 12-hour or 24-hour urine collection in which urinary amylase levels have declined 50% or more from baseline suggests rejection or pancreatitis. When confronted with this situation, further evaluation and probable biopsy are warranted, whether percutaneously via ultrasound or computed tomography guidance, or transcystoscopically, assisted by ultrasound guidance.[1,7,48]

The development of hematuria in bladder drainage pancreas recipients also warrants further evaluation and may necessitate the initiation of continuous bladder irrigation through a three-way Foley catheter to prevent obstructive thrombus formation. Cystoscopy is usually necessary to determine the etiology or remove the clot or both. Urethritis or cystitis owing to enzymatic irritation, the most common cause of hematuria, may resolve with increased bicarbonate supplementation.[106] Enteric conversion may be required for refractory irritation; however, such an extreme intervention is rarely required in the early postoperative period.[107] Bleeding from the duodenal-bladder anastomosis may arise, especially when a stapled anastomosis is performed. This complication can be avoided by oversewing the staple line at the time of the anastomosis. If a problem does develop, staples can be removed cystoscopically, although enteric conversion ultimately may be required to alleviate the bleeding.

Serum amylase and lipase levels provide additional means for following pancreas function, especially for enterically drained grafts.[47,116] These markers lack the sensitivity and specificity of urinary amylase, however. Serum human anodal trypsinogen has been shown to complement serum amylase and lipase levels in the determination of

graft dysfunction.[20,86] Few laboratories are equipped to monitor this factor, however.

Anticoagulation

Some centers advocate low-dose intravenous (partial thromboplastin time no greater than 1.5 × normal) or subcutaneous heparin. Low-dose aspirin is overlapped for 2 days before cessation of heparin and continued long term on hospital discharge. Frequent monitoring of coagulation parameters (partial thromboplastin time, international normalized ratio, prothrombin time, and hemoglobin) is required to avoid overanticoagulation. After segmental pancreas transplantation, from either a living related or a deceased donor, initial systemic heparinization followed by warfarin (Coumadin) therapy (for ≤6 months) is recommended. This approach is mandated by the more narrow caliber of the vascular anastomoses and the associated higher risk of thrombosis.[5,41]

Antimicrobial Prophylaxis

The literature clearly shows that early infection results in the highest incidence of graft loss and in serious patient morbidity and mortality.[6,24,84,88] Various single agents or combinations are available and should be given over the first 24 to 48 hours after transplantation. Recipients with positive urine cultures (from preoperative specimens) or positive intraoperative duodenal stump cultures should have antibiotic coverage for 3 to 7 days. Retrospective studies have shown that pancreas recipients are at high risk for losing a second pancreatic allograft to the same infectious agent when their first graft was lost to infection. A detailed microbial history of an individual transplant candidate is imperative so that appropriate antibiotic coverage can be initiated intraoperatively.

Because of the duodenal anastomosis in pancreas transplantation and the potential contamination of the operative field with small bowel contents, many centers also recommend antifungal prophylaxis with fluconazole. Calcineurin inhibitor serum levels must be monitored closely when azoles are administered because of decreased metabolism of the immunosuppressant and resultant higher systemic concentrations. As shown in several articles (referenced earlier), fungal infections result in the highest rates of graft loss and patient mortality.

Cytomegalovirus prophylaxis is recommended for any positive combination of a donor-recipient pair.[29,52] Controversy exists as to whether negative-to-negative combinations require prophylaxis. When antilymphocyte therapy is used, cytomegalovirus prophylaxis is almost always administered. Ganciclovir and, more recently, valganciclovir are presently the antiviral agents of choice in pancreas transplantation and can be initiated intravenously or per nasogastric tube in the immediate postoperative period, and then orally when the patient tolerates a diet. Patients intolerant to ganciclovir may tolerate valaciclovir, which provides adequate prophylaxis against cytomegalovirus infection in renal-only transplantation.[63] The efficacy of valganciclovir in pancreas transplantation is currently under investigation. Most centers begin trimethoprim/sulfamethoxazole immediately postoperatively and continue long-term prophylaxis against *Pneumocystis carinii* and *Nocardia* infections.

PANCREAS TRANSPLANT OUTCOMES

Changes over Time of Pancreas Transplant Outcomes

The changes of outcomes with deceased donor pancreas transplantation according to recipient categories, surgical technique, and immunosuppression protocol for U.S. cases as reported to UNOS are summarized here. From December 16, 1966, to December 31, 2005, about 25,000 pancreas transplants were reported to the IPTR, including more than 18,000 from the United States and almost 6000 from outside the United States. The annual number of U.S. and non-U.S. cases reported is shown in Figure 34-1. The annual number of U.S. pancreas transplants from 1988 through 2005 for those identified by major recipient category (SPK, PAK, and PTA) is shown in Figure 34-8. Most have been SPK transplants, but the number of PAK and PTA transplants has increased significantly in recent years. In 2005, of the 1367 pancreas transplants in which a major recipient category was designated, 896 were SPK (66%), 339 were PAK (25%), and 132 were PTA (10%). In the PAK category, there has been a significant change in the percentage of recipients whose kidney came from a living donor from 37% for 1988-1989 to 70% for 2004-2005.

Recipient age at the time of transplant increased significantly over time from mid-30s to early 40s. This trend can be seen in all three categories. Reporting of the diabetes type also began in 1994. The percentages of recipients labeled as having type 2 diabetes mellitus has continuously increased and in 2004-2005 was 7% for SPK recipients.

Figure 34-4 shows the changes in duct management over the years. Less than 10% of all pancreas transplants were done using enteric drainage before 1995-1996. Since then, the proportion of pancreas transplants that were managed by enteric drainage has steadily increased. Of the 2004-2005 pancreas transplants, 88% in the SPK, 83% in the PAK, and 80% in the PTA categories were enteric drainage.

Portal vein drainage of the pancreas graft venous effluent for enteric drainage transplants has been done since the early 1980s but not in large numbers until the mid-1990s. The proportion of enteric drainage transplants with portal drainage has varied by category and year (see Fig. 34-6). Although the proportion of enteric drainage SPK transplants with portal drainage has been constant at around

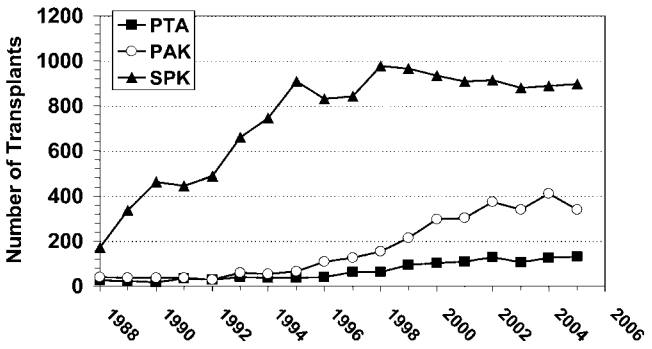

Figure 34–8 Number of pancreas transplants performed annually in the United States from 1988 through 2005 by recipient category. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

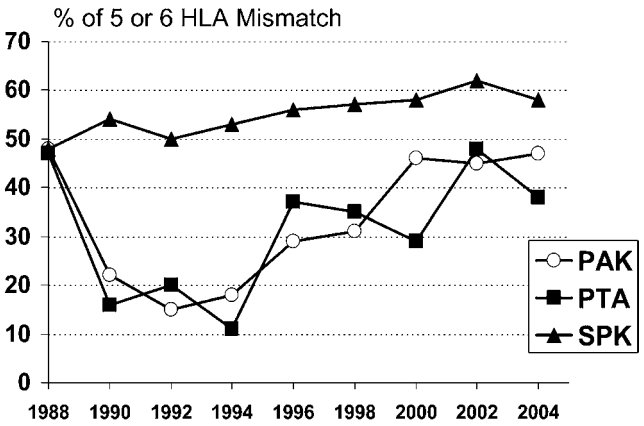

Figure 34–9 Percentage of U.S. pancreas transplant recipients mismatched for five or six HLA-A, HLA-B, and HLA-DR donor antigens, by category and era, in 2-year intervals, 1988 through 2005. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

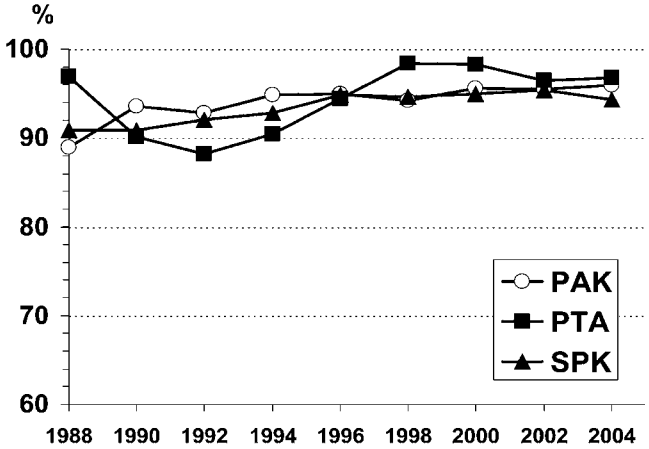

Figure 34–10 Patient 1-year survival rates for U.S. deceased donor primary pancreas transplant recipients by category and era, in 2-year intervals, 1988 through 2005. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

20% since 1994, the proportion in the PAK category has decreased steadily from the high-water mark of 35% in 1994-1995. In 1998-1999 (peak era), 60% of all enteric drainage PTA cases had portal drainage, but the proportion has since declined as well. In 2004-2005, 23% of enteric drainage SPK, 17% of enteric drainage PAK, and 18% of enteric drainage PTA cases had portal drainage.

There has been a progressive decline in the degree to which pancreas recipients have been matched for HLA, more so in the SPK than in the PTA and PAK categories (Fig. 34-9). For 2004-2005 cases, 58% of SPK recipients were mismatched for five or six HLA at the A, B, and DR loci (out of a possible six). A high proportion of solitary pancreas recipients in the latest era also were highly mismatched, however. In 2004-2005, 47% of PAK and 38% of PTA recipients were mismatched for five or six antigens.

Besides the changes in maintenance immunosuppression from cyclosporine to predominantly tacrolimus and from azathioprine to predominantly mycophenolate mofetil during the years 1994 and 1996, a change in the usage of anti–T cell agents for induction therapy has occurred over time. In all three categories, the proportion of recipients given induction therapy was the lowest between 1990 and 1993 but thereafter increased significantly. In 2004-2005, more than 80% of all patients received some sort of anti–T cell induction therapy.

Improvements in Pancreas Transplant Outcomes by Era

The results of U.S. primary deceased donor pancreas transplants analyzed by 2-year intervals are given to show changes in outcome over time. Long-term and short-term patient survival rates improved constantly over the years in all three categories (Figs. 34-10 and 34-11). Survival rates at 1 year have been greater than 90% in all recipient categories since the earliest era and are now around 95% for transplants performed in 2004-2005 (see Fig. 34-10). Overall, patient survival rates at 5 years can be calculated only up to the 2000-2001 era, but they also have improved and are greater than 80% in all categories, including 90% for 2000-2001 PTA recipients (see Fig. 34-11).

In contrast to patient survival rates, which have been high in all eras, pancreas graft survival rates improved even more over time, particularly in the solitary (PAK and PTA) categories (Figs. 34-12 and 34-13). In the earlier eras, graft survival rates were much higher in the SPK than in the PAK and PTA categories. In 2004-2005, the differences are much smaller, although still significant. One-year pancreas graft survival rates were 85% for SPK versus 79% for PAK and 78% for PTA (see Fig. 34-12). One-year kidney graft survival rates in the SPK category also improved significantly for many years, reaching 92% in 1998-1999 but plateauing since then.

Graft survival rates at 5 years can be calculated only for the years preceding 2000-2001, but in the solitary categories (PAK and PTA), they more than doubled to 57% for PAK and 49% for PTA in 2000-2001 era (see Fig. 34-13). For that era, in SPK recipients, the 5-year pancreas graft survival reached 70%, and the kidney graft survival reached 77%.

The technical failures are primarily early graft losses attributed to vascular thrombosis or removal because of bleeding, anastomotic leaks, pancreatitis, or infection. Technical failure rates decreased significantly over time in all three categories. In the early years, the technical failure rates were higher in the solitary (PAK and PTA) categories

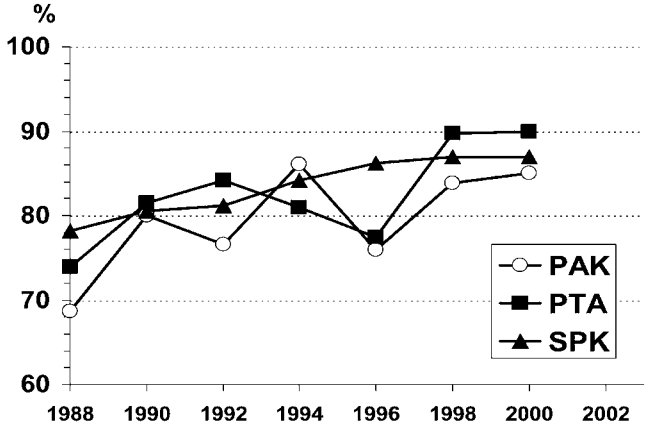

Figure 34–11 Patient 5-year survival rates for U.S. deceased donor (DD) primary pancreas transplant recipients by category and era, in 2-year intervals, 1988 through 2005. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

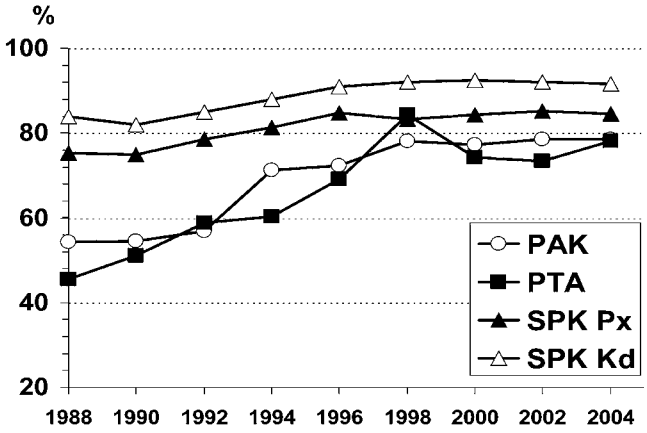

Figure 34–12 Pancreas and simultaneous pancreas-kidney (SPK) 1-year graft survival rates for U.S. deceased donor primary pancreas transplant recipients by category and era, in 2-year intervals, 1988 through 2005. Kd, kidney; PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; Px, pancreas.

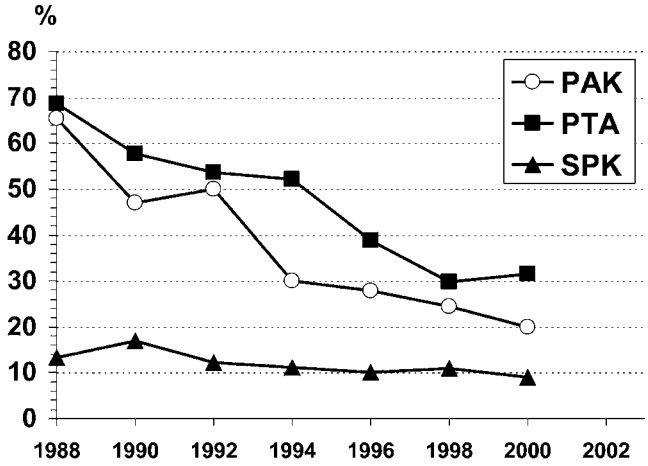

Figure 34–14 Immunological 5-year graft loss rates for U.S. deceased donor primary pancreas transplants by recipient category and era, in 2-year intervals, 1988 through 2005. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

than in the SPK category, which, we hypothesize, may be due partly to misclassifying some thromboses as technical when they were actually secondary to early rejections. In 2004-2005, the technical failure rates were similar in all three categories, with 6.4% for SPK, 8.9% for PAK, and 3.9% for PTA. The technical failure rate is significantly higher in the SPK category for enteric drainage versus bladder drainage transplants, 6.5% versus 3.2% in the 2002-2003 era ($P = .02$).[37]

The improvement in pancreas graft survival rates is due not only to a decline in the technical failure rate but also to declines in the rejection loss rates (Fig. 34-14). The rejection loss rates at 1 year declined fourfold to fivefold from the earliest to the most recent years, and in 2004-2005 were 5.4% for PAK, 11% for PTA, and 2% for SPK. The decline in the long-term rejection loss rates in the solitary (PAK and PTA) categories were more than halved from the years 1988-1989 and were 20% for PAK and 31% for PTA in the latest era for which a calculation can be made, 2000-2001 (see Fig. 34-14).

Pancreas Transplant Outcome for Contemporary (2000 to 2005) U.S. Cases

Current outcomes with deceased donor pancreas transplantation according to recipient categories, surgical technique, and immunosuppression protocol for U.S. cases as reported to UNOS from January, 2000, to December, 2005, are summarized here. During this period, greater than 7500 pancreas transplants were reported to UNOS, including greater than 5300 SPK transplants, greater than 1600 PAK transplants, and greater than 600 PTAs.

The primary transplant patient survival rates in the three recipient categories are shown in Figure 34-15. At 1 year, 94.9% of the SPK, 95.6% of the PAK, and 96.9% of the PTA recipients were alive; at 3 years, 90.8% of the SPK, 90.2% of the PAK, and 93.4% of the PTA recipients were alive ($P > .06$). The highest patient survival rate was in the PTA category, presumably because this group had less advanced complications before transplantation.

The primary pancreas graft survival rates in the three recipient categories are shown in Figure 34-16. At 1 year, 84.7% of the SPK, 78% of the PAK, and 75.9% of the PTA

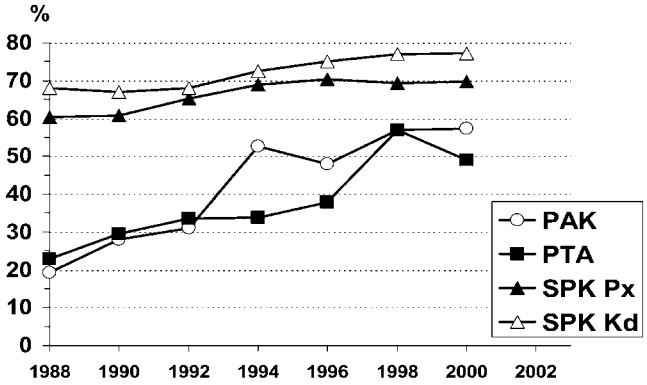

Figure 34–13 Pancreas and simultaneous pancreas-kidney (SPK) 5-year graft survival rates for U.S. deceased donor primary pancreas transplant recipients by category and era, in 2-year intervals, 1988 through 2005. Kd, kidney; PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; Px, pancreas.

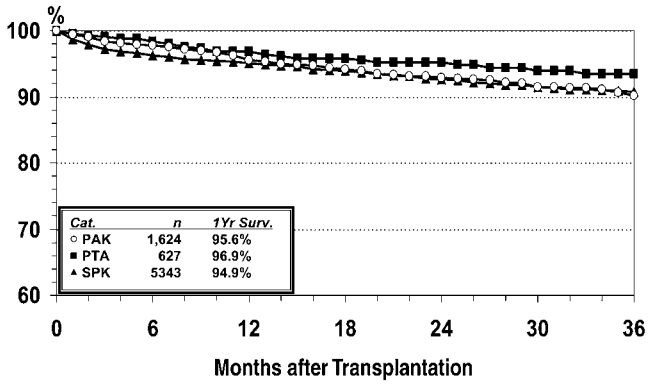

Figure 34–15 Patient survival rates for 2000 to 2005 U.S. deceased donor primary transplants by recipient category. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

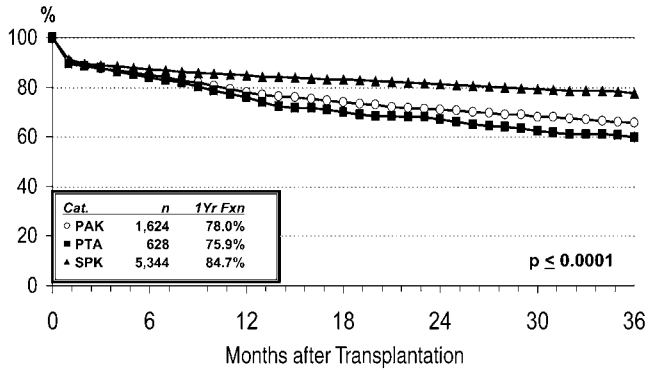

Figure 34–16 Pancreas graft functional survival rates (insulin independence) for 2000 to 2005 U.S. deceased donor primary transplants by recipient category. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

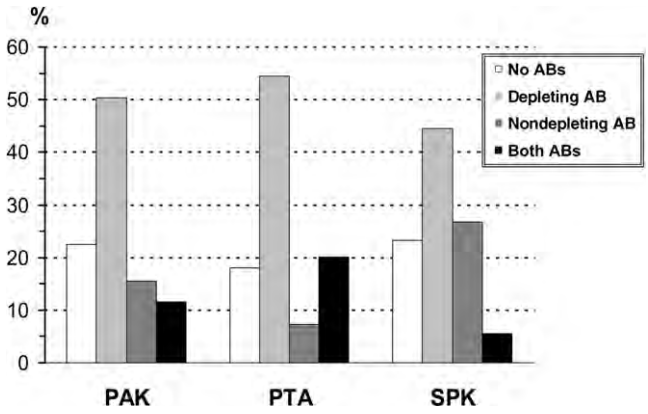

Figure 34–17 Frequency distribution of use of and type of anti–T cell antibody (AB) induction therapy by recipient category for U.S. deceased donor primary pancreas transplants, 2000 to 2005 cases. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

recipients were insulin-independent; at 3 years, 77.7% of the SPK, 65.6% of the PAK, and 59.9% of the PTA recipients were insulin-independent ($P < .0001$). The highest pancreas graft survival rates are in the SPK category, presumably because the kidney graft (usually from the same donor as the pancreas) can be used to detect rejection episodes earlier than in the other categories, where only the pancreas can be monitored. Support for this hypothesis comes from registry data showing no significant differences in graft technical failure rates between categories but large differences in rejection loss rates.

Of the primary pancreas grafts between 2000 and 2005, 8% failed for technical reasons, with thrombosis being the biggest risk for technical loss (5%). Infection, pancreatitis, and anastomotic leak constituted the rest. There were no significant differences between categories in regard to technical losses.

In regard to management of pancreatic duct exocrine secretions for cases between 2000 and 2004, enteric drainage predominated for SPK transplants (81%); for PAK and PTA, the proportion of cases that were enteric drainage was slightly lower (67% and 56%). Overall, the technical failure rate was slightly higher with enteric drainage than with bladder drainage (8% versus 6%). Pancreas graft survival rates were not significantly different, however, for enteric drainage versus bladder drainage transplants in any of the categories: at 1 year, 85% ($n = 3047$) versus 79% ($n = 707$) for SPK; 77% ($n = 733$) versus 80% ($n = 364$) for PAK; and 72% ($n = 238$) versus 79% ($n = 184$) for PTA cases. For PTA cases between 2000 and 2005, the failure rate from rejection for technically successful grafts was 8% ($n = 185$) for bladder drainage, 10% ($n = 250$) for enteric drainage with systemic drainage, and 13% ($n = 101$) for enteric drainage with portal venous drainage at 1 year (P NS .71).

In the SPK category, bladder drainage and enteric drainage would be expected to give similar results because in most cases both grafts come from the same donor, and monitoring of serum creatinine serves as a surrogate marker for rejection in the pancreas transplant, allowing easy detection and reversal by treatment. In contrast, for solitary pancreas transplants (PAK and PTA), serum creatinine cannot be used as a marker of pancreas rejection; hyperglycemia is a late manifestation of rejection, and exocrine markers must be used. Although serum amylase and lipase may increase during a rejection episode, this does not occur in all cases, but for bladder drainage grafts, a decrease in urine amylase eventually always accompanies rejection (100% sensitive, although it is not specific), and nearly always precedes hyperglycemia, so a rejection episode is more likely to be diagnosed in a bladder drainage graft and lead to treatment and reversal.

For enteric drainage grafts in all categories, the pancreas graft survival rates were slightly lower when a Roux-en-Y loop of recipient bowel was used for the enteric anastomosis rather than not.[36] Approximately one third of enteric drainage pancreas grafts reported to UNOS were done with a Roux-en-Y loop, but the outcomes are not improved by the additional surgery, and at least in PTA recipients, the technical failure rate was higher when a Roux loop was used.[36]

Another variation in surgical techniques is portal drainage of the venous effluent for enteric drainage grafts.[99] It establishes normal physiology, a theoretical metabolic advantage over systemic venous drainage, and some groups have reported that portal venous–enteric drainage grafts are less prone to rejection than systemic venous–enteric drainage grafts.[87,115] The latest registry analysis shows that portal venous drainage was used for one fifth of enteric drainage transplants, but there were no significant differences in pancreas graft survival versus systemic venous–enteric drainage transplants in any of the categories: at 1 year, 85% ($n = 610$) versus 85% ($n = 2437$) for SPK; 78% ($n = 168$) versus 77% ($n = 564$) for PAK; and 71% ($n = 85$) versus 72% ($n = 153$) for PTA enteric drainage cases.

In regard to immunosuppression, according to the latest registry analysis, anti–T cell agents were used for induction therapy in about three fourths of U.S. pancreas recipients in each category between 2000 and 2005 (Fig. 34-17). The agents available can be divided into two groups: (1) T cell–depleting polyclonal (e.g., antithymocyte gamma globulin [Atgam], antithymocyte globulin [Thymoglobulin]) or monoclonal (e.g., OKT3, alemtuzumab [Campath]) antibodies or (2) nondepleting (monoclonal anti-CD-25–directed, daclizumab, or basiliximab) antibodies.

The most frequently used regimen for maintenance immunosuppression (two thirds of the recipients in each category) was tacrolimus and mycophenolate mofetil in combination (Fig. 34-18), with or without prednisone (Fig. 34-19). In recipients of primary deceased donor pancreas grafts

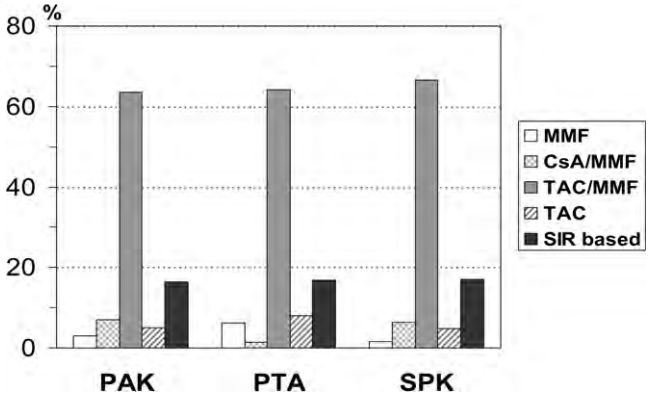

Figure 34–18 Frequency distribution of use of and type of major maintenance immunosuppressive protocols by recipient category for U.S. deceased donor primary pancreas transplants, 2000 to 2005 cases. CsA, cyclosporine; MMF, mycophenolate mofetil; PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SIR, sirolimus; SPK, simultaneous pancreas-kidney; TAC, tacrolimus.

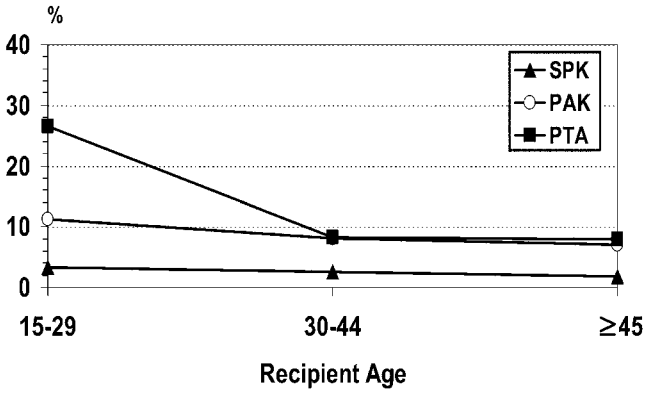

Figure 34–20 Immunological graft loss rates at 1 year for U.S. deceased donor primary pancreas transplant recipients by age and category, 2000 to 2004 cases. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

given anti–T cell agents for induction and tacrolimus and mycophenolate mofetil for maintenance immunosuppression, the 1-year graft survival rates in the SPK, PAK, and PTA categories were 87% ($n = 2728$), 80% ($n = 817$), and 79% ($n = 328$). Sirolimus was used as a maintenance immunosuppressive drug in about one sixth of recipients in each category with comparable outcomes. The 1-year pancreas graft survival rates in the SPK, PAK, and PTA categories were 90% ($n = 527$), 84% ($n = 170$), and 82% ($n = 79$).

Outcome by Recipient and Donor Risk Factors

In regard to the logistics of pancreas transplantation, more recent registry data[37] showed a slight increase in technical failure rates and a slight decrease in graft survival rates with increasing preservation time. In the SPK category, 1-year pancreas graft survival rates were 86% with 4 to 7 hours of preservation versus 81% with 28 to 31 hours of preservation. HLA matching had virtually no impact on SPK graft survival rates, but matching at least at the class I loci had a beneficial effect in the PAK and the PTA categories.

In regard to pancreas recipient age, the registry analysis of 2000-2004 cases showed an effect on outcome mainly in PTA recipients, with rejection more likely in the youngest recipients (Fig. 34-20). In the PAK category, all recipients were older than 20 years, and in analysis of rejection rates by decade of age, at 1 year the rates varied from 4% to 7%; in the SPK category, the rejection rate at 1 year was 2% to 4% in the various age groups older than 20 years but 0% for recipients younger than 20 years ($n = 4$). In contrast, in the PTA category, the rejection rate at 1 year was 50% for recipients younger than 20 years ($n = 14$) and 13% for recipients 20 to 29 years old ($n = 39$); for PTA recipients older than age 30, the 1-year rejection loss rates were 4% to 6%, similar to the other two recipient categories.

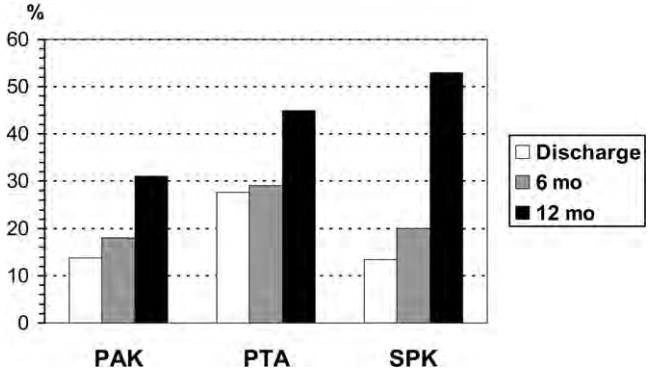

Figure 34–19 Frequency distribution of patients off steroids by recipient category for U.S. deceased donor primary pancreas transplants, 2000 to 2005 cases. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

The young nonuremic diabetic patient is highly immunocompetent and more prone to reject a pancreas graft, consistent with an earlier analysis of outcomes in U.S. pediatric pancreas transplant recipients from 1988 to 1999.[68] In that analysis, of slightly more than 8000 pancreas transplants, only 49 were in recipients younger than 21 years old (<1%)—34 in the SPK, 2 in the PAK, and 13 in the PTA category; all were deceased donor pancreas transplants except for two PTA segmental grafts from living donors. Less than half of the pediatric pancreas recipients were younger than 19 years old. In the PTA recipients, the 1-year graft survival rate was only 15%, with all but one loss being from rejection in less than 1 year. The registry data do not include the indications for a PTA in the pediatric recipients, but presumably they had extremely labile diabetes justifying placement on immunosuppression in an attempt to gain control. In the pediatric SPK recipients, the 1-year patient, pancreas, and kidney graft survival rates were 96%, 78%, and 71%, outcomes comparable to that of adult SPK recipients for the entire period. Of the pediatric SPK recipients, most had a renal disease other than diabetic nephropathy.

In regard to donor age, in the registry analysis of 2000 to 2004 primary deceased donor pancreas transplants, graft survival rates in all recipient categories tended to be highest with younger donors and lowest with older donors, principally because technical failure rates increased with increasing donor age.[37] Only 3.4% of all donors were 50 years old or older, and those donors were also mainly used in SPK.

With respect to outcome measures other than insulin independence—prevention and reversal of secondary complications, improvement in QOL, expansion of life span, and reduction of health care costs per quality-adjusted

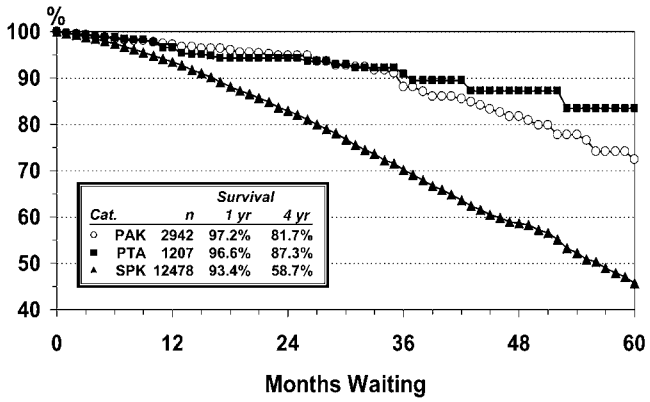

Figure 34–21 Patient survival rates on the pancreas waiting lists for 1995 to 2003 U.S. deceased donor primary transplants by recipient category. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney. (Modified from Gruessner RW, Sutherland DE, Gruessner AC: Mortality assessment for pancreas transplants. Am J Transplant 4:2018, 2004.)

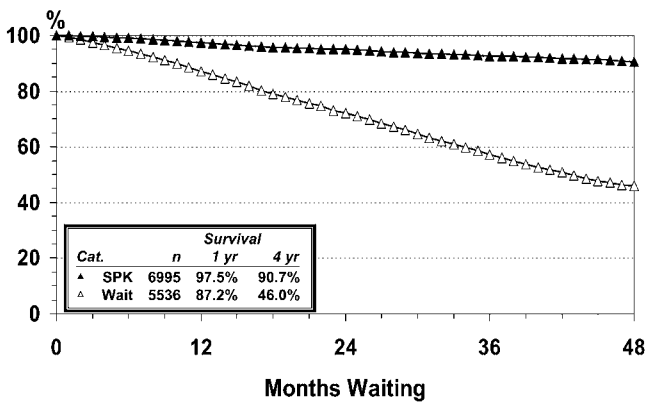

Figure 34–22 Patient survival rates on the pancreas waiting lists and after pancreas transplants for 1995 to 2003 U.S. deceased donor primary transplants in the simultaneous pancreas-kidney (SPK) category. (Modified from Gruessner RW, Sutherland DE, Gruessner AC: Mortality assessment for pancreas transplants. Am J Transplant 4:2018, 2004.)

life-year—these all have been positively shown in type 1 diabetic pancreas transplant recipients. [21,27,32,82,113,131,136] In patients with labile diabetes and hypoglycemic unawareness, a pancreas transplant can resolve an otherwise intractable and life-threatening problem.[54,80,96]

Survival Probabilities for Patients Who Remained on the Waiting List

Whether a pancreas transplant has an effect on survival probabilities for the diabetic patients selected for the procedure is controversial. Two separate analyses of U.S. data from the Organ Procurement and Transplantation Network (OPTN)/UNOS for pancreas transplant candidates and recipients compared the survival probabilities for patients who remained on the waiting list with patients who received a transplant by category between 1995 and 2000[133] and between 1995 and 2003.[44] In the first analysis,[133] SPK recipients were found to have significantly higher probability of survival than patients who remained on the waiting list for the procedure, but for solitary (PAK or PTA) recipients, just the opposite was the case. There is an explanation for the different results between the two studies. In the second analysis (Figs. 34-21 and 34-22),[44] multiple listings were eliminated. However, in the first study, they were not. By eliminating the multiple listings, patients were counted only once—from the first date of listing—increasing the accuracy of the waiting list mortality calculations.

Figure 34-23 shows hazard ratios of death among transplant recipients compared with patients who remained on the waiting list in the second study. For all categories, the hazard ratio in the early post-transplant period was greater than 1 because the surgical procedure itself increases the mortality hazard. In all three recipient categories, the hazard ratio was significantly decreased, however. Pancreas transplantation does not entail a higher risk than staying on exogenous insulin for patients on the waiting list and may improve survival probabilities for solitary and SPK recipients.

Expected Life-Year Gains from an Extra Deceased Donor

Understanding the additional life-years given to patients by deceased organ donors is necessary because substantial investments are being proposed to increase organ donation. Data were drawn from the United States Scientific Registry of Transplant Recipients. All patients placed on the waitlist as eligible to receive or receiving a deceased donor solid organ transplant between 1995 and 2002 were studied.[100] The average expected gain in life-years for kidney-pancreas waitlisted patients from an extra deceased organ donor was 12.9 life-years. Average benefit given average frequency of transplants in 2002 was 1.9 life-years.

PANCREAS RETRANSPLANTS

The following data are from the University of Minnesota. In our series of pancreas transplants from 1978 to 2005 (n = 1835), 321 (17%) were retransplants (14% second

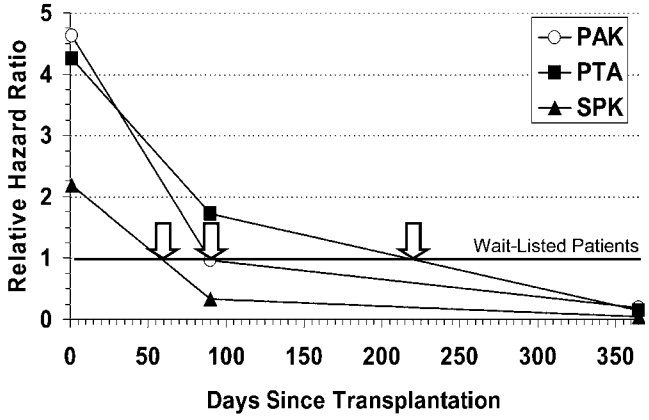

Figure 34–23 Mortality hazard ratios by recipient category. PAK, pancreas after kidney transplant; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney. (Modified from Gruessner RW, Sutherland DE, Gruessner AC: Mortality assessment for pancreas transplants. Am J Transplant 4:2018, 2004.)

Table 34–1 **Primary Simultaneous Pancreas-Kidney Transplantation Living Donor versus Deceased Donor Outcomes from 1994 to 2005**

	Patient Survival (%)		Pancreas GSR (%)		Kidney GSR (%)	
Years Post-Transplantation	LD	DD	LD	DD	LD	DD
1	100	90	86	78	100	87
3	100	86	78	74	91	86
5	100	85	74	69	86	73
7	95	79	67	62	79	67
10	79	72	67	55	65	57
	P = .01/.03		P = .31/.41		P = .09/.19	

P = Wilcoxon/log-rank tests.
DD, deceased donor; GSR, graft survival rate; LD, living donor.

transplants, 2.5% third transplants, 0.5% fourth transplants); all but 3 were from deceased donors. From 1985 to 2005, we performed 53 deceased donor SPK retransplants (38 second transplants). The overall 1-year pancreas graft survival rate was 62% for all SPK retransplants together and 66% for second SPK transplants only; at 3 years, survival rates were 45% for all and 52% for second transplants only. From 1978 to 2005, 163 deceased donor PAK retransplants (135 second transplants) were done. From 1994 to 2005 cases combined, the 1-year graft survival rate for deceased donor PAK retransplants with second, third, and fourth transplants included (n = 117) was 67% and for second transplants only (n = 99) was 65%; at 3 years, survival rates were 51% for all and 50% for second transplants only. From 1978 to 2005, there were 99 deceased donor PTA retransplants (86 second transplants). From 1998 to 2005 cases combined, the 1-year graft survival rate for deceased donor PAK retransplants with second, third, and fourth transplants included (n = 49) was 67% and for second transplants only (n = 43) was 66%; at 3 years, survival rates were 50% for all and 48% for second transplants only.

LIVING DONOR PANCREAS TRANSPLANTS

The following data are from the University of Minnesota. Nearly all of the living donor solitary (PAK and PTA) pancreas transplants were done from 1978 to 1994. All but two of the living donor SPK transplants (n = 38) were done from 1994 to 2005.[125]

We initiated living donor SPK transplants in March 1994.[42] Of the 38 donors, 6 were HLA-identical siblings, 25 were HLA-mismatched relatives, and 7 were unrelated. Two donors were ABO-incompatible; antibody reduction was successfully accomplished with plasmapheresis,[67] and both grafts are currently functioning at more than 6 years. In the overall series of 38 living donor SPK transplants, the 1-, 5-, and 10-year patient survival rates were 100%, 100%, and 84%; the 1-, 5-, and 10-year segmental pancreas graft survival rates (technical failures included, death with functioning graft counted as a graft failure) were 84%, 70% and 60%; and the 1-, 5-, and 10-year kidney graft survival rates are 100%, 86%, and 67%. We used duct injection technique in four living donor SPK transplants—the first two SPK segmental pancreas grafts (one still functioning at >12 years; one failed at >10 years) and in two later cases (one pancreas failed at 4 months, the kidney is still functioning at > 10 years; in the other case, both grafts are functioning at > 1 year). We used enteric drainage in 2 cases (both organs are functioning at > 2 and > 7 years) and bladder drainage in the other 32.

A comparison of outcomes from 1994 to 2005 cases combined was made for primary living donor SPK (n = 36) versus primary deceased donor SPK (n = 324) transplants (Table 34-1). The patient survival rates were significantly higher (P = .01 Wilcoxon and P = .03 log rank) in the living donor versus deceased donor cases—at 1, 3, and 7 years after transplantation, 100%, 100%, and 95% in living donor versus 90%, 86%, and 79% in deceased donor recipients. Pancreas graft survival rates were not significantly different between the living donor and deceased donor SPK recipients—at 1, 3, and 7 years after transplantation, 86%, 78%, and 67% in living donor versus 78%, 74%, and 62% in deceased donor cases. Kidney graft survival rates were marginally significantly higher (P = .09 Wilcoxon, P = .19 log rank) in living donor versus deceased donor SPK recipients—at 1, 3, and 7 years after transplantation, 100%, 91%, and 79% in living donor versus 87%, 86%, and 67% in deceased donor cases.

QUALITY-OF-LIFE STUDY

At University of Minnesota, from 1985 to 2003, 316 SPK, 204 PAK, and 98 PTA recipients enrolled in a prospective study of QOL changes after pancreas transplantation.[125] For QOL assessment, we used four dimensions of the Karnofsky Index: status of health, management of life, life satisfaction, and health satisfaction. Each recipient's response was recorded on a scale of 1 (low) to 5 (high) for each parameter. A total score was calculated from the sum of the four parameters (maximum score possible, 20). The impact of a successful or failed transplant was assessed by the changes in scores from baseline in annual follow-up evaluations.

The baseline (before pancreas transplant) median total scores were significantly higher (P < .0001) in the PAK (13.3) than in the SPK (11.3) and PTA (10.9) candidates. The ranges of baseline scores for the two midquarters in each recipient category are provided in Table 34-2. The mean baseline scores in these eras were 9.5 ± 2.6 (n = 109), 12.3 ± 3.9 (n = 131), and 13 ± 3.7 (n = 62) for SPK (P = .0001); 10.9 ± 2.6 (n = 32), 13.9 ± 3.3 (n = 82), and 15.2 ± 2.8 (n = 46) for PAK (P = .0001); and 9.9 ± 2.9 (n = 26), 10.3 ± 3.6 (n = 30), and 12.7 ± 3.3 (n = 24) for PTA (P = .009) candidates.

Table 34–2 **Pretransplant Baseline Quality-of-Life Scores from 1985 to 2003: Pancreas Transplant Recipient Study Patients (Range of Middle Two Quartiles)***

Category (*N*)	Q1	Median	Q2
SPK (316)	8.4	11.3	14.6
PAK (204)	11.7	13.3	15.9
PTA (98)	8.1	10.9	13.4

*Scores are summation of four parameters from Karnofsky Index: status of health, management of life, life satisfaction, and health satisfaction.

Q1 = highest sum in first quartile.

Q2 = highest sum in third quartile.

PAK, pancreas after kidney; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

Possibly, diabetic patients are coming to pancreas transplantation in better health condition than in the past.

It is not the absolute QOL score but rather the change (Δ) in score from the pretransplant baseline to the post-transplant evaluation that is important. The total score Δ for each recipient category according to graft function at 1 year are shown in Tables 34-3 and 34-4. SPK recipients were divided into four groups by graft status: (1) both grafts had sustained function ($n = 130$); (2) the pancreas had sustained function, but the kidney graft failed ($n = 5$); (3) the kidney graft had sustained function, but the pancreas graft failed ($n = 24$); or (4) both grafts failed ($n = 2$).

At 1-year after transplantation, the mean increase from baseline in total QOL scores was highly significant ($P = .0001$) in the SPK recipients with both grafts functioning but not in recipients with a functioning pancreas but a failed kidney. In recipients with a functioning kidney, but a failed pancreas graft, there was virtually no change from baseline. Only two recipients in whom both grafts failed completed the follow-up evaluation at 1 year. The total score did not change in one; it was lower compared with the pretransplant baseline in the other. The results in the SPK recipients in whom only one graft failed suggest that achieving insulin independence improves QOL more than becoming dialysis-free. At 1 year, the mean total QOL score increased significantly ($P = .0001$) from baseline in PAK recipients with sustained graft function ($n = 55$) (but not in recipients with failed grafts ($n = 16$) (see Table 34-4). At 1 year, the mean total QOL score increased significantly ($P = .0001$) from baseline in PTA recipients with sustained graft function ($n = 25$) but not in recipients with failed grafts ($n = 12$) (see Table 34-4).

LONG-TERM QUALITY OF LIFE

The increase in mean total points from pretransplant baseline was sustained in succeeding years in patients with functioning grafts. At 2 years, the mean increases were 4.3 ± 0.8 points for SPK ($n = 100$), 3.7 ± 5.6 for PAK ($n = 32$), and 6.4 ± 4.3 for PTA ($n = 8$) ($P = .0001$). For 50 SPK study patients who completed the evaluation at 4 years, the mean increase in total points from baseline was 6.2 ± 4.6 ($n = 50$) ($P = .0001$). Overall, our study showed that diabetic patients who become insulin independent perceive their QOL as having improved despite immunosuppression. The data presented here are original and complement past QOL studies, done by independent investigators,[31-34,135,136] of the Minnesota pancreas recipients.

METABOLIC STUDIES

Formal metabolic studies of the Minnesota pancreas recipients and living donors have been conducted since the inception of our program[127] and are still ongoing.[98] The initial studies were very basic: 24 metabolic profiles of glucose and insulin values before and after meals, and standard oral or intravenous glucose tolerance tests in pancreas recipients who were insulin independent as a result of a functioning graft.[127] The profiles usually resembled those of nondiabetic individuals, or at least those of nondiabetic kidney allograft recipients, with or without portal drainage of the graft venous effluent.[121] The metabolic profile and glucose tolerance test studies were used to compare post-transplant endocrine function by duration of pancreas graft preservation[76] and to compare function in recipients who did or did not have reversible rejection episodes.[72] Metabolic profile and glucose tolerance test results were similar regardless of preservation time or occurrence of rejection episodes in recipients with sustained insulin independence; short-term[73] and long-term[74] glycosylated hemoglobin levels[75] were normal.

More sophisticated metabolic studies using new methods were introduced[94] and carried out by a series of fellows and associate faculty members in the Division of Endocrinology.* These studies not only examined pancreatic graft beta cell function but also alpha cell function, glucose counterregulatory mechanisms, and the impact of the site of venous drainage (systemic or portal) of a pancreas graft.

Diem and colleagues[18] were the first to establish systemic venous drainage as the principal cause of systemic venous hyperinsulinemia after pancreas transplantation. A smaller portion of the hyperinsulinemia could be attributed to recipients' glucocorticoid use. Despite this metabolic abnormality, virtually all measures of carbohydrate metabolism

*References 2, 18, 19, 50, 54, 55, 94, 101, 102, 129.

Table 34–3 **One-Year Post-Transplant Mean (±SD) Change (Δ) in Quality-of-Life Scores from Pretransplant Baseline in Simultaneous Pancreas-Kidney Recipients According to Graft Function or Failure**

	Graft Status (*N*)			
	Pancreas Fxn, Kidney Fxn (130)	Pancreas Fxn, Kidney Fail (5)	Pancreas Fail, Kidney Fxn (24)	Pancreas Fail, Kidney Fail (2)
Quality-of-life score change	5.2 ± 4.0	2.4 ± 1.5	0.2 ± 3.7	≤ 0
P value	.0001	.12	> .5	NA

Fail, failure; Fxn, function; NA, not applicable.

Table 34–4 **One-Year Post-transplant Mean (±SD) Change (Δ) in Mean Quality-of-Life Score from Pretransplant Baseline in Solitary Pancreas after Kidney (PAK) Transplant Recipients with Functioning or Failed Grafts**

	Graft Status	
≈	Functioning	Failed
PAK (N)	3.7 ± 4.1 (55)	0.9 ± 2.5 (16)
P value	.0001	.009
PTA (N)	5.9 ± 4.2 (25)	2.8 ± 4.8 (12)
P value	.0001	.07

PAK, pancreas after kidney; PTA, pancreas transplant alone.

in the fasting state and after a mixed meal remained normal.[50]

Possible adverse effects of immunosuppressive drugs on beta cell function and glucose tolerance also were studied. Many of the drugs are known to interfere with insulin synthesis or secretion, or action. Teuscher and coworkers[129] assessed insulin secretory reserve in pancreas transplant recipients by measuring glucose potentiation of arginine-induced insulin secretion and observed abnormally low insulin responses. Because diminished insulin secretory reserve also was observed in nondiabetic kidney recipients, the immunosuppressive drugs were the likely causes of this metabolic abnormality. A similar defect was observed in psoriasis patients treated with cyclosporine but not in arthritis patients treated with glucocorticoids; cyclosporine was the likely cause of diminished insulin secretory reserve.[129] Despite the hyperinsulinemia consequent to systemic drainage and glucocorticoids, and despite the diminished insulin secretory reserve attributable to cyclosporine, we have reported normal levels of fasting plasma glucose and hemoglobin A_{1c} in a group of pancreas recipients followed for 10 to 18 years.[97]

Defective glucagon and epinephrine counterregulatory responses to hypoglycemia are serious consequences of type 1 diabetes. These abnormalities can lead to dangerous levels of hypoglycemia that incapacitate patients and seriously compromise their QOL. This scenario is worsened because such patients lose normal symptom recognition of hypoglycemia, which prevents them from taking early corrective measures. Studies by Diem and colleagues[19] showed that a successful pancreas transplant restores normal glucagon responses. Later studies by Kendall and associates[56] concluded that the transplanted pancreas, rather than the alpha cells in the native pancreas, provided the restored glucagon response. Barrou and colleagues[3] used isotopic infusions and hypoglycemic clamp methodology to show that the restored glucagon response normalized hepatic glucose production during hypoglycemia. Kendall and associates[54] showed that a successful pancreas transplant partially restored epinephrine response during hypoglycemia. More important, these studies also documented that recipients of a successful pancreas transplant re-establish normal symptom recognition.

More recently, Paty and coworkers[85] have shown that restored hypoglycemic counterregulation is stable in pancreas recipients with functioning grafts for at least 2 decades after transplantation. The effect of the occurrence of post-transplant obesity in pancreas recipients was studied, and a detrimental effect on metabolism was shown similar to that in the general population.[95]

Although most of our pancreas transplants were from deceased donors, nearly 10% were segmental grafts from living donors. The metabolic responsitivity of the transplanted hemipancreas is generally indistinguishable from that of whole-pancreas grafts. Donors of the pancreatic segments generally maintain normal glucose levels, but follow-up studies of the donors (before we established our current criteria to be a living donor) show that about 25% had metabolic evidence of acquired glucose intolerance several years after donation.[55] Studies by Seaquist and Robertson[102] established that beta cell and alpha cell responses were compromised in hemipancreatectomized donors during measurements of insulin secretory reserve. Later studies by Seaquist and colleagues[101] showed that hemipancreatectomy also was associated with elevated circulating levels of proinsulin, presumably owing to release of immature insulin granules in which cleavage of C peptide from proinsulin was not yet complete.

The results of these studies prompted us to modify our criteria to be a living donor. Now, all living donors must have a body mass index less than 28 kg/m^2, in addition to having normal glucose tolerance test results, and plasma insulin levels must increase by 300% within 1 to 2 minutes after intravenous stimulation with glucose or arginine. Living donors who meet these criteria have so far remained euglycemic and insulin independent, but they need to be carefully studied over time. More recent studies of living hemipancreatectomized donors and their recipients during the second decade after surgery have shown a relationship between the development of obesity and occurrence of diabetes,[95] and the potential for weight gain in donors and recipients must be taken into account when selecting living donors and recipients for hemipancreatectomy and segmental pancreas transplantation. Most living segmental pancreas donors retain normal hormonal responses to metabolic challenges, however.[98]

STUDIES OF DIABETIC SECONDARY COMPLICATIONS

Formal studies on the course of preexisting diabetic secondary complications after pancreas transplantation were initiated.[117,118] Until the multicenter DCCT[17] was completed in 1993, the best evidence that a constant euglycemic state mitigated the progression of secondary complications was from our studies[9,57,81,92,132] and those of others.[104,134] These studies were done by members of our faculty from ophthalmology,[92] pediatric nephrology,[9,27] and neurology.[57,80-82] The failure rate of pancreas transplants was high, generating a control group for these studies. Recipients were studied at baseline, and subsequently divided into two groups: (1) recipients with early pancreas graft failure (<3 months) and (2) recipients with sustained graft function for more than 1 year.

Retinopathy

Ramsay and colleagues[92] studied solitary pancreas recipients. Retinopathy and visual acuity were quantitated before and serially after transplantation. Most candidates had advanced proliferative retinopathy. At 2 years after transplantation, the incidence of progression to a higher grade of retinopathy

was the same (approximately 30%) in the eyes of recipients with versus without graft function. After 3 years, no further progression occurred in the recipients with functioning grafts. Seventy percent with failed transplants advanced to a higher grade by 5 years, however. Only a few recipients had no retinopathy at the pretransplant baseline examination, but disease has not emerged in the subgroup with continuously functioning pancreas grafts.

Nephropathy

Studies of diabetic nephropathy focused on disease recurrence or on preventing it in the kidney grafts of diabetic KTA, SPK, or PAK recipients[9,71,77] and on disease progression, stabilization, or regression of disease in the native kidneys of PTA recipients.[27,28] Mauer and associates[69-71] documented the recurrence of diabetic nephropathy (vascular lesions[69] and an increase in glomerular and tubular basement membrane and mesangial matrix[71]) in nearly half of kidneys transplanted without a pancreas in uremic diabetic recipients.[70]

Initial evidence that a successful pancreas transplant can influence the course of diabetic nephropathy came from kidney allograft biopsy studies in PAK recipients by Bilous and colleagues.[9] At the time of the pancreas transplant, 1 to 7 years (mean 4 years) after the kidney transplant, the graft glomerular mesangial volume was moderately increased and glomerular basement membrane was moderately thickened. There was no progression; there was regression of glomerular lesions in follow-up biopsy specimens obtained 2 to 10 years later (mean 4.5 years). These findings contrasted to the findings in the KTA recipients in whom progressive diabetic glomerulopathy occurred,[70] leading to kidney graft failure and the need for a kidney retransplant in some recipients.[78]

The most dramatic and surprising findings came from studies by Fioretto and colleagues[27,28] of native kidneys in PTA recipients. We obtained baseline biopsy specimens of native kidneys in most of the PTA recipients.[25] Follow-up biopsy samples in some have shown cyclosporine-induced lesions that were associated with a progressive decline in kidney function, independent of the diabetic lesions already present.[26,77,130] The diabetic kidney lesions were distinct. In eight PTA recipients who were nonuremic at the time of the pancreas transplant, but who had mild to moderately advanced lesions of diabetic nephropathy at baseline, 10-year follow-up biopsy specimens showed that glomerular basement membrane and tubular basement membrane thickness and mesangial fractional volume of the glomerulus had decreased and returned to normal.[27] In follow-up studies, Fioretta and colleagues[28] also showed remodeling of renal interstitial and tubular lesions in the kidneys of the pancreas transplant recipients. Although these studies were in patients with diabetic nephropathy, the fact that structural lesions could be reversed shows in principle that the kidney has the capacity for remodeling if the environmental perturbations responsible for the lesions originally are removed, having implications for renal disease in general, and not just that secondary to diabetes.

Although it takes at least 5 years of normoglycemia, a pancreas transplant can reverse the lesions of diabetic nephropathy. Such reversal does not guarantee normal function because independent damage to the kidney may occur from the calcineurin inhibitors needed to prevent pancreas rejection[26]—hence the need for attempts to develop effective non-nephrotoxic immunosuppressive regimens.[38] Nearly all patients with early diabetic nephropathy would benefit from a pancreas transplant if successful.

Neuropathy

As with the eye and kidney, our pancreas recipients had baseline neurological studies with serial follow-up.[57,82,92] More than 80 of our recipients had symptomatic neuropathy, and more than 90% had an abnormal neurologic examination at baseline.[58] Kennedy and associates[57] showed significant improvement in motor and sensory indices and autonomic function 1 to 4 years after transplantation; we concluded that progression of diabetic neuropathy is halted, and that an improvement is possible with sustained normoglycemia.

Navarro and coworkers[81] found mortality rates were higher in patients with autonomic dysfunction or abnormal nerve conduction studies compared with patients with minimal disease. The mortality rate also was high in nontransplanted diabetic patients with neuropathy. In neuropathic patients with a successful pancreas transplant, the mortality rate was significantly lower, however, even if neuropathy improved only minimally.[80] The combination of diabetes and severe neuropathy is lethal; correction of diabetes improves survival even if neuropathy persists. Navarro and coworkers[82] did follow-up studies at 10 years of diabetic pancreas recipients. In control patients (patients with a failed transplant), neuropathy progressively worsened, whereas in recipients with sustained graft function, the improvement in neuropathy was sustained.

SUMMARY

Pancreas transplantation should be in the armamentarium of every transplant center for the treatment of diabetic patients. Likewise, every endocrinologist should consider pancreas transplantation in the treatment of patients in whom type 1 diabetes is complicated by hypoglycemia-associated autonomic failure[16] or progressive microvascular complications or both. Continued clinical research on pancreas transplantation is needed to identify the most appropriate recipient population, the optimal timing of transplant in the course of diabetes, and the most suitable donor tissue and transplant protocol for a given patient. Pancreas transplantation needs to be made as economical as possible.[114] Studies such as those done in pancreas-kidney transplant recipients showed the efficiency in the treatment of complicated diabetes.[21] Currently, pancreas transplantation has a well-defined clinical role for diabetic patients, and it is expected to remain an important option in the treatment of diabetes.

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Chapter 35

Kidney Transplantation in Children

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It has become axiomatic that kidney transplantation is the optimal treatment for children and adolescents with end-stage renal disease (ESRD). Approximately two thirds of children with ESRD receive at least one transplant in their lifetime. Successful transplantation ameliorates uremic symptoms and allows for significant improvement of skeletal growth, appetite and nutrition, sexual maturation, cognitive performance, quality of life, and psychosocial functioning. Survival in pediatric patients with kidney transplants exceeds that seen with dialysis. For pediatric patients of all ages, transplantation results in better survival than dialysis. Five-year survival rates in transplanted patients range from 94% to 97%; in dialyzed patients, the survival rate ranges from 75% to 87%.[115]

Data from the 2006 North America Pediatric Renal Transplant Cooperative Study (NAPRTCS) annual report show that, at every age, patient survival at 4 years with either living donor or deceased donor transplantation is markedly superior to that seen in dialysis patients (Fig. 35-1). In addition, long-term survival of pediatric patients with ESRD has increased over 20 years. Prolonged dialysis remains a strong mortality risk factor over a functioning renal graft, however, with cardiovascular disease and infections accounting for almost 70% of patient mortality in pediatric ESRD.[61] A child with a well-functioning kidney can have a quality of life that cannot be achieved by any dialysis therapy.

Current success in pediatric renal transplantation can be attributed to improvements in transplantation surgery, the choice of donor organs for pediatric patients, improvements in immunosuppressive therapy, and the provision of age-appropriate clinical care.[115] Nevertheless, success in pediatric kidney transplantation is still a challenging undertaking. Children and adolescents are constantly growing, developing, and changing. Each developmental stage produces a series of medical, biological, and psychological challenges that must be appropriately addressed if truly successful graft outcome and rehabilitation are to be realized.

Much of the statistical data reviewed in this chapter come from databases that have provided an invaluable resource for the advancement of pediatric transplantation. These databases have permitted the evaluation and extrapolation of data from multiple pediatric renal transplant programs that tend to be small compared with their adult counterparts. Major databases referred to are the NAPRTCS, the Scientific Registry of Transplant Recipients (SRTR), and the United States Renal Data System (USRDS) annual report.

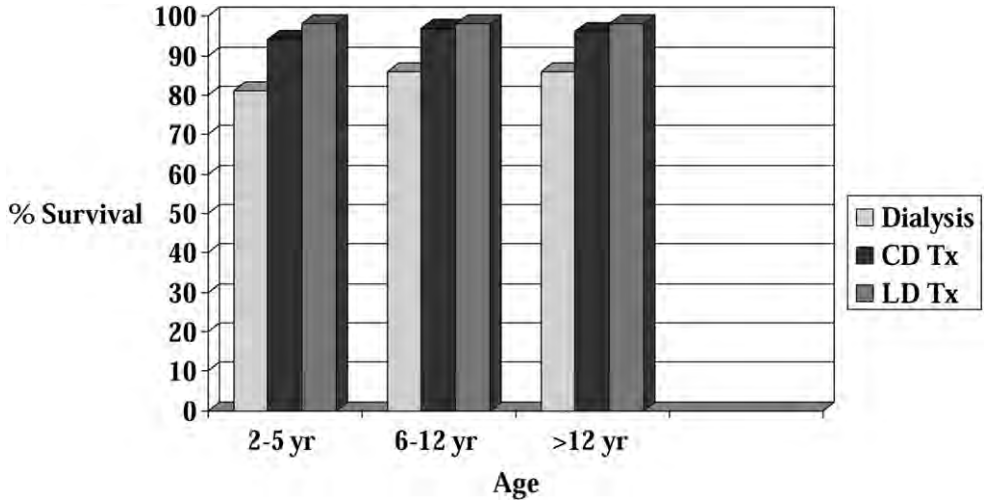

Figure 35–1 Pediatric end-stage renal disease patient survival at 4 years after transplantation versus dialysis. CD Tx, cadaver donor transplant; LD Tx, live donor transplant. (Data from North American Pediatric Renal Transplant Cooperative Study (NAPRTCS), Rockville, Md., 2006.)

EPIDEMIOLOGY OF END-STAGE RENAL DISEASE IN CHILDREN

Incidence

The incidence and prevalence of treated pediatric ESRD patients have been increasing since 1989. As of 2000, the incidence of new cases of ESRD in children 0 to 19 years old was 15/1 million U.S. children per year (Table 35-1). The point prevalence of ESRD in this population is 70/1 million child population. The incidence of ESRD increases with age, with the highest incidence observed in children 15 to 19 years old (28/1 million). Adolescents compose about 50% of treated pediatric ESRD patients.

There is a wide variation by race and gender in the incidence rates of treated ESRD. African-American children have the highest incidence of 27/1 million compared with 12/1 million white, 15/1 million Asian and Pacific Islander, and 17/1 million Native American children. The incidence is higher in African Americans across all age groups but is most prominent in the 15- to 19-year age group (60/1 million African Americans compared with 20/1 million whites). Over 20 years, incidence rates for white pediatric patients have remained constant, but for African-American patients and patients of races other than white, the rates of ESRD have more than doubled. The incidence of glomerulonephritis as a cause of ESRD is two to three times higher in African-American children than in white children; there is no racial predilection in patients with congenital, hereditary, or cystic diseases. According to the NAPRTCS dialysis registry, patients with focal segmental glomerulosclerosis (FSGS) compose almost 24% of all African-American dialysis patients and more than 30% of adolescent African-American dialysis patients. Boys have a higher incidence of treated ESRD than girls in all age groups.

Table 35–1 **End-Stage Renal Disease in Children**

Incidence of new cases/1 million child population	
0-19 yr old	15
Incidence by age/1 million child population	
0-4 yr old	9
5-9 yr old	7
10-14 yr old	14
15-19 yr old	28

Etiology

Glomerular diseases account for about 30% and congenital, hereditary, and cystic diseases account for 26% of cases of pediatric ESRD (Table 35-2). Although incidence rates for glomerular diseases have remained steady in the pediatric population, the incidence rates for patients with congenital, hereditary, and cystic diseases have trended upward over 20 years.

Table 35–2 **Common Causes of End-Stage Renal Disease in Pediatric Transplant Recipients (*N* = 8435)**

	%
Obstructive uropathy	16.1
Aplasia/hypoplasia/dysplasia	16
Focal segmental glomerulosclerosis	11.5
Reflux nephropathy	5.2
Chronic glomerulonephritis	3.5
Polycystic disease	2.9
Medullary cystic disease	2.8
Hemolytic-uremic syndrome	2.7
Prune-belly syndrome	2.6
Congenital nephrotic syndrome	2.5
Familial nephritis	2.2
Cystinosis	2.1
Crescentic glomerulonephritis	1.9
MPGN type I	1.9
MPGN type II	0.9

MPGN, membranoproliferative glomerulonephritis.
Data from North America Pediatric Renal Transplant Cooperative Study (NAPRTCS), Rockville, Md., 2005.

Pediatric ESRD has different causes compared with adults. In contrast to adults, ESRD secondary to diabetes mellitus or hypertension is rare in children. The etiology of ESRD varies significantly by age. Congenital, hereditary, and cystic diseases cause ESRD in more than 52% of children 0 to 4 years old, whereas glomerulonephritis and FSGS account for 38% of cases of ESRD in patients 10 to 19 years old. The most common diagnosis in transplanted children is structural disease (49%), followed by various forms of glomerulonephritis (14%) and FSGS (12%) (see Table 35-2). Children also seem to start ESRD therapy with a higher estimated glomerular filtration rate (GFR) than do adults; in 2001, approximately 50% of patients 0 to 19 years old had an estimated GFR greater than 10 mL/min compared with approximately 38% in patients 20 years old.

ACCESS TO TRANSPLANTATION

As of 2005, the NAPRTCS registry reported that 8435 pediatric recipients have had kidney transplants performed since 1987. Data from the 2006 SRTR show that approximately two thirds of transplants are performed in children 11 to 17 years old, whereas 17% are done in patients 6 to 12 years old, and 17% are done in patients 1 to 5 years old. NAPRTCS registry data show that about 5% of pediatric transplants are performed in children younger than 2 years old. Approximately 60% of recipients are male, 62% are white, 16% are African American, and 16% are Hispanic.

Pediatric transplants constitute only 4% to 6% of all transplants in the United States. The number of kidney transplants has been gradually increasing since 2000 (Fig. 35-2). Data from the SRTR indicate that in 2005, almost 900 pediatric transplants were performed in the United States. As shown in Figure 35-2, the number of living donor transplants over the past 5 years has been consistent at approximately 400 per year.

Historically, more than half of all pediatric kidney transplants came from living donors. From 1998 to 2003, 58% of pediatric transplants had come from living donors. This trend was probably a result of the awareness on the part of families that transplantation is the best therapeutic option for children with ESRD.

The kidney transplant community, through the Organ Procurement Transplant Network (OPTN), has consistently supported the concept of expedited kidney transplantation for pediatric patients. The increasing number of deceased donor transplants in children (see Fig. 35-2) indicates that these efforts apparently are succeeding. In 2005 for the first time in a decade, the number of deceased donor transplants exceeded the number of living donor transplants. Children continue to represent an increasing percentage of the waiting list for deceased donors. In 1992, there were 630 patients younger than 18 years old on the waiting list for a deceased donor organ, and in 2001 that number had increased to 701, representing an increase of 11%. For comparison, in the same time period, the number of adult patients increased by almost 30,000, or more than 100%, from 21,443 to 50,443.

The rates for living related and deceased donor renal transplantation are now higher in children than in adults. According to the USRDS, for children 0 to 19 years old, there were 29 living donor transplants and 27 deceased donor transplants per 100 dialysis patient-years. These figures are more than double the corresponding rates for adults 20 to 44 years old. The highest rates of transplantation are in the 5- to 9-year-old group, with 40 living donor transplants and 46 deceased donor transplants performed per 100 dialysis patient-years.

Median waiting times have remained roughly constant for pediatric patients. Since the 1990s, the size of the active pediatric waiting list has consistently remained in the range of 500 to 650. The median waiting time for all pediatric transplants is approximately one half of the time for adults to receive a transplant.

TIMING OF TRANSPLANTATION

Renal transplantation is considered when renal replacement therapy is indicated. In children, dialysis may be required before transplantation to optimize nutritional and metabolic conditions, to achieve an appropriate size in small children, or to keep a patient stable until a suitable donor is available. Many centers want a recipient to weigh at least 8 to 10 kg, to minimize the risk for vascular thrombosis and to accommodate an adult-sized kidney. In infants with ESRD, a target weight of 10 kg may not be achieved until 12 to 24 months of age. At experienced centers, transplantation with an adult-sized kidney has been successful, however, in

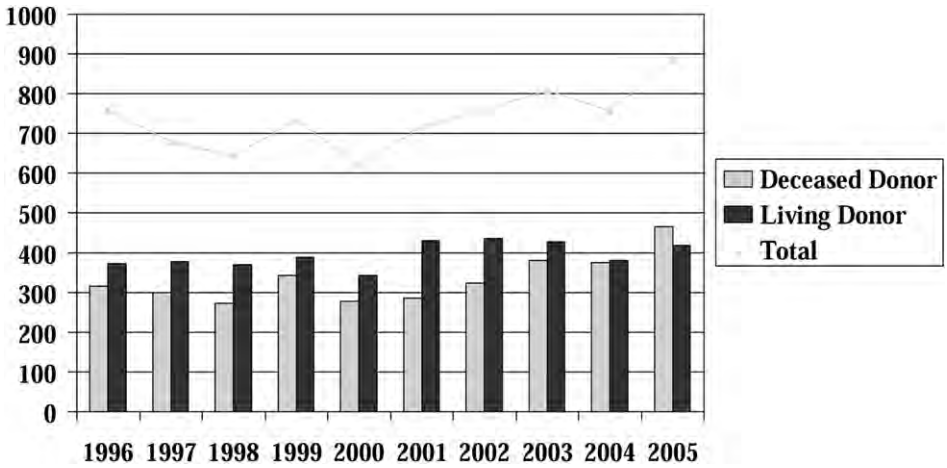

Figure 35–2 Pediatric (1 to 18 years old) kidney transplants performed annually. (Data from Scientific Registry of Transplant Recipients: Preliminary Data, Ann Arbor, Mich., 2006.)

children who weighed less than 10 kg or were younger than 6 months old.

Preemptive transplantation (i.e., transplantation without prior dialysis) accounts for approximately one quarter of all pediatric renal transplants. The major reason cited by patients and families for the decision to undertake preemptive transplantation is the desire to avoid dialysis.[49] There seems to be no impairment in graft outcome in pediatric recipients who have undergone preemptive transplantation compared with recipients who have undergone dialysis before transplantation, and some data suggest a small improvement in allograft outcome.[84,171] The reasons for the improved graft survival are unknown but may relate to the shorter time in ESRD, with its attendant risk factors for cardiovascular and infectious morbidity. Because of the prolonged waiting time for deceased donors, most kidneys for preemptive transplants are from living donors. With the increased efforts of the OPTN to expedite pediatric transplantation, however, more children on the waiting list are receiving transplants before dialysis is instituted.

PATIENT AND GRAFT SURVIVAL

Patient survival after transplantation is superior to that achieved by dialysis for all pediatric age groups. The 1-year, 2-year, and 5-year patient survival rates are 97.4%, 96.5%, and 95.7% for all primary transplants. Survival rates for recipients of primary transplants are excellent for deceased and living donor groups: the 1-year, 2-year, and 5-year rates for recipients of living donor kidneys are 98%, 97%, and 95%; comparable values for deceased donor kidneys are 97%, 96%, and 92%.

The patient survival for pediatric transplant recipients has improved over 15 years. From 1987 to 1994, the 5-year patient survivals were 92.8% and 94.9% in recipients of kidneys from deceased and living donors. From 1995 to 2002, the comparable figures are 95.5% and 95.9%. Infection accounts for 31% of patient deaths. Other causes of death include cardiopulmonary disease (16%), malignancy (11%), and cardiopulmonary (16%) and dialysis-related complications after graft failure (3%). About 45% of patients who die do so with a functioning graft.

Historically, pediatric kidney allograft survival was consistently inferior to that observed in adults. This is no longer the case. Serial data analysis shows that, at every time point up to 7 years after transplantation, there is a marked improvement in graft survival in recipients of deceased donor and living donor transplants (Fig. 35-3). Over the past 15 years, the graft survival has been 93% at 1 year and 80% at 5 years for living donor transplant recipients and 84% and 66% for deceased donor transplant recipients. Transplants performed more recently have even better outcomes. Since the late 1990s, 1-year and 5-year graft survivals are 95% and 83% in living donor transplants. In deceased donor transplants, these values are 91% and 73%.[13]

Improvements in graft survival can be correlated with recipient age. Patients younger than 2 years old were previously reported to have the lowest graft survival rates: 90% and 81% at 3 years for recipients of living and deceased donor kidneys. This situation has improved more recently, with the increasing use of adult-sized kidney donors.[137] A review of the UNOS database revealed that younger recipients (< 5 years old) when receiving an optimal donor (an adult-sized kidney with minimal or no acute tubular necrosis), regardless of living or deceased donor source, had projected graft half-life expectancy rates almost double that of young adult recipients and equivalent or slightly better even than the "gold standard" HLA-identical adult transplant. Graft outcome, drawn from the 2005 SRTR report, is shown for recipients of deceased donor transplants for all ages in Figure 35-4. The results at 3 months and 1 year for all three pediatric age groups are comparable to the results in adults of all ages. Graft survival continues to be excellent out to 5 years in the two youngest groups. There is a falloff in graft survival in adolescents compared with the results in the other age groups. Similar results are seen in recipients of living donor allografts, although the differences are less pronounced (Fig. 35-5).

Incidence and Causes of Graft Failure

Of the more than 8000 pediatric kidney transplantations reported to NAPRTCS since 1987, about 26% have failed. Twenty-three percent of primary transplants and 37% of retransplants have failed. Of the transplants that failed, 75% resulted in a return of the patient to dialysis; 6% were retransplanted preemptively, and 9% died with a functioning graft.

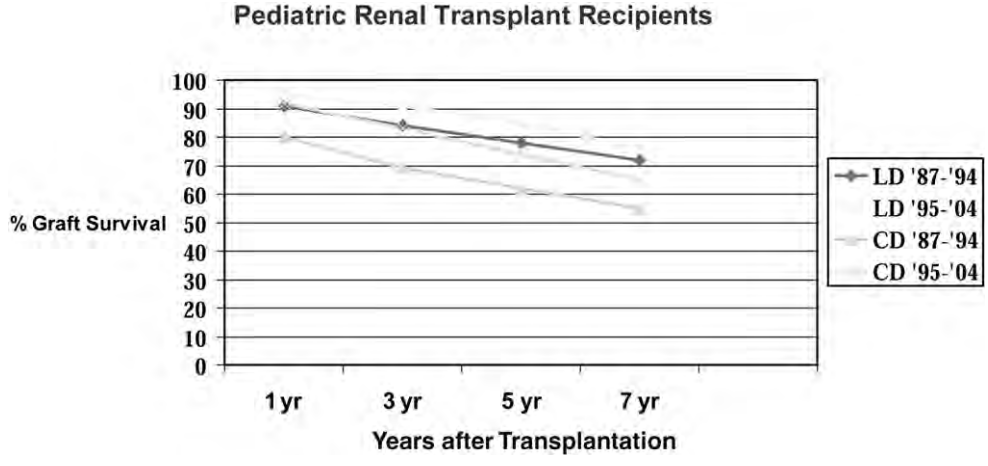

Figure 35–3 Improvement of graft survival. CD, cadaver donor; LD, live donor

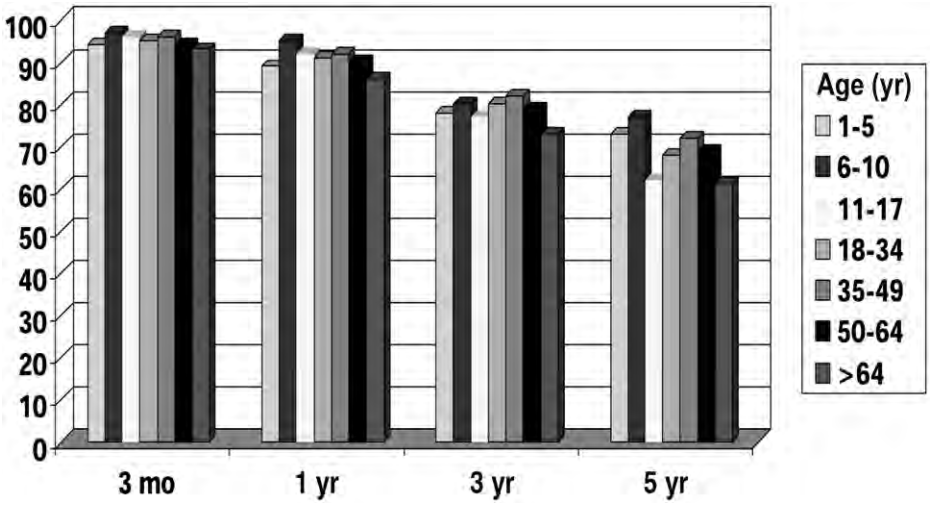

Figure 35–4 Percent graft survival of cadaver donor kidney transplants by age. (Data from Scientific Registry of Transplant Recipients, Ann Arbor, Mich., 2005.)

Figure 35-6 shows the causes of graft failure. With increasing length of follow-up, chronic rejection continues to be the leading cause of graft failure in pediatric patients. Chronic renal allograft dysfunction accounts now for approximately 33% of graft failures, with acute rejection accounting for 15%. Other causes include vascular thrombosis (11%), recurrence of original disease (7%), patient noncompliance (4.5%), primary nonfunction (2%), infection (2%), malignancy (1%), and death due to other causes (9%). Although some causes of graft failure, such as graft thrombosis and recurrence of the original disease, have remained constant over 10 years, loss from acute rejection has decreased dramatically. Technical issues remain a challenge. Approximately 3.8% of all grafts performed are lost to a combination of vascular thrombosis, primary nonfunction, and miscellaneous technical causes.

Prognostic Factors Influencing Graft Survival

Dramatic improvements have been made in short-term and long-term graft survival rates. The following factors are important determinants of improving graft survival in pediatric patients.

Donor Source

Short-term and long-term graft and patient survival rates are better in recipients of living donor transplants in all pediatric age groups (Table 35-3). Registry data show that recipients of kidneys from living donors have a 10% to 20% advantage in graft survival at 1, 3, and 5 years (see Fig. 35-3). Younger transplant recipients benefit the most from live donor transplantation and have a 20% to 30% better graft survival rate 5 years after transplantation. Shorter cold ischemia time and less acute tubular necrosis, better HLA matches, lower acute rejection rates, and better preoperative preparation help account for the better outcome in recipients of live donor kidneys.

Recipient Age

In the past, children younger than 6 years old, especially those younger than 2 years old, have had lower graft survival rates than older children, especially with deceased donor kidneys. Now that trend seems to be reversed. Some studies suggest that infant recipients of adult kidneys with immediate function may have the longest half-lives of any type of kidney transplant.[19,30,137] Data from the SRTR document that pediatric recipients younger than age 11 had 5-year graft

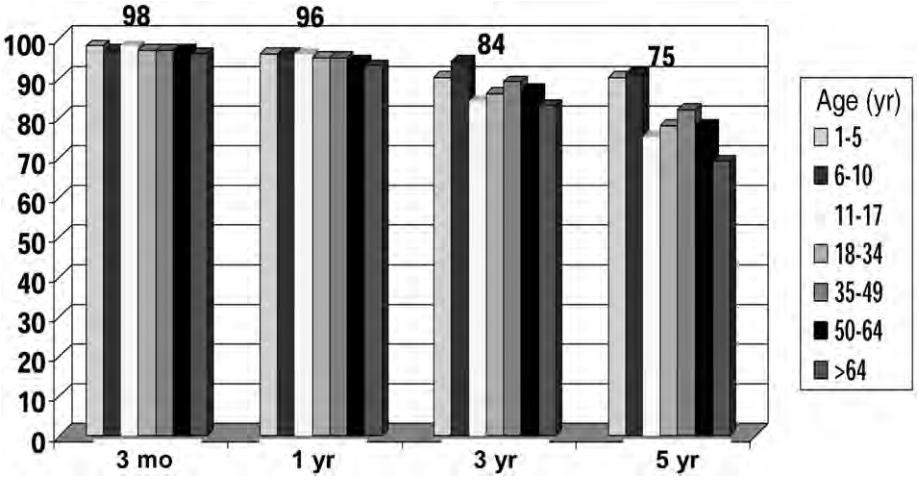

Figure 35–5 Percent graft survival of live donor kidney transplants by age. (Data from Scientific Registry of Transplant Recipients, Ann Arbor, Mich., 2005.)

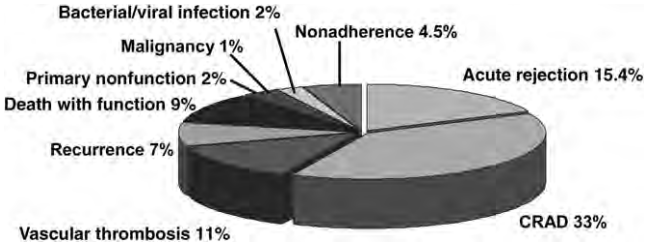

Figure 35–6 Causes of renal allograft failure in pediatric renal transplantation. CRAD, chronic renal allograft dysfunction. (Data from North American Pediatric Renal Transplant Cooperative Study, Rockville, Md., 2005.)

survival rates that were as good as, if not better than, the rates in most other older age groups (see Figs. 35-4 and 35-5). The long-term graft survival rates in adolescents are not as good as the rates seen in younger children, even though the shorter term outcome is equivalent. The 1-year, 3-year, and 5-year graft survival rates for adolescent recipients of living donor kidneys are 96%, 84%, and 75%. The 5-year outcome in adolescents is inferior to the graft survival of every group except recipients older than 65 years, where the two results are virtually the same.[33] With regard to deceased donor kidneys, the graft outcomes in adolescents were 92%, 77%, and 62%. The results for 5 years are the poorest of all age groups. Higher rates of medication noncompliance, an unexplained high incidence of graft thrombosis,[153] and a high recurrence rate of FSGS,[11] which is the most common acquired cause of ESRD in this age group, all have been cited as potential causes for these poor outcomes in adolescents.

Donor Age

For deceased donor recipients, kidneys from donors 11 to 17 years old provide optimal graft survival and function. This group is followed next by donors 18 to 34, 6 to 10, and 35 to 49 years old. Grafts from patients younger than 5 years old fare more poorly, and grafts from patients older than 50 years fare most poorly.

Although transplanted kidneys grow in size with the growth of the recipient, transplantation with kidneys from deceased donors younger than 5 years old is associated with markedly decreased graft survival. Children younger than 5 years old receiving a kidney from a donor younger than 6 years old have the highest relative risk of graft failure.[124]

Deceased donor kidneys from donors older than 50 years old are more likely to result in suboptimal long-term outcome. The older the donor, the greater is the decline of renal function with time. This finding is consistent with more recently generated data that link chronic allograft dysfunction with limited repair capacities because of tissue injury. This long-term renal dysfunction is an important consideration in pediatric renal transplantation because graft function has an important effect on post-transplantation growth.

Recipient Race

In recipients of live donor kidneys, African-American race is the most significant factor associated with poor outcome. African-American race is second only to young recipient age (< 2 years) as a predictor of graft failure in recipients of deceased donor kidneys. At 5 years after transplantation, African Americans have graft outcomes of 53% and 69% for recipients of deceased donor and living related kidneys. For white and Hispanic recipients, graft survivals at 5 years are 70% and 64% for recipients of deceased donor kidneys and 82% for both groups for living donor grafts. When taken as a group, African-American patients not only have poorer graft survival but also have poorer renal function, likely owing to the higher rate of acute rejection.

HLA Matching in Children

In pediatric transplantation, most living donor transplants come from parents and, as noted previously, these transplants are being done with increasing frequency and have excellent graft outcome. Long-term graft survival is best when the donor is an HLA-identical sibling. When considering transplants from HLA-haploidentical sibling donors, more recent studies suggest that there is improved outcome when donor and recipient share "noninherited maternal antigens," as distinct from "noninherited paternal antigens."[25] With regard to deceased donor transplantation, NAPRTCS data suggest improved outcome with the sharing of HLA-B and HLA-DR antigens.[154]

Presensitization

Blood transfusions have become less common since human recombinant erythropoietin became an integral part of ESRD therapy. It is surprising, however, that more recent USRDS data find that hemoglobin levels in children on dialysis are lower than their adult counterparts, and evidence currently exists for more aggressive management of anemia to forestall transfusions. Repeated blood transfusions expose the recipient to a wide range of HLA antigens and may result in sensitization to these antigens, leading to higher rates of rejection and graft failures. Data from NAPRTCS suggest that the graft failure rate increases in living donor and deceased donor transplant recipients with more than five blood transfusions before transplantation compared with recipients who had five or fewer transfusions. There is a 41% increase in the likelihood of graft failure in living donor recipients with more than five transfusions. For recipients of deceased donor transplants with similar transfusion exposure, there is an increased risk of 32%.

Table 35–3 **Graft Survival (%) in Patients Transplanted between 1999 and 2004**

	Living Donor			Deceased Donor		
Recipient Age (yr)	**1 yr**	**3 yr**	**5 yr**	**1 yr**	**3 yr**	**5 yr**
1-5	95	92	90	91	81	76
6-10	96	91	86	93	78	73
11-17	94	88	77	93	79	65

Data from Scientific Registry of Transplant Recipients (SRTR), Ann Arbor, Mich., 2006.

Sensitization also may result from rejection of a previous transplant. The 5-year graft survival rate for repeat deceased donor transplantation in pediatric patients is about 20% lower than for primary transplants.

Technical Factors and Delayed Graft Function

The surgical techniques of kidney transplant for older children and adolescents are similar to techniques used in adults (see Chapter 11). Placement of the vascular anastomosis depends on the size of the child and the vessels. An extraperitoneal approach usually is accomplished with the venous anastomosis done to the common or external iliac vein, and the arterial anastomosis done to the common or external iliac artery. These vascular anastomoses are more cephalad than what is usual for adult transplants.

Small children present difficult operative challenges. The relatively large size of the graft may result in longer anastomosis times, longer ischemia time, and subsequently higher rates of early graft dysfunction. When possible, the transplanted kidney usually is placed in an extraperitoneal location, although with very small children, the placement can be intra-abdominal. The aorta and inferior vena cava usually are used for anastomosis to ensure adequate blood flow, but smaller vessels may be used. Vascular anastomosis may be problematic in a child with a previous hemodialysis access placed in the lower extremities or with a previous kidney transplant. Children should be evaluated thoroughly by abdominal imaging before transplantation to identify any potential anastomotic difficulties. Unidentified vascular anomalies may lead to prolonged anastomosis times and subsequently higher rates of delayed graft function (DGF) and graft thrombosis.

Occasionally, native kidney nephrectomy is necessary at the time of transplantation. Although this operation can be done routinely in living donor transplantations where there is little cold ischemia time, it is preferable to avoid this, when possible, in recipients of deceased donor transplants. Native nephrectomy at the time of deceased donor transplantation often prolongs the surgical procedure and predisposes to "third spacing," which can complicate fluid management and contribute to an increase in DGF. Nevertheless, native nephrectomies are indicated as a staged procedure before transplantation for optimizing the recipient for transplantation, or at the time of transplantation for certain indications, as discussed subsequently.

DGF occurs in about 5% of live donor and 18% of deceased donor transplants and is associated with a reduced graft survival. In children with DGF (defined by the requirement for dialysis within the first week of transplantation), the 3-year graft survival rates are reduced by about 20% and 30% in recipients of deceased and live donor kidneys. In living donor transplants, risk factors for DGF are more than five prior transfusions, prior transplantation, native nephrectomy, and African-American race. In deceased donor transplants, cold ischemia time greater than 24 hours is an additional risk factor.

Antibody Induction

Antibody induction with either polyclonal or monoclonal antibodies is used for prophylaxis against rejection or in a sequential manner to avoid nephrotoxicity resulting from early use of calcineurin inhibitors. Although the NAPRTCS database continues to show a 13% to 14% reduction in the proportional hazard of graft loss in living and deceased donor transplantation, the effect of antibody induction has decreased over time. Evaluations of its use from registry databases are hampered by confounding variables and selection factors. In addition, the agents used for induction have changed markedly. In the United States, two commercially available monoclonal antibodies are directed against CD25 (the interleukin-2 receptor). When taken together, these are used in approximately 50% of all pediatric transplants done presently in the United States. Rabbit antithymocyte globulin (Thymoglobulin) is the most frequently used biological agent in pediatrics, with a frequency of approximately 20%.

Transplantation Center Volume

Transplant outcome in high-volume pediatric renal transplant centers has been reported to be superior to that found in lower volume centers. High-volume centers (defined by the performance of >100 pediatric transplants between 1987 and 1995) reported a lower incidence of graft thrombosis and DGF, improved long-term graft survival, and more frequent use of antibody induction.[143]

CONTRAINDICATIONS TO TRANSPLANTATION

There are very few absolute contraindications to kidney transplantation. Preexisting malignancy, especially with metastasis, precludes patients from transplantation. Nevertheless, patients with remission of malignancy, off maintenance treatment, for a minimum of 2 years may be reconsidered on an individual basis for transplantation and its incumbent immunosuppression, with the caveat that patients would require close post-transplantation surveillance. Similarly, patients with autoimmune diseases resulting in ESRD are candidates for transplantation after a period of immunological quiescence of the primary disease, usually defined as "burnout" of the original disease acuity, on minimal or no immunosuppression, for at least 1 year before transplantation. Patients with severe devastating neurological dysfunction may not be suitable candidates. The wishes of the parents and the potential for long-term rehabilitation must be considered, however.

RECURRENCE OF ORIGINAL DISEASE

Recurrent disease in the renal graft accounts for graft loss in almost 7% of primary transplantations and 10% of repeat transplantations.[8] On a percentage basis, this far exceeds the figure for adult transplantation, which is on the order of 2%. Glomerular and metabolic diseases can recur after transplantation, with most recurrences caused by glomerular disease. The most common causes of recurrence in children are discussed next.

Glomerular Diseases

Focal Segmental Glomerulosclerosis

FSGS is the most common cause of graft loss owing to recurrent disease.[162] In patients whose original disease was steroid-resistant nephrotic syndrome or confirmed FSGS, the disease recurs in 30% to 40% of patients undergoing primary transplantation. When the first transplant was lost to recurrence, FSGS recurs in 50% to 80% of patients undergoing

subsequent transplantation.[26,32,34,60,89,118,122,145,178] The NAPRTCS database has found that grafts in approximately 20% to 30% of patients with the diagnosis of FSGS fail because of recurrence. In patients with the original disease of FSGS whose grafts fail, the mean time to failure is 17 months.

Recurrence usually is characterized by massive proteinuria, hypoalbuminemia, and often the full-blown picture of nephrotic syndrome with edema or anasarca and hypercholesterolemia. It may present immediately or weeks to months after transplantation.

Predictors of recurrence include rapid progression to ESRD from the time of initial diagnosis (<3 years),[8,64,118,132] poor response to therapy, younger age at diagnosis (but >6 years old), African-American race, and presence of mesangial proliferation in the native kidney.[28,55,64,145] In recent years, a protein permeability factor has been isolated from sera of patients with FSGS, and its concentration was found to correlate with recurrence and severity of disease in the transplanted kidney.[140] The precise nature of this factor is unclear.[108,180] More recent data suggest that this factor is a protein, difficult to characterize, which is 30 to 100 kD in size. Paradoxically, isolates of this factor seem to be normal components of plasma. It has been suggested that this recurrence is actually due to an absence or loss of an inhibitor of a factor that is present in normal sera.[53,173]

Early post-transplant recognition of recurrent FSGS is important because plasmapheresis or high-dose calcineurin inhibitors or both may lead to significant reduction in graft losses owing to recurrent FSGS. In vitro studies using rat glomeruli have shown that cyclosporine or tacrolimus, incubated with sera from FSGS patients, inhibits the proteinuric effect of such sera. Thrice-daily cyclosporine may be used in doses that maintain whole-blood trough levels, as measured by fluorescence polarization immunoassay or enzyme-multiplied immunoassay technique, of 200 to 400 mg/mL or higher and tapered slowly after achieving remission of the nephrotic syndrome and as cholesterol concentration decreases, or if significant toxicity develops. Some centers have used high-dose continuous intravenous cyclosporine with similar improvement. Still others have used high-dose or thrice-daily tacrolimus. Each of these regimens has been associated with remission. Cyclophosphamide has been found to induce remission by some investigators. Plasmapheresis is generally used with a frequency that matches disease severity and occasionally is required on a weekly basis for prolonged periods.[66]

Living related donor transplant recipients have been reported in some studies to have a higher rate of recurrence. More recent registry data from NAPRTCS also have suggested that the graft outcome in recipients of living donor grafts with FSGS recurrence is no better than the outcome observed in recipients of deceased donor grafts who have not experienced recurrence. These data have led many pediatric transplant centers to reduce or discontinue the use of living related donation in patients with FSGS. The controlled settings of living donor transplantation may benefit patients with FSGS recurrence, however. Specifically, it has been suggested that repetitive pretransplant plasma exchange may preempt the immediate onset of recurrent nephrotic syndrome.[56]

Living donation may dramatically reduce the incidence of post-transplant DGF. In the setting of FSGS recurrence, it is important to avoid DGF so that the dose of cyclosporine or tacrolimus can be augmented. The preplanning implicit in living donation permits preoperative and early postoperative plasmapheresis. Our experience suggests that this approach may prevent or decrease the severity of recurrent disease,[127] but this approach must be tested in a controlled clinical trial. At our centers, the potential for recurrence of FSGS is not regarded as a contraindication to living donor transplantation.

Alport's Syndrome

Alport's syndrome, or hereditary glomerulonephritis, is a progressive disease often associated with neurosensory hearing loss and ocular abnormalities, such as anterior lenticonus and cataracts. Its inheritance pattern can be X-linked, autosomal recessive, or autosomal dominant. The abnormality in almost all patients stems from mutations in the α3, α4, or α5 helices of type IV collagen. In more than 80% of patients, Alport's syndrome results from mutations in the *COL4A5* gene on the X chromosome.

Strictly speaking, Alport's syndrome itself does not recur; however, anti–glomerular basement membrane (GBM) glomerulonephritis may occur in approximately 3% to 4% of patients after transplantation and lead to graft loss. The antibodies causing the anti-GBM nephritis are usually directed against the α5 chain of the noncollagenous portion of type IV collagen in the GBM, but antibodies against the α3 chain also have been described. The risk seems to be greatest in patients with mutations of *COL4A5* that prevent synthesis of the α5 chain.

Anti-GBM glomerulonephritis manifests as rapidly progressive crescentic glomerulonephritis with linear deposits of IgG along the basement membrane and most commonly leads to graft loss. It usually occurs in the first post-transplant year but does not have to occur in the early post-transplant period. Asymptomatic cases with linear IgG deposits also have been reported. This complication is rare. Treatment consists of plasmapheresis and cyclophosphamide, but such treatment is of only limited benefit. Retransplantation is associated with a high recurrence rate.

Membranoproliferative Glomerulonephritis

Histological evidence of recurrence of membranoproliferative glomerulonephritis (MPGN) type I varies widely, with reported rates of 20% to 70%. Graft loss occurs in 30% of cases.[63] There is no proven treatment for recurrence of MPGN type I in children. Anecdotal case reports describe success with high-dose corticosteroids, mycophenolate mofetil (MMF), or plasma exchange.

Histological recurrence of MPGN type II occurs in virtually all cases. Often this recurrence is benign, however, without causing graft dysfunction or loss. Some studies suggest that graft loss from recurrent MPGN type II may be 30% to 50% of cases.[4,39] In the 2000 NAPRTCS database, 78 patients with MPGN type II received allografts, and 24 (13%) of these grafts failed at a mean time after transplantation of 29 months. Ten (42%) of these grafts failed because of recurrent disease. Presence of crescents in the native kidney may predict severe recurrence that often leads to graft loss. As with MPGN type I, plasmapheresis, MMF, and high-dose corticosteroids have been reported to be beneficial in a few cases of recurrent MPGN type II. These cases are at best anecdotal, however, and full-blown recurrence with hematuria,

proteinuria, and graft dysfunction has a high likelihood of graft failure. This notwithstanding, it has been reported that after an initial graft failure from recurrence, subsequent allografts may not manifest this recurrence.[3]

IgA Nephropathy and Henoch-Schönlein Purpura

Histological recurrence with mesangial IgA deposits is common and occurs in about half of patients with IgA nephropathy and in about 30% of patients with Henoch-Schönlein purpura.[23,52,86,111,112] Most recurrences are asymptomatic, but graft loss may occur, often associated with crescent formation. Data from adult centers suggest that a fulminant presentation of IgA nephropathy as the original cause of ESRD predicts poor outcome in the transplanted kidney with disease recurrence. In the NAPRTCS database, only 5% to 8% of graft failures were due to recurrence in patients with IgA nephropathy or Henoch-Schönlein purpura nephritis.

Hemolytic-Uremic Syndrome

Hemolytic-uremic syndrome (HUS) accounts for 2.5% to 4.5% of primary renal disease in children leading to ESRD. In children, the most frequent form of HUS is diarrhea-associated (D^+), or "typical," and is caused by verotoxin-producing *Escherichia coli* (VTEC), usually O157: H7. This is the most common form of HUS in childhood, but it results in ESRD in only approximately 10% of cases. So-called D^- HUS is far less frequent in children. This is a heterogeneous group of entities that is characterized by (1) a prodrome that lacks diarrheal association (D^-), (2) a relapsing course, and (3) a very poor renal prognosis. Although rare (European prevalence of 3.3/1 million child population), this group is medically devastating.[179]

When considering transplantation in patients whose original cause of ESRD was HUS, care must be directed to the form of HUS that the patient had. The diarrhea-associated, or typical, form does not usually recur after transplantation, whereas atypical HUS has a high propensity for recurrence. There are pitfalls, however, in assessing recurrence of HUS. The D^+/D^- terminology sometimes can be misleading. Occasionally, patients with verotoxin-producing *E. coli*–associated HUS do not have diarrhea and may be mistakenly labeled as D^-. Similarly, diarrheal disease can trigger HUS in a patient who is genetically predisposed to HUS and erroneously be characterized as D^+ HUS. In addition, it has been known for decades that it may be difficult to distinguish antibody-mediated vascular rejection from recurrent HUS, which manifests histologically as thrombotic microangiopathy (TMA).

Finally, the calcineurin inhibitors, cyclosporine and tacrolimus, occasionally have caused TMA in the transplanted kidney. In some of these cases, there is a clinical picture that resembles D^- HUS. Despite these caveats, it is reasonable to conclude that D^+ HUS has a recurrence rate of less than 1%, whereas the recurrence rate in D^- HUS varies with different studies and can range from 20% to 73%.[18,27,41,120,167,179] When the reports are taken in aggregate, D^- HUS recurs in approximately 21%.

A review of the literature by Loirat and Niaudet[97] of verotoxin-producing *E. coli*–associated D^+ HUS in children suggests that not only is the recurrence rate surpassingly small but also that renal transplantation in children with this disease is not associated with an increased incidence of allograft failure. The use of cyclosporine in these D^+ patients also is not associated with a triggering of HUS recurrence.

As noted earlier, recurrence is frequent in patients with D^- HUS, or HUS without diarrheal prodrome. It had been previously recommended that at least 1 year of clinical quiescence occur before transplantation was attempted in patients with D^- HUS. More recent experience suggests, however, that a prolonged interval between initial HUS and transplantation does not reduce the risk of recurrence. It is difficult to ascertain the effect of calcineurin inhibition on recurrence of D^- HUS[97]; avoidance of cyclosporine or tacrolimus did not prevent recurrence and graft loss in two children with this condition.

Atypical HUS is a heterogeneous group of conditions with multiple pathogenic mechanisms; many of these are currently either poorly defined or undefined. Some forms of atypical HUS can be subdivided further based on the condition's pathogenesis or genetics. The definition of atypical HUS is only an operational one. Atypical HUS can clinically resemble thrombotic thrombocytopenic purpura. This latter entity is characterized by the absence or low activity of von Willebrand's factor cleavage protease ADAMTS13; this can result from a genetic mutation in the *ADAMTS13* gene or antibodies to ADAMTS13.[109] It has been shown that genetic defects of complement factor H, complement factor I, and membrane cofactor protein 1 production are associated with severe forms of atypical D^- HUS. Factor H deficiency induces continuous complement activation resulting in low C3 and C4 levels. This form of D^- HUS seems to have an associated rate of recurrence of greater than 50%.[97]

The patient and graft outcomes in recurrent atypical HUS are poor. In a report of a European registry, only 18% of patients had a successful transplant, and 73% have lost the graft.[179] The standard treatment for thrombotic thrombocytopenic purpura, in original kidney disease and in transplant recurrence, has been repetitive infusion of fresh frozen plasma, with or without plasma exchange.[131] High-dose fresh frozen plasma with plasma exchange also has been advocated in atypical D^- HUS with factor H deficiency.[97] Liver transplantation or combined liver-kidney transplantation has been attempted with mixed results,[126] but some success in a few patients.[133] The rationale for these approaches is that factor H is synthesized in the liver. The recurrence rate in the few reported patients with factor H gene mutations but normal factor H concentrations seems to be markedly less than in patients with factor H deficiency. Currently, the following tests are recommended for the workup of genetic disorders of complement regulation[179]:

1. Plasma C3 and a measure of the alternative pathway (e.g., C3d or C4—C4 is normal when the alternative pathway is involved)
2. Factor H concentration, Western blot
3. Factor H gene mutational analysis (this is done regardless of the results of a normal C3 or factor H concentration)
4. Membrane cofactor protein 1
5. Factor I
6. Acquired disorders of complement regulation (e.g., anti–factor H antibodies)

In children with D^- HUS and a presumed autosomal recessive inheritance, the risk of recurrence seems to exceed 60%. This risk is as high in children as it is in adults. The use

of cyclosporine or the type of donor (living related donor versus deceased donor) does not seem to affect the rate of recurrence. In patients with the putative autosomal dominant form of D^- HUS, the recurrence rate seems to be similar to patients with autosomal recessive D^- HUS.[97]

The diagnosis and management of recurrent HUS is made even more challenging by the clinical entity of TMA that may accompany the use of calcineurin inhibitors, such as cyclosporine or tacrolimus, in some patients. Other rarer causes in the post-transplant patient may include valacyclovir, viral infections such as parvovirus, human immunodeficiency virus, and possibly cytomegalovirus (CMV), and antibodies against the von Willebrand factor–cleaving metalloproteinase ADAMTS13. In calcineurin-associated TMA, pathological features may be localized only to the kidney without evidence of systemic hemolysis or thrombocytopenia in greater than 50% of cases. TMA in this situation typically manifests shortly after starting treatment with cyclosporine or tacrolimus but can occur at any time after transplantation. This form of TMA manifests with a decline in urine output, a decrease in the rate of decline in serum creatinine, or an elevated serum creatinine level, with or without hematuria or proteinuria. Because of the nonspecific clinical course, a renal biopsy may be necessary to confirm the diagnosis. The most important aspects of therapy are stopping the calcineurin inhibitor and starting plasmapheresis/fresh frozen plasma, in addition to augmenting the rejection prophylaxis regimen to compensate for the discontinuation of the calcineurin inhibitor.[114] Restarting cyclosporine or tacrolimus after recovery of graft function has been reported to be successful, but recurrent TMA rates are 20% to 30%. In some series, substitution of cyclosporine for tacrolimus (or vice versa) has been successful.

Living donor transplantation is not contraindicated in patients whose original disease was D^+ HUS. Living donor transplantation is not advocated for patients with D^- HUS[179] because of the high recurrence rate in such patients. In addition, it has been noted that some parental carriers of D^- HUS might not manifest the disease until later in life, and organ donation would put such carriers at excessive risk.

Anti–Glomerular Basement Membrane Disease

Anti-GBM disease is rare in children. A high level of circulating anti-GBM antibody before transplantation is thought to be associated with a higher rate of recurrence. A waiting period of 6 to 12 months with an undetectable titer of anti-GBM antibody is recommended before transplantation to prevent recurrence. Reappearance of anti-GBM antibody in the serum may be associated with histological recurrence. Histological recurrence has been reported in half of the cases, with clinical manifestations of nephritis in only 25% of these cases. Graft loss is rare, and spontaneous resolution may occur.

Congenital Nephrotic Syndrome

Congenital nephrotic syndrome occurs in the first 3 months of life. It can be classified as either congenital nephrotic syndrome of the Finnish type (CNSF) or diffuse mesangial sclerosis.

CNSF is an autosomal recessive disease that occurs as a result of a mutation in the *NPHS1* gene. Although it is seen most commonly in Finnish patients, it also is found in other countries.[40] The *NPHS1* gene is located on chromosome 19 and has as its gene product the protein nephrin. Nephrin is a transmembrane protein, which is a member of the immunoglobulin family of cell adhesion molecules. It is characteristically located at the slit diaphragms of the glomerular epithelial foot processes. More than 50 mutations of *NPHS1* have been identified in CNSF, but greater than 90% of all Finnish patients have one of two mutations—the so-called Fin major and Fin minor mutations.

Infants with CNSF are usually born prematurely and exhibit low birth weight and placentomegaly. CNSF manifests as heavy proteinuria, edema, and ascites, often in the first week of life and always by 3 months of age. Untreated, these children have malnutrition, poor growth, frequent infections, and thromboembolic complications. ESRD occurs invariably in mid-childhood. Corticosteroids do not ameliorate CNSF, but in mild forms, angiotensin-converting enzyme inhibition together with indomethacin may be successful.[72,85] The best therapeutic success has come from the approach of early dialysis, nephrectomy, and transplantation.

CNSF rarely recurs after transplantation, and most cases (approximately 25%) of nephrotic syndrome after transplantation are likely de novo. This nephrotic syndrome manifests with proteinuria, hypoalbuminemia, and edema that may start immediately or 3 years after transplantation. All of the patients with post-transplant nephrotic syndrome have been reported to have the homozygous Fin major genotype. Antibodies against fetal glomerular structures are found in most patients with post-transplant nephrotic syndrome, and antibodies to nephrin are found in more than 50%.[116] Approximately half of the patients with this nephrotic syndrome respond to steroids and cyclophosphamide, but in the patients who do not respond, the graft is usually lost.[51] Within the NAPRTCS database, vascular thrombosis and death with a functioning graft (mostly owing to infectious complications) occur in 26% and 23% of cases and account for higher rates of graft failure in this particular group.

Diffuse mesangial sclerosis can be found in isolated form or as part of Denys-Drash syndrome. The latter is a syndrome composed of progressive renal disease with nephrotic syndrome and diffuse mesangial sclerosis, Wilms' tumor, and male pseudohermaphroditism. Most patients with diffuse mesangial sclerosis have been found to have mutations of the *WT-1* gene located on chromosome 11p13.[80,142] Patients with diffuse mesangial sclerosis who have received kidney transplants have not been observed to develop nephrotic syndrome.

Membranous Nephropathy

Recurrence of membranous nephropathy is rare in children because it is unusual for membranous nephropathy to cause ESRD in children. The NAPRTCS database reports that of 7651 pediatric patients who developed ESRD since 1987, only 36 (0.5%) had membranous nephropathy as a diagnosis. In adults, some series have reported a recurrence rate of approximately 25%, with the clinical hallmark being proteinuria. Although some reports suggest that recurrence leads to graft dysfunction, other reports suggest that there is no effect on graft outcome. In the 500 transplants performed in pediatric patients at the Mattel Children's Hospital at UCLA, and a similar number at the Pediatric Kidney Transplant Program at Stanford University, a combined group of five had membranous nephropathy, and in each of those, we have observed recurrence of the biopsy picture, mild nephrotic syndrome, and stability of graft function.

De novo membranous nephropathy occurs more frequently. It affects less than 10% of all transplanted children. It usually manifests later (4 months to 6 years after transplantation) than recurrent membranous nephropathy, which usually becomes apparent within the first 2 years (the mean follow-up at the time of diagnosis is 10 months in de novo disease compared with 22 months in recurrent disease). The occurrence of de novo membranous nephropathy does not seem to affect graft outcome in the absence of rejection.

Systemic Lupus Erythematosus

In the pediatric transplant literature, recurrence of systemic lupus erythematosus had been considered rare, with minimal clinical sequelae. More recent data suggest that this is not the case. The NAPRTCS 2000 registry database showed only one graft failure from recurrence in 117 patients with systemic lupus erythematosus. Studies in adults have reported clinically significant recurrence, however, in approximately 10% to 30% of transplant recipients.[58] Recurrence and subsequent graft failure do not usually manifest until 4 to 7 years after transplantation; this is important because in pediatric nephrology, it is most common to observe lupus nephritis progress to ESRD in adolescence. Because it is standard clinical practice to defer transplantation until the systemic lupus erythematosus has become "quiescent" for at least 6 to 12 months,[59] it is likely that the patient with systemic lupus erythematosus who receives a kidney transplant in the pediatric transplant program may not experience recurrence until he or she transfers to an internal medicine nephrologist. Pediatric and adult transplant physicians have a unique opportunity to develop cooperative approaches in such areas as transplantation immunosuppression, clinical monitoring, and follow-up to examine which factors have an impact on recurrence.

C-ANCA–Positive and P-ANCA–Positive Glomerulonephritis

Cytoplasmic antineutrophilic cytoplasmic antibody (C-ANCA)–positive and perinuclear antineutrophilic cytoplasmic antibody (P-ANCA)–positive glomerulonephritis can recur in the transplanted kidney. Wegener's granulomatosis and pauci-immune glomerulonephritis recur in a few patients and can cause graft loss. Cyclophosphamide seems to be beneficial in the treatment of recurrent Wegener's granulomatosis. There is similar anecdotal experience with cyclophosphamide and corticosteroids in P-ANCA–positive pauci-immune glomerulonephritis, and a similar quiescent period of 6 to 12 months is desired before proceeding with renal transplantation.

Metabolic Diseases

Primary Hyperoxaluria Type I (Oxalosis)

Primary hyperoxaluria type I results from a deficiency or a mistargeting of hepatic peroxisomal alanine glyoxylase aminotransferase. Alanine glyoxylase aminotransferase is normally made only in the liver and excreted primarily by the kidney. Absence or functional deficiency of this enzyme leads to hyperoxaluria, renal deposition of calcium oxalate, kidney damage, and evolving renal failure. Deposition of oxalate occurs in virtually all body tissues, including the kidneys, myocardium, bone, retina, nerves, and blood vessels. Although most patients with primary hyperoxaluria type I experience renal insufficiency in the later first or second decade of life, approximately 10% develop ESRD in infancy; in these patients, the clinical picture can be quite debilitating in the absence of successful transplantation.

Renal transplantation alone does not correct the enzymatic deficiency, and graft loss is frequent in these cases because of oxalate mobilization from tissue deposits and subsequent deposition in the graft. Therapy with combined or two-stage liver-kidney transplantation has led to higher rates of success. The transplanted liver corrects the enzymatic deficiency and prevents further oxalate production. The well-functioning transplanted kidney excretes the mobilized plasma oxalate. Success of this approach is greatly facilitated by immediate renal allograft function with a good diuresis.

In practice, aggressive long-term hemodialysis before transplantation is used to decrease the patient's body oxalate load to safe levels, preventing as much as possible tissue oxalate deposition. Hemodialysis is superior to peritoneal dialysis. During this preparatory period, one aims to bring the plasma oxalate level to less than 50 mg/mL. Usually this goal is impossible, particularly in patients with the infantile form of primary hyperoxaluria type I, and, as a practical matter, the medical/surgical teams try to minimize dialysis and expedite transplantation.[104] At transplantation, a large donor kidney is used whenever possible to excrete vigorously the body oxalate burden. Early use of a calcineurin inhibitor is deferred until the serum creatinine decreases to 1 to 2 mg/dL. Until this reduction occurs, immunosuppression is accomplished with MMF, corticosteroids, and antibody induction. If early renal transplant dysfunction occurs, daily hemodialysis is continued. When good renal function is established, calcineurin inhibitor therapy can be initiated. In addition, post-transplant treatment may include pyridoxine, neutral phosphate, citrate, and noncalciuric diuretics. If possible, liver or combined liver-kidney transplantation early in the course of renal disease, preferably before the GFR becomes less than 20 to 25 mL/min/1.73 m^2, optimizes outcome and prevents severe complications of the disease that may lead to irreversible morbidity and handicap.

Nephropathic Cystinosis

Transplantation in children with cystinosis corrects the transport defect in the kidney but not other organs affected by the disease. Hypothyroidism, visual abnormalities, and central nervous system manifestations are not corrected by transplantation and require ongoing therapy with cysteamine and thyroid hormone. Cystine crystals can be found in the renal graft interstitium within macrophages of host origin. This does not result in recurrence of Fanconi's syndrome or graft dysfunction.

Methylmalonic Acidemia

Methylmalonic acidemia is a rare autosomal recessive inborn error of metabolism that typically manifests in infancy with recurrent episodes of metabolic acidosis, developmental delay, and failure to thrive. The disease course is complicated by the development of chronic tubulointerstitial nephritis progressing to ESRD in adolescence. Rare case reports have described good outcomes with combined liver-kidney transplantation[106,170] with liberalization of protein intake and improved quality of life after transplantation.

Sickle Cell Anemia

The graft survival rate in patients with sickle cell disease is low, with only about 25% of grafts functioning beyond 1 year after transplantation. The improvement in the hematocrit results in higher numbers of abnormal red blood cells, leading to sickling episodes in the renal graft.

Wilms' Tumor

The recurrence rate after kidney transplantation for patients who have been treated for Wilms' tumor is about 13%. Most patients who develop recurrences after kidney transplantation have been transplanted less than 2 years after therapy for their tumors. Factors associated with recurrence include incomplete tumor removal and metastasis.[117] Mortality for recurrent Wilms' tumor after kidney transplantation is approximately 80%. The recommendations are to wait at least 2 years after completion of therapy of Wilms' tumor before proceeding with kidney transplantation. Because of the high risk of developing Wilms' tumor, patients with Denys-Drash syndrome should undergo bilateral nephrectomy before transplantation.[83]

PRETRANSPLANTATION EVALUATION

Evaluation of the Potential Living Donor

The evaluation and preparation of a living donor for a child is essentially the same as for an adult. Generally, it is possible to consider an adult donor of almost any size for a child, no matter how young. Live donation from siblings usually is restricted to donors who are 18 years old, although the courts have given permission for younger children to donate under extraordinary circumstances.

Histocompatibility matching considerations are not different for pediatric recipients of kidneys from live donors. HLA-identical transplants are optimal and enable the lowest amount of immunosuppression to be used, minimizing steroid and other side effects. The first living donor for a child is most frequently a one-haplotype-matched parent. Siblings may become donors as they reach the age of consent. When considering transplantation from siblings, data suggest that kidneys from haploidentical donors with noninherited maternal HLA antigens fare better in the long term than do kidneys from donors with noninherited paternal HLA antigens.[25] Second-degree relatives and zero-haplotype-matched siblings also may be considered as donors. The excellent results of nonbiologically related live donor transplants do not depend on high degrees of HLA matching.

Evaluation of the Recipient

The evaluation of the potential pediatric transplant recipient is similar to that performed in adults, but because certain problems occur with more frequency in children, the emphasis may be different. It is important to establish the precise cause of ESRD in children whenever possible. Surgical correction may be required for certain structural abnormalities before transplantation. The precise cause of metabolic or glomerular disease also should be established if possible because of the possibility of post-transplant disease recurrence. Common medical, surgical, and psychiatric issues in pediatric transplant candidates are discussed next.

Neuropsychiatric Development

INFANTS

Infants with ESRD during the first year of life may have neurological abnormalities. These abnormalities include alterations in mental function; microcephaly; and involuntary motor phenomena, such as myoclonus, cerebellar ataxia, tremors, seizures, and hypotonia. The pathogenesis is unclear, although aluminum toxicity had been incriminated when aluminum-rich dialysates were in wide use. Preemptive kidney transplantation or institution of dialysis at the earliest sign of reduction in head circumference growth rate or developmental delay may ameliorate the problem. Psychomotor delay improves in many infants with successful transplantation, with a significant percentage of infants regaining normal developmental milestones. Tests of global intelligence show increased rates of improvement after successful transplantation.

OLDER CHILDREN

It is often difficult to assess to what extent uremia contributes to cognitive delay and impairment in older children. Uremia has an adverse, but often reversible, effect on a child's mental functioning, and it may often cause psychological depression.[102] It may be necessary to institute dialysis and improve the uremic symptoms before making a precise assessment of the child's mental function. Initiation of dialysis often clarifies the picture and permits progression to transplantation in situations in which it might otherwise have not seemed feasible. Severely retarded children respond poorly, however, to the constraints of ESRD care. A child with a very low IQ cannot comprehend the need for procedures that are often confusing and uncomfortable. In this situation, the family must be involved and supported in the decision to embark on a treatment course that does not include long-term dialysis or transplantation.

Seizures

A seizure disorder requiring anticonvulsant medication may be present in 10% of young pediatric transplant candidates. Before transplantation, seizures should be controlled, whenever possible, with drugs that do not interfere with calcineurin inhibitors, sirolimus, or prednisone metabolism. Carbamazepine reduces calcineurin inhibitor and prednisone levels, but its effect is not as strong as that of phenytoin (Dilantin) or barbiturates. Some of the more recently developed anticonvulsant agents do not interfere with immunosuppressive drug metabolism, but it is always wise to consider thoroughly all possible drug interactions. If it is necessary to use a drug that reduces immunosuppressive drug levels, a moderately augmented dose of prednisone may be given. The calcineurin inhibitor may need to be administered three times per day or the dose adjusted upward to achieve the desired trough levels, which should be monitored closely.

Psychoemotional Status

Psychiatric and emotional disorders are not by themselves contraindications to dialysis and transplantation; however, the involvement of health care professionals skilled in the care of affected children is mandatory. Primary psychiatric problems may be amenable to therapy and should not exclude children from consideration for transplantation.

Experience with psychotropic drugs, such as selective serotonin reuptake inhibitors, has been positive. As with antiseizure medications, it is important to recognize that certain drugs may interfere with the metabolism of some immunosuppressive medications. This interference has not been found to be an issue with the selective serotonin reuptake inhibitors citalopram, escitalopram and sertraline.

Nonadherence

Nonadherence is a particularly prevalent problem in adolescent transplant recipients and can be driven by myriad reasons.[129,147] Patterns of medication and dialysis compliance should be established as part of the transplant evaluation. Psychiatric evaluation should be performed in high-risk cases to identify preexisting risk factors. If noncompliance is identified or anticipated, interventions should be in place before transplantation; these should include social and psychiatric interventions, where possible. Psychosocial support systems must be identified and nurtured. Frequent medical and social work monitoring is crucial if the patient is to be rehabilitated medically and psychosocially to the point where the patient is a candidate for transplantation.[128] The best outcomes are achieved when there is close coordination between medical and mental health providers. It is particularly important for the transplant and dialysis teams to stay in close communication as they prepare the patient for transplantation.

Cardiovascular Disease

Children and adolescents are unlikely to have overt cardiovascular disease that requires invasive diagnostic workup. Hypertension and chronic fluid overload during dialysis may predispose to left ventricular hypertrophy, and severe hypertensive cardiomyopathy and congestive heart failure may supervene. Even at this late stage, kidney transplantation may be beneficial to cardiac function. Occasionally, the degree of cardiac compromise is so severe, however, that heart transplant must accompany kidney transplantation.

The importance of hypertension control in children with ESRD cannot be overemphasized. In performing the pretransplant evaluation, blood pressure profiles and dialysis management must be scrutinized carefully. In a child who is hypertensive and on dialysis, echocardiograms need to be examined every 6 months to 1 year to assess ventricular hypertrophy and valve competence. In patients who require multiple antihypertensive drugs, bilateral nephrectomy may be required before transplant.

Premature cardiovascular disease is a common feature of adults who have had childhood ESRD, and attention to "adult" cardiovascular disease risk factors in childhood may minimize long-term morbidity and mortality. It has been reported that the coronary vessels of young adult dialysis patients have significant premature calcification.[77,88] This calcification may be the harbinger of atherosclerotic lesions and focuses attention on control of calcium/phosphorus metabolism and hyperhomocysteinemia in the pretransplant period as a potential way to ameliorate post-transplant coronary heart disease.

Infection

COMMON BACTERIAL PATHOGENS

Urinary tract infections and infections related to peritoneal dialysis are the most common sources of bacterial infection in children with ESRD. Aggressive antibiotic therapy and prophylaxis of urinary tract infections in children may effectively suppress infection, although pretransplant nephrectomy occasionally is required for recalcitrant infections in children with reflux. Peritonitis and related infections with peritoneal dialysis are discussed later.

CYTOMEGALOVIRUS

The incidence of CMV infection increases with age, and young children are unlikely to have developed CMV seropositivity. CMV IgM and IgG levels should be obtained with the pretransplant evaluation, and these studies should be considered when planning post-transplant CMV prophylaxis.

EPSTEIN-BARR VIRUS

It is important to establish the Epstein-Barr virus (EBV) antibody status of the child. As with CMV, EBV infections and resultant seropositivity increase with age. Primary EBV infection, in the context of potent immunosuppression, may predispose to a particularly aggressive form of post-transplant lymphoproliferative disease.

BK VIRUS

Polyomavirus (BK virus) is a relatively new entrant into the post-transplant monitoring pool, and with the increasing use of potent immunosuppression combinations after transplantation, polyomavirus nephropathy is being increasingly recognized. This virus resides in the urinary tract, and its presence has been detected with sporadic pretransplant screens of the urine in patients immunosuppressed because of treatment of their primary disease. Donor-derived polyomavirus infection in the transplanted kidney also is a possibility for post-transplant dissemination (see Chapter 29).

IMMUNIZATION STATUS

Immunizations must be brought up to date whenever possible. Live viral vaccines are contraindicated in immunosuppressed patients. Every effort must be made to complete these vaccinations before transplantation, including measles/mumps/rubella (MMR) and varicella vaccination. Vaccination of the immunosuppressed host may fail to induce an adequate immune response, especially with the use of agents, such as MMF, that suppress antibody production.

Diphtheria and tetanus vaccine and hepatitis B vaccine can be given safely after transplantation, although pretransplant administration is preferred. *Haemophilus influenzae*–type b vaccine also is safe. Influenza and pneumococcal vaccines are recommended for pediatric transplant recipients. Most of the available data on their effectiveness come from transplant recipients treated with cyclosporine or azathioprine.[123] Studies are needed to address the immune responsiveness to vaccines under immunosuppression with newer agents.

Hemostasis

If a careful history yields any suggestion of hypercoagulability or hypocoagulability, a full clotting workup should be performed. Approximately 11% to 13% of graft loss in pediatric patients is due to graft thrombosis.[169] For this reason, it is particularly important to search for clues to a tendency to hypercoagulability. Such clues include clotting of hemodialysis access. In pediatric patients, a coagulation workup consists of the following: prothrombin time, partial thromboplastin

time, platelet count, protein S level, protein C level, activated protein C resistance (monitors for factor V Leiden), antithrombin III, G20210A prothrombin mutation, homocysteine level (5,10-methylene tetrahydrofolate reductase T677 mutation), antiphospholipid antibody,[92,166,174] anticardiolipin antibody, β_2-glycoprotein-1 level, lipoprotein (a), and factor VIII level.

Workup in Patients with Glomerulonephritis of Unknown Etiology

Pediatric patients often are referred for a pretransplant evaluation without having had the diagnosis of their ESRD established. As noted previously, recurrence of glomerulonephritis or glomerulopathy is a significant concern in pediatric and adolescent recipients. For this reason, any patient with significant proteinuria or hypertension accompanying ESRD should have serological tests that can help classify the diagnosis of ESRD. This testing includes C3, C4, antinuclear antibody, anti–single-stranded and anti–double-stranded DNA, and P-ANCA and C-ANCA.

Urological Problems

Children with ESRD as a result of urological diseases account for a significant proportion of transplanted patients. Obstructive uropathy is the cause of ESRD in nearly 16% of transplanted children. Other causes of ESRD that are commonly associated with abnormalities of the urinary tract, such as reflux nephropathy, neurogenic bladder, prune-belly syndrome, and renal dysplasia, account for another 20% of transplanted children.

The presence of an abnormal lower urinary tract is not a contraindication to transplantation. Urological problems are best addressed before transplantation. (See Chapter 12.) Malformations and voiding abnormalities (e.g., neurogenic bladder, bladder dyssynergia, remnant posterior urethral valves, and urethral strictures) should be identified and repaired if possible. Children with urological disease and renal dysplasia often require multiple operations to optimize urinary tract anatomy and function. Such procedures include ureteric reimplantation to correct vesicoureteral reflux, bladder augmentation or reconstruction, creation of a vesicocutaneous fistula using the appendix to provide a simple, continent, and cosmetically acceptable way for intermittent catheterization (Mitrofanoff procedure), and excision of duplicated systems or ectopic ureteroceles that may cause recurrent infections.

BLADDER AUGMENTATION

Urodynamic studies usually provide important information about bladder capacity and function, and help to define situations that require bladder augmentation. Bladders that have high intravesical pressures are at risk to produce serious hydronephrosis in a transplanted kidney. Bladder augmentation may be required in numerous patients with obstructive uropathies and some other select patients with small bladder capacity. Augmentation can be done using dilated ureter tissue, small intestine, or large intestine. Ureteric augmentation provides the best results because the ureteric mucosa is identical to the urinary bladder mucosa. Intestinal or colonic augmentation often requires frequent bladder irrigation and is often complicated by significant mucus secretion that can cause intermittent obstruction of the bladder stoma and lead to frequent urinary tract infections. Augmentation using gastric tissue causes severe dysuria because of the acidity of gastric secretions and has been abandoned in most centers.

After bladder augmentation, most children require long-term intermittent catheterization. Forceful hydrodilation as a substitute to bladder augmentation is used at some centers, but most clinicians agree that it is very painful and futile, especially in children awaiting deceased donor transplantation.

If a child has a neurogenic bladder, a bladder augmentation, or other voiding abnormality, it is usually possible to teach a parent or the patient clean, intermittent self-catheterization. This self-catheterization can be done in transplant recipients safely and successfully. Urinary tract infection may occur, however, when catheterization technique is poor. In addition, noncompliance with self-catheterization may lead to partial obstruction and subsequent graft dysfunction.

In some studies, graft outcome in children with urological problems is inferior to that in patients with normal lower urinary tracts.[2,110,168] In addition, in recipients with an abnormal bladder, there is an increased incidence of post-transplant urological complications and urinary tract infection. Nevertheless, in centers with skilled pediatric urologists, children with ESRD caused by urological malformations can be transplanted successfully. Excellent outcomes often can be achieved in posterior urethral valve bladders by following a staged procedure of initial valve resection to limit any injury to the posterior urethra and bladder rehabilitation, without the requirement of augmentation, by a process of regimented double voiding.[10]

Renal Osteodystrophy

Aggressive diagnosis and treatment of hyperparathyroidism, osteomalacia, and adynamic bone disease are important in the pretransplantation period. Control of hyperparathyroidism with vitamin D analogues, or even parathyroidectomy, may be required. Failure to control hyperparathyroidism may predispose to post-transplantation hypercalcemia and limit the growth potential of a successful transplant recipient. When evaluating pretransplant patients, the clinician must examine the trend in parathyroid hormone levels and serum calcium and phosphorus levels. We have designated an arbitrary cutoff of 500 for intact parathyroid hormone levels as acceptable in dialysis patients who are being considered for transplantation.

Children Receiving Peritoneal Dialysis

It has been generally accepted that children being treated with peritoneal dialysis have graft and patient survival rates that are similar to those of children receiving hemodialysis. A more recent retrospective study by the NAPRTCS concluded, however, that children treated with peritoneal dialysis are at significantly higher risk of graft thrombosis than children treated with hemodialysis or children who received preemptive transplants independent of the age of the transplant recipient.[99] The cause of this observation is unclear. In adults, there is increased production of coagulation factors in patients on peritoneal dialysis as a result of loss of albumin in the peritoneal fluid, similar to that seen in nephrotic patients. Center volume effect, which has been proposed as a risk factor for graft thrombosis, especially in deceased donor transplants, may be significant because most small-volume centers tend to rely more on peritoneal dialysis than on hemodialysis.

In contrast to the previously cited study, our experience suggests that peritoneal dialysis may facilitate transplant

surgery, especially in very young and small infants. Repeated peritoneal fluid cycling expands the abdomen and creates adequate space for extraperitoneal placement of the large adult kidney. Extraperitoneal placement of the graft is desirable because it may allow for continued peritoneal dialysis after transplantation in the event of DGF, and patients can tolerate oral feeds and medications sooner owing to minimal bowel manipulation. Intraperitoneal graft placement is not an absolute contraindication to post-transplant peritoneal dialysis, however, should it become necessary.

A recent episode of peritonitis or exit-site infection in a child awaiting a transplant does not preclude transplantation. Potential transplant recipients should be appropriately treated for 10 to 14 days and have a negative peritoneal fluid culture off antibiotic treatment before contemplating transplantation. In addition, the preoperative peritoneal cell count should not suggest peritonitis. If a chronic exit-site infection is present at the time of surgery, the catheter should be removed, and appropriate parenteral antibiotics should be administered. An overt tunnel infection should be treated before transplantation. The incidence of post-transplantation peritoneal dialysis–related infections is low.[96] Peritonitis and exit-site infection should be considered, however, in the differential diagnosis in any child with unexplained fever after transplantation, and early sampling of the peritoneal fluid should be pursued. Such infections typically respond to appropriate antibiotic therapy, although catheter removal may be necessary for recurrent infections. In the absence of infections, the peritoneal catheter may be left in place until good graft function has been established for 2 to 3 weeks.

Nephrotic Syndrome

In children with glomerular diseases, proteinuria usually diminishes as kidney function deteriorates and ESRD ensues. Occasionally, florid nephrotic syndrome may persist, particularly in children with focal glomerulosclerosis. Persistence of heavy proteinuria may cause a hypercoagulable state and increase the risk of graft thrombosis and thromboembolic complications at the time of surgery. In addition, the presence of the nephrotic syndrome can make fluid management difficult because of leakage of fluids into the extravascular space, which may lead to DGF and adversely affect graft outcome. Control of heavy proteinuria before transplantation is important and sometimes can be achieved with prostaglandin inhibitors, although renal embolization or bilateral laparoscopic nephrectomy may be required.

In a child with CNSF, unilateral or bilateral nephrectomy usually is performed early in the course of the disease to allow for better skeletal growth while on dialysis, and to prevent infectious and thromboembolic complications. Congenital nephrotic syndrome resulting from diffuse mesangial sclerosis usually requires early bilateral nephrectomy as part of the treatment of Wilms' tumor or its precursor commonly present at the time of diagnosis (Denys-Drash syndrome).

Nephrectomy

Nephrectomy is indicated in severely hypertensive patients in whom blood pressure control is suboptimal despite optimal fluid removal and use of multiple antihypertensive agents. Intractable urinary tract infection, in the presence of hydronephrosis or severe reflux, also may require nephrectomy before transplantation. Nephrectomy should be avoided if possible because leaving the kidneys in situ may facilitate fluid management during dialysis, an important consideration for small children in whom fluid balance may be tenuous. Nevertheless, in patients with high-output renal failure where the 24-hour urine volume is greater than 3 L/day, fluid management in the postoperative period may become difficult because of the demands of high fluid intake to support the perfusion of an adult-sized kidney in the infant recipient. Failure to maintain adequate perfusion of the adult-sized kidney, secondary to a "perfusion steal" by the native kidneys, results in a histological picture of "chronic" acute tubular necrosis[135] and a negative impact on graft function.[134]

Occasionally, nephrectomy is required to create adequate space for placement of the adult graft in a small infant. This is frequently the case in autosomal recessive polycystic kidney disease, where the enlarged kidneys occupy the abdominal cavity and may impair diaphragmatic movement causing respiratory difficulty.

Portal Hypertension

Portal hypertension may occur in certain forms of ESRD common in children, such as that resulting from congenital hepatic fibrosis, which may accompany autosomal recessive polycystic kidney disease and nephronophthisis. The manifestations of congenital hepatic fibrosis must be controlled. Esophageal varices require sclerotherapy or portosystemic shunting. If neutropenia and thrombocytopenia are present as a result of hypersplenism, partial splenectomy or splenic embolization may be required, although these are often able to be avoided.

Prior Malignancy

Wilms' tumor is the most common renal malignancy in children, and it is the principal malignancy producing ESRD in children. An analysis of NAPRTCS and U.S. transplant registries from 1987 through 2002 included 80 children with Wilms' tumor and 76 with Denys-Drash syndrome.[90] Among both groups, there was only one recurrent Wilms' tumor, and this led to death of the patient. Patients not transplanted but maintained on dialysis ($n = 13$) all died. A disease-free period of 2 years from the time of remission should be observed before transplantation. Premature transplantation also has been associated with overwhelming sepsis, which may be related to chemotherapy for the tumor. The presence of a primary nonrenal malignancy is not an absolute contraindication to transplantation, although an appropriate waiting time of approximately 2 years malignancy-free or in remission may be observed between tumor extirpation and transplantation.

Preemptive Transplantation

Nearly 25% of all pediatric transplantations performed between 1987 and 2002 proceeded without the institution of dialysis. Most of these transplants were from living donors. Thirty-three percent of living donor transplants and 13% of deceased donor transplants were performed preemptively. The rates of preemptive transplantation differ moderately for different age groups (20%, 24%, 28%, and 22% for recipients 0 to 2, 2 to 5, 6 to 12, and 13 to 17 years old). The incidence of preemptive transplantation also differs according to race and ethnicity. In white, African-American, and Hispanic recipients, the rates are 30%, 14%, and 16%.

Many studies suggest that there is a significant improvement in graft survival in patients who have not received pretransplant dialysis; this is true for pediatric and adult patients and seems to be true regardless of the estimated GFR before the preemptive transplant.

Nutrition

Poor feeding is a prominent feature of uremia in children. Aggressive nutritional support is essential. Early gastrostomy or nasogastric tube feeding is often employed to improve caloric intake and promote growth, especially in children started on dialysis therapy at a young age. Such aggressive nutritional therapy may allow infants to achieve the minimal weight to perform a transplant. Because of technical difficulty and a resultant possibility of graft loss, a weight of 8 to 10 kg is used as a target weight for transplantation at most centers. This weight may not be reached until 2 years of age, even with the most aggressive nutritional regimens. Transplantation in children weighing less than 5 to 8 kg has been successfully performed at some centers.

PERIOPERATIVE MANAGEMENT OF PEDIATRIC RENAL TRANSPLANT RECIPIENTS

Preparation for Transplantation

Living donor transplantation allows a pretreatment period with immunosuppression. MMF, 600 mg/m^2 twice daily, and prednisone, 0.5 mg/kg, may be used beginning 1 week before the transplant date in some centers. With the more recent advent and success of steroid avoidance regimens in pediatric renal transplantation,[138,139] steroids are being avoided completely for the transplant process. A final crossmatch is performed within 1 week of transplantation, and the patient is evaluated clinically to ensure that he or she is stable, and that there is no active infection. For living and deceased donor transplantation, a final set of laboratory tests is obtained at admission to detect any metabolic abnormalities that require correction by dialysis. Aggressive fluid removal is discouraged in the immediate preoperative period to reduce the risk for DGF.

The current immediate preoperative immunosuppressive regimen for transplant recipients at the Mattel Children's Hospital at UCLA combines an intravenous infusion of a humanized anti–interleukin-2 receptor monoclonal antibody and MMF. If the use of a calcineurin inhibitor is planned, it is not begun until after transplantation.

Intraoperative Management

Methylprednisolone sodium succinate (Solu-Medrol), 10 mg/kg, is given intravenously at the beginning of the operation in steroid-based immunosuppression protocols. Close attention is paid to blood pressure and hydration status in an attempt to reduce the incidence of DGF. Typically, a central venous catheter is inserted to monitor the central venous pressure throughout the operation. To achieve adequate renal perfusion, a central venous pressure of 12 to 15 cm H_2O should be achieved before removal of the vascular clamps; a higher central venous pressure may be desirable in the case of a small infant receiving an adult-sized kidney. Dopamine usually is started in the operating room at 2 to 3 μg/kg/min and increased as required and is continued for 24 to 48 hours postoperatively. It is used to facilitate diuresis and perhaps to effect renal vasodilation.

The mean arterial blood pressure is kept at greater than 65 to 70 mm Hg by adequate hydration with a crystalloid solution or 5% albumin and, if necessary, the use of dopamine at higher doses. Blood transfusion with packed red blood cells may be required in very small recipients because the hemoglobin may decrease as a result of sequestration of about 150 to 250 mL of blood in the transplanted kidney. Mannitol or furosemide or both may be given before removal of the vascular clamps to increase the effective circulatory volume and facilitate diuresis. Mannitol also may act as a free radical scavenger and, together with renal dose dopamine, is a crucial factor for minimizing ischemia-reperfusion injury in steroid avoidance regimens. After the transplanted kidney starts to produce urine, volume replacement should be immediately started with ½ normal saline. Occasionally, an intra-arterial vasodilator, such as verapamil, is used intraoperatively to overcome vasospasm that may impair renal perfusion.

POSTOPERATIVE MANAGEMENT

Fluid management in children must be particularly fastidious because of their small size. Urine output replacement with 0.45% or 0.9% normal saline is started in the recovery room and continued in the intensive care unit for 24 to 48 hours. In addition, insensible water losses are replaced with a dextrose-containing crystalloid. Potassium chloride may be added to the insensible water loss replacement if required. Dextrose is not added to the replacement solution and is used only as part of the insensible water loss replacement solution. Withholding dextrose in the urine replacement solution helps to prevent post-transplant hyperglycemia and osmotic diuresis. The lack of concentrating ability of the newly transplanted kidney accounts for obligatory high urine output that may be observed in the first few days after transplantation.

As the kidney function improves, and the serum creatinine levels decline close to normal values, urinary concentrating ability recovers, and urine output decreases from several liters per day to amounts that begin to match daily fluid intake. At this time, urine output replacement can be stopped, and daily fluid intake usually is set to provide about 150% to 200% of the normal daily maintenance needs, preferably administered orally.

Hypertension is commonly observed. Pain is an important cause of hypertension in the immediate postoperative period, and adequate analgesia may be all that is required to control blood pressure. Hypertension is rarely aggressively corrected in the immediate postoperative period to avoid sudden swings in blood pressure that may impair renal perfusion. Electrolyte disorders encountered early in the postoperative course are discussed elsewhere.

IMMUNOSUPPRESSIVE PROTOCOLS AND DRUGS

Figure 35-7 summarizes current trends in the use of the various immunosuppressive agents. Most pediatric renal transplant centers employ combination drug therapy consisting of a calcineurin inhibitor and corticosteroids with or without

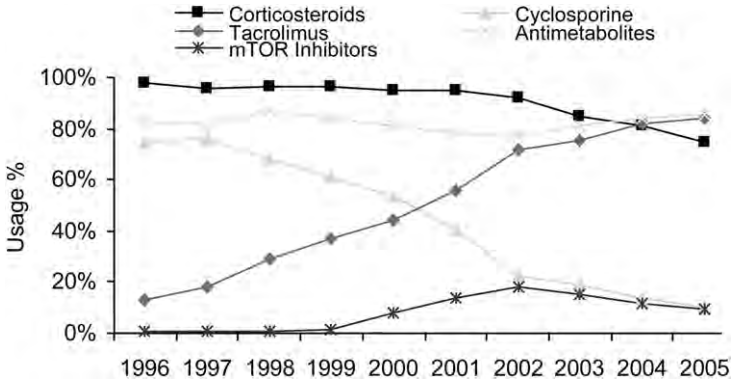

Figure 35–7 Maintenance of pediatric kidney recipients before discharge. (Data from Scientific Registry of Transplant Recipients: Preliminary Data: Draft 2006 AR, Special Analysis. Data as of May 1, 2006, Ann Arbor, Mich.)

an antiproliferative agent. In 2003, the NAPRTCS reported that approximately 80% of transplanted patients were receiving a three-drug regimen at 6 months after transplantation. The rationale for combination therapy in children is to provide effective immunosuppression while minimizing the toxicity of any single drug. Induction therapy with a biological agent is currently employed in approximately 60% of transplant recipients according to the latest NAPRTCS report.

In pediatric transplantation, the choice of the immunosuppressive regimen is usually center-specific, but individualization of therapy is often necessary to address the specific clinical circumstances. Induction therapy with an antilymphocytic agent can be used to provide adequate initial immunosuppression and allow delayed introduction of the calcineurin inhibitor in cases of DGF, or to provide intensified immunosuppression in a highly sensitized transplant recipient. When transplantation is contemplated in a child with prior malignancy, a two-drug regimen or even monotherapy may be considered to minimize the effect immunosuppressive drugs may have on immune surveillance. In this situation, the use of antibody induction is generally avoided, and living donation is encouraged to provide the best HLA matches. Tacrolimus may be preferred to cyclosporine when there is concern about medication nonadherence because of the cosmetic side effects of cyclosporine.

Central to many current pediatric immunosuppressive regimens is a calcineurin inhibitor (cyclosporine or tacrolimus) in combination with steroids and an adjunctive antiproliferative agent (azathioprine, sirolimus, or MMF). MMF is used as the adjunctive agent in more than two thirds of the pediatric kidney transplants performed. Sirolimus is used in 10% to 15%, whereas azathioprine is used in only about 2%. Corticosteroids continue to be used in approximately 80% to 85% of transplant recipients. There has been a steady increase, however, in the percentage of patients treated with steroid minimization or steroid avoidance protocols.

Corticosteroids

Corticosteroids remain an integral part of many immunosuppressive protocols despite their toxicity. (See Chapter 15.) The emergence of more powerful immunosuppressive agents has led to a dramatic improvement in acute rejection rates. Consequently, lower daily doses of steroids have come into use in pediatric renal transplantation.

In children, retarded skeletal growth is the most noteworthy side effect of corticosteroid usage. Concerns remain about familiar side effects, such as hypertension, obesity, diabetes mellitus, hyperlipidemia, osteopenia, and aseptic necrosis of bone (particularly the femoral heads). Cosmetic side effects, such as cushingoid facies and acne, are significant additional problems of long-term steroid use. Such side effects often tempt children and adolescents to stop taking their immunosuppressive drugs.[43]

Steroid withdrawal trials in children have been conducted with variable degrees of success. Many of these trials have been uncontrolled and anecdotal. Most patients reported have received cyclosporine as the maintenance immunosuppression, although more recent reports discuss steroid withdrawal under tacrolimus, MMF, or sirolimus immunosuppression. Generally, steroid withdrawal has led to improvements in blood pressure, lipid profiles, and statural growth. In the reports with cyclosporine as the base immunosuppression, the benefits of steroid withdrawal have been overshadowed by high rates of acute rejection occurring in 25% to 70% of children. Late acute rejections (>1 year in some cases) and graft loss have occurred with enough frequency to dissuade pediatric nephrologists from this approach. Even if graft loss does not occur, the acute rejections that have been reported impair skeletal growth as a result of the renal insufficiency that persists after the rejection and from the high doses of corticosteroids that have been used to reverse the rejection episodes.

Several investigators have reported single-center experience on the successful withdrawal of steroids using tacrolimus-based regimens.[67,81,95] Benefits of steroid withdrawal include skeletal growth in children. Long-term data on late rejection episodes and renal function are still lacking, however.

More recently, NAPRTCS conducted a controlled pediatric trial of steroid withdrawal using sirolimus (Rapamune). Although the incidence of acute rejection was low, the trial was halted prematurely because of a much higher than expected rate of post-transplant lymphoproliferative syndrome. Because of the paucity of data in controlled trials, and with an understanding of the damage that late rejection episodes can cause, prednisone continues to be used in many centers, with an increasing tendency toward the use of lower daily maintenance doses or alternate-day dosing. There are currently no reliable immunological or clinical indicators to predict in which pediatric transplant recipients steroids can be safely withdrawn.

Complete steroid avoidance is emerging as an alternative strategy to prevent steroid-associated morbidities in children. Some data suggest that the use of steroids may render the recipient sensitive to an immunological response on steroid withdrawal. Building on such observations, investigators at Stanford University have shown that complete steroid avoidance can be achieved successfully using tacrolimus in combination with MMF and an extended course of daclizumab. After a mean follow-up of 16 months, 48 patients treated with this protocol had an acute rejection rate of 4.6% versus 27.9% (P = .02) in historic controls treated with tacrolimus and steroids. Growth was significantly greater in the steroid-free group at 6 months and 1 year after transplantation. Patients 5 to 15 years old, classically reported to have poor improvement in growth parameters, also had better growth with steroid-free immunosuppression at 6 months and 1 year after transplantation. There was significant improvement in graft function in the steroid-free group, with mean GFR of 95 mL/min/1.73 m^2 in the steroid-free group versus 77 mL/min/1.73 m^2 in the control group ($P = 0.006$).

There is similar experience at Cincinnati,[151] where the steroid avoidance protocol from Stanford was effective and safe despite more African-American recipients and deceased donors included in the study. A National Institutes of Health–sponsored controlled randomized trial of such an approach is under way. Birkeland[16] reported a series of 100 transplants including 7 pediatric patients treated successfully with a largely steroid-free regimen, with some intraoperative and perioperative steroid exposure.[16] Acute rejection and graft survival rates were good using Thymoglobulin induction followed by maintenance therapy with cyclosporine and MMF. Finally, the Pediatric Nephrology Program at the University of Utah has used a short induction protocol with Thymoglobulin and maintenance immunosuppression with tacrolimus and MMF and achieved excellent results in a small group of pediatric transplant recipients.[156] Preliminary experience suggests that there may be many ways to accomplish steroid minimization and avoidance.

Calcineurin Inhibitors

Cyclosporine

Cyclosporine has been the cornerstone of most immunosuppressive regimens in pediatric kidney transplantation since the 1980s. (See Chapter 16.) When the idiosyncrasies of cyclosporine in children were mastered, its use was associated with a marked improvement in allograft outcome. Cyclosporine's popularity has decreased, however, in recent years (see Fig. 35-7). When it is used, cyclosporine microemulsion, rather than an oil-based formulation, is now used in virtually all patients. The replacement of the oil-based Sandimmune preparation with cyclosporine microemulsion has reduced many of the pharmacokinetic difficulties of cyclosporine in children of different ages.

Cyclosporine microemulsion has many advantages in pediatric transplantation. It is associated with an acute rejection rate of 20% to 40%, depending on the graft source and the adjunctive immunosuppressive agents that are used. Because of the long experience with this drug, the pediatric medical community is quite familiar with the pharmacokinetics, pharmacodynamics, and drug interactions of this drug. In addition, more recent studies have suggested that the pharmacokinetics of cyclosporine microemulsion can be assessed in pediatric patients regardless of their age by the use of C2 monitoring or abbreviated (limited sampling) pharmacokinetic analysis. It has been suggested that improvements in monitoring may result in a reduced incidence of rejection episodes. Trough level measurement is still used in many centers to guide cyclosporine therapy despite the lack of correlation between trough levels and drug exposure as measured by the area under the concentration-time curve (AUC). Abbreviated AUC_0 and C2 have been reported to have improved correlation with AUC.[35,74] In contrast to C2 monitoring in adults, the correlation with drug toxicity and efficacy has not yet been established using these methods.

In the past, there have been some important differences in the use of cyclosporine between adults and children. When Sandimmune was the formulation in use, children required higher doses than adults when calculated on a milligram-per-kilogram of body weight basis; this was especially true in children younger than 2 years old. This increased dosing requirement is believed to be due to a higher rate of metabolism by the hepatic cytochrome P-450 CYP3A4 and decreased gastrointestinal absorption. This increased dosing requirement is present with cyclosporine microemulsion, but it is far less pronounced than with the oil-based cyclosporine preparations. Dosing based on surface area, or thrice-daily dosing, seems to provide better therapeutic levels in smaller children and in children in whom metabolism is accelerated (e.g., patients receiving certain anticonvulsant medications). The reduced variability in drug levels and enhanced bioavailability seen with cyclosporine microemulsion may be particularly beneficial in children by permitting easier dosage reduction and monitoring, which may be reflected by a reduced incidence of rejection episodes.

The side-effect profile of cyclosporine in children is similar to that seen in adults, but the impact of these side effects on children is more pronounced. Hypertrichosis, gingival hyperplasia, and coarsening facial features may be particularly troublesome in children. We have observed gingival hyperplasia in 73% of pediatric patients on cyclosporine. Hispanic and African-American children seem to be at higher risk for significant hypertrichosis. In adolescents, especially girls, these side effects may cause severe emotional distress, possibly leading to dangerous noncompliance. Seizures, although uncommon, are observed more commonly in children treated with cyclosporine than in adults. Children, similar to adults, are likely to develop hypercholesterolemia and hypertriglyceridemia and may be candidates for lipid-lowering agents. Hyperglycemia is less common in children than in adults and occurs in less than 5% of children (<1% in some series) treated with cyclosporine.

Tacrolimus

Although tacrolimus is a more potent immunosuppressive agent, cyclosporine and tacrolimus have similar mechanisms of action, similar renal toxicity profiles, and generally similar efficacy. Of importance in pediatrics, the cosmetic side effects associated with cyclosporine are not seen with tacrolimus. The hyperlipidemia associated with cyclosporine and other immunosuppressive agents also is absent with tacrolimus. Post-transplantation glucose

intolerance, tremor, alopecia, and mild sleep disturbances are more common with tacrolimus. Historically, post-transplant lymphoproliferative disease has been significantly more common in children receiving tacrolimus, but with the reduced doses of tacrolimus that are currently in use, there is essentially no difference.

The mere lack of cosmetic side effects makes tacrolimus an attractive alternative in children and especially young adolescents and girls, in whom the cosmetic side effects can lead to dangerous noncompliance. Many centers have now adopted tacrolimus as the primary calcineurin inhibitor (see Fig. 35-7). (See Chapter 17.)

In contrast to cyclosporine, tacrolimus drug level monitoring in pediatrics is straightforward. Trough levels seem to correlate well with drug exposure. The pharmacokinetic drug interactions of tacrolimus are similar to those of cyclosporine. One notable difference is the effect of diarrhea on drug exposure. With cyclosporine, blood levels are reduced; in children and adolescents on tacrolimus, blood levels are elevated, sometimes remarkably so. As the diarrhea abates, the blood levels return to prediarrhea levels. If tacrolimus doses are modified because of the effect of the diarrhea, it is important to follow the levels closely as the diarrhea improves to avoid underimmunosuppression.

Direct comparative data in pediatrics between cyclosporine and tacrolimus are limited. Trompeter and coworkers[165] published the results of the only randomized controlled multicenter clinical trial in pediatric renal transplantation comparing these two agents. About 85% of the patients in this study received kidneys from deceased donors. Both treatment arms received prednisone and azathioprine in addition to either cyclosporine (93 patients) or tacrolimus (103 patients).

The overall acute rejection rates at 6 months were 59.1% for cyclosporine versus 36.9% for tacrolimus ($P = .003$). The differences also were significant for biopsy-confirmed acute rejection (16.5% versus 39.8%; $P < .001$). The incidence of corticosteroid-resistant rejection was significantly lower in the tacrolimus group compared with the cyclosporine group (7.8% versus 25.8%; $P = .001$). Numerically superior 1-year graft survival rates were observed in tacrolimus-treated patients, with 17 graft losses in cyclosporine-treated patients and 10 graft losses in tacrolimus-treated patients ($P = .06$). In the tacrolimus group, graft function (as determined by creatinine clearance calculations using the Schwartz formula) was better at 1 year after transplantation, with a clearance of 62 mL/min/1.73 m^2 versus 56 mL/min/1.73 m^2 in the cyclosporine group. The mean total steroid dose from time of transplant to 6 months after transplantation was significantly lower in the tacrolimus group (112 mg/kg versus 141 mg/kg; $P = .009$). The overall safety profiles of the two calcineurin inhibitors were equivalent, with essentially no difference in post-transplant lymphoproliferative disease or diabetes requiring insulin treatment.[165]

A retrospective analysis of the NAPRTCS database compared cyclosporine with tacrolimus when used in combination with MMF and prednisone.[107] In this study, 766 cyclosporine-treated patients were compared with 220 tacrolimus-treated patients. In contrast to the findings of the above-mentioned study, there was no difference between the two treatment groups with respect to the time to first rejection, the risk for rejection, or graft survival. These investigators concluded that, in combination with MMF and prednisone, both calcineurin inhibitors were equally effective in preventing acute rejection and facilitating graft survival at 1 and 2 years after transplantation. Graft function at 1 and 2 years after transplantation, as determined by the Schwartz formula, was significantly better in the tacrolimus-treated patients. In addition, the requirement for antihypertensive medications was higher in the cyclosporine-treated group.[107]

Adjunctive Immunosuppressive Agents

Adjunctive immunosuppressive agents are generally antiproliferative drugs that are deemed (correctly or incorrectly) to be unsuitable as cornerstone immunosuppression or monotherapy because of perceived shortcomings in potency, efficacy, side effects, or specificity directed against rejection. They are often used in combination with a calcineurin inhibitor and prednisone to reduce the incidence of acute rejection episodes. There has been a significant change in the use of these agents over the past 10 years, with mycophenolic acid (MPA) compounds replacing azathioprine.

Mycophenolate Mofetil

MMF is the morpholinoethylester prodrug of MPA, an inhibitor of de novo purine synthesis. MMF is part of the initial maintenance immunosuppression regimen in about two thirds of U.S. pediatric renal transplant recipients. It has largely replaced azathioprine, which in 2002 was used as initial therapy in less than 2%. (See Chapter 18.)

The capacity of MMF to reduce the incidence of acute rejection episodes relative to azathioprine is similar in children to that described in adults. According to the NAPRTCS database, deceased donor transplant recipients seemed to benefit most from MMF, with acute rejection rates of 18% compared with 60% for historical controls taking azathioprine. In living donor transplant recipients, the relative benefits of MMF were smaller. At the Mattel Children's Hospital at UCLA, the acute rejection rate using cyclosporine, MMF, and prednisone was 19% in 69 pediatric patients followed for a mean of 33 months, and in the steroid avoidance regimen at Stanford, on MMF and tacrolimus alone, the rate of acute rejection was 8% in 50 children followed for a mean of 44 months after transplantation.

In our experience, the rates of infectious complications and malignancy are comparable to children who did not receive MMF. The benefit of MMF in treatment of chronic allograft nephropathy in children has been evaluated on a limited scale with encouraging results. More data are required, however, before this strategy can be widely adopted. Similarly, MMF has been found to be successful in reversing steroid-resistant rejection in children who were not previously on MMF, but more data also are required for this use of MMF.

MMF has proved to be popular in pediatric renal transplantation for many reasons. An international multicenter open-label study that included 100 pediatric renal transplant recipients on MMF, cyclosporine, and prednisone found a 25% incidence of acute rejection in the first 6 post-transplant months, with an additional 4% in the next 6 months.[24] These and other data suggest that the acute rejection rates with MMF are approximately 20% to 30% when used with cyclosporine and corticosteroids. When MMF is used

with tacrolimus, humanized monoclonal antibodies to the interleukin-2 receptor, or both, lower rejection rates are usually seen. At many centers, the use of MMF has facilitated the use of a lower dose of corticosteroids after transplantation. It also has proved useful in calcineurin inhibitor–sparing protocols, wherein MMF is combined with sirolimus and corticosteroids.

The absence of nephrotoxicity, hyperlipidemia, and hepatotoxicity also has contributed to the usefulness of MMF. In children, as in adults, gastrointestinal and hematological side effects can be troublesome. Most of these instances can be treated with dosage reduction or brief discontinuation of the drug, with resumption after 7 to 14 days at a lower dose. Our first pharmacokinetic and safety and tolerability studies found that within the first 6 months of treatment with MMF, dosage reduction was most frequently necessary for diarrhea (37% of patients) and for leukopenia (30% of patients).[45] In the large multicenter study discussed earlier,[24] leukopenia was found in 22% of patients, diarrhea in 13%, infection in 10%, anemia in 6%, and abdominal discomfort in 5%.

Many of the side effects of MMF seem to be more frequent in younger children.[24] Diarrhea requiring an MMF dosage change in the first year after transplant occurred in 24% of children younger than 6 years old, 12% in children 6 to 12 years old, and only 3% in children older than 12 years old. Similarly, anemia was seen in 24% of the youngest patients and 12% and 6% of the older two groups. In this study, an infection of any kind was seen in 48% of the children younger than 6 years old, whereas infection was seen in only 32% of children 6 to 12 years old and 24% of children older than 12 years old. In all pediatric studies, the incidence of abdominal discomfort is usually underreported because the use of an H_2 blocker or a proton-pump inhibitor is virtually universal in pediatric patients receiving MMF.

In an attempt to improve the "window" for MMF in pediatrics, therapeutic drug monitoring of MPA has been attempted on a limited scale. The German study group on MMF therapy conducted a pharmacodynamic-pharmacokinetic study of MPA in pediatric renal transplant recipients treated with cyclosporine, MMF, and steroids.[175] This group found that the $AUC_{0\text{-}12}$ MPA value of less than 33.8 mg × hr/L was predictive of acute rejection with diagnostic sensitivity of 75% and specificity of 64%. The relative risk of acute rejection was 0.41 in patients with MPA $AUC_{0\text{-}12}$ values less than 33.8 mg × hr/L versus only 0.14 in patients with values greater than 33.8 mg × hr/L.

This group also reported that 12-hour trough levels could be used to monitor drug exposure and propensity to rejection. These trough levels were not as predictive as $AUC_{0\text{-}12}$ determinations, however. A 12-hour trough MPA level of 1.2 mg/L or lower also was predictive of acute rejection, with sensitivity and specificity of 83% and 64%; the upper bound for trough level monitoring has been identified as 4 mg/L. Although these values may allow clinicians to assess whether the MMF dose is in the therapeutic range, it has been impossible to correlate high total MMF levels with side effects. The only relationship that has been described is that between high free MPA AUC 1evels (as determined by high-performance liquid chromatography) and leukopenia. A value for the free MPA $AUC_{0\text{-}12}$ greater than 0.4 mg × hr/L predicted toxicity, with sensitivity and specificity of 92% and 61%.[175]

Therapeutic drug monitoring of MMF/MPA in children has been criticized because of the high interindividual and intraindividual variations that are present in these determinations. Nonetheless, some important dosing guidelines have emerged. The $AUC_{0\text{-}12}$ for MPA differs according to the other immunosuppressive agents that are used concurrently. In patients receiving cyclosporine, the MPA $AUC_{0\text{-}12}$ is reduced by 20% to 40%. Compared with the AUC that results when MMF is given alone or in conjunction with tacrolimus or sirolimus, it has been shown that cyclosporine may decrease the bioavailability of MPA in a dose-dependent fashion, owing to inhibition of MPA glucuronidation. Generally, the starting pediatric dose of MMF is 600 mg/m^2 given twice a day for patients on cyclosporine; in patients on tacrolimus or on no calcineurin inhibitor, the starting dose ranges from 300 to 400 mg/m^2 given twice daily. Dosing guidelines for combinations of MMF with other immunosuppressive agents, such as tacrolimus, are still not well defined for pediatric patients. Table 35-4 outlines current dosing guidelines in children.

It has been shown that corticosteroids can induce hepatic enzymes that control glucuronidation. Studies in adult renal transplant recipients suggest that the use of steroids with MMF is associated with reduced MPA exposure.[29] More data are needed to confirm this association, particularly because MMF is being used with increasing frequency for indications other than transplantation (e.g., glomerulonephritis, systemic lupus erythematosus, nephritic syndrome) in conjunction with corticosteroids. Our preliminary studies suggest that these interactions may not be as prominent in pediatric patients.

Table 35–4 Guidelines for Drug Dose Tapering in Pediatric Renal Transplant Recipients

Cyclosporine and Tacrolimus
Minimal or no change in the first 4 wk to allow for faster tapering of prednisone
Individual dose reduction should not exceed 10%-20%
Cyclosporine/tacrolimus and prednisone doses should not be reduced on the same day (risk of precipitating acute rejection)
Serum creatinine and cyclosporine/tacrolimus levels should be checked 2-3 days after each change and before the next change is made
Prednisone
Start tapering the dose 2-3 wk after transplantation if stable and cyclosporine/tacrolimus level is within desired range
Initial dose tapering is by 2.5 mg each time, about 10% (may reduce by 5 mg if total dose is >2 mg/kg); when a 10-mg dose is reached, dose reduction is by 1 mg each time
Longer periods should elapse before further tapering at the lower dose range
Cyclosporine/tacrolimus and prednisone doses should not be reduced on the same day
Serum creatinine and cyclosporine/tacrolimus levels should be checked 2-3 days after each change and before the next change is made
Mycophenolate Mofetil
Dose reduction is indicated only if hematologic or gastrointestinal side effects develop
Dose reduction is done in 30%-50% increments
Mycophenolate mofetil can be safely withheld for a few days up to 2-3 wk for severe side effects

More recently, a new formulation of MPA has been introduced. This formulation is an enteric-coated MPA. This enteric-coated MPA has been shown to decrease the upper gastrointestinal side effects of MMF in adult transplant recipients. Data on this formulation are limited in pediatric patients. We have studied the single-dose pharmacokinetics of this agent in 24 pediatric kidney transplant recipients on cyclosporine microemulsion and prednisone for a minimum of 6 months after transplantation. We found that a dose of 450 mg/m^2 yielded an AUC of MPA that was comparable to that found with a dose of MMF at 600 mg/m^2.[44]

Sirolimus

Sirolimus, an inhibitor of the mammalian Target of Rapamycin (mTOR), is used primarily as an adjunctive immunosuppressive agent in combination with a calcineurin inhibitor. (See Chapter 19.) It is used in approximately 10% to 15% of pediatric renal transplant recipients (see Fig. 35-7). Preliminary experience with sirolimus in pediatric renal transplantation is encouraging. In a single-center, open-label study, the rate of acute rejection was quite low at 1-year after transplantation in 20 pediatric renal transplant recipients treated with sirolimus, tacrolimus, and prednisone in addition to induction with basiliximab.[42] Limited anecdotal experience with sirolimus as a rescue agent in cases of refractory acute rejection, chronic allograft nephropathy, calcineurin inhibitor nephrotoxicity, and post-transplant lymphoproliferative disease has been promising.[152] Optimal dosing is still being investigated, however.

The pharmacokinetics of sirolimus in children has been only incompletely delineated. The data that have been emerging suggest that young children have a more rapid apparent clearance, reduced AUC, and shorter half-life of sirolimus than do adolescents and adults.[160] Limited data on the use of sirolimus without calcineurin inhibitors suggest that higher doses (corrected for body surface area) and more frequent dosing are appropriate in children; the mean drug half-life was approximately 12 hours in pediatric patients, in contrast to a half-life of 96 hours in adults. All of these data suggest that twice-daily dosing may be advisable in pediatric renal transplant recipients. Preliminary data in pediatric patients also suggest that the correlation is good between 12-hour trough concentrations of sirolimus and AUC, suggesting that therapeutic drug monitoring is an appropriate way to adjust dosage.

There is no consensus on the starting dose of sirolimus in pediatric patients, but studies suggest that body surface area should be used to determine the dosing.[46,141] An examination of the existing reports has suggested initial dosing at a range of 1.15 to 6 mg/m^2/day. The dose can be modified on the basis of 12-hour trough levels. We have not found a need to load the patient with a large dose at the outset of therapy but have attempted to keep 12-hour trough concentrations in the range of 5 to 12 ng/mL.

Everolimus

Everolimus, another mTOR inhibitor, has been studied in renal transplant recipients. Its use has been approved in Europe and some parts of South America, but as of this writing, it has not been approved for use in the United States. Limited studies have been performed in pediatric renal transplant recipients. In an initial pharmacokinetic study, the apparent clearance of everolimus in pediatric patients was lower than that in adult patients, probably because of a small apparent distribution in the children, rather than because of a difference in the elimination half-lives.[94] An open-label study in 19 pediatric renal transplant recipients showed three acute rejection episodes in the first 6 post-transplant months. The initial dose of everolimus was 0.8 mg intramuscularly twice daily with a maximal dose of 1.5 mg twice a day.[73] As with sirolimus, therapeutic monitoring seems to be crucial for individualizing everolimus exposure, assessing regimen adherence, and adjusting doses as the child matures.

Induction Therapy Agents

NAPRTCS has consistently reported better graft survival rates in patients treated with antilymphocyte induction therapy. In pediatric deceased donor transplantation, NAPRTCS registry data report that there is close to a 10% advantage in the 5-year graft survival rate when antibody induction is used. Acute rejection episodes are about 30% less frequent and tend to occur later. These data are subject to the caveat that, as registry data, they do not represent randomized controlled trials but only a historical reporting of experience.

The use of antibody induction therapy in pediatrics has increased dramatically since 1997 (Fig. 35-8). Most of this increase seems to be due to the use of the humanized or chimeric monoclonal anti-CD25 antibodies. The use of OKT3 as an induction regimen in pediatrics has been waning over the past decade, in part because of the undesirable side effects that accompanied its use and its perceived lack of efficacy. In 1997, OKT3 was used in only approximately 15% of new pediatric recipients, and that figure was reduced to virtually 0% since 2000. Thymoglobulin, the rabbit polyclonal antithymocyte antibody, was the most used agent in 2005 (see Fig. 35-8). The humanized or chimeric monoclonal

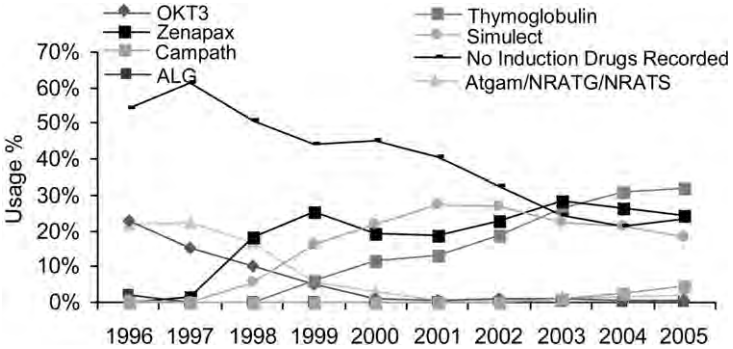

Figure 35–8 Immunosuppression use for induction in pediatric kidney recipients. (Data from Scientific Registry of Transplant Recipients: Preliminary Data: Draft 2006 AR, Special Analysis. Data as of May 1, 2006, Ann Arbor, Mich.)

anti-CD25 antibodies, daclizumab and basiliximab, are the most popular agents when taken together.

There does not seem to be a clinical benefit to induction with OKT3 in pediatric kidney transplant recipients. A retrospective analysis of the NAPRTCS database comparing the different induction agents showed that the relative risks of acute rejection and graft failure at 1-year after transplantation were significantly higher in OKT3-treated patients compared with patients treated with an anti-CD25 antibody or no induction.[14,113,155] Graft function at 1-year after transplantation was significantly better in patients treated with either anti-CD25 antibody compared with OKT3. In addition, in a multicenter collaborative trial, there was no advantage in either rejection frequency or graft survival between induction with OKT3 and cyclosporine.[14]

Biological immunological agents in major use today in pediatric renal transplantation are the monoclonal antibodies daclizumab and basiliximab, and the polyclonal antibodies Thymoglobulin and, to a lesser extent, antithymocyte globulin (Atgam) (see Fig. 35-8). There also is growing experience with alemtuzumab with excellent results.[9,91] The anti-CD25 monoclonal antibodies may be beneficial in children because of their effectiveness, ease of administration, and absence of side effects. In addition, they are unique in that they target only activated T lymphocytes and, theoretically, should not cause overimmunosuppression. The Cochrane Library has authored a meta-analysis of all published trials (primarily in adult kidney transplantation) on induction with humanized monoclonal anti-CD25 antibodies.[176] This meta-analysis found that the use of these agents, when added to standard therapy, significantly decreased the incidence of acute rejection episodes and steroid-resistant rejection episodes. There were no differences when comparing the efficacy of basiliximab and daclizumab. Anti-CD25 antibodies were equally as effective as other monoclonal and polyclonal antibodies in preventing acute rejection but were associated with a significantly lower incidence of adverse side effects. This latter point is important in children because they tolerate the adverse effects of many biological preparations poorly.[1]

A novel extended use of daclizumab for 6 months after transplantation, instead of its standard 2-month induction usage, has allowed for successful steroid avoidance in children,[138] with a very low incidence of acute rejection, lower than with steroid-based programs (8% versus 28%; $P < .001$), and without any incumbent burden of increased infections or post-transplant lymphoproliferative disease. The novel double pretransplant dose of 2 mg/kg also is likely to contribute to the very low rate of acute rejection seen with this protocol.

The two polyclonal antilymphocyte preparations in current use for induction in pediatrics are equine antithymocyte globulin (Atgam) and rabbit antithymocyte globulin (Thymoglobulin). Both of these agents have been shown to produce similar suppression of CD3-bearing, CD4-bearing, and CD8-bearing T cells in pediatric patients, although the suppression with Thymoglobulin may be more profound and long lasting. The lymphocyte-depleting effects of Thymoglobulin used as induction therapy in pediatric patients may last many months. In pediatric patients, lymphocytes are suppressed effectively for long periods with Thymoglobulin without increasing the risk of viral infection.

In a more recently published observational study, Thymoglobulin induction seemed to be safe and effective in preventing acute rejection episodes in the short-term in pediatric recipients.[20] Thymoglobulin has been reported to be more effective than Atgam for rejection prophylaxis (in addition to rejection reversal) in adult transplant recipients. At the Mattel Children's Hospital at UCLA, we have successfully used Thymoglobulin during periods of post-transplant graft dysfunction, when the nephrotoxic effect of calcineurin inhibitors makes either cyclosporine or tacrolimus challenging to use. (See Chapter 20.)

Donor Bone Marrow or Stem Cell Infusion and Renal Transplantation

Current immunosuppressive agents and regimens are highly effective in preventing acute rejection. Improvement in acute rejection rates has not been met with significant improvement in long-term graft survival, however. Adverse effects of immunosuppression, particularly calcineurin inhibitor nephrotoxicity, are largely responsible for this dissociation between the improved acute rejection rates and lack of improvement in long-term graft survival. Donor-specific blood transfusion has been shown to be effective in preventing acute rejection and improving long-term graft outcome. Infusion of donor bone marrow–derived stem cells has been shown to create a state of donor-specific immune tolerance, with the ability to withdraw or minimize immunosuppression at variable intervals after transplantation.

Trivedi and coworkers[164] reported their experience with high-dose peripheral blood stem cell infusion. Twenty-four pediatric renal transplant recipients who received peripheral blood stem cell infusion and treatment with cyclosporine and low-dose prednisolone (experimental group) were compared with 20 patients treated with cyclosporine, azathioprine, and prednisolone and no stem cell infusion (control group). In the experimental group, no acute rejection episodes occurred compared with 25% in the control group, and they had superior graft survival and function after 18 months of follow-up. Prednisolone was successfully discontinued in the experimental group without inducing acute rejection. Such tolerance-inducing protocols are promising and are especially important in pediatric patients; however, the choice of the initial immunosuppressive regimen, the timing of drug withdrawal, and the significance of chimerism in this setting need further study.

ACUTE REJECTION IN PEDIATRIC TRANSPLANTATION

Acute rejection episodes in pediatric renal transplantation account for about 15% of graft failures. With today's standard immunosuppressive therapy, an acute rejection episode is experienced in about 27% of recipients of living donor transplants and 31% of deceased donor recipients. The first rejection episode occurs within the first 3 months after transplantation in about half of patients, with higher frequency and earlier recurrence in recipients of deceased donor transplants. African-American race, DGF, and poor HLA matching may predispose to rejection episodes. In children, as in adults, acute rejection (particularly late acute rejection and multiple acute rejection episodes) is the most

important predictor of chronic rejection. Acute rejection precedes graft failure from chronic rejection in more than 90% of cases. Chronic rejection is the most common cause of graft loss in children.

Diagnosis of Acute Rejection

Diagnosis of acute rejection in very young transplant recipients is often not straightforward. Because most small children are transplanted with adult-sized kidneys, an elevation in serum creatinine may be a late sign of rejection as a result of the large renal reserve compared with the body mass. Significant allograft dysfunction may be present with little or no increase in the serum creatinine level. One of the earliest and most sensitive signs of rejection is the development of hypertension along with low-grade fever. In children, any increase in serum creatinine, especially if accompanied by hypertension, should be considered a result of acute rejection until proved otherwise.

Late diagnosis and treatment of rejection are associated with a higher incidence of resistant rejections and graft loss. More recent genomic studies in pediatric renal transplantation have shown molecular heterogeneity for different acute rejection episodes, not distinguishable by pathological grading, with a key evolving role for B cells as antigen-presenting cells for aggressive T cell–mediated, recalcitrant rejection.[136] These studies emphasize the need for mechanistic counterparts to ongoing clinical trials in organ transplantation, to study better and identify surrogate markers for monitoring diagnosis and prognosis of acute rejection.

The differential diagnosis of acute allograft dysfunction in children is similar to that in adults. Renal biopsy is the "gold standard" for diagnosis. The procedure has been shown to be safe in pediatric patients,[172] with a very low complication rate. In our practice, desmopressin acetate (DDAVP) is given 1 hour before the procedure in any child with even mild allograft dysfunction to correct any potential bleeding tendency; the dose is 0.3 μg/kg given intravenously. Urinalysis and urine culture, viral cultures and polymerase chain reaction (PCR) studies, and ultrasound and radionuclide imaging studies are used to diagnose other causes of graft dysfunction and should be performed without delay before allograft biopsy.

The role of a protocol biopsy specimen is still not well established, although data in children suggest that graft outcome may be improved by detecting early pathology.[15,144,149] Additionally, this biopsy specimen may give valuable information for monitoring for drug nephrotoxicity.[138]

Treatment of Acute Rejection

The techniques used to treat acute rejection are similar in children to the techniques used in adults. Complete reversal of acute rejection, as judged by a return of the serum creatinine level to baseline, is achieved in about half of children; 40% to 45% achieve partial reversal, and graft loss occurs in the remainder. Complete reversal from acute rejection is even less likely with late rejection episodes (>1 year after transplantation) or with repetitive rejection episodes. In past years, younger transplant recipients were at higher risk for graft loss from acute rejection; with the current knowledge of immunosuppression, younger children do as well as adults after treatment for acute rejection.

Corticosteroids

In children, as in adults, high-dose corticosteroid pulses are the first line of treatment of acute rejection, and about 75% of episodes are responsive to treatment. After the diagnosis is made, intravenous methylprednisolone is given in doses that range from 5 to 10 mg/kg/day for 3 to 5 days. After completing therapy, the maintenance corticosteroid is resumed at the prerejection level or is increased and then tapered to baseline levels over a few days. The serum creatinine level may increase slightly during therapy and may not return to baseline until 3 to 5 days after therapy is completed.

Thymoglobulin

The polyclonal rabbit antithymocyte globulin, Thymoglobulin, may be used to treat steroid-resistant rejection. Thymoglobulin has been shown to be effective in the reversal of steroid-resistant rejection in adults, and this has been reported more recently.[57] Thymoglobulin can be used successfully in children to treat rejection even if the patients received it for induction therapy.[68] It is usually used at an intravenous dose of 1.5 mg/kg.

Refractory Rejection

Refractory rejection usually refers to episodes of acute rejection that do not respond to, or recur after, treatment with high-dose corticosteroids. There is no standard of treatment for such rejections in pediatric renal transplantation. With increasing experience with new immunosuppressive medications, the treatment for refractory rejection usually is tailored to the patient's previous immunosuppression under which the rejection developed and the severity of the rejection episode. In patients who are receiving cyclosporine-based immunosuppression, tacrolimus can be substituted for the cyclosporine, and the MMF can be adjusted into the optimal range. About 75% of refractory rejection episodes can be reversed by switching to tacrolimus and adding or adjusting MMF. High doses and trough levels may be required to reverse the rejection adequately. Sirolimus is now another potential treatment option, although experience with this drug for refractory rejection is limited.

If the refractory rejection episode is severe, OKT3 or Thymoglobulin may be required. Both agents are equally effective, but OKT3 is associated with more severe acute side effects. Alemtuzumab also has been used and is well tolerated, but the experience is small. If a renal biopsy specimen shows that the refractory rejection has a component of humoral rejection (as manifested by positive staining for C4d or the presence of donor-specific antibody in the peripheral blood), empirical therapy with a regimen that has efficacy against antibody generation may be indicated. There are reports of success using high-dose intravenous immunoglobulin, humanized monoclonal antibody against CD20 (rituximab), and plasma exchange. These experiences are largely anecdotal; however, controlled trials need to be conducted in pediatric transplantation.

Whenever aggressive therapy for refractory rejection is employed, the risk for opportunistic infections and post-transplant lymphoproliferative disease increases. This is particularly true for pediatric patients, who are often seronegative against opportunistic illnesses. *Pneumocystis jiroveci* viral prophylaxis and infection surveillance are crucial (see Chapter 29).

NONADHERENCE IN PEDIATRIC TRANSPLANTATION

Nonadherence with immunosuppressive medications is one of the most important and, at the same time, one of the most elusive problems facing the medical team. By one estimate, using as an assessment direct reporting to the medical team, at least half of the pediatric deceased donor transplant recipients exhibited significant medication nonadherence in the post-transplantation period.[87,103] This figure exceeded 60% in adolescents.[47,147] Because direct reporting of nonadherence may significantly underestimate its true incidence, this analysis points out the potential magnitude of the problem.

The frequency of consequences of medication nonadherence also is difficult to assess because of the imprecision of the diagnosis of nonadherence. Nonadherence seems to be the principal cause of graft loss in 10% to 15% of all pediatric kidney transplant recipients; for retransplanted patients, this figure may exceed 25%.[12,17,30,47,87,93,103] Reversible and irreversible episodes of graft dysfunction related to noncompliance occur in 40% of adolescents and are less frequent in younger children.

Risk factors that suggest an increased propensity toward medication nonadherence include female sex, adolescent age, family instability, insufficient emotional support, lower social economic class, and maladaptive behavior.[12] In addition, factors related to the health team and health care delivery may contribute to nonadherence, such as lack of continuity of care, lack of communication between the health care provider and the patients or their families, and degree of mutual trust and satisfaction between the health care team and the patient.[12,177]

Patterns of medication nonadherence vary from partial compliance to complete noncompliance. Partial compliance ranges from the occasional missed dose to an occasional extra dose. It is most commonly the result of forgetfulness, distractions, misunderstanding of a dose change or modification, or the presence of events that lead to the belief that medications are not helping. In children and adolescents, complete nonadherence is often the result of underlying emotional or psychosocial stress in the patient, the caregivers, or both.[129]

Measuring Adherence

Currently available methods to measure adherence are crude and provide only a general estimate. The easiest method is asking patients directly about their compliance. Patients tend to tell physicians what they want to hear, however. Assessments made by patients of failure to take medications are often accurate, whereas denials of noncompliance are not. Serum drug level monitoring is helpful only when the drug level is either inexplicably low or inexplicably high.

Other methods to measure nonadherence include pill counts[12] and assessment of prescription refill rates. A continuous microelectronic device, usually attached to the cap of the medication bottle, records each opening of the bottle as a presumptive dose and records the time and frequency of taking the medication. Recorded data can be retrieved, and an assessment of compliance can be made. Data using this device have been reported in renal transplant recipients.[17] Studies strongly suggest that acute rejection episodes occur when "drug holidays" are prolonged.

Predicting Compliance

Pretransplantation prediction of post-transplantation noncompliance is difficult. Risk factors include a disorganized family structure, female sex, adolescence,[12] and a history of previous graft loss owing to noncompliance. Personality problems related to low self-esteem and poor social adjustment are found with higher frequency in noncompliant patients. A linear decline in compliance rates has been shown with increasing number of doses per day. Frequent clinic visits may improve compliance. Noncompliance in children must be suspected when there are unexplained swings in graft function or trough blood levels of the immunosuppressive agents. When higher doses of corticosteroids were used, changes in cushingoid features or sudden unexplained weight loss were indicators of potential nonadherence, but with newer immunosuppression regimens with less or no steroids, these findings have become less reliable.[93]

Strategies to Improve Compliance

Education, attention to planning the dose regimens, clinic scheduling, communication, and involving patients in medical management are the main strategies to improve compliance. The child and family members should know that the physician is their advocate and is interested in how they take their medications. This knowledge implies that the medical team and the patient and family have a shared health belief system.

Providing patients with specific reminders or cues to which the medication can be tied can be helpful.[129] These cues should be simple and preferably part of the patient's daily activities, such as meal times, daily rituals, specific clock times, a certain television program, tooth brushing, or shaving. Contracting with pediatric patients and rewarding them is another strategy to enhance compliance. Finally, asking the same questions about compliance each visit and explaining the consequences of noncompliance repeatedly reinforces the compliance message and physician interest.

Despite all of these measures, the medical team must be prepared to concede that these strategies may prove insufficient, especially over time, as the frequency of scheduled post-transplant visits diminishes. It is incumbent on the transplant team to maintain support and vigilance as the post-transplant patient transitions into new developmental stages.

Psychological Intervention to Improve Compliance

Behavior modification programs and other means of psychological intervention may be beneficial in some patients, particularly in light of the maturing process from childhood to adolescence and then to young adulthood. In the pretransplantation period, the high-risk patients must be identified, and an ongoing program of counseling should be undertaken. Clearly defined therapeutic goals should be set while the patient is receiving dialysis, and family problems that are recognized in the pretransplantation period should be addressed before activation on the transplant list. The presence of at least one highly motivated caretaker is a helpful factor in long-term graft success.

Adolescence brings with it rapid behavioral, emotional, and physical changes. The adolescent's strong desire to be normal conflicts with the continued reminder of chronic disease that the taking of medication engenders; this tendency is particularly true when medications are taken many times a day or alter the physical appearance. Ambivalence between the desire for parental protection and autonomy, combined with a magical belief in his or her invulnerability, may set the stage for adolescent experimentation with noncompliance.[129] Adolescents with psychological or developmental problems may use impulsive noncompliance during self-destructive episodes. The transplantation team members must be aware of these developmental issues so that they can initiate appropriate psychological intervention before the onset of significant noncompliant behavior.

GROWTH AFTER TRANSPLANTATION

Retarded skeletal growth is a constant feature in children with chronic renal failure. The severity of growth retardation is directly related to the age of onset of renal failure; the earlier the onset, the more severe is the growth retardation. Renal osteodystrophy, metabolic acidosis, electrolyte disturbances, anemia, protein and calorie malnutrition, delayed sexual maturation, accumulation of uremic toxins, and peripheral resistance to insulin-like growth factor-I all have been implicated in growth retardation. Growth retardation is typically assessed by the standard deviation score (SDS) or height deficit score (also known as the Z score). These measure the patient height compared with that of unaffected children of similar age.

Determinants of Growth

Growth improves after transplantation. Catch-up growth, defined as a gain of +1 SDS from baseline, is not realized in most patients, however. The following factors have a major influence on post-transplant growth.

Age at Transplantation

Children younger than 6 years old have the lowest SDS before transplantation, and these patients exhibit the best improvement in their SDS after transplantation.[75,101,161,163] Two years after transplantation, infants younger than 1 year old have an improvement in their SDS by 1 full standard deviation (SD) compared with an improvement of only 0.5 SD for children 2 to 5 years old, and 0.1 SD for children 6 to 12 years old. Children older than 12 years tend to have minimal or no growth after transplantation. Older children occasionally continue to grow into puberty; however, the growth spurt experienced by most growing children at this age may be blunted or lost.

The fact that youngest children benefit the most in statural growth from early transplantation provides a strong argument for expedited transplantation in an attempt to optimize and perhaps normalize stature. In addition, earlier transplantation allows less time for growth failure while receiving dialysis and fewer requirements for catch-up growth.

Corticosteroid Dose

The precise mechanism by which steroids impair skeletal growth is unknown. They may reduce the release of growth hormone, reduce insulin-like growth factor activity, directly impair growth cartilage, decrease calcium absorption, or increase renal phosphate wasting. Strategies to improve growth include the use of lower daily doses of steroids, the use of alternate-day dosing, dose tapering to complete withdrawal and, more recently, steroid avoidance (see earlier).

Alternate-day steroid dosing has gained acceptance in pediatric renal transplantation; at 5 years after transplantation, this is the regimen used in almost one third of all patients. This dosing schedule has been shown to improve linear growth significantly without increased rates of rejection or graft loss. Conversion to alternate-day dosing should be considered in selected, stable patients with well-organized home support in whom compliance can be ensured.[22,78,100]

Ideally, steroids could be withdrawn completely, as they may be for some other solid organ transplants in pediatrics. In tacrolimus-based immunosuppressive regimens, withdrawal of steroids has been successfully accomplished in more than 70% of patients, usually by 5 months after transplantation. The effect of this approach on growth has been significant, with improvement in the SDS at 2 years after transplantation in children younger than 13 years of 3.62 SD in the withdrawn group compared with 1.48 SD in the nonwithdrawn group.[146] The reported rates of acute rejection in the withdrawn group were high, however.[125] If acute rejection occurs, it can adversely affect growth by virtue of a decline in graft function and the need for high-dose steroids to treat rejection. In adults in whom steroids were withdrawn, a decline in graft function has been observed. Long-term follow-up of steroid-withdrawn children is required before this regimen can he adopted on a widespread basis. Numerous steroid withdrawal studies and trials are currently under way in pediatric renal transplantation, using the immunosuppressive agents discussed in previous sections, and long-term data are eagerly awaited.[43]

In uncontrolled trials, complete avoidance of steroids has been successfully achieved (see earlier). The effect of complete steroid avoidance on growth seems to be dramatic, and improvement can be detected 6 months after transplant and in children older than 5 years of age. The rejection risk in these steroid-avoidance regimens seems to be low.[139] If a dramatic growth rate could be coupled with a low rate of rejection in controlled trials, many concerns in pediatric renal transplantation would be allayed.

Growth Hormone

The use of recombinant growth hormone (rhGH) in pediatric renal transplant recipients significantly improves growth velocity and SDS.[48,65,70,71,82] The NAPRTCS reports that growth velocity almost tripled 1 year after starting rhGH therapy, with a slight slowing after 2 and 3 years of therapy. There is some evidence to suggest that rhGH increases allogeneic immune responsiveness, leading occasionally to acute rejection and graft loss in addition to direct adverse effects on graft function.[21,31,76,79] These adverse effects were not observed in the NAPRTCS data but were observed in earlier studies in high-risk patients (e.g., patients who had had prior acute rejection episodes or who were on alternate-day steroid therapy). Growth hormone therapy is generally started in prepubertal children at least 1 year after transplantation and continued until catch-up growth is achieved or until puberty ensues. We have found that cyclosporine levels may decrease after initiation of

rhGH therapy; we follow drug levels closely, along with using adjunctive therapy, such as MMF, at appropriate doses.

Allograft Function

An allograft GFR of less than 60 mL/min/1.73 m^2 is associated with poor growth and low insulin-like growth factor levels; optimal growth occurs with a GFR greater than 90 mL/min/1.73 m^2. Graft function is the most important factor after a high corticosteroid dosage in the genesis of post-transplantation growth failure. The immunosuppressive properties of corticosteroids needed to control rejection and preserve kidney function must be balanced against the need to minimize steroids to maximize growth. An excessive steroid dose leads to impairment of growth, whereas an inadequate dose leads to impairment of graft function. Against the background of this equation, the minimization or avoidance of steroids with new immunosuppressive agents is so important in the evolution of pediatric renal transplantation. Administration of high-dose rhGH may induce acceleration of growth even in the presence of chronic graft dysfunction.

SEXUAL MATURATION AFTER TRANSPLANTATION

Restoration of kidney function by transplantation improves pubertal development. This occurs most likely by normalization of gonadotropin physiology. Elevated gonadotropin levels and reduced gonadotropin pulsatility are observed in chronic renal failure, whereas children with successful kidney transplants show a higher nocturnal rise and increased amplitude of gonadotropin pulsatility.

Female patients who are pubertal before transplantation typically become amenorrheic during the course of chronic renal failure. Menses with ovulatory cycles usually return within 6 months to 1 year after transplantation. Potentially sexually active adolescents should be given appropriate contraceptive information.

Adolescent female transplant recipients have successfully borne children. The only consistently reported neonatal abnormality has been an increased incidence of prematurity. Adolescent boys should be made aware that they can successfully father children. No consistent pattern of abnormalities has been reported in their offspring.

INFECTIONS AFTER TRANSPLANTATION

With new immunosuppressive agents, the incidence of acute rejection has decreased, but the incidence of infections after transplantation has been increasing. In a more recently published study that accessed the NAPRTCS database, rates of hospitalization for acute rejection were compared with rates of hospitalization for infection.[36] For patients transplanted in 1987, the acute rejection–associated hospitalization rate exceeded the equivalent hospitalization rate for post-transplant infections at 1 to 6 months and at 6 to 24 months. In contrast, for patients transplanted in 2000, the infection-associated hospitalization rate was twice that for rejection-associated hospitalization during each time period. In the 6- to 24-month period after transplant, the risk of bacterial infection–related and viral infection–related hospitalization increased significantly from 1987 to 2000. Infections associated with transplantation are addressed in detail in Chapter 29, but some issues that warrant emphasis in children are summarized here.

Viral Infections

The herpesviruses (CMV, herpesvirus, varicella zoster, and EBV) pose a special problem in view of their common occurrence in children. Many young children have not yet been exposed to these viruses, and because they lack protective immunity, their predisposition to serious primary infection is high. The incidence of these infections is higher in children who receive antibody induction therapy and after treatment of acute rejection, and prophylactic therapy is advisable where available.

Cytomegalovirus

The incidence of CMV seropositivity is about 30% in children older than 5 years old and increases to about 60% in teenagers. Younger children are at greater potential risk for serious infection when a CMV-positive donor kidney is transplanted.

CMV infection may have the same devastating effect on the course of pediatric transplantation as on adult transplantation, and various strategies have been proposed to minimize its impact. It has been suggested that seronegative children receive only kidneys from seronegative donors. Given the frequency of seropositivity in the adult population, however, this restriction would penalize seronegative children with a prolonged wait for a transplant at a period crucial for growth. CMV hyperimmune globulin, high-dose standard immunoglobulin, high-dose oral acyclovir, and oral ganciclovir all are potentially valuable therapeutic options.[6,50,69,98,130,157,158] Ganciclovir is effective therapy for proven CMV infection in children, as in adults. Valacyclovir and valganciclovir are new antiviral agents with activity against CMV. These are still under study in pediatric renal transplantation.

Varicella-Zoster Virus

Varicella vaccination is now considered the standard of care in transplant candidates and children with chronic renal failure who are seronegative for varicella zoster antibody. Two doses in such patients may be required. We attempt to confirm seropositivity after the administration of the varicella vaccine. Because varicella vaccine is a live virus vaccine, we wait a minimum of 6 weeks before undertaking transplantation.

The most commonly seen manifestation of varicella-zoster virus infection in older pediatric transplant recipients is localized disease along a dermatomal distribution. In younger children, primary varicella infection (chickenpox) can result in a rapidly progressive and overwhelming infection, however, with encephalitis, pneumonitis, hepatic failure, pancreatitis, and disseminated intravascular coagulation. It is important to know a child's varicella zoster antibody status because seronegative children require prophylactic varicella zoster immunoglobulin within 72 hours of accidental exposure. Varicella zoster immunoglobulin is effective in favorably modifying the disease in 75% of cases. A child with a kidney transplant who develops chickenpox should begin receiving parenteral acyclovir without delay; with zoster infection, there is less of a threat for dissemination, although acyclovir also should be used. In both situations, it is wise to discontinue azathioprine, MMF, or

sirolimus until 2 days after the last new crop of vesicles has dried. The dose of other immunosuppressive agents depends on the clinical situation and response to therapy.

Epstein-Barr Virus

About half of children are seronegative for EBV, and infection occurs in about 75% of these patients. Most EBV infections are clinically silent. Post-transplant lymphoproliferative disease in children, as in adults, may be related to EBV infection in the presence of vigorous immunosuppression. Seronegative patients receiving a kidney from a seropositive donor are at greater risk to develop EBV. For this reason, we constantly observe children for manifestations of early EBV infection (e.g., pharyngitis, lymphadenopathy, fever), using laboratory tests to diagnose EBV (e.g., PCR) at an early stage of symptoms. Should the EBV PCR test show positivity, we discontinue adjunctive immunosuppression. Other centers perform periodic EBV PCR surveillance.

Herpes Simplex Virus

Typical perioral herpetic ulcerations are common in immunosuppressed children and usually respond to oral acyclovir therapy. Disseminated herpes infection is rare.

Polyomavirus

Polyomavirus nephropathy is emerging as an important cause of allograft dysfunction.[37] In one study, surveillance for virus in the urine of transplanted children detected the virus in 26%; however, allograft dysfunction was observed in only 5%. The increased incidence of polyomavirus nephropathy is thought to be the result of more potent immunosuppressive regimens.

Polyomavirus nephropathy usually manifests with allograft dysfunction after treatment of presumed or biopsy-proven acute rejection. The distinction of polyomavirus nephropathy from acute rejection is difficult because both pathologies may coexist. Occasionally, ureteric stenosis is associated with polyomavirus infection and polyomavirus nephropathy. Specific testing for polyomavirus is required to confirm infection. The presence of decoy cells in the urine is highly predictive of viral replication in the uroepithelial cells. The urinary PCR for polyomavirus seems to be more sensitive, but the PCR for blood seems to be more specific for polyomavirus nephropathy. Renal biopsy, with identification of polyoma by immunoperoxidase staining, may be required to make the diagnosis with certainty. Reducing immunosuppression is the main form of therapy. The antiviral agent cidofovir has been used anecdotally in children but is associated with significant toxicity. Children who have lost kidneys to polyomavirus nephropathy may be transplanted safely without a high likelihood of recurrence.

POST-TRANSPLANTATION HYPERTENSION AND CARDIOVASCULAR DISEASE

More than two thirds of transplanted children treated with cyclosporine are hypertensive, and many require multiple medications for blood pressure control.[7] The differential diagnosis of hypertension is the same as that for adults, and much of the discussion of hypertension and cardiovascular issues from Chapter 28 pertains to children as well. Late-onset hypertension may be a sign of acute rejection, however, and may be present before any change in the serum creatinine level.

Calcium channel blockers generally are well tolerated in children. They are often our initial agents of choice for blood pressure management.[148,159] They do not tend to alter the serum creatinine or cause drowsiness. Calcium channel blockers accentuate the tendency to gingival hyperplasia that is seen with cyclosporine, and this can be a concern with children. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are theoretically attractive as agents that may be able to delay allograft fibrosis. Although these can be used effectively in pediatric patients, the occasional increase in serum creatinine, particularly when a patient is even mildly volume depleted, can make clinical management challenging. Children and adolescents also seem to be occasionally troubled by the characteristic cough that sometimes occurs and a mild tendency to anemia.

Concern regarding long-term post-transplant cardiovascular morbidity and mortality has generally been directed toward adult patients.[38] Risk factors also should be addressed in children who, it is hoped, will grow to adulthood with their transplants. Serum cholesterol levels are frequently higher than the age-adjusted limits for children with transplants. The use of lower doses of corticosteroids and tacrolimus/MMF combinations (in contrast to regimens that use cyclosporine or sirolimus) have helped improve the lipid profiles of pediatric patients. Dietary measures are often appropriate to reduce hyperlipidemia. Data are currently insufficient to make firm recommendations for the use of pharmacological measures in children,[150] but the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) have been generally effective and safe.[62] Much more research is necessary to identify and moderate adequately the risk factors for atherosclerosis later in life.

REHABILITATION OF TRANSPLANTED CHILDREN

With today's transplantation technology, medical results have improved so markedly that diligent attention to the pediatric patient's psychosocial, educational, vocational, and developmental rehabilitation is mandatory. Much of the preparation for this multifaceted rehabilitation must be begun in the pretransplant period.

After surgery, we usually recommend that the patients avoid school and crowds for 4 to 6 weeks. Immunosuppression is usually the strongest during this period, and there is concern about exposure to common viral pathogens. Most patients can reenter school and social activities after this short recovery time. Successful reentry into school after transplantation requires coordinated preparation of the child, family or caregivers, classmates, and school personnel. Treatment side effects, social and emotional difficulties, academic difficulties, school resources, and caregiver attitudes all play a role in the adequacy of the reentry process and should be addressed.

Within a year of successful transplantation, the social and emotional functioning of the child and the child's family seems to return to preillness levels. Pretransplantation personality disorders continue to manifest themselves, however. More than 90% of children attend school, whereas less than 10% are not involved in any vocational or education programs within 1 year after transplantation. Three-year follow-up shows that nearly 90% of children are in appropriate school or job placement. Surveys of 10-year survivors of

pediatric kidney transplants report that most patients consider their health to be good; they engage in appropriate social, educational, and sexual activities, and they experience a very good or excellent quality of life.[105,119]

These favorable data must be tempered by the fact that survey instruments for quality-of-life measures in pediatric renal transplant recipients have not yet been developed. As a result, generic pediatric assessment tools are often used. Although such instruments may be flawed, studies using these instruments suggest that patients with a functioning kidney transplant have an overall better quality of life than do dialysis-dependent children[54]; however, when compared with a population of normal children, children with a renal transplant have a lower ranking quality of life and report fewer school and physical activities.[5,121] Although this finding may not be surprising, the challenge of the transplant team today is to try to ensure an optimal quality of life.

A challenge for the pediatric transplant team is to prepare the adolescent transplant recipient for adulthood. There is much to be learned about the transition process and the adult outcome of transplant in the pediatric years. This transition is a challenge despite studies that suggest that most adult patients who received transplants as children or adolescents are rehabilitated in regard to education and socioeconomic status, with less than 15% being unemployed. More effort and collaborative studies need to be devoted to optimal transition coping strategies.

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Chapter 36

Renal Transplantation in Developing Countries

M. Rafique Moosa

Renal transplantation is the only viable therapeutic option for most patients with irreversible renal failure in developing countries. The demand for kidney transplantation has grown inexorably as the number of patients with end-stage renal disease (ESRD) has rapidly escalated worldwide. To aggravate the already dire situation, there has been a decline in the number of cadaver kidney transplants being performed.

ESRD is taking its toll particularly on patients from developing countries. The main reason is the rampant epidemic of diabetes mellitus in developing countries and the aging of the population.[156,289] The diabetes pandemic is threatening developing countries more than developed countries; it is estimated that the within the next generation the number of people with diabetes will increase by 88% in Latin America, 98% in Africa, and 91% in Asia compared with 18% in Europe, and by 2030 greater than 80% of diabetics will be from developing countries. Type 2 diabetes has now overtaken glomerulonephritis as the major cause of end-stage kidney failure worldwide in the developed and developing world.[17]

It has been estimated that between 2001 and 2010, the number of patients on dialysis worldwide will have doubled to more than 2 million, and the aggregate cost of treatment during this decade would be greater than U.S. $1 trillion.[144] The average annual cost of dialysis is currently U.S. $66,000. This amount exceeds the gross national income per capita (GNIPC) of every country in the world (in 2002, the GNIPC of the United States was $37,610). The World Bank classification (2004) of countries by economic groupings, based on gross national income per capita is as follows: (1) low income, $825 or less (U.S. dollars); (2) lower middle income, $826 to $3255; (3) upper middle income, $3256 to $10,065; and (4) high income, greater than $10,066.

Countries with low-income and middle-income economies are referred to as developing countries. The disparity between emerging market economies of developing countries and established market economies of developed countries continues to widen.[87] Nowhere is this disparity more striking than in the differential resources spent on health care. Spending on health care in industrialized countries far exceeds that committed by developing countries (10% to 15% versus 0.8% to 4%).[223] The situation is aggravated further by the lack of access of the patients to basic facilities, such as potable water, sanitation, and electricity; cultural and societal constraints, such as low literacy rates, poverty, and poor governance; and natural and man-made disasters.

Although the powerful economies of the developed countries permits almost universal access to renal replacement treatment for their populace, the struggling economies of the developing countries fail to provide even basic medical care for their citizens. The high cost of dialysis limits this form of treatment to a privileged few, making a successful renal transplant a greater necessity than in rich countries.[133] The economies of most developing countries have many other priorities, and generally less than 5% of gross national product is spent on health (Table 36-1). The question of whether dialysis and transplantation are justified at all has been raised but remains largely rhetorical. If transplants are to be done, the timing of transplantation must be optimized, graft function maximized, and costs and complications minimized.

Worldwide, the number of patients with ESRD beginning dialysis increased by more than 33-fold in the years 1975 to

Table 36–1 **Expenditure on Health by African Countries Compared with United States (2002)**

Country	Per Capita Expenditure (U.S. $)*	Total expenditure (% of GNI)†
Algeria	182	4
Angola	92	5
Benin	44	5
Botswana	387	6
Burkina Faso	38	4
Burundi	16	3
Cameroon	68	5
Central African Republic	50	4
Chad	47	7
Congo	25	2
Côte d'Ivoire	107	6
DR Congo	14	4
Djibouti	78	6
Egypt	192	5
Equatorial Guinea	139	2
Eritrea	36	5
Ethiopia	21	6
Gabon	248	4
Gambia	83	7
Ghana	73	6
Guinea	105	6
Guinea-Bissau	38	6
Kenya	70	5
Lesotho	119	6
Liberia	11	2
Libyan Arab Jamahiriya	222	3
Madagascar	18	2
Malawi	48	10
Mali	33	5
Mauritania	54	4
Morocco	186	5
Mozambique	50	6
Namibia	331	7
Niger	27	4
Nigeria	43	5
Rwanda	48	5
Senegal	62	5
Sierra Leone	27	3
South Africa	689	9
Sudan	58	5
Swaziland	309	6
Togo	163	11
Tunisia	415	6
Uganda	77	7
Tanzania	31	5
Zambia	51	6
Zimbabwe	152	9
United States	**5274**	**15**

*Average per capita expenditure is $114.4 (international U.S. $ rate).

†Average total expenditure is 5.2% of GNI.

GNI, gross national income.

Data from World Health Organization: WHO Global Atlas. Available at: http://www.who.int/globalatlas/. Accessed January 17, 2006.

1989, but the number of transplants increased only 5-fold in the same period. The marked discrepancy between the number of patients with ESRD and the number of patients who receive transplants continues to grow at an alarming rate. Of all the transplants worldwide, less than 10% are performed in developing countries, which rely heavily, and in some cases exclusively, on living related donors. With many developing countries having virtually no dialysis activity or cadaver donor programs, the only hope for patients with irreversible renal failure is a living related donor transplant.

The availability and rate of transplant activity vary considerably; however, demand exists even in the poorest nations. Kidney transplants were performed in 95% of 44 countries in a more recent survey—most in the more developed countries.[130] Since the 1990s, there also has been burgeoning "transplant tourism," which has become an important factor in the medical economies of some poorer countries, such as Peru, South Africa, India, the Philippines, Iraq, China, Russia, and Turkey.[251]

END-STAGE KIDNEY DISEASE IN DEVELOPING COUNTRIES

Incidence

In the absence of formal registries, the true incidence of ESRD is difficult, if not impossible, to determine. Estimates put the incidence of ESRD at 48 to 240 per 1 million population (pmp) in developing countries compared with 88.9 to 338 pmp in the developed regions of North America,[159] Europe, and the Asia-Pacific region.[188] ESRD in developing countries seems to be at least as common, if not more common, than in developed countries. With the anticipated increase in the incidence of diabetes alluded to earlier, ESRD is likely to become an increasing problem. A community-based study in India put the point prevalence of ESRD at an incredible 785 pmp.[4]

Demographics

Age

In some developing countries, the mean age of patients starting renal replacement therapy is 30 years. In contrast, in 2002, the mean age of a patient starting renal replacement therapy in the United States was 62.2 years; the incidence of ESRD is increasing most rapidly in the older than 75 years age group, suggesting that the mean age is set to increase in industrialized countries.[282] A possible explanation for the presentation at a younger age in developing countries is that inadequate preventive and curative medical care allows more rapid development of ESRD.[29]

Gender

Another important difference that has emerged is the marked male predominance in the incidence of renal replacement therapy compared with industrialized countries. In the United States, men account for 53% of patients started on treatment. In developing countries, men account for up to 93% of patients receiving treatment.[53] The incidence of chronic kidney failure is unlikely to be considerably higher in men, and the marked discrepancies probably reflect social and cultural factors in paternalistic societies that favor men, who often are the sole breadwinners.[223]

DIALYSIS OPTIONS IN DEVELOPING COUNTRIES

It has been estimated that 85% of all patients with ESRD reside in developing countries, and prevalence of treatment is generally proportionate to the economic strength of individual countries.[24,74,158,234,236] Of all patients on dialysis treatment

Table 36–2 **Factors Contributing to the Poor Rate of Transplantation in Developing Countries**

Community Factors
Illiteracy (58% in men and 29% in women)
Poverty (⅓ live on <U.S.$1/day)
Lack of access to potable water and sanitation
Cultural and societal constraints
Donor shortage
Post-Transplant Issues
High cost of immunosuppression
Post-transplant infections
Governmental Issues
Lack of funding and will
Commercialization of transplantation
Inadequate health infrastructure
Lack of public awareness of importance of donation
Health Professionals
Apathy and ignorance among health professionals
Lack of team spirit
Lack of planning for organ procurement

Data from references Rizvi et al[223] and Shaheen and Souqiyyeh.[255]

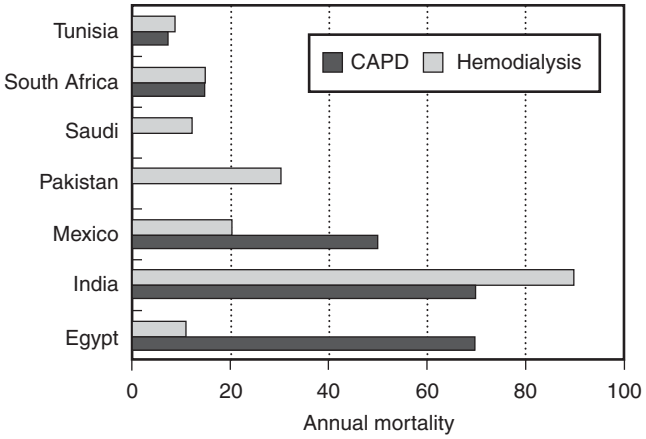

Figure 36–1 Mortality rates on two forms of dialysis. The annual mortality of patients started on both forms of dialysis in a range of developing countries is shown. The average annual survival is 73.4% on hemodialysis and 62% on continuous ambulatory peritoneal dialysis (CAPD).[26]

worldwide, 52% are being treated in just four countries (United States, Japan, Brazil, and Germany) that constitute only approximately 11% of the world population.[86] By contrast, less than 1% of patients with ESRD in sub-Saharan Africa receive dialysis treatment.[20]

Because of limited resources, the management of ESRD poses complex medical, social, moral, and economic challenges for patients and communities in developing nations (Table 36-2). On the Indian subcontinent, only 3% to 5% of all patients with ESRD receive any form of renal replacement therapy at all. Of patients who start hemodialysis, about 60% are lost to follow-up within 3 months as the economic realities of the treatment come into play.[133] In West Africa, the situation is even more dire, with only 2.7 pmp receiving dialysis; most of these are patients able to pay for treatment themselves.[76] In sub-Saharan Africa, the prevalence of treatment is 9.9 pmp,[20] but the situation is better in North Africa and the Middle East with average dialysis rates of 171 pmp (North Africa)[26] and 140 pmp (Middle East).[86] In Asia, the dialysis rate is 60 pmp.[86] Of the developing regions, Latin America seems to be faring best with the prevalence of dialysis treatment 320 pmp in 2000 and projected to increase to 450 pmp in 2005.[227] The political and economic emancipation of the former communist bloc countries in Central and Eastern Europe has resulted in a significant increase in the prevalence of treatment, with an average rate of 220 pmp, ranging from 500 pmp in Slovenia to 77 pmp in Lithuania.[158,234]

Hemodialysis

Globally, hemodialysis is the preferred form of dialysis despite the many advantages of peritoneal dialysis in the developing country setting (see later).[86] The access to long-term hemodialysis varies from region to region; it is readily available to patients in Latin America but virtually nonexistent in most developing countries in Asia and Africa. The major difference is that programs in Latin America are state subsidized, whereas very few programs in the rest of the developing world are, and only affluent patients or patients with ready access to funds are able to afford dialysis treatment.[20] Besides high costs, the initiation of hemodialysis programs is restricted by lack of trained staff[155] and lack of infrastructure, among other constraints.[20]

In an international survey, the mean annual survival of patients on hemodialysis in several developing countries was comparable to results in the West, indicating that treatment of an adequate quality can be achieved (Fig. 36-1).[25] The survival rates in dialysis patients from Central and Eastern Europe now approach those of Western counterparts and range from 91% in Romania[158] to 81% in the Czech Republic.[236] The poor outcome in other developing countries is because many dialysis units treat patients only one to two times weekly and largely use cuprophane-based or cellulose acetate–based, hollow-fiber dialyzers, which are the most affordable.[25] Dialyzer reuse, usually performed manually, is extensively practiced in developing countries out of economic necessity,[86,133] although the practice varies widely and is even banned in certain countries, such as Egypt.[25]

As their financial situation worsens, many patients reduce the frequency of treatment, which results in progressive uremia and ultimately in death.[115] Another problem in developing countries is that a greater proportion of the population lives in rural areas far from existing dialysis centers; because of the expense and work disruption, patients attend infrequently for treatment, which ultimately leads to poor outcome on dialysis.[20,133] The lack of access of ancillary treatment, such as erythropoietin and iron, also contributes to poor outcome; in a survey reported in 2002, less than 25% of patients received erythropoietin therapy, and in many the dose was suboptimal.[25]

Late referral of patients with ESRD also is an important consideration in the outcome of dialysis.[26,244] In the absence of an established renal transplant program, it is not economically viable to maintain large numbers of patients on hemodialysis in a developing country.[155] In South Africa,

national guidelines set by the Department of Health preclude patients from the renal replacement therapy program if they are not suitable candidates for kidney transplantation for any reason.[176]

Peritoneal Dialysis

Peritoneal dialysis is an efficient form of renal replacement therapy[186] that is often the preferred form of treatment in developed countries. Worldwide, only 8.5% of ESRD patients receive peritoneal dialysis, but the annual growth rate is 5% with growth rates significantly greater in developing countries.[163] Advantages of peritoneal dialysis are that it is more physiological than intermittent hemodialysis, and it requires less stringent dietary and fluid restrictions. It is more appropriate for certain categories of patients, such as diabetics and children, and can be taken to remote regions that have limited facilities. Peritoneal dialysis would be an ideal form of renal replacement treatment in a developing country setting. The chief reason for its limited use is the high cost of treatment in developing countries because peritoneal dialysis solutions have to be imported.[25] Peritoneal dialysis is three times more expensive than hemodialysis, precluding the widespread use of this promising modality of therapy.[115]

Although peritoneal dialysis holds tremendous promise, failure to ensure adequate standards of care can result in dismal outcomes (as in the Mexican model, where the annual mortality rate was >50%) (see Fig. 36-1).[279] In developing countries, the dose of treatment is related to available resources, and many patients are unable to afford the standard three to four exchanges per day. As residual renal function fails, patients become increasingly uremic and consequently die.[1] The introduction of new connection technology, such as the Y-system and twin-bag system, has resulted in a significant reduction in acute peritonitis rates.[264] Malnutrition, which may be present in 77% of patients with ESRD in developing countries, is aggravated by cultural practices that promote the restriction of dietary animal protein intake[2] and by inadequate dialysis that results in loss of appetite.[177]

RENAL TRANSPLANTATION

A kidney transplant is the treatment of choice for patients with ESRD, with preemptive transplantation being the ideal option.[133,244] A striking feature of renal replacement therapy programs in developing countries is the emphasis, and in some cases exclusive reliance, on living donor transplantation, predominantly related living donors but increasingly unrelated living donors.[244] Transplantation often occurs without the benefit of backup dialysis facilities and largely in the absence of a cadaver donor program. Lack of resources, cultural factors, and ignorance all contribute to the ongoing shortage of organs.[221]

Barriers to transplant activity in developing countries have been identified (see Table 36-2). The number of transplants done also correlates with the socioeconomic status of a country (Fig. 36-2).[27] The transplant rate in developing countries is less than 10 pmp compared with 45 to 50 pmp in industrialized countries (Fig. 36-3). Developed countries are able to satisfy 30% to 35% of their needs, in contrast to developing countries, where only 1% to 2% of the estimated need for organ transplantation is met.[223] Of all renal transplants that have been done around the world, almost 90% have been in developed countries that constitute only 20% of the world population (Table 36-3).[133] Under these circumstances, the purchase of kidneys from living unrelated donors has flourished.

Donors

Living donors form the backbone of transplant programs in developing countries, accounting for 85% to 100% of donations compared with 1% to 25% in the West.[223] Most living donors are members of the extended family or marital partners. Despite the large size of extended families, with on average six genetically related members being available at initial workup, almost half of potential donors are eliminated because of hypertension, diabetes, urological problems, and other medical issues; another one quarter may refuse to donate.[107] Families also refuse to allow breadwinners

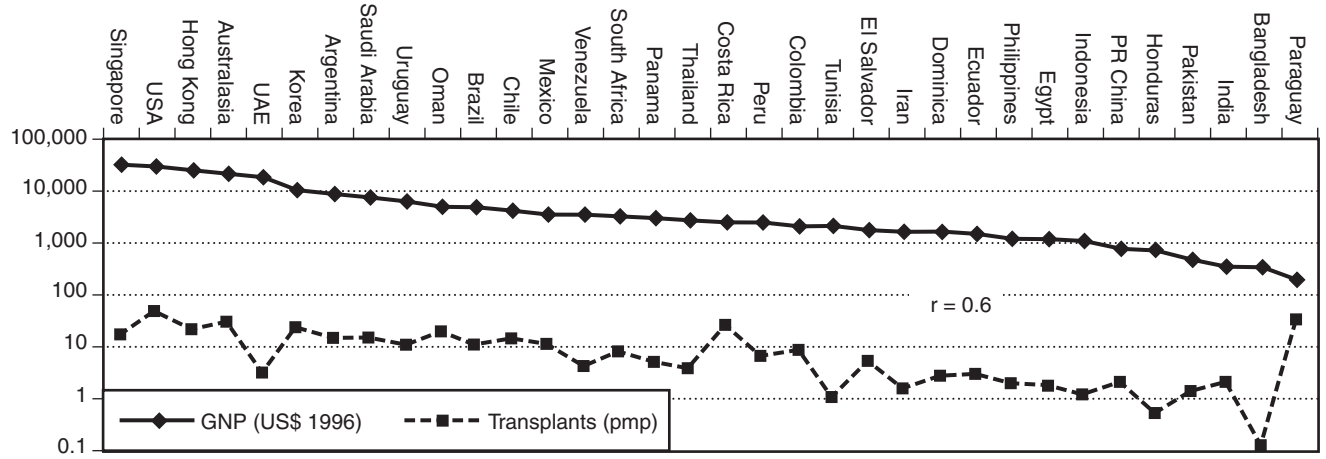

Figure 36–2 Correlation between transplant activity and the economic strength of a country. Renal transplant activity and gross national product (GNP) per capita for selected developing countries and the United States shows a significant relationship between these two parameters. (Transplant data obtained from published data and the economic data from the World Bank Atlas [1997].)

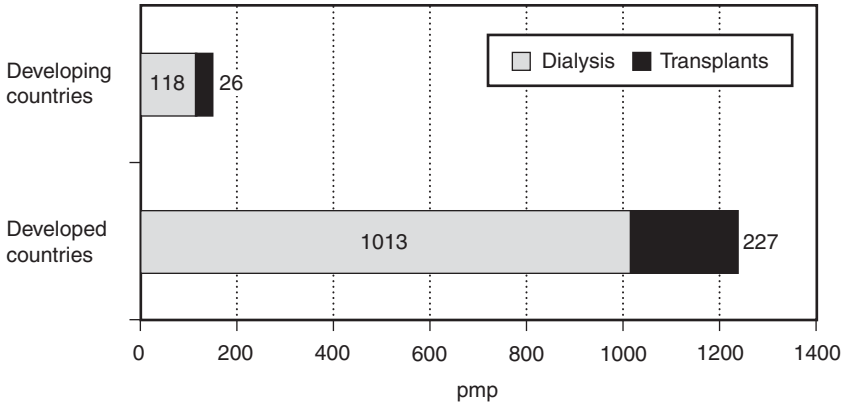

Figure 36–3 Difference in replacement activity in developed and developing countries (2001). The point prevalence rates of dialysis and kidney transplantation in the world show significantly inferior delivery of both forms of renal replacement treatment in developing countries. (Data from Kher V: End-stage renal disease in developing countries. Kidney Int 62:350, 2002; and Rizvi SAH, Naqvi SAA, Ahmed E: Renal transplantation in developing countries. In El Nahas M [ed]: Kidney Diseases in the Developing World and Ethnic Minorities. New York, Taylor & Francis, 2005, pp 211-245.)

to donate.[185] In the final analysis in one report, only 1.6 donors were available per recipient.[107]

Spousal donations are an important source of kidneys in developing countries.[33,190,273] About two thirds of donations are from wives to husbands, which is approximately the same ratio as in Western countries.[182] Spousal donation accounts for 28% of all living unrelated transplants in Asia[190] and can be a rewarding experience with donors expressing satisfaction with their decision and improvement in family relationships. The results of spousal transplants also are superior to the results of parental donors and living unrelated donors; the 3-year survival rates for spousal transplants were 85% compared with 81% for living unrelated donors and 82% for parental donors.[274,275] Results of spousal transplants can be improved by a further 10% if the recipient receives donor-specific blood transfusions.[275] The passage of the Transplantation of Human Organs Act in India in 1994 resulted in an upsurge of spousal transplants, which now constitute greater than 20% of all living transplants. Most donors (94%) are wives. A dilemma faced by physicians is to ensure that women, who live in a male-dominated society, are not being coerced into donation.[33]

With the exception of some Latin American countries, cadaver donations are limited in most developing countries for a variety of reasons, including religious and cultural issues. After some initial resistance, most religious commentators, including Islamic, Christian, Hindu, Judaic, and Buddhist, support solid organ transplantation. Saudi Arabia is an excellent example of a conservative Muslim country that has implemented a cadaver donor program successfully.[151] The growth of the cadaver program in Latin America is another example of what can be achieved with the combined effort of the medical community and governmental involvement (see later).[297] The United States is the only country in the world that performs more transplants than Brazil, which reached an absolute number of 3400 in 2001.[297] The success of such a cadaver donor program requires several factors to be addressed.[50]

Table 36–3 **Vital Differences between Different Regions of the World That Influence the Delivery of Renal Replacement Treatment (2001)**

Regions	Developed	Developing
Population (million)	1242	4932
Population of world (%)	20	80
Annual increase (%)	0.5	1.9
Urban (%)	74	31
GNI per capita (U.S. $)	26,366	2275

GNI, gross national income.

Data from Kher V: End-stage renal disease in developing countries. Kidney Int 62:350, 2002.

Education

A concerted education campaign is required to increase public awareness of the need for organ donation to change negative public attitudes that hinder discussion of this subject by family members. In developing countries, low adult literacy rates hinder education drives.[244] In many Southeast Asian countries, organ donation is considered a Western concept that has not yet gained acceptance in these communities.[47]

Attitude

The attitude of indifferent health care professionals has been identified as a major limiting factor, and changing such indifferent attitudes should be given priority.[47,184,255]

Legal Aspects

Recognition of the concept of brain death and the enactment of laws that allow the use of organs from cadaver donors are important. Many developing countries do not have such laws, including Pakistan, Bangladesh, and Malaysia. The Transplantation of Human Organs Act of India banned trade in organs, recognized brain death, and simultaneously promoted cadaver organ donation.[184] Singapore has had a progressive law in place since 1987, which allowed the removal of organs in the case of accidental death, unless the person had opted out during his or her lifetime. Muslims were excluded from this arrangement. The Human Organ Transplant Act was amended in 2004 to include death from all causes and extended to include organs other than kidneys. It also regulated living unrelated donors.

Resources

Adequate resources in terms of capital, personnel, and services are crucial. Cadaver donor programs tend to be more expensive than living donor transplants and are constrained in countries where health resources are stretched to the limit.[50] Access to intensive care facilities is required to allow the ventilation

of donors. The severe shortage of intensive care unit beds in developing countries can be a major limitation.[62,184,244] A reliable tissue-typing laboratory also is an essential service for the success of a cadaver transplant program. In Saudi Arabia, the government undertook a leading role and established a national procurement agency responsible for the supervision of organ donation and transplantation emphasizing the importance of government will and involvement.[255]

Transplant Tourism and Living Unrelated Transplants

Controversy with regard to the moral, social, economic, and ethical issues surrounding sale of organs for transplantation has raged unabated, and despite strong arguments from both sides of the divide, there seems to be little chance of the debate being settled. In a provocative article, Cameron and Hoffenberg[40] eloquently argued the case for paid donation and listed the main reasons for and against the practice. They identified the abuses of the commercial aspects of donation as a major problem and supported the findings of the Bellagio Task Force Report.[230]

A growing scourge is the rampant trafficking in organs that continues unchecked in countries around the world to the detriment of all parties involved and despite the laws prohibiting the practice.[251] Although a case for the sale of organs can be made on grounds of economic benefit, the major problem is undoubtedly exploitation of these individuals, especially by the "middleman." The overwhelming reason motivating sale of kidneys by unrelated donors in India is the reduction of debt.[85] In one study, 95% of donors admitted that helping a patient with kidney failure was not a major motivation. The economic benefit of the sale was not realized by most donors, with family income declining by one third, and almost three fourths continuing to have significant debt after kidney donation. There was a decline in reported overall health status in 87% of patients after donation, which may explain the deterioration in economic status in donors. Most donors would not recommend donation after their experience,[85] suggesting that patients may not have been adequately informed of all the consequences of organ donation.

The lack of significant donor benefit and the development of other hardship have previously been noted.[251] In a survey from Iran where living unrelated transplants have eliminated waiting lists, the experience of donors has been noted in a damning report that severely questions the feasibility of continuing the practice. In this report, 75% of donors thought the practice should be banned.[296] As long as the demand for organs exists, however, market forces, rather than ethical and moral considerations, will continue to dictate current practice. Although patients from developed countries have the option of remaining on dialysis, for patients from developing countries who do not have access to these facilities, a transplant from a living donor may be their only lifeline. While the debate continues, the exploitation of the poor by unscrupulous operators should be addressed by the relevant authorities as a matter of urgency, as has been the case in South Africa, where criminal charges have been levied against alleged perpetrators of illegal transplant activity as reported in the popular press.

TRANSPLANT ACTIVITY IN DIFFERENT DEVELOPING REGIONS OF THE WORLD

No country in the world can claim to have enough donors for its transplantation needs. At best, 45% to 50% of the prevalent ESRD population has functioning grafts in developed countries. In developing countries, the situation is considerably worse, but significant growth has occurred in many regions. Growth in transplant activity has been particularly good in the former Soviet bloc countries, the Middle East, and Latin America, but renal replacement treatment has lagged in Africa and Asia.

Latin America

Latin America is economically, socially, and racially a heterogeneous region, which is manifested in widely differing wealth and health indicators. The incidence of ESRD in this region has increased progressively, in 1992, 1997, and 2001, the rates were 27.8 pmp, 57 pmp, and 91.4 pmp.[60] In common with the trends elsewhere in the world, the incidence of diabetic nephropathy has increased, and diabetic nephropathy together with hypertension accounts for almost two thirds of all ESRD in this region (Fig. 36-4A).[31,60,79] The annual growth in ESRD patients is almost sevenfold greater than the population growth of Latin America.[60] A second important observation, seen especially in developed countries, has been the aging of the incident population with patients older than 65 years accounting for 38% of patients starting treatment in 2001 compared with 20% a decade previously.[60] Argentina pioneered kidney transplantation in the region in 1957.[60] Since then, Latin America has experienced a phenomenal increase in transplant activity. In the period 1987 to 2001, the number of kidneys transplanted in Latin America increased by 370% (see Fig. 36-4B).[60]

This region is the fastest growing in terms of number of transplants, new units established, and progress with cadaver organ donation. What makes the achievement more remarkable is that it occurred during a decade of economic recession. The region with 8.5% of the world population has performed 12.7% of all kidney transplants.[78] The Latin American Registry, created in 1991 and including 20 countries and a regional population of 509 million, had recorded a cumulative number of 5947 kidney transplants in 2001; in 2003, 6357 transplants were performed of which 55% were from living donors. Cadaver kidney transplants have increased, and in at least eight countries these exceed living donor transplants.[60] The overall cadaver donor rate in Latin America was 2.5 pmp in 2001 but averaged 10 pmp in Uruguay, Puerto Rico, Chile, and Cuba.[78] Part of the success of the cadaver donor program is due to the "Punta Cana" group formed by the Latin American transplant coordinators trained in Spain on the "Spanish model," who spread their expertise throughout the region.[78]

Brazil, which has the region's largest population and strongest economy, performed the largest number of transplants, second only to the United States. Brazil performed 20 pmp transplants in 2001, which is almost double the average of 11 pmp for Latin America but less than one half of developed countries.[297] In terms of transplants relative to the size of the population, Costa Rica was the most active with 27.7 pmp, followed by Puerto Rico with 23.2 pmp. With diabetic nephropathy one of the primary causes of ESRD in this

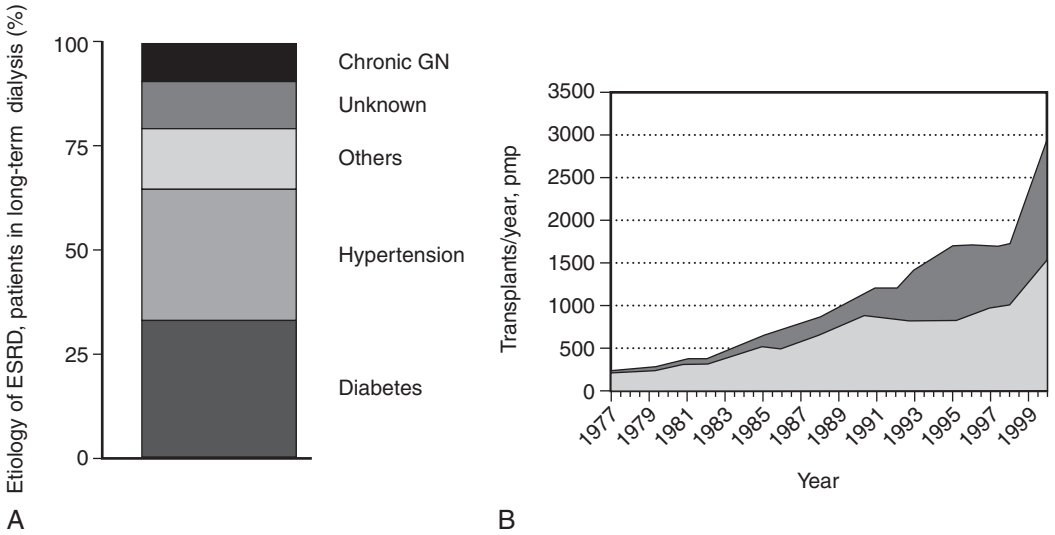

Figure 36–4 **A,** Etiology of patients on long-term dialysis in Latin America. Hypertension (32%) and diabetic nephropathy (33%) together account for two thirds of all-cause end-stage renal disease (ESRD) in this region. GN, glomerular nephritis. **B,** Transplant activity in Latin America. Brazil has a successful cadaver transplant program and a progressive increase in the number of living and cadaver transplants being done. This trend is typical of that in Latin America. Living (*light gray shading*) and cadaver (*dark gray shading*) renal transplants done in Brazil since 1977. (**A** from Rodriguez-Iturbe B, Bellorin-Font E: End-stage renal disease prevention strategies in Latin America. Kidney Int 57[Suppl 74]:S30, 2005. **B** from Zatz R, Romao JE, Noronha IL, et al: Nephrology in Latin America, with special emphasis on Brazil. Kidney Int 63[Suppl 93]:S131, 2003.)

region now, increasing numbers of combined kidney-pancreas transplants are being performed in Argentina and Brazil for patients with type 1 diabetes.[60]

The treatment of ESRD places an enormous economic burden on countries that spend on average U.S. $391 per capita per year on health. This amount is 10 times less than that spent by industrialized countries.[227] In addition, there are large disparities within countries between the rich and poor even in the more affluent countries.[297] Less than 5% of funds for renal replacement are met by private funds, and costs are covered by public funds in most countries.[79,227] In Brazil, the cost of a kidney transplant of U.S. $10,000 is paid by the government. The cost of triple therapy (cyclosporine, azathioprine, and steroids) is borne by the Health Ministry, which also allows the use of mycophenolate mofetil and tacrolimus.[297] The poor who have limited access to health care facilities are often discriminated against and receive significantly less treatment. In Mexico, the treatment prevalence rate among the poor was 166 pmp compared with 939 pmp among the insured; the transplant rate was 7.5 pmp among the poor and 72 pmp among the insured.[79] As in other parts of the world, efforts should be made to prevent and treat kidney disease, especially in diabetics and elderly patients.[39,227] In addition, the growth in the pool of donors, especially deceased donors, should be supported and encouraged.[60,79]

Asia-Pacific Region

The Asia-Pacific region probably represents the greatest spectrum of social, cultural, economic, and ethnic diversity of all the areas of the developing world. The true incidence and etiology of ESRD in this region are unknown because of the absence of regional or national registries. It has been estimated that the incidence may be 240 pmp. More recent data on the etiology are unavailable,[244] but older reports from the Indian subcontinent suggest that chronic glomerulonephritis accounts for more than one third of cases of ESRD, and diabetes accounts for one quarter.[220,242] With diabetes in this region increasing, diabetic nephropathy is becoming more common, however, and is the most common cause of ESRD in patients 40 to 60 years old.[161]

This region falls in the Afro-Asian stone belt that stretches across North Africa and the Middle East to South Asia, in which nephrolithiasis is an important cause of ESRD.[226] The mean age of patients is 42 years.[244] Of the approximately 100,000 patients who have ESRD in India, only 10,000 consult a nephrologist, and less than 10% of patients with ESRD receive renal replacement therapy. Of the patients who do receive therapy, 60% are no longer on treatment after 3 months.[133] Only 5% of all patients with ESRD receive a kidney transplant.[265] Between 1996 and 2000, 48,420 kidney transplants were performed in 13 countries in this region[190]; of these, 53.5% were living donor transplants, and 46.5% were cadaver donor transplants (Fig. 36-5).

The People's Republic of China is exceptional in undertaking the greatest number of cadaver donor transplants in the region[190]; its main source of organs is reported to be from judicially executed prisoners.[38] Excluding China's controversial contribution, living donor transplantation accounts for 86% of transplants in the region; 62% were living related donor transplants and 27% were living unrelated donor transplants,[190] considerably increased from that previously reported.[189] Several countries reported patients being transplanted abroad, but these numbers were small.[190] Cadaver donation is underdeveloped in most of Asia, where brain death has not yet been generally accepted. Efforts are being made to increase this source in the face of social and cultural inhibitions and the lack of organ procurement organizations that are supported by the necessary legislation.[49,188,192,255] Singapore revised its Human Organ Transplant Act in an effort to improve organ procurement (see earlier).

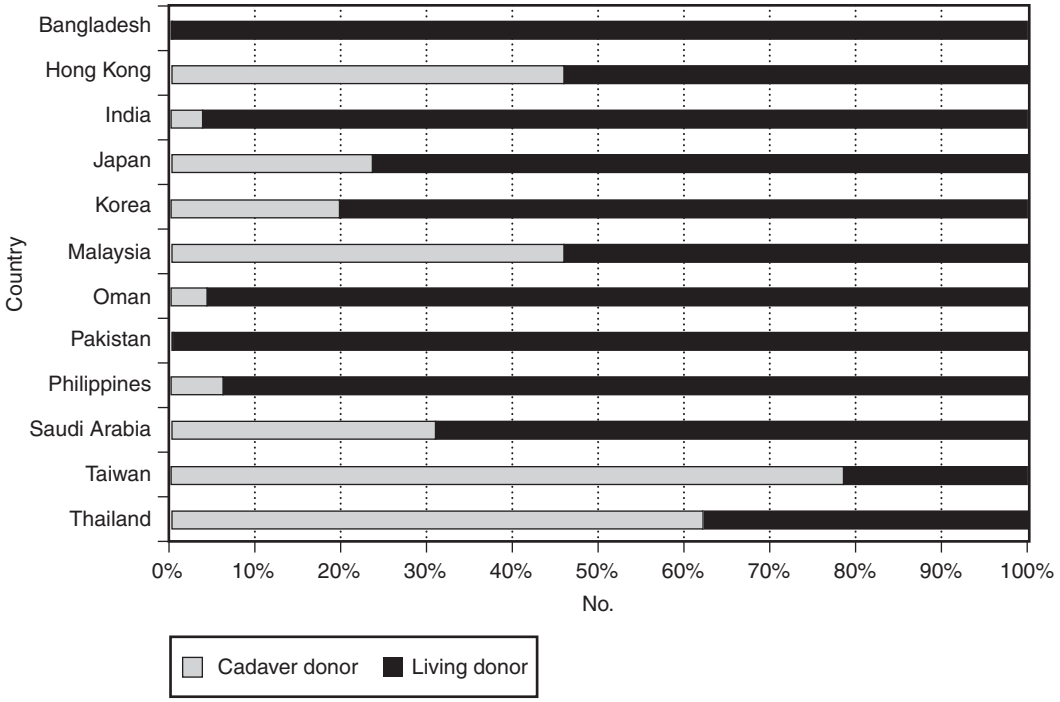

Figure 36–5 Source of donor kidneys in Asia (1996 to 2000) excluding China, Singapore, UAE, and Indonesia. The main source of kidneys in this region, as in other developing regions, is living donor transplants. Spousal transplants account for 11% of all living transplants. India performed 52% of all living donor transplants and 68.5% of all spousal transplants in this region. Cadaver donation remains an underused source in most countries in this region. (Data from Ota K: Current status of organ transplantations in Asian countries. Transplant Proc 35:8, 2003.)

Health delivery in most of the countries is via a two-tier system with few countries able to fund transplantation; as a result, burgeoning private clinics perform transplants on a fee-for-service basis.[113,133] Of the approximately 100 dialysis units in India, 75% are in the private sector.[133] The cost of a kidney transplant in India was U.S. $1000 at a public hospital, whereas the cost in a private clinic in Pakistan was U.S. $6000 to U.S. $10,000.[133,223] The cost of triple immunosuppression (cyclosporine, azathioprine, and steroids) was an additional U.S. $2500 per year. Cyclosporine is often tapered after 1 year and discontinued purely for economic reasons with the consequent risk of acute rejection.[244] In addition, some centers reduce the dosage of cyclosporine by the addition of ketoconazole.[49] The high costs, together with the shortage of trained nephrologists and transplant surgeons, remain a major disincentive to the further growth of transplant programs.

China with its enormous population and rapidly growing economy is set to dominate this region economically. The most common cause of glomerular disease is IgA nephropathy, followed by lupus nephritis. In the future, the rising tide of diabetes almost certainly will escalate diabetic nephropathy to a position of greater prominence as cause of irreversible renal failure from the 17.6% in 2000. Funding for transplants is not provided by the government, and for patients on medical insurance, the sum of U.S. $12,000 is reimbursed in the first year. The principal source of kidneys are brain-dead donors (see earlier).[139] Cyclosporine is locally produced, which helps in containing costs.

The annual number of renal transplants has increased over the years in Asia, but great potential remains for further growth. This growth can be achieved through legal and social acceptance of the brain death concept, the establishment of organ procurement organizations, and, most important, education of the public and health care providers through systematic support from the authorities.

Middle East and Afro-Arab Region

Although kidney transplants were performed sporadically in the 1970s, this region made dramatic progress in the 1980s with the introduction of cyclosporine but perhaps more importantly with the issuance of the Amman Declaration in 1986. Muslim clergy recognized the concept of brain death, which permitted the retrieval of organs from deceased donors and living donors. All countries in this region with the exception of Egypt, Iran, and Iraq, have adopted laws that permit use of organs from cadavers and regulate live donations.[151] Despite the passage of these laws allowing cadaver transplantation being operative for 10 years, living donor transplantation still predominates and accounts for 85% of total transplants.

The Middle East Society for Organ Transplantation (MESOT) registry was established in 1987 to document transplant activity in the region.[151] The Registry represents about 29 countries from the Middle East, North Africa, and neighboring areas, with a total population of 635 million.[80] The number of patients receiving kidney transplants is only 9 pmp; the regional ESRD incidence ranges from 34 to 200 pmp (Fig. 36-6).[151] Economically, the region is divided into three income groups. The 12 low-income countries have limited health budgets and small or nonexistent dialysis and transplant programs. The incidence of ESRD in these countries is 101 pmp; 95% of patients die because of lack of treatment options. The nine medium-income countries in this region have a similar incidence of ESRD (99.3 pmp).

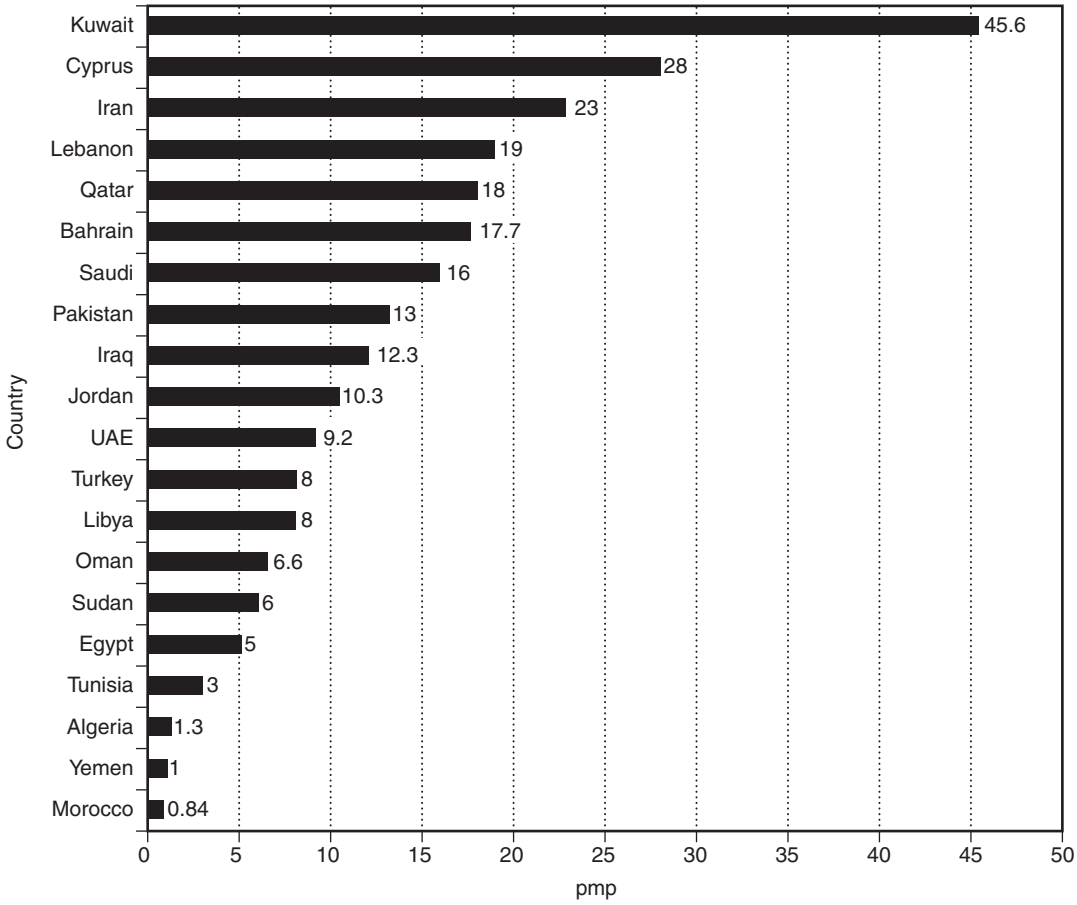

Figure 36–6 Kidney transplants performed in Middle East Society for Organ Transplantation countries (2003). The estimated average annual need for kidneys is 200 pmp; on average 10% to 15% of patients die on dialysis annually.[255] (Data from Masri MA, Haberal MA, Shaheen FA, et al: Middle East Society for Organ Transplantation (MESOT) Transplant Registry. Exp Clin Transplant 2:217, 2004.)

Renal replacement programs are available but limited. Only half of patients are started on dialysis treatment, and because of limited transplant activity, these patients remain permanently on dialysis, placing an enormous burden on already strained health budgets. The eight high-income countries have an incidence of ESRD of 111 pmp. Dialysis facilities are provided by the government, but transplantation is limited.[80]

The history of renal transplantation in the region has followed fairly distinct patterns.[61] Initially, transplantation of cadaver donors was undertaken in Europe and North America. Local living related transplant programs were then established, followed by local experience with imported cadaver kidneys. During this period, commercialized living unrelated donor transplantation undertaken in neighboring countries thrived. The region has seen considerable progress, with almost all Middle Eastern countries now having successful transplant programs, including several with active cadaver donor programs.

There are three predominant models of organ donation and transplantation in this region.[255] In the Saudi model, a quasi-government organization is responsible for all aspects of organ donation, from increasing awareness in the medical fraternity and public education to organ procurement and allocation.[256] This organization has enjoyed considerable success as evidenced by a remarkable increase in the number of transplant centers and organs transplanted.[255] Despite this success, in 2003, of 1022 patients transplanted, 71% were living unrelated transplants performed outside the Kingdom, whereas 228 living related transplants and 71 cadaver transplants were performed locally.[151]

Iran performs the most kidney transplants (24 pmp) in this region, of which 77% are from living unrelated donors.[20] The Iranian model allows for living unrelated donors to be compensated by the recipient or a charitable organization, and the cost of the transplant is carried by the government.[82] The model has been so successful that the waiting list for kidney transplantation in Iran has been eliminated.[81] Although several ethical problems remain unresolved, improvements to the model have been suggested that would mandate that all compensation be made by the government, and that compensation be substantial and "life-changing."[81] Despite the success of the program, the feedback from living donors clearly indicates that much remains to be done to improve the process of donation.[255]

Finally, the Pakistani model pioneered by the Sind Institute of Urology and Transplantation involves community and government partnership in the care of patients, in which the latter contributes 40% toward the cost of the transplant and the community the remainder.[220,223] The center has averaged 110 kidney transplants per year. The free supply of medication to the patient is an important factor in the success of the initiative. The program prides itself on its transparency, accountability, and quality of care.[223]

Sub-Saharan Africa

The lack of registries in sub-Saharan Africa makes it difficult to establish the prevalence and etiology of ESRD in this region. The most reliable data from this region were contained in the South African Dialysis and Transplant Registry, although it was last updated in the 1990s. The most common causes of ESRD were chronic glomerulonephritis and hypertension.[239] A more recent report suggested that diabetes was, as elsewhere in the developing world, likely to become an ever-increasing problem.[176]

Of all the developing regions, sub-Saharan Africa has the lowest transplant activity, averaging less than 5 pmp, and in contrast to most other regions where renal replacement is increasing even modestly, activity in this region seems to be declining (Fig. 36-7). It has been estimated there are less than 4000 ESRD patients on treatment in sub-Saharan Africa, constituting less than 1% of dialysis patients in the world.[21] Reasons for this are not hard to find. This region is beset by poverty (according to the World Bank, 41% of the population of sub-Saharan Africa live in extreme poverty, i.e., < U.S. $1/day), poor governance, migration of skilled health personnel, and lack of resources, among other serious problems.[20]

The major health problems facing this region are tuberculosis, malaria, and hypertension, but the biggest problem is the acquired immunodeficiency syndrome (AIDS) pandemic, which is decimating the population indiscriminately and consuming valuable health resources.[176] Sub-Saharan Africa, with a population of 752 million, or 11.6% of the world population, has two thirds of all human immunodeficiency virus (HIV) cases. Health authorities are under pressure to fight this scourge with the result that other areas of health care, such as renal replacement therapy, are neglected. AIDS has caused life expectancy in this region to decline to 48 years. South Africa, with the only transplant program in sub-Saharan Africa, performs 300 transplants per annum with 85% of transplants being cadaver in origin.[175,239] South Africa presently provides transplant expertise to patients from Namibia, Botswana, Zimbabwe, Lesotho, Swaziland, and Mauritius.

Central and Eastern Europe

Of all developing regions, Central and Eastern Europe has shown the most development in renal replacement treatment in recent years. This region represents 18 countries

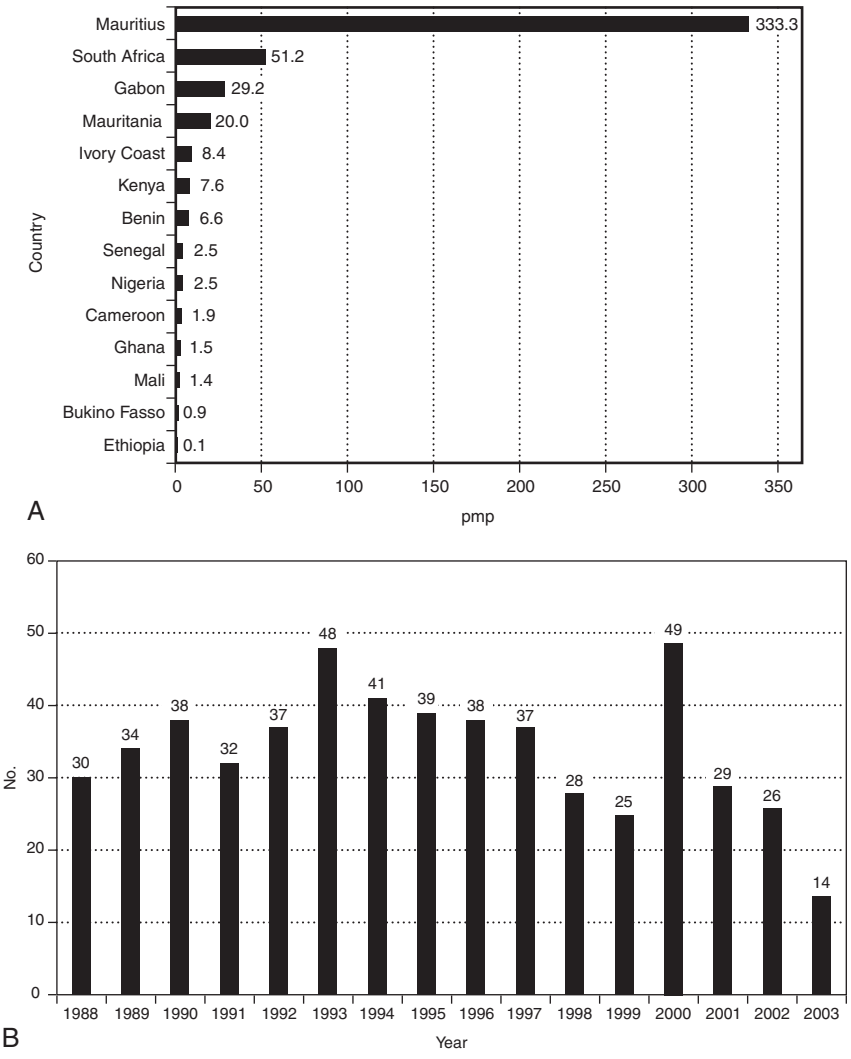

Figure 36–7 **A,** Estimated dialysis activity in sub-Saharan Africa (2000). This region is the poorest in the world and is the only one in which renal replacement activity is declining; there are many reasons for this, but the lack of resources and other health priorities are the main ones.[21] **B,** Kidney transplantation at a single center in South Africa. There is a progressive decline in transplant activity (unpublished data). In contrast to other developing regions, these were mostly cadaver transplants. Living donor transplants all were related; no unrelated transplants were performed at this center. The number of living donor transplants is increasing. (**A** from Bamgboye EL: Hemodialysis: management problems in developing countries, with Nigeria as a surrogate. Kidney Int 63(Suppl 83): S93, 2003.)

with more than 330 million inhabitants. The epidemiology of ESRD is changing here as it is elsewhere in the world. The main cause of ESRD is still chronic glomerulonephritis followed by interstitial kidney disease.[141] Diabetes is accounting for an increasing proportion of patients with ESRD averaging 10% to 14%, but in Czech Republic, it accounts for 31% of all dialyzed patients.[235] Another important epidemiological observation is the aging of the population, which may explain the increase in hypertensive renal disease.[235]

After the collapse of communism, many of the Soviet bloc countries experienced adverse socioeconomic conditions, and this was reflected in the effective renal replacement therapy rates.[237] In the years since, the region has experienced phenomenonal socioeconomic growth and political stability with dramatic improvements in the prevalence of dialysis treatment.[236] Although dialysis has grown dramatically (average of 51.6%), the rate of increase in transplantation has been less spectacular (Fig. 36-8)[236]; since 1990, the number of transplant units in the region has increased by 148%, but the number of kidney transplants has increased by only 44%. The Baltic states, Poland, Lithuania, and Romania, have recorded the most progress with regard to developing dialysis facilities.

Russia has been least successful in developing facilities. Although the treatment rates in some of the other Central and Eastern European countries exceed the European mean,[158] the rates in Russia are similar to those in India and China.[236] Most patients receive hemodialysis, but there has been a satisfying increase in peritoneal dialysis, with almost 10% of patients in this region receiving this treatment.[236] From having no patients on peritoneal dialysis in the early 1990s, in 2003, 18% of patients were receiving peritoneal dialysis in Romania, and the plan is to allow this to grow to 30% by 2008.[158] Romania has enjoyed astounding development and is exemplary of what can be achieved with the necessary pressure from clinicians, the support of a strengthening economy, and, perhaps most importantly, the correct political decisions and support.

Transplantation collapsed in many of the Balkan states after the political upheaval in that region. Currently, renal transplantation is well developed in only half of the Central and Eastern European countries.[236] The Baltic countries, especially Estonia and Latvia, have been performing exceptionally, whereas in some larger countries, such as Russia, transplant activity is less satisfactory.[236] In contrast to the substantive growth in dialysis, growth in kidney transplantation in Romania has been less successful. Most transplants are from living related donors, whereas the cadaver donor program has failed to grow significantly for numerous reasons. Although the growth in dialysis is commendable, the

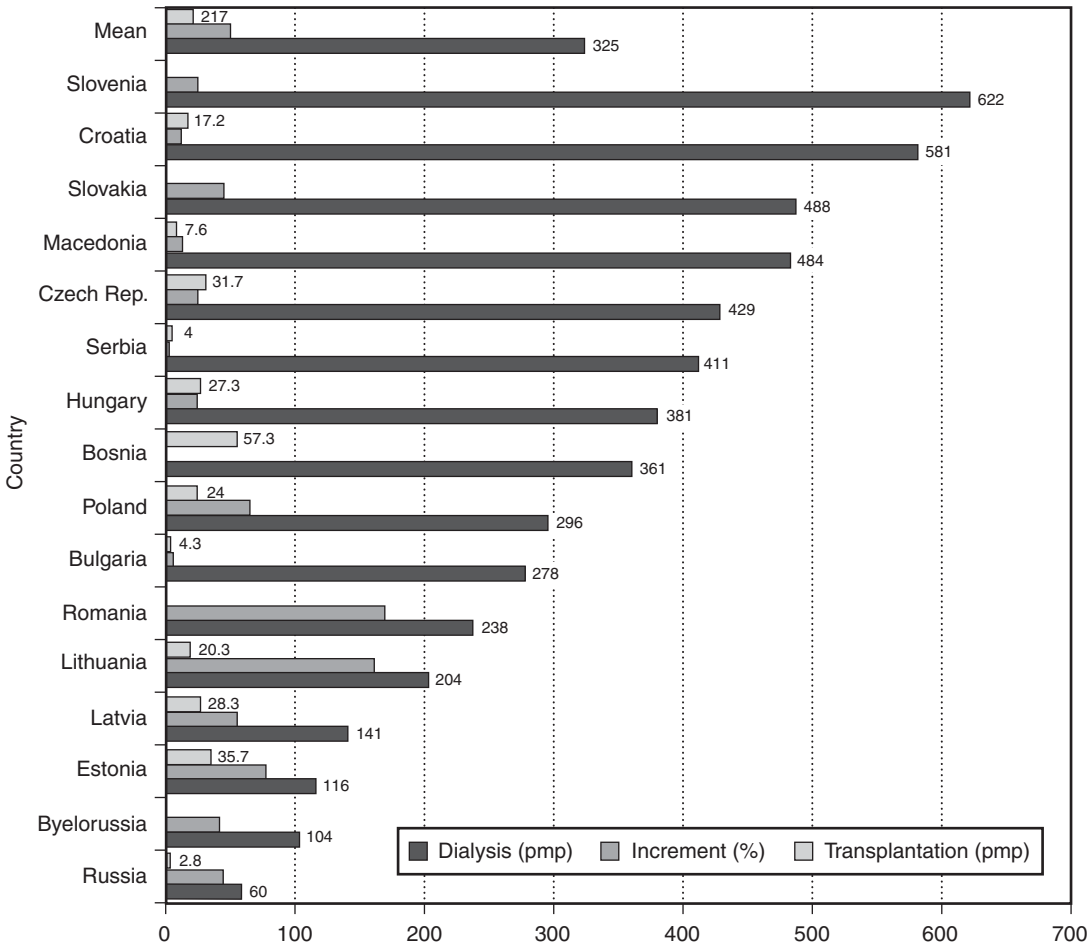

Figure 36–8 Renal replacement activity in Central and Eastern Europe (2002). The prevalent dialysis rates are indicated, and the percent (%) increase in the number of patients since 1998 is shown. The growth in transplant activity in this region has failed to keep pace with the increase in dialysis activity.[236] Data on Bosnia-Hercegovina (Bosnia) are unavailable. For Romania, 2003 data are used for comparison.

lack of a commensurate growth in the transplant program has the potential to overwhelm available dialysis resources rapidly.

A large part of the growth in renal replacement therapy can be ascribed to countries adopting free market systems of economy and allowing significant investment by private companies. The most successful countries, such as Hungary, Slovakia, and Lithuania, have allowed private facilities to proliferate, whereas Russia and Byelorussia have no private facilities. Romania is the exception, having developed without private sector input until 2004.[236]

IMMUNOSUPPRESSION

Cyclosporine-based prophylaxis remains the mainstay of immunosuppressive regimens in developing countries (Table 36-4). The availability of safe, efficacious, and cheaper generic versions of cyclosporine and extensive experience with the drug make it a popular choice.[150,183] Steroids and azathioprine were standard treatment up to the early 1980s, and are still used in some living related donor transplants with very good matches.[23,258] Acute rejection is treated with pulses of methylprednisolone and polyclonal or monoclonal antibodies if the rejection is resistant.[23] Antibodies also occasionally are used in induction, especially in high-risk patients, such as the elderly[183,271]; some centers use antibodies routinely in cadaver donor transplants to reduce early cyclosporine nephrotoxicity.[13]

In Latin America, there has been a shift in immunosuppression from cyclosporine-based therapy to regimens increasingly using mycophenolate mofetil and tacrolimus. In addition, the full spectrum of biological antisera is used in induction and treatment of rejection.[249] Sirolimus is the only newer agent that has failed to gain widespread acceptance.[249] In other parts of the developing world, costs limit the use of mycophenolate mofetil and tacrolimus. If chronic allograft nephropathy is diagnosed, some centers substitute mycophenolate mofetil for azathioprine and reduce the dose of cyclosporine.[183]

In many countries, several strategies are employed to reduce the cost of immunosuppression. First, cyclosporine is withdrawn at 3 to 12 months after transplantation, especially in patients with well-matched living donor kidneys, who have had no acute rejection episodes.[125] The risk of acute rejection is greatest the earlier withdrawal occurs; withdrawal after 1 year seems to be safer and reduces the risk of cyclosporine nephrotoxicity whereas graft and patient survivals are comparable.[65] Slow withdrawal of cyclosporine over several months is associated with less acute rejection than rapid withdrawal.[117] Even with careful cyclosporine withdrawal, more than 25% of patients have acute rejection[65]; in addition, the mortality and renal function of patients who undergo rejection are much worse.[65,117] After 1 year, there are no additional benefits in terms of patient and graft survival; long-term outcome may be compromised by sustained use of cyclosporine.[166]

The second strategy employed to reduce the dose of immunosuppression and effect significant cost savings is combining use of cyclosporine with either ketoconazole or diltiazem.[3,196] Ketoconazole elevates the blood level of cyclosporine, allowing 75% to 80% reduction in cyclosporine dose with commensurate cost savings.[75] Additional benefits are reduction of chronic allograft nephropathy and better blood pressure control. In contrast to the first strategy, the risk of acute rejection is not increased with the coadministration of ketoconazole.[70] The savings must be weighed against the cost of additional monitoring of cyclosporine drug levels required, potential hepatotoxicity of ketoconazole, and the danger of nonadherence.

TRANSPLANT OUTCOMES

Patient and graft actuarial survivals (see Table 36-4) serve as crude indicators of the success of transplant programs. Comparisons of outcomes of kidney transplantation are confounded by an array of variables that make comparisons between regions especially difficult. These factors include differing experiences of centers, patient mix (e.g., in terms of age, ethnicity), donor source, immunosuppressive regimens, follow-up periods, and compliance.[103] Many centers in developing countries can boast results that compare favorably with the best in the world.[13] The introduction of low-dose steroid regimens resulted in the reduction of patient mortality to less than 10% by the end of the 1970s when few developing countries were involved in transplantation. Graft survival remained at 60%, however, until the introduction of cyclosporine in the early 1980s, which resulted in dramatic improvements in 1-year graft survival rates.[173]

In most countries transplanting in the 1990s and since 2000, patient survival at 1 year was greater than 90% (see Table 36-4).[104,140] At 5 years, patient survival ranged from 70% to 95%, with most centers reporting survival rates of 80%. At 10 years, patient survival decreased to 43% to 80%.[193] The graft survival of living donor transplants, the major source of kidneys in developing countries, was generally very good, and 1-year graft survival rates compared favorably with reports from developed countries. Actuarial survival averaged 88% at 1 year in the reports in Table 36-4 and decreased progressively with longer follow-up. Graft survivals in HLA-identical donor transplants of 95% at 5 years have been reported, whereas survival in HLA-haploidentical and poorly matched donor transplants was equally impressive with 5-year survival rates of 90%.[127,222]

Few developing countries have well-established cadaver donor transplant programs. Latin America has the most active cadaver donor transplant program among developing regions of the world. The 1-year and 3-year graft survival rates of transplants performed between 1987 and 1997 were 74% and 60%.[187] Of the new programs, that of Saudi Arabia reported good results,[13] whereas promising results have been reported from India in a few patients (see Table 36-4).[262]

Although much vilified and without entering into the ethical debate surrounding commercial living unrelated transplantation, the results of graft survival are comparable to the results of living related transplants. In one of the earliest reports from the Middle East on 130 recipients transplanted abroad, the actuarial patient and graft survivals were 81.5% and 77%, with graft loss resulting mostly from patient mortality.[245] Of the 24 patients who died in the first year, 56% of deaths were ascribed to infections. In a Saudi study, patient survival of 86% at 2 years was reported in patients transplanted abroad; this rate compared with 100% and 95% 2-year patient survivals for living related donor and cadaver donor transplants in patients transplanted locally.[46] In other reports from the same period, 2-year actuarial graft survival was 82%, which was slightly better than that of living related donor transplants.[276]

Table 36–4 **Immunosuppressive Regimens Used in Selected Developing Countries and Outcomes of Kidney Transplantation**

Country (Period)	No. Transplants	Donor Type	Immunosuppression*	% Survival (at Year Indicated)		Reference
				Patient	Graft	
Australian Aborigines (1991-2000)	161	CD/LD	NS	94 (1), 86 (5)	93 (1) + 63 (5)[†]	154
Bangladesh	68	LRD	Aza	—	96 (1) + 81 (3)	212
(1982-1992)	26	LURD	CsA[‡]			
Brazil	687	LRD	NS	—		84, 253
(1970-1989)	60	LURD	CsA (42%)	—	70 (1), 49 (5)	
(1987-1989)	239	CD	CsA (75%)	—	76 (2)	
	1051	LD		89 (2)	61 (2)	
	467	CD		80 (2)		
	45	?				
China (2002)	2016	LD	—		83 (1), 66 (5), 48 (10)	201
Egypt (1994)	45	LRD	Aza (good matches)	92 (1), 86 (5)	89 (1), 73 (5)	22, 23
(1992)	130	LURD(C)	CsA	88 (1), 80 (4)	86 (1), 58 (4)	
	15	CD	CsA			
	30	LRD	CsA			
	124	LURD(C)	CsA			
(1976-2001)	82	Preemptive	Aza/CsA	95 (1), 88 (5)	94 (1), 81 (5)	69
	1197	LD	Aza/Csa	96 (1), 88 (5)	92 (1), 74 (5)	
India (1985-1988)	153	LRD	Aza	—	83 (1)	276
	303	LURD(C)	CsA (low dose)	—	83 (1)	
(1981-1989)	144	LRD	Aza	53 (10)	47 (10)	5
(1995-2001)	100	CD	NS	86 (1), 80 (2)	82 (1), 74 (2)	262
(1994-2004)[§]	39	LRD	CsA[‡]	89 (1), 70 (3)	89 (1), 50 (5)	89
(1984-1996)[§]	63	LRD	CsA	92 (1), 90 (3)	88 (1), 86 (3)	157
Iran (1986-2000)	478	LRD[¶]	CsA/MMF	93 (1), 84 (5), 73 (10) overall	89 (1), 82 (5), 70 (10)	82
	942	LURD	CsA/MMF		87 (1), 64 (5), 44 (10)	
(1996-1999)	207	LD	CsA	94 (1), 90 (3), 77 (10)	89 (1), 83 (3), 54 (10)	129
(1992-2002)	242	LD	—	—	87 (1), 84 (5), 71 (7)	92
Iraq (1979-1999)	182	LD	Csa/Aza	83 (1), 80 (5)	84 (1), 64 (5)	10
Korea (1979-1996)	1500	LD/CD	Aza/CsA	91 (5), 80 (10)	81 (5), 61 (10)	193
Kuwait	53	LURD(C)	NS	90 (2)	90 (2)	122, 247,
(1985-1990)	151	LRD	CsA (MMF)	94 (1), 92 (5)	89 (1), 85 (5)	248
(1993-1998)	158	CD	NS	93 (1), 89 (7)	81 (1), 75 (7)	
(1996-2004)	402	LRD	NS	97 (1), 95 (5)	95 (1), 91 (5)	
Latin America	5347	CD	CsA*	NS	74 (1), 60 (3)	187
(1987-1997)		LRD	CsA*	NS	86 (1), 74 (3)	
Mexico	282	LRD	CsA 1984[‡] (Aza in HLA-identical LRD)	86 (1), 68 (5)	77 (1), 60 (5)	34
(1967-1991)	10	LURD				
	46	CD				
Macedonia	16	LURD (C)	CsA/Aza/MMF	78 (1), 70 (5)	78 (1), 33 (5)	104
(2004)	14	LURD	As above	100 (1), 86 (5)	100 (1), 78 (5)	
Myanmar (Burma) (1997-2003)	21	LRD	CsA	95 (1)	95 (1)	277
Pakistan (2002)	1000	LRD	CsA 1990	95 (1), 85 (5)	90 (1), 75 (5)	223
(1992-2000)	711	LRD	CsA/MMF	90 (1), 78 (5)	90 (1), 75 (5)	183
(1986-1999)[§]	75	LRD	CsA	90 (1), 75 (5)	88 (1), 65 (5)	225
Philippines	1024	LRD	CsA[‡] (1983)	90 (1)	90 (1)	140
(1969-1992)		CD	CsA[‡]	75 (1), 71 (3)	62 (1), 56 (3)	
Saudi Arabia	~2500	LRD	CsA	96 (1)	90 (1)	8, 11, 13
(1999)	910	CD	CsA	95 (1)	78 (1)	
	60	LURD(C)	CsA	94 (1), 81 (3)	93 (1), 60 (5)	
(1991-1996)	172	LD	CsA	99 (1), 93 (5), 86 (10)	97 (1), 86 (5), 70 (10)	
(1987-1996)	188	CD	CsA (ATG)	98 (1), 94 (5), 91 (10)	86 (1), 72 (5), 58 (10)	
Singapore	47	LRD	CsA	95 (1), 88 (7)	86 (1), 77 (7)	285
(1985-1992)	157	CD	CsA		98 (1), 92 (6)	

Table 36–4 **Immunosuppressive Regimens Used in Selected Developing Countries and Outcomes of Kidney Transplantation—cont'd**

Country (Period)	No. Transplants	Donor Type	Immunosuppression*	% Survival (at Year Indicated) Patient	Graft	Reference
Slovenia	83	CD	CsA	91 (1), 88 (3)	73 (1), 73 (3)	128
(1986-1991)	65	LRD	CsA	95 (1), 93 (5)	90 (1), 90 (3)	
South Africa (1976-1999)	542	CD	Aza/CsA	81 (1), 60 (5)	50 (1) Aza, 72 (1) CsA	166
(1984-2003)§	282	LRD/CD	CsA	97 (1), 84 (5), 68 (10)	82 (1), 44 (5), 23 (10)	200
Sri Lanka (1985-1992)	105	LRD	CsA‡	71 (1), 47 (4)	71 (1), 47 (4)	259
Taiwan (1968-1992)	~1000	LRD/CD	NS	92 (1)	82 (1)	135
Tunisia (1986-2005)	330	LRD/CD	CsA/Aza	NS	85 (1), 30 (5), 16 (15)	35
Turkey (1985-1989)	80	LURD	NS	95 (1-3)	80 (1-3)	61
(1975-1993)	766	LRD	CsA (1985)	Aza: 60 (10)	Aza: 42 (10)	91
	230	CD	CsA	CsA: 87 (1), 72 (5)	CsA: 66 (1), 37 (5)	
(1985-1992)	391	LRD	DST + Aza or + CsA	DST 98 (1) – DST 94 (1)	92 (1) 72 (1)	94
(1992-1999)	115	LURD(C)	CsA	90 (2), 80 (5)	84 (2), 66 (5)	254
	NS	LRD	NS	90 (2), 85 (5)	86 (2), 78 (5)	
(1991-1995)	127	LURD(C)	CsA	93 (1), 92 (5)	83 (1), 57 (5)	55
UAE/Oman (1984-1988)	130	LURD(C)	CsA	82 (1), 81 (3.75)	77 (1), 75 (3.75)	246
Venezuela (2002)	NS	All	NS	NS	83 (1), 50 (10)	31
		LRD	NS	NS	90 (1), 64 (10)	

*Regimen predominantly used.
†Primary graft survival rate censored for patient survival.
‡Cyclosporine discontinued at 3-12 mo.
§Pediatric cases
¶HLA-identical matched donor.
ATG, antithymocyte globulin; Aza, azathioprine; CD, cadaver donor; CsA, cyclosporine as part of triple or dual therapy; DST, donor-specific blood transfusion; LD, living donor; LRD, living related donor; LURD, living unrelated donor; LURD(C), commercial living unrelated donor; NS, not specified.

The superiority of unrelated transplants was confirmed in subsequent reports comparing outcome with living related donor transplants done at the same center.[23,276] In a comparison of patients who received commercial transplants abroad with patients who received living unrelated transplants locally in Macedonia, the former had a slightly higher mortality,[104] whereas a Turkish report found comparable survival rates.[254] Significant morbidity was reported in most studies of commercially transplanted recipients, but these studies were uncontrolled. Infection was the most common reported complication and was the most common cause of mortality; surgical problems also were common.[11,51,104] The unique Iranian program of living unrelated transplantation has been well described, and the long-term results have been good; 5-year patient survival in Iranian living unrelated donor transplants was 91%[207] and at 10 years was 73% (see Table 36-4).[82]

POST-TRANSPLANT COMPLICATIONS

Optimal immunosuppression in the transplanted patient is a delicate balance between maximizing graft survival and minimizing complications. Most complications arise from immunosuppression, but other post-transplant diseases may occur as a result of the underlying chronic disease that caused renal failure. Recipients of renal allografts in developing countries may be more prone to certain complications, such as infections, which are the most common cause of post-transplant mortality.[60,247] Contributing to the risk for infections are protein-calorie malnutrition, tropical climate, lower socioeconomic status, lack of hygiene, lack of potable water, presence of parasites, and perhaps genetic factors.[219] Cardiovascular disease is the second most common cause of mortality in transplanted patients[60] and may become the primary cause as infections are conquered.

Infections

Although patients in developed countries have experienced a dramatic reduction in the rate of post-transplant infections from 70% in the early days to 40% currently, and a concomitant reduction in mortality from 40% to 5%, their counterparts in developing countries continue to battle with this problem.[114,133] Infections complicate the post-transplant course of 50% to 75% of recipients in these regions, and mortality ranges from 20% to 60%.[114] Because a successful transplant is the only viable treatment for most of these patients, graft retention is crucial, and immunosuppression

is often maintained in the presence of serious infection. Other factors contributing to the high incidence of infections and resulting mortality are delayed presentation and diagnosis, and the high cost of vital antimicrobials.[114] Limited availability and the expense of diagnostic tools, such as tissue biopsy, antigen testing, polymerase chain reaction, and facilities for the culture of unusual organisms, further aggravate the situation.[116,133] Immunosuppressed patients are more prone to develop infections endemic to the region, and dormant infections, such as tuberculosis, *Strongyloides stercoralis, Leishmania*, and herpesviruses, may flare.[114]

Bacterial Infections

Most infections are of bacterial origin and are commonly encountered in the early postoperative periods. The urinary tract and lungs are the most common sites infected. The classic symptoms of urinary tract infection are almost consistently absent, with the diagnosis being made on the presence of bacteriuria.[229,280] The most common organisms isolated are *Escherichia coli* and *Klebsiella*. Although the response to antibiotic treatment is good, relapses are common. Organisms resistant to commonly employed antibiotics are prevalent.[19] Their eradication is often problematic because these organisms respond only to expensive and parenteral antibiotics that are impractical to use.

Emphysematous pyelonephritis is a serious complication that may necessitate graft nephrectomy.[114] Pneumonia (excluding tuberculosis) occurred in 16% of 110 South African renal allograft recipients at a mean of 91 days post-transplantation[67]; this is comparable to the 18% reported from the Indian subcontinent. Causative organisms range from community-acquired *Streptococcus pneumoniae* and *Haemophilus influenzae* to dreaded multidrug-resistant nosocomial organisms.[114] With appropriate intervention, patients with lung infections respond very well.[67]

Tuberculosis

In developing countries, the incidence of tuberculosis post-transplantation is considerably higher than in industrialized countries; malnutrition, overcrowding, HIV/AIDS epidemic, poverty, and illiteracy contribute to this high incidence (Table 36-5).[169] In countries of the Indian subcontinent, 12% of renal transplant patients[179,243] develop tuberculosis compared with 1.7% in the United Kingdom.[96] In Turkey, tuberculosis is 8.5 times more common than in the general population.[43]

The interval between development of tuberculosis post-transplantation varies from 1 month to 10 years, but 50% to 80% occur within 1 year of transplantation.[179,243,293] Transplant recipients who have had treatment for acute rejection with steroids or monoclonal or polyclonal antibodies are at greater risk of tuberculosis.[16,28,169] The disease typically manifests with the classic symptoms of cough, fever, night sweats, and weight loss,[28] but the classic features of tuberculosis are often obscured by immunosuppression.[58,146,169] Transplant patients are prone to developing extrapulmonary and disseminated forms of tuberculosis; these forms of tuberculosis may account for 12% to 46% of all cases of post-transplant tuberculosis.[169,181,204,243]

The diagnosis of tuberculosis, especially extrapulmonary forms, may be challenging, and a high index of suspicion should be maintained in the appropriate setting.[58] The chest x-ray fails to show the typical apical cavitary disease in 90% of cases and may show pulmonary opacification or effusions instead.[118,169,283] Diagnosis of pulmonary tuberculosis is made by examination of the sputum for acid-fast bacilli using appropriate staining techniques and culture, although the latter is time-consuming and expensive. The diagnostic yield can be enhanced by bronchoscopy and bronchoalveolar lavage.[118] The polymerase chain reaction test for tuberculosis is used increasingly in the diagnosis of tuberculosis,[28,181] but it has a high false-positive rate.[252] The tuberculosis skin test has limited diagnostic value in developing countries, where tuberculosis is endemic, and most of the population has been exposed to the tubercle bacillus.[204,278] A positive skin test, regardless of degree, implies infection and not disease. Most renal transplant patients are anergic.[28,118,278] For extrapulmonary forms, bone marrow biopsy and liver biopsy should be considered.[133]

The treatment of tuberculosis in kidney transplant recipients poses no less challenge, mainly because of drug interaction. Most transplant patients receive triple-immunosuppressive therapy based on cyclosporine, whereas rifampicin and isoniazid are the mainstays of antituberculous treatment. Rifampicin and, to a lesser extent, isoniazid are potent inducers of the liver cytochrome P-450 enzyme system, markedly increasing the elimination of cyclosporine and steroids. The dose of steroids should be doubled, but cyclosporine may need to be increased severalfold to maintain therapeutic

Table 36–5 **Incidence and Mean Latent Period to Diagnosis of Tuberculosis after Renal Transplantation in Selected Developing Countries**

Country/Region	Incidence (%)	Latent Period (mo) (Range)	Reference
Iran	1	15.7 (1-110)	28
South America	2.3	—	41
South Africa	1.7, 4.5, 6.6	15.3 (2-78)	169
India	11.8	20.7 (1-84)	243
Pakistan	15	(1-108)	181
Saudi Arabia	3.5	16.6 (1-84)	204
Turkey	3.1	15 (2-33)	43
Mexico	5	45.4	295
Philippines	3.1	13.4 (3-38)	88
China	6.3	[37% within 1 yr]	143

blood levels. The cost of treatment is increased, and the risk of acute rejection is enhanced.[114,169,181]

Patients with renal allografts who develop tuberculosis respond well to conventional therapy.[43,56] The duration of therapy is determined by the choice of drugs. If the combination of isoniazid and rifampicin is used with another agent, usually pyrazinamide, 6 months of therapy should be adequate,[169] although some centers treat for 9 months.[114] If a rifampicin-free regimen is used, treatment should be continued for a minimum of 9 to 12 months[169,283] and possibly extended to 18 months.[114,243] With prolonged therapy, compliance is always a potential problem, and multidrug resistance is an ever-increasing concern.[292] A developing country innovation, directly observed therapy, has ensured the success of intermittent therapy where other techniques have failed.[19,132] The use of chemoprophylaxis is unresolved in the absence of controlled studies. Many centers use isoniazid when a transplant patient has historical or radiological evidence of tuberculosis.[23,57,96,169,181] Other centers believe the small risk of tuberculosis when low doses of steroids are used does not justify use of chemoprophylaxis, and that drug resistance is a risk.[133,204] The mortality of disseminated tuberculosis is high in transplant recipients in developing countries—almost 40% compared with 11% in the isolated form.[204]

Protozoan Infections

MALARIA

Malaria, caused by *Plasmodium*, is the most common parasitic infection in developing countries, where it continues to have a major influence on social and economic development.[41] Malaria may occur in renal transplant patients after the bite of an infected female anopheline mosquito, from the transfusion of infected blood,[41] or rarely from an infected kidney.[48] Most reported cases have occurred in recipients of living unrelated transplants who received their grafts in India and were diagnosed when they returned home after transplantation.[88,281] A high index of suspicion should be maintained in patients who have traveled in malaria endemic areas who present with high fever weeks after the visit; these patients should have examination of thick and thin blood films for the parasite. This is the most cost-efficient diagnostic test in a developing country. Repeated examinations of blood films are essential, preferably by a skilled technologist. An indirect fluorescent antibody test for malaria also is available.

Patients respond well to standard antimalarial treatment, and the prognosis is good.[281] There generally are no contraindications to the use of malaria chemoprophylaxis in renal transplant patients. Recommendations of specific prophylaxis vary from region to region and from time to time. Expert advice should be sought before visiting a particular region. Patients traveling to malaria endemic areas should be advised that personal protection measures, such as covering of arms and legs, use of insect nets and repellents, and avoiding nocturnal excursions, to avoid mosquito bites are important to prevent malaria.[66]

CHAGAS' DISEASE

American trypanosomiasis (Chagas' disease) is endemic in South America, where an estimated 16 to 18 million people are infected with the extracellular protozoan *Trypanosoma cruzi*. Chagas' disease is usually transmitted by the feces of blood-sucking insects or by blood transfusion. The disease may manifest with acute, subacute, or chronic clinical features. The acute presentation is a febrile illness in children associated with vomiting, diarrhea, and chagomas. The subacute and chronic forms manifest with myocarditis and heart failure, with the chronic form being complicated by megacolon and megaesophagus.

The infection has been transmitted with donor organs.[45] Liberalization of use of organs from donors with Chagas' disease was controversially instituted in the late 1980s in Argentina.[215] All recipients were very carefully monitored serologically and for parasitemia. The disease occurred in 19% of uninfected kidney recipients; it manifested with fever and patent parasitemia 1 to 5 months after transplantation. Reactivation of the disease occurred in 22% of chagasic recipients 1 and 29 months after transplantation. Almost half of recipients became serologically nonreactive at a mean of 78 days after initiation of immunosuppressive treatment. Patients responded well to benznidazole, the specific therapy available. In view of the low transmission rate and availability of effective treatment, the use of organs from potential seropositive donors should not be excluded. Chemoprophylaxis in seropositive patients who receive heavy immunosuppression is controversial, but serial monitoring for parasitemia and serology should be standard practice in endemic areas.[41,215]

VISCERAL LEISHMANIASIS (KALA-AZAR)

Visceral leishmaniasis is caused by *Leishmania donovani* and is endemic in parts of India, Africa, and Southwest Asia. Full-blown visceral leishmaniasis manifests clinically with fever, weight loss, hepatosplenomegaly, cytopenias, and hypergammaglobulinemia, although it is suspected that most human infections are subclinical. Kidney transplant patients are at risk of visceral leishmaniasis because of impaired cellular immunity.[15,32,257] The disease has been reported in men who had lived in or traveled to endemic areas. The patients develop clinical features of disease 3 months to 8 years after transplantation and manifest typically with the full-blown clinical picture of the disease. Diagnosis is confirmed by examination of bone marrow aspirate for the intracellular *Leishmania* amastigotes. Serology also may useful. The treatment of choice is sodium stibogluconate for 20 to 30 days.[114] Reported mortality is 28%, with all fatalities related to superinfection by other microbes. Relapse can occur in 31% of the patients 2 to 6 months later but responds to retreatment with antimonials with or without allopurinol.[32]

Helminthic Infestations

SCHISTOSOMIASIS

Schistosomiasis is a major public health problem in many parts of the developing world. Schistosomiasis may cause kidney disease either directly through chronic glomerulonephritis with the deposition of immune complexes in *Schistosoma mansoni* infection[268] or indirectly after damage to the urinary tract by *Schistosoma haematobium*.[260] Patients with urinary schistosomiasis can be transplanted successfully. Graft and patient survivals are comparable with controls even with prolonged follow-up,[145] but urological complications

can occur in 15% of schistosomal patients.[261] Patients with schistosomiasis require 67% more cyclosporine to achieve the same blood levels as uninfected recipients because intestinal disease impairs absorption of cyclosporine.[145,266] Schistosomal reinfection occurs in approximately one quarter of patients, but this does not have an impact on graft function if the disease is adequately treated.[260] These patients may be at increased risk of bladder carcinoma, and cystoscopy should be part of long-term follow-up.[21,260]

In endemic areas, potential live donors should be screened carefully. The question of whether live donors with uncomplicated, treated disease should be accepted is controversial. Hefty and McCorkell[95] suggest that donors with a history of infection, but no cystoscopic or radiological abnormalities should be accepted; potential donors showing structural changes—even small "sandy" patches on cystoscopy—probably should be excluded because progression may lead to further urinary tract damage. Sobh and colleagues[267] failed, however, to find any adverse outcome in living donors who had uncomplicated schistomiasis. The mean follow-up was only 3.5 years, however.

STRONGYLOIDIASIS

Strongyloidiasis is an intestinal nematode infestation endemic in Southeast Asia, sub-Saharan Africa, and Central and South America. It is an uncommon but potentially devastating disease in immunosuppressed patients.[41,114] Because of the organism's capacity to multiply repeatedly within the host without external reinfection (in contrast to *Schistosoma*), a state of hyperinfestation may occur years after exposure. In recipients, this hyperinfestation may take a fulminant course (e.g., pneumonia, respiratory failure, severe diarrhea, or intestinal obstruction) accompanied by infection by other microbes.[269] Eosinophilia should alert the clinician to possibility of *Strongyloides* infestation because the worm may not be found in the stool unless it is concentrated after incubation. The worm also occurs in duodenal aspirates, and in severe cases larvae occur in sputum or bronchial aspirates.

In severely ill patients, supportive treatment may be needed, in addition to specific therapy with thiabendazole or mebendazole. In endemic areas, it is advisable to give prophylactic thiabendazole or mebendazole on several occasions to ensure eradication of migrating larvae and adult worms. *Strongyloides* may be transmitted with a kidney graft.[41,172]

Fungal Infections

Renal allograft recipients may develop either mucocutaneous or systemic fungal infections. Risk factors for fungal infections include hot humid climate, poor personal hygiene, and use of broad-spectrum antibiotics.[114] In the Indian experience, superficial fungal infection occurred in 60% to 72% of all renal transplant patients.[54,286] Tinea accounted for two thirds of all these infections, and *Candida* accounted for 7% to 9%. Topical treatment is effective in cutaneous infections, although prolonged griseofulvin or fluconazole may be required for widespread skin or nail involvement. *Candida* infection of the gastrointestinal tract occurred in 10% of patients and generally responded well to nystatin or clotrimazole, although fluconazole may be effective if there is no response to local treatment. *Candida* urinary tract infection is related almost invariably to the prolonged use of an indwelling urinary catheter. These patients respond well to removal of the catheter and amphotericin B bladder irrigation.[52]

Invasive fungal infections complicate the course of 1.4% to 10% of patients after renal transplantation, with a high mortality of 60% to 100% (Table 36-6).[53,178] The most commonly encountered pathogens are opportunistic organisms, such as *Candida* and *Cryptococcus*, but there has been a more recent increase in infection by angioinvasive *Aspergillus* and *Mucor*.[90,120] Infections also rarely have been caused by geographically restricted mycoses, such as histoplasmosis.[77] Almost two thirds of systemic fungal infections in the tropics occur more than 12 months post-transplantation, contradicting Rubin's timetable, which suggests that most fungal infections occur within 6 months.[52] The most common risk factors for the development of these infections are diabetes mellitus and cytomegalovirus (CMV) infection.[120] The most common presenting feature of systemic fungal infection is fever unresponsive to antibiotics.[52]

Systemic candidiasis, the most common invasive fungal infection in patients after renal transplantation in developing countries, manifests most commonly with clinical features of pyelonephritis affecting the graft.[53] Prolonged urinary catheterization, use of broad-spectrum antibiotics, and diabetes enhance the risk of infection. The diagnosis can be confirmed with culture of *Candida* in blood or urine.

Cryptococcus is common in renal transplant recipients in the tropics and is typically present in pigeon droppings and spread by aerosol. Patients with cryptococcal infection present most commonly with features of meningitis, and India ink staining of cerebrospinal fluid shows the presence of the organism.[53,108,119] Dissemination to other organs, such as the skin and eye, can occur. The diagnosis is confirmed on positive latex agglutination test or culture of the organism from cerebrospinal fluid, blood, or urine.[114]

Rhinocerebral mucormycosis typically manifests with cavernous sinus thrombosis. The diagnosis of mucormycosis is suspected clinically when patients have periorbital cellulitis and black necrotic pus discharging from the nasal mucosa and palate that characteristically shows *Mucor*.[52] Approximately 70% of renal transplant patients who develop mucormycosis are diabetic.[42] The disease also may manifest as a necrotizing pneumonia.[52]

Aspergillosis is an uncommon but serious fungal infection that carries a very high mortality in renal allograft recipients. It also most commonly manifests as a necrotizing pneumonia or disseminated infection.[114] Rare cases of infective endocarditis and allograft disease have been reported.[14,121] Diagnosis is made by culture of sputum or histology, but the diagnostic yield can be enhanced by bronchial lavage with or without transbronchial biopsy. The fungus is angioinvasive and produces extensive tissue infarction, reducing the efficacy of treatment.[114]

Treatment of invasive fungal infections can be challenging because of the limited range of effective drugs available and their toxicity. Amphotericin B is the drug of choice for these infections because it controls infections sooner, although fluconazole is less toxic. Fluconazole also increases cyclosporine levels.[77] Liposomal amphotericin B can be substituted for amphotericin B because although it is equally efficacious, it is less nephrotoxic.[83] The prohibitive cost of this agent often precludes its use in developing countries, however.[14]

Table 36–6 **Main Systemic Fungal Infections Reported from Developing Countries and Recommended Treatment***

	Nampoory[178] (*N* = 512)	Jayakumar[108] (*N* = 362)	Gupta[90] (*N* = 850)	Chugh[53] (*N* = 310)	John[120] (*N* = 1476)	Treatment[77, 90]
Frequency (%)	3.5	19	9.8	1.3	6.6	—
Mortality (%)	55.6	60	—	63	85	—
Candidiasis (%)	1.5	13.8	2.8	2.2	1.2	Ampho-B/Lipo-Ampho-B, fluconazole
Cryptococcosis (%)	0.6	0.8	1.9	2.9	1.5	Ampho-B/Lipo-Ampho-B
Aspergillosis (%)	1.2	3	2.3	0.6	1.8	Ampho-B/Lipo-Ampho-B, itraconazole
Mucormycosis (%)	0.4	1.5	2	0.6	0.9	Ampho-B/Lipo-Ampho-B

*Surgical resection may be warranted in certain infections, such as aspergillosis, to reduce organism.
Ampho-B, amphotericin B; Lipo, liposomal.
Data from Jha V, Chugh KS: Posttransplant infections in the tropical countries. Artif Organs 26:770, 2002.

Viral Infections

The herpes group of viruses takes an immense toll on the health of renal transplant patients in developing countries.[110,112] The ability of herpesviruses to establish latent infections that can be reactivated after primary infection to result in disease is key to the success of this virus group. The development of potent new antiviral agents and improved diagnostic and monitoring techniques has offset the challenge posed by these viruses.[110] The main culprit is CMV, which occurs in 60% to 90% of recipients in the first year post-transplantation on serological testing in a developing country setting. Of these, about one third develop overt disease, and 28% die as a result of CMV-related complications.[22,59,148]

Reactivation and de novo infection are the two epidemiological patterns of CMV infection recognized. Transmission of CMV from an infected donor to an unexposed recipient may occur. Symptomatic CMV disease occurs in the first 4 months post-transplantation when immunosuppression is most intense.[210] It usually manifests as a febrile illness, with neutropenia, thrombocytopenia, pneumonia, hepatitis, or gastrointestinal ulceration.[263] It also may predispose to other opportunistic fungal and bacterial infections.[214] In developing countries, the clinical diagnosis may be confounded by coinfection with hepatitis viruses, tuberculosis, and fungal infections.[210]

Detection of infectious virus can be established by either conventional cell culture or shell vial assay. The presence of CMV based on the pp-65 antigen also is used to detect acute viral infections with a high degree of sensitivity and to detect early disease.[119] Polymerase chain reaction for CMV DNA in peripheral blood also can be used to detect and monitor disease. Serology, although suggestive, is not always a reliable guide to active infection.[214] The treatment of CMV infection is with intravenous ganciclovir; if this fails, foscarnet or cidofovir may be used. Prophylaxis with intravenous ganciclovir in patients at risk has been shown to be effective at not only reducing the onset and severity of CMV disease,[174,240] but also reducing the incidence of acute rejection.[216] Oral valganciclovir is becoming available for prophylaxis but is prohibitively expensive. Diagnosed and treated early, CMV infection has a good outcome.

HEPATITIS INFECTIONS

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are cause for major concern in the management of ESRD patients in developing countries. The prevalence of these infections, which are usually acquired before transplantation, ranges from 12% to 53% for HBV and 4% to 68% for HCV in the dialysis populations of developing countries. The prevalence is higher than in the general population but similar in dialysis and renal transplant patients.[73] The outcome of kidney transplantation in terms of patient or graft survival in patients infected with either HBV or HCV is controversial.[152] Immunosuppression results in rampant viral replication that can result in acute hepatitis, chronic liver disease, and hepatocellular carcinoma.[63,152,206] In early reports, acute hepatitis occurred in 60% of HBV recipients with high mortality owing to acute liver failure.[231] In both forms of viral hepatitis, the presence of chronic liver disease is associated with a poorer outcome, and a biopsy specimen of the liver before transplantation may be valuable in guiding management of patients.[180,211]

Currently, no other clinical or laboratory markers are available that assist in identifying patients who are at risk of chronic liver disease, making liver biopsy a very important tool.[36,152] In HBV, hepatitis e antigen (HBeAg) and HBV DNA, although initially reported to be associated with increased mortality due to liver disease,[72] in subsequent studies have been found to bear no relationship to the later development or progression of liver disease.[194] Similarly, in HCV, no correlation was found between the development of fibrosis and any clinical or laboratory parameter, including HCV RNA titers and serum alanine aminotransferase levels.[36] More recent studies of the long-term outcome of hepatitis virus–infected patients indicate 10-year patient and graft survivals are compromised, although 5-year survival rates are comparable to those of uninfected patients, with HBV patients doing worse than HCV patients.[152] Controlled studies have shown that HBV patients had poorer graft and patient outcomes regardless of whether or not they had evidence of viral replication, such as HBeAg and HBV DNA.[152]

The treatment of transplant patients with hepatitis infection is fraught with difficulty and is unsatisfactory. Interferon should be used only before transplantation because of its propensity to trigger acute rejection of transplanted kidneys, enhancing graft loss. Treatment with interferon must be maintained for about 12 months to be effective in 30% to 70% of HCV patients. The high cost of treatment, the duration of treatment, and the unpredictability of response make it unlikely to be used widely in many developing countries.[101] Lamivudine has been used in HBV patients after transplantation and can result in clearance of HBeAg and HBV DNA in a significant number of patients with improvement of liver enzymes. Lamivudine is well tolerated and has no significant impact on graft survival.[194,250] The emergence of resistance and expense are major limiting factors in the use of this agent in developing countries.[194]

In view of the morbidity and costs associated with the development of viral hepatitis, prevention of these infections should be a priority. Recommended specific infection control measures should be implemented in all hemodialysis units, together with HBV vaccination preferably before the initiation of hemodialysis. HBV-positive patients should be physically separated from susceptible patients and have dedicated dialysis machines, instruments, supplies, and staff. Although isolation of patients with HCV is unnecessary, staff need to be encouraged to follow standard hemodialysis precautions because the virus undergoes nosocomial transmission.[131] Reducing unnecessary blood transfusions is important, especially in developing countries where the prevalence of HCV infection is higher.

Although screening and testing donors for HCV has now become standard in the West, the quality and extent of this practice in developing countries is uncertain. With vaccination against HBV[111] and the implementation of strict isolation practices, the risk of HBV infection has been considerably reduced.[114] In practice, however, many centers in developing countries do not have dedicated units for HBV patients, and immunization is often incomplete; in addition, because of the expense of dialysis, most HBV and HCV patients are offered transplantation unless they have active viral hepatitis.[114]

OTHER VIRAL INFECTIONS

HIV infection has reached epidemic proportions worldwide and is particularly rampant in developing countries. Of the 40 million people infected with HIV worldwide, greater than 95% live in developing countries. Despite this prevalence, HIV infection is not a major problem yet in dialysis populations in developing countries.[133] A partial explanation is that HIV-associated nephropathy is a late complication of HIV disease,[290] with many patients dying of other HIV-related complications, and in many countries, patients living with HIV are not offered transplantation.

Patients can acquire HIV as a result of organ transplantation either from unscreened blood products or from a contaminated kidney, usually following commercial transplants.[12] Of 540 Saudi patients transplanted in India, 4.3% became infected with poor survival. Expressed differently, organ transplantation accounted for 1.5% of all cases of HIV infection in Saudi Arabia.[12] In a report from South Africa, one of the countries with the highest prevalences of HIV infection, 1% of all recipients seroconverted at a mean of 5 years after transplantation, most likely through high-risk behavior.[126] The patients were not offered antiretroviral treatment and survived, on average, for 6 months after diagnosis. Management of these patients is uncertain. Although the use of antiretroviral agents would seem to be intuitively correct, in practice limited availability and potential drug interactions increased the complexity and cost of treating these patients.[105,134] The risk of transplanting an HIV-infected organ from a donor still seronegative (the window period) is a real concern in developing countries.[160]

Polyomavirus (BK virus)-induced nephropathy is a novel disease that occurs in approximately 5%[98] of all cases in developed countries and results in graft loss in 50% of affected patients.[64,284] Most cases have been associated with the use of tacrolimus with or without mycophenolate mofetil, although it would seem that the infection is associated with excessive immunosuppression rather than any specific agent.[37,99] It mimics acute rejection except that it occurs 10 to 13 months after transplantation.[208,284] It can be diagnosed with confidence only on histology, although the presence of decoy cells in urine provides a valuable clue.[208] Its prevalence in developing countries is uncertain. In a report from Korea, BK virus infection occurred in 4.7% of all patients. All the patients who developed disease were receiving tacrolimus and mycophenolate mofetil treatment suggesting a role for the intensity of immunosuppression.[102] Based on anecdotal cases, the therapeutic recommendation is that immunosuppression be reduced.[44]

Malignancies

Malignancies are an important complication of renal transplantation, occurring in 1% to 25% of renal allograft recipients. With patients surviving longer, the risks of malignancies is set to increase, and malignancies are the third most common cause of mortality after infections and cardiovascular disease.[223] The overall incidence of post-transplant malignancies is lower in developing countries; this could be related to the shorter duration of follow-up in developing countries that have relatively new transplant programs, younger patients, and lower intensity of immunosuppression in programs that perform predominantly living related transplants.[168] The pattern of malignancies in developed and developing countries also differs (Fig. 36-9).

Kaposi's Sarcoma

Kaposi's sarcoma is the most common malignancy in renal transplant patients in most developing countries, accounting for 80% of all malignancies in transplant recipients.[205] The incidence more than doubled under cyclosporine, and the disease occurs earlier than it did in the azathioprine era.[199] The mean time to the development of Kaposi's sarcoma is 21 months, but it may occur within months post-transplantation.

Kaposi's sarcoma is one of the earliest malignancies to develop after transplantation. The disease typically affects skin and usually manifests on the legs, with painless, reddish blue eruptions that may ulcerate.[167] Besides skin, lesions also may occur in the oropharynx and conjunctivae.[299] Visceral involvement, especially of lungs and gastrointestinal system, is usually a serious complication with an adverse prognosis.[167] Human herpesvirus-8 has been causally linked to all forms of Kaposi's sarcoma.[171,287]

In post-transplant Kaposi's sarcoma, the immunosuppression makes recipients more susceptible to the disease, as evidenced by remission of lesions with the reduction or

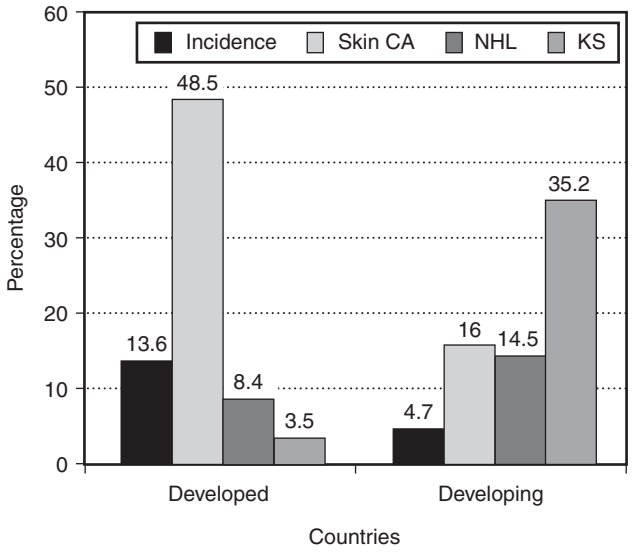

Figure 36–9 The incidence and pattern of malignancies in developed and developing countries. The most common malignancy in Western countries is skin cancer, whereas the most common malignancy in developing regions is Kaposi's sarcoma. The reason for this difference may be due to geographical or ethnic/genetic factors. Black and white patients in the same geographical region have patterns of cancer that epitomize that seen in the West and developing countries, emphasizing the importance of ethnogenicity. CA, cancer; KS, Kaposi's sarcoma; NHL, non-Hodgkin's lymphoma. (From Moosa MR: Racial and ethnic variations in incidence and pattern of malignancies after kidney transplantation. Medicine [Baltimore] 84:12-22, 2005.)

withdrawal of these agents; this is the primary form of treatment of the disease. Reduction of immunosuppression may be achieved safely with successful maintenance of graft function.[167] Success of this treatment varies, with 24% to 75% of patients undergoing partial or complete remission of Kaposi's sarcoma.[165,203] Radiotherapy, antiviral drugs, and a variety of cytotoxic agents have been used with varying success.[149]

Patients cured of Kaposi's sarcoma face an uncertain future. If grafts are rejected, patients need to be maintained on dialysis; retransplantation and further immunosuppression should not be undertaken without careful consideration because Kaposi's sarcoma recurs when immunosuppression is reintroduced.[9] Sirolimus, an immunosuppressant with antiproliferative properties, had been used successfully to treat Kaposi's sarcoma in renal transplant recipients,[270] and reports from developing countries are now starting to appear in which cutaneous and visceral disease have been successfully treated.[164,299]

Post-Transplantation Lymphoproliferative Disease

Post-transplantation lymphoproliferative disease is a syndrome that includes a spectrum of abnormal hyperplastic and neoplastic lymphocyte growths from a benign self-limited form of lymphoproliferation to aggressive, widely disseminated disease.[202] Approximately 85% to 90% of these growths are of B cell origin,[197] and 90% to 95% contain the Epstein-Barr virus.[202] Patients with post-transplantation lymphoproliferative disease have different histological findings, have a more aggressive clinical course (more extranodal disease, especially intestinal involvement), respond poorly to conventional treatment for lymphoma, and have a poorer prognosis (70% mortality) compared with immunocompetent individuals who develop lymphomas.[198,202]

On a worldwide basis, non-Hodgkin's lymphoma is the second most common post-transplant malignancy after skin and lip cancers. In developing countries, post-transplant lymphomas are more common than in industrialized countries, accounting for 14.5% of malignancies in developing countries and 8.5% of malignancies in industrialized countries.[168] These lymphomas are the major cause of cancer-related mortality and morbidity after transplantation.[18,168] In reports from developing countries, the latent period from transplantation to the diagnosis of post-transplantation lymphoproliferative disease was long (range 2.6 to 7 years).[106,202,298] The latent period was shorter when patients were receiving cylosporine-based treatment or OKT3 monoclonal antibody.[202]

SPECIAL CONSIDERATIONS IN TRANSPLANTATION

Pregnancy after Renal Transplantation

Pregnancy is uncommon in women on dialysis, and when it occurs, it is associated with a high rate of complications and fetal wastage.[147] Correction of the uremic state by a functioning renal allograft often restores fertility in women of reproductive age, and 2% to 3% become pregnant in Western countries.[139a, 271a] Reported pregnancy rates in women of childbearing age in developing countries are 14% (Brazil),[238] 31% (Oman),[7] and almost 50% in Saudi Arabia.[7]

All reports suggest that with extra care, pregnancy can be undertaken successfully after renal transplantation. Reported problems include hypertension in 67%, but this is controlled easily.[238] Preeclampsia is rare. Infections, predominantly of the urinary tract, can occur in 86% of pregnancies.[136,272] Graft and patient survivals are comparable to controls,[238,272] even after repeated pregnancies.[191] The incidence of obstetrical problems is high, however. Prematurity occurred in 67% of patients compared with 5% in the general population in one report.[238] The incidence of cesarean sections (76%)[238] and small-for-dates infants (64%) is increased,[8] but no congenital abnormalities were detected in any of the infants reported.[7,191,238,272] Available information suggests that pregnancy after successful renal transplantation is safe if the patient has normal renal function and delays conception for 1 year post-transplantation. Careful management by a multidisciplinary team is essential.

Transplantation in Children

A well-functioning renal allograft is the best treatment for a child with ESRD—perhaps even more so than in an adult—but children in developing countries constitute less than 5% of all renal allograft recipients.[89] The incidence of ESRD of 7 per 1 million child population in these countries is similar to or slightly higher than that reported from developed countries.[8,68] The causes of ESRD in children are most commonly chronic glomerulonephritis, chronic interstitial nephritis, and congenital abnormalities.[6,89,241]

Resources in developing countries for treating uremic children with dialysis are severely constrained and prioritized for the care of adults.[87,218] Transplantation offers the

recipient the opportunity of a better quality of life, improved growth and psychomotor development, and the re-establishment of social and psychological functioning. With the low incidence of cadaver donor transplantation in developing countries, living related donor transplantation is the main option for these children. Mothers are the donors in more than two thirds of cases.[89,241] Cyclosporine forms the basis of immunosuppression in developing countries.[200,225]

One-year actuarial graft and patient survival rates of 89% and 5-year survival rate of 50% have been reported from India.[89] In South Africa, where the option of dialysis exists, patient survival rates of 97% and 84% and graft survivals of 82% and 44% have been reported at 1 and 5 years.[200] Generally, these results are poorer than in developed countries and bear testimony to the challenges of undertaking this complex multidisciplinary intervention in a developing resource-constrained environment.[200] See Table 36-4 for results of pediatric transplantation.

Table 36–7 **Ethnic Differences in End-Stage Renal Disease Incidence, Delay in Transplantation, and Outcome in Americans (2002)**

	White	African American
Incidence (pmp)	256	982
Transplant rate (ppd)	3.5	2.2
Waiting time (mo)	817	1382
1-yr graft survival (%)	90.6	87.3
10-yr graft survival (%)	39	25

ppd, per 100 patient-years on dialysis; pmp, per 1 million population.

Data from U.S. Renal Data System: USRDS 2004 Annual Data Report. Atlas of Endstage Renal Disease in the United States. Bethsda, Md, National Institutes of Diabetes and Digestive and Kidney Disease, 2004.

Race and Ethnicity

Ethnic minorities and indigenous groups around the world share several characteristics: a higher incidence of ESRD (often strikingly so), an excess of comorbidities (e.g., hypertension but particularly diabetes mellitus), younger age of presentation with ESRD, greater delay and difficulty in accessing transplantation, poorer tissue matching, and paucity of cadaver donor organs from the group.[93,124,138,142,154,213,294] In many groups, allograft survival rates were inferior,[154,294] but not in all cases.[142,170]

In the United States, the incidence of ESRD is considerably higher in racial and ethnic minorities. Despite their greater propensity for ESRD, the kidney transplant rate is lower and the waiting time for transplantation is longer in minority groups because of differences in clinical appropriateness and underuse of transplantation (Table 36-7).[71,93] Early graft survival in African-American patients has improved as a result of improved immunosuppressive regimens, but long-term graft survival has remained significantly lower than in white counterparts. The inferior outcome in African-American patients remains largely unexplained, although a variety of immunological and nonimmunological factors have been described that may conspire together to prevent better results.[294]

In Australia and New Zealand, the incidence of ESRD is greater by eightfold among Aborigines; the mean age is 48 years (compared with 60 years); and the incidence of coronary heart disease, obesity, and diabetes is significantly higher. Indigenous ESRD patients are less likely to be waitlisted and even when accepted have lower rates of transplantation, and grafts were less well matched.[154] Overall mortality among recipients of all forms of renal replacement therapy, including kidney transplantation, is greatest in Australian Aborigines. Treatment of ESRD in this population by dialysis and transplantation is difficult for social, cultural, geographic, and economic reasons that together with the comorbid diseases and possible genetic factors contribute to the poorer results.[153,154]

Early experience with transplantation in South Africa revealed poor graft survival in black patients, especially patients receiving cadaver donor transplants (5-year survival was 28%).[162] Later reports failed, however, to find significant differences in outcome between nonwhite and white patients in South Africa,[170] although in black patients, a significant difference was observed in graft survival between living related and cadaver donor transplants at 3 years: 83% versus 43%. In black renal transplant recipients, the graft survival at 5 years was similar regardless of the race of the donor organ.[124]

Many transplant units in developed countries have a significant number of patients from developing countries. The impact of ethnicity and race of these immigrant communities on renal replacement therapy in their adopted countries is strikingly apparent.[213] Patients from South Asian immigrant communities are overrepresented on renal transplant waiting lists in the United Kingdom. They represent 2.5% of the population of England and Wales but 7% of all patients receiving renal replacement therapy.[142] The annual growth rate of the waitlist in Birmingham in the period 1990 to 1996 was 6.4%, but the rate was 24% for South Asian patients.[213] The rate of transplantation was significantly lower with an important contributing factor being the lack of suitable cadaver donors as a result of ethnic disparities in ABO blood group and HLA tissue types between the predominantly white donors and Indo-Asian recipients.[138] A solution to these biological differences is to increase the rate of organ procurement within the South Asian community.[209] More recent reports suggest that the rejection rates and graft survival in Asians and non-Asians are comparable.[109,142]

Similarly, a study from the Netherlands, which has a socialized health service providing uniform access to all, revealed no differences in overall graft survival between European and non-European recipients of primary cadaver renal transplants.[228] Analysis of the non-European recipients, predominantly first-generation immigrants from developing countries, revealed particularly good graft survival in Asian recipients. This finding parallels results in Asian patients in the United States, who have superior graft survival compared with other ethnic groups, although their access to transplantation also is limited.[43a, 93]

IMPORTANCE OF EARLY DETECTION AND PREVENTION OF CHRONIC RENAL DISEASE

Kidney transplantation is not only the best biological replacement for an irreversibly damaged kidney but also the most economical throughout the developing world.[100,133,288]

Kidney transplantation is considerably cheaper in developing countries, but in contrast to developed countries where the state contributes significantly to the costs, patients are personally responsible for costs in most developing countries.[133] In these countries, the annual cost of renal replacement is more than tenfold the GNIPC compared with twice the GNIPC in United States.[133]

With ESRD escalating worldwide, a paradigm shift was required especially in developing countries that bear the brunt of the problem. The emphasis was on treatment in the previous decades, whereas the 21st century ushered in a renewed interest in the early detection and prevention of chronic kidney disease, with clear demonstration of the benefit of angiotensin-converting enzyme inhibitors[232,233] and angiotensin receptor blockers.[137,195] Adding to the urgency was the recognition that proteinuria and chronic kidney disease were risk factors for cardiovascular disease, having a major impact on the overall health of the population.[97,217]

A strategy of early detection and prevention of chronic kidney disease especially in developing countries would not only reduce the overall burden of kidney disease but also cardiovascular disease associated with diseases such as diabetes and hypertension.[30] Primary prevention consists of lifestyle modifications, such as weight reduction, exercise, smoking cessation, and dietary changes, combined with tight control of blood pressure and diabetes mellitus.[30,156] For patients with established chronic kidney disease, in addition to the aforementioned measures, of which blood pressure is the most important, pharmacological agents are used. The most important are angiotensin-converting enzyme inhibitors, to which may be added angiotensin receptor blockers or nondihydropyridine calcium channel blockers.[156]

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Chapter 37

Results of Renal Transplantation

Stuart J. Knechtle • Peter J. Morris

Outcome data for renal transplantation in the United States represent one of the best available examples of medical care supported by a local and national database to allow evidence-based decisions in the field. According to requirements directed by the United Network for Organ Sharing (UNOS), a federal government–authorized body, all transplant centers must submit transplant data to the Scientific Registry of Transplant Recipients (SRTR), where such data are collated and analyzed on a center-specific basis and cumulative national basis. Much of the data from the United States summarized in this chapter is substantially derived from the 2006 SRTR report on kidney and pancreas transplant outcomes,[5] which is available in published form in the *American Journal of Transplantation* and available online at http://www.blackwell-synergy.com/loi/ajt.

The massive amount of data in the 2006 SRTR report has been reduced to that which is included in this chapter for the purpose of greater usefulness and readability. The source of the data is acknowledged in figures and tables. In addition, other data have been added to supplement the SRTR report, including individual center reports and multicenter trial data, and data from Europe through the Collaborative Transplant Study (CTS) and the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry. These data inform decisions regarding patient access and outcomes and organ allocation. These data refer to transplantation in the Western world; results from less well-developed countries are discussed in Chapter 36.

RENAL FAILURE TREATMENTS—DIALYSIS VERSUS TRANSPLANTATION

Renal failure is known to increase mortality from cardiovascular disease and from causes directly resulting from renal failure itself, including fluid and electrolyte imbalance and uremia.[34] Although dialysis addresses the immediately life-threatening complications of renal failure, it does not provide the fluid and electrolyte homeostasis comparable to a well-functioning kidney. Several additional metabolic functions of the kidney, such as vitamin D synthesis and erythropoietin synthesis, also are not regulated appropriately in the absence of a well-functioning kidney. This reality is reflected by the well-documented finding that patients with end-stage renal disease have improved survival with transplantation compared with dialysis therapy.[31,55,92,105,121,126] In addition, kidney transplantation is cost-effective compared with dialysis and offers improved quality of life.[32,57,102] Studies have shown an increasing cardiovascular risk proportional to the increase in serum creatinine, suggesting that renal failure at least correlates with, if not causes, accelerated vascular and metabolic defects that predispose to cardiovascular death. Dialysis patients are known to experience accelerated atherosclerosis,[47,58,123] and several inflammatory and atherogenic factors may account for this.[40,59,120,122,127] Given these facts, it is not surprising that analysis of the USRDS revealed that longer time on the waitlist for renal transplantation correlates with poorer death-censored graft survival after renal transplantation (Fig. 37-1). There is a clear advantage of preemptive renal transplantation, and this should be the first choice of patients and physicians where such a choice is available.[68,97]

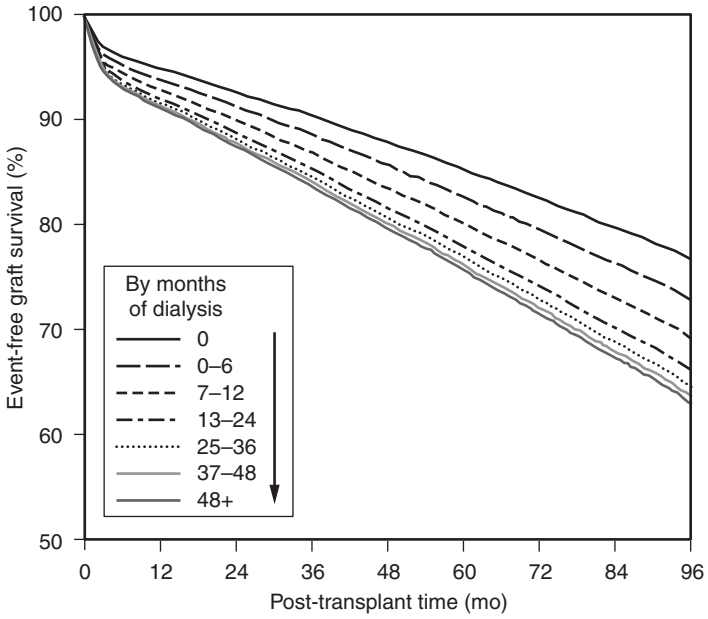

Figure 37–1 Death-censored graft survival estimated by Cox proportional hazard analysis in the United States. (From Meier-Kriesche HU, Port FK, Ojo AO, et al: Effect of waiting time on renal transplant outcome. Kidney Int 58:1311, 2000.)

The better outcomes of patients with preemptive transplants and with shorter time on dialysis underscore the importance of early referral and evaluation for renal transplantation. The racial disparities in the United States for patients awaiting renal transplantation (longer waiting time for African Americans versus whites) partially explain the poorer outcomes of African-American recipients versus other racial groups, although there are multiple additional explanations for the disparate results.[126] Patients with end-stage renal disease would benefit from transplantation as early as possible to maximize their potential for long survival after transplantation.

KIDNEY DONATION

The total number of kidneys donated increased 2.5% between 2004 and 2005 in the United States, from 15,674 to 16,072. In the United States, the introduction of the Organ Donation Breakthrough Collaborative has led, for the first time in many years, to an increase in deceased donor transplantation; this represented a 5.3% increase in deceased donor kidney transplants from 9027 in 2004 to 9509 in 2005. Standard criteria donors accounted for the largest component of this increase in 2004 and increased by 7% compared with 2003. After a significant increase annually in living kidney donors, the number of kidneys transplanted from living donors decreased by 1.3% from 2004 to 2005; these totals are 6647 in 2004 and 6563 in 2005. In contrast, in Europe, there is a wide variation in deceased donor rates between countries, but deceased donation has generally remained stagnant or even decreased over recent years, with the exception of Spain and Austria, where donor rates are the highest in the world.[22] There has been a steady increase in living donors but overall not approaching the rate in the United States.

Expanded Criteria Donors

Expanded criteria donors (ECDs) are defined as all deceased donors older than age 60 or deceased donors between ages 50 and 59 who have two of the following three criteria: (1) a history of hypertension, (2) death caused by cerebrovascular accident, and (3) creatinine greater than 1.5 mg/dL at the time of procurement. ECDs have increased dramatically throughout the world in recent years. In 1996 in the United States, ECD kidneys accounted for 14% of kidneys transplanted from deceased donors; in 2005, this increased to 17% or 1609 kidneys. Between 1995 and 2004, the number of ECD kidney transplants increased at an average annual rate of 4%. In contrast, standard criteria donor kidneys increased at an average annual rate of only 1% per year until more recently, as described earlier. Outcomes for ECD kidney transplants are addressed later.

Donation after Cardiac Death

Donation after cardiac death (DCD) has increased substantially since 2000, as has been the case in Europe, and represented 7% of all organ donors in the United States in 2006. DCD kidneys are kidneys procured after cessation of cardiac activity (in Europe often referred to as non–heart-beating donors); this also is discussed in Chapters 6 and 9. Between 2004 and 2005, the number of kidneys transplanted from DCD donors increased by 43%. Seventy-four kidneys that were transplanted in 2005 from DCD donors also were ECDs. Donors who are both ECD and DCD represent 0.7% of all deceased donor kidney transplants. Growth in DCD donors for kidney transplantation represents the largest increase in a type of donor kidney available for recipients in the United States. The ethics and methods of DCD recovery have been discussed at length by D'Alessandro and colleagues,[23] and single-center experiences have resulted in outcomes not significantly different from standard criteria donor kidney transplantation.[20] The use of DCD donors, normal practice in the early days of transplantation, was pioneered in the modern era by Kootstra's team at Maastricht some years ago,[52,53] but the concept was only reluctantly accepted as the shortage of kidneys grew.[124]

RECIPIENT POOL

At the end of 2005, there were 62,294 patients awaiting renal transplantation in the United States. New registrations for kidney transplantation in 2005 numbered 29,135 (Table 37-1), an increase of 8% or net addition of 4905 patients since 2004 and a 53% increase since 1995. In contrast, patients receiving kidney transplants increased only 45% over the same 10-year period. The largest demographic increase in this population was in the 50- to 64-year-old age range. Since 2003, the age group with the greatest percentage increase in registration for renal transplantation comprised patients 65 years old and older, with a 20% increase. Children younger than 18 years old remain stable at 2% of the list over 10 years. Factors contributing to the increase of older patients on the waiting list include the aging general population of the United States, the increased incidence of end-stage renal disease with aging, and improvements in transplantation outcomes in the elderly. This disproportion between the increase in the waiting list and the number of patients receiving a transplant is similar throughout the Western world. In developing countries, where access to deceased donor transplantation is low, the disparity between need and provision of kidneys is even greater.

The racial representation on the United States waitlist includes 39% white and 35% African American, with the remaining 26% comprising an increasing percentage of Hispanics, Asians, and others. Gender representation remains unchanged with males accounting for 58% and females 42% of the active waiting list. The proportion of patients undergoing retransplantation in 2005 was 10.9% of living donor and 13.6% of deceased donor transplants. The length of time on the waiting list continues to increase, with 22% of active patients at the end of 2004 having waited 3 years or more compared with 14% at the end of 1995.

Glomerular disease, diabetes, and hypertension are the most common primary diseases among active waiting list patients at 22% (glomerular disease), 27% (diabetes), and 21% (hypertension) (see also Chapters 3 and 4). Diabetes is likely to remain the most common diagnosis of patients awaiting renal transplantation in the United States; in most European countries, diabetes is not the major cause of renal failure in patients on the waiting list. The median time from listing to transplantation was considerably different among ethnic minorities and whites. For registrants added to the waiting list in 2000, the median time to transplant was 1814 days for African Americans, 1372 days for Hispanics, 1694 days for Asians, and 796 days for whites. Reasons for these racial disparities in waiting times have been addressed in several publications[7,54] and relate to HLA typing and antigen representation in the donor population, social networks, and presence of comorbid conditions.

ABO blood groups significantly influence median time to transplant with blood group B registrants waiting the longest, or 1848 days for registrants listed in 2000. Blood group AB registrants had the shortest waiting time at 469 days. Patients with a previous organ transplant wait nearly twice as long as registrants awaiting their first kidney transplant, owing to sensitization and presence of comorbidities.

Death on the waiting list for children 11 to 17 years old was approximately half that of children 1 to 10 years old (Table 37-2). Death on the waiting list increases in probability with increasing age, although death rates for patients younger than age 50 have decreased over 10 years. Death rates for patients 65 years old and older are approximately four times the rate for patients 18 to 34 years old.

FACTORS INFLUENCING OUTCOME

Many factors influence the outcome of renal transplantation as illustrated by an earlier analysis of consecutive deceased donor kidney transplants in the United Kingdom between 1994 and 1998.[75] Factors such as HLA matching, donor age, cause of death, and cold ischemic time were found to have a significant impact on outcome. This section looks at these factors and others that influence outcome.

Donor Age

Analysis of 5-year outcomes by Gjertson[37] showed that donor age was the most important factor governing the survival rates of living donor and deceased donor renal transplants. Logistic regression analysis of Organ Procurement and Transplantation Network (OPTN)/UNOS Registry data from 1996 to 2003 was used to calculate the impact of 21 prognostic factors in 85,270 recipients whose grafts survived beyond 1 year and were followed for 5 years. This result underscores the importance of the quality of the donor kidney with respect to long-term function. The European data from the CTS shows the same impact of donor age on graft outcome (Fig. 37-2).

Recipient Age

Since the first report of an acceptable outcome to renal transplantation in the elderly[84] and the widespread introduction

Table 37–1 **Time to Transplant: New Waiting List Registrations in the United States, 1996 to 2005**

	Year of Waiting List Registration									
	1996	*1997*	*1998*	*1999*	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*
No. Registrations	18,330	19,051	20,175	21,002	22,285	22,340	23,495	24,419	27,126	29,135
10th percentile of TT (days)	91	98	106	106	113	115	107	107	111	108
25th percentile of TT (days)	285	295	323	314	337	347	338	353	357	355
Median TT (days)	1036	1051	1148	1124	1198	1175	1136	+	+	+
Median TT 95% CI lower bound	1000	1022	1111	1092	1168	1138	1110	+	+	+

CI, confidence interval; TT, total time.
Data from OPTN/SRTR Data, as of May 1, 2006.

Table 37–2 **Reported Deaths and Annual Death Rates per 1000 Patient-Years at Risk, 1996 to 2005: Kidney Waiting List in the United States**

		Year									
Age This Year		***1996***	***1997***	***1998***	***1999***	***2000***	***2001***	***2002***	***2003***	***2004***	***2005***
Total	Patients	45,312	49,211	53,330	57,079	60,567	64,200	68,230	71,874	76,941	82,582
	Deaths	1949	2184	2528	3320	3125	3375	3729	3853	4038	4156
	Rate	64.2	65.2	69.1	84	73.6	73.6	76.6	74.7	73.1	70.2
>1 yr	Patients	1	2	3	3	2	—	—	4	6	3
	Deaths	—	—	—	—	—	—	—	—	1	—
	Rate	*	*	*	*	*	—	—	*	*	*
1-5 yr	Patients	143	134	127	124	127	149	150	164	181	200
	Deaths	6	3	7	4	5	3	4	4	3	5
	Rate	84.1	46.9	111.5	66.9	85.9	45.1	64.2	56.2	35.9	54.9
6-10 yr	Patients	178	203	194	175	182	213	231	209	219	234
	Deaths	—	—	2	3	7	1	2	3	4	4
	Rate	—	—	18.8	32.6	75.6	9.2	16.7	28	36.2	34.6
11-17 yr	Patients	639	626	664	686	647	724	787	856	931	960
	Deaths	5	8	5	15	6	5	18	12	9	15
	Rate	14.3	22.5	12.9	40.9	16.5	12.9	41.7	26.1	17.7	28.6
18-34 yr	Patients	8657	8935	9091	9271	9106	9151	9330	9521	9868	9991
	Deaths	213	197	226	258	226	215	242	232	207	217
	Rate	36.6	32.6	36.2	40.2	35	33.3	36.4	34.1	29.6	30.6
35-49 yr	Patients	17,446	18,385	19,233	19,690	20,256	20,690	21,247	21,915	22,778	23,971
	Deaths	693	715	773	969	831	817	827	845	853	820
	Rate	58.4	56.3	57.5	69.8	57.7	54.4	53.6	52.7	51.2	46.9
50-64 yr	Patients	14,690	16,724	18,961	21,177	23,272	25,268	27,410	29,076	31,492	34,220
	Deaths	766	916	1130	1464	1455	1649	1816	1839	1923	2054
	Rate	78.2	80.3	87.1	99.6	89.1	91	92.9	87.8	84.6	83.3
65 yr	Patients	3558	4202	5057	5953	6975	8005	9075	10,129	11,466	13,003
	Deaths	266	345	385	607	595	685	820	918	1038	1041
	Rate	113.6	122.2	113.4	151	124.7	120.5	128.1	128.5	127.9	113

Data from OPTN/SRTR Data, as of May 1, 2006.

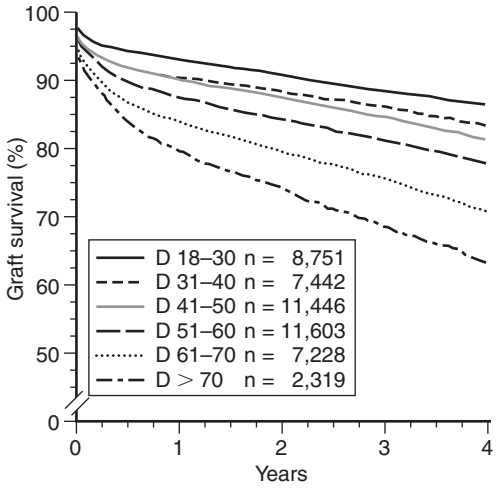

Figure 37–2 Impact of donor age (D) on graft outcome. Donor age and graft survival of first cadaver kidney transplants, 1997 to 2005, in Europe. (Data from Collaborative Transplant Study, available at http://www.ctstransplant.org.)

of cyclosporine-based immunosuppressive protocols, all units adopted a much more liberal approach to the selection of elderly recipients for transplantation. The results of renal transplantation in the elderly (arbitrarily defined as >55, >60, or >65 years old in various reports) have continued to confirm the validity of such policies (Fig. 37-3).* Although there is a higher mortality rate in the early years after transplantation, which is reflected by a poorer graft survival, rejection is less common than in younger patients and rarely a major problem.[49] Cardiovascular disease, including pulmonary embolism, and infection are the two major causes of death in this age group. It is unusual for a graft to be lost from irreversible rejection.

Bearing in mind the shortage of deceased donor kidneys for renal transplantation, it is important to select elderly patients who are relatively low-risk recipients[79,106] and to use lower levels of immunosuppression. Nyberg and coworkers[79] pointed out that some of their elderly patients lost muscular strength after transplantation, which they did not regain, emphasizing that rehabilitation after transplantation is not as good as that in the younger patient. The study by Wolfe and associates,[126] referred to earlier, points out that older patients have a survival advantage with a transplant compared with survival on dialysis. This study confirmed the same suggestion from an earlier Canadian study.[104] A more recent analysis from the SRTR examined the outcome of renal transplantation in patients on the waiting list who were 70 years old or older, the fastest growing group in the United States.[96] This analysis showed that transplantation offered a significant reduction in mortality compared with dialysis.

*References 9, 12, 14, 45, 48, 90, 91, 100, 101, 115.

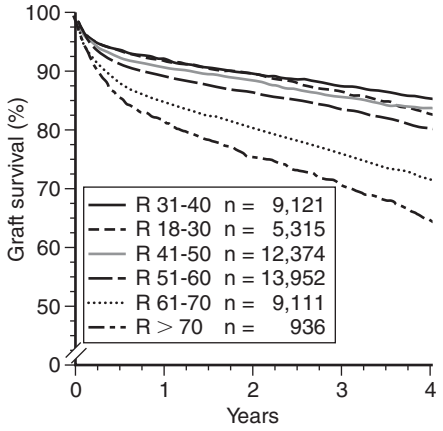

Figure 37–3 Impact of recipient age on graft outcome. Recipient age and graft survival of first cadaver kidney transplants, 1997 to 2005, in Europe. (Data from Collaborative Transplant Study, available at http://www.ctstransplant.org.)

Obesity

Obesity has reached epidemic proportions in the United States, reaching a prevalence in 2003 of greater than 20% of the population in 35 states.[72] Based on body mass index criteria, 65% of the U.S. population is obese.[44] Between 1987 and 2001, renal transplant patients classified as obese increased by 11.6%.[35]

Obesity in renal transplantation is a risk factor for wound infections,[39,89] delayed graft function,[29,39,46,66,70,83,89] acute rejection,[39,66] increased radiographic monitoring, and need for biopsy,[39,50] and is associated with worse graft survival (Fig. 37-4).[39,66] Although analysis of USRDS data by Meier-Kriesche and associates[66] suggested a higher risk of patient death after renal transplantation in the obese, a subsequent study by Gore and colleagues[39] showed that comorbidities, including hypertension, diabetes, and hyperlipidemia, accounted for the increased risk of death in obese patients. Donor obesity does not seem to have an impact on recipient outcomes. Voluntary weight loss and bariatric surgery before renal transplantation[3] may achieve significant long-term weight loss and relief of comorbidities in obese patients anticipating renal transplantation.

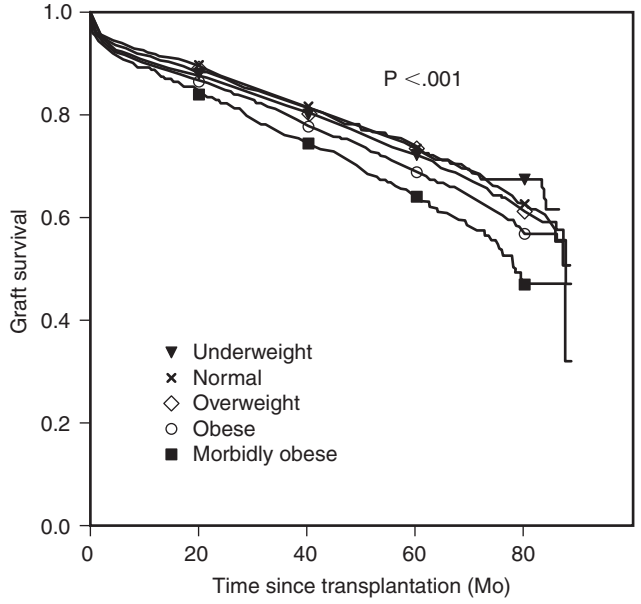

Figure 37–4 Graft survival after renal transplantation stratified by recipient body mass index at the time of transplantation. (From Gore JL, Pham PT, Danovitch GM, et al: Obesity and outcome following renal transplantation. Am J Transplant 6:357-363, 2006.)

Race

Much has been written about the influence of race on outcome with respect to the donor and the recipient involved in kidney transplantation. In the United States, outcomes have been best for individuals of Asian background with respect to less rejection and graft loss; outcomes are worst for African Americans for these same parameters. Much effort has been expended on determining why these differences exist. An analysis of a huge experience of deceased donor transplantation from the University of Alabama where more than half the recipients are African American has shown a continuing improvement in graft survival in the non–African-American population and in the African American population with the use of more potent immunosuppressive regimens. Long-term graft survival remains inferior, however, and the authors suggest that their data reinforce the importance of nonimmunological variables, such as time on dialysis before transplantation, diabetes, and access to medical care.[30] In support of this hypothesis, a study by Pallet and coworkers[86] of black recipients transplanted between 1987 and 2003 in France suggested that there was not a difference between white and black recipients. The authors suggest that the origin of the difference is not so much genetic, immunological, or pharmacological as it is related to universal access to immunosuppressive drugs (i.e., compliance and social and economic factors). A study by Lunsford and associates[60] from the University of South Carolina suggested that from a study of 333 patients awaiting transplantation, of which 61% were African American, African Americans are less accepting of their renal failure and more likely to deny the need for renal transplantation than their counterparts. Similar to the findings for African Americans, Press and colleagues[93] have reported that Hispanics also have a higher rate of graft failure compared with whites after adjustment for poverty and other covariates, and that poverty, but not race or ethnicity, is related to functional status after renal transplantation.

HLA Mismatch and Prior Sensitization

There continues to be an advantage of receiving a well-matched kidney, meaning fewer donor-recipient HLA mismatches, as illustrated by the CTS registry data (Fig. 37-5) (see also Chapter 10). In the United States, 14% of kidney transplant recipients in 2004 received a zero-mismatched kidney versus 12% in 1995. In 2004, there were 1343 recipients of zero-mismatched deceased donor kidneys in the United States, representing 17% of the deceased donor, non-ECD transplants. Transplants into patients with four or more HLA antigen mismatches in 2005 accounted for two thirds of deceased donor, non-ECD transplants, reflecting decreased emphasis on HLA matching in allocation policy and increased accrued waiting time emphasis.

In other words, most recipients in the United States of deceased donor kidneys are not well matched, if defined as

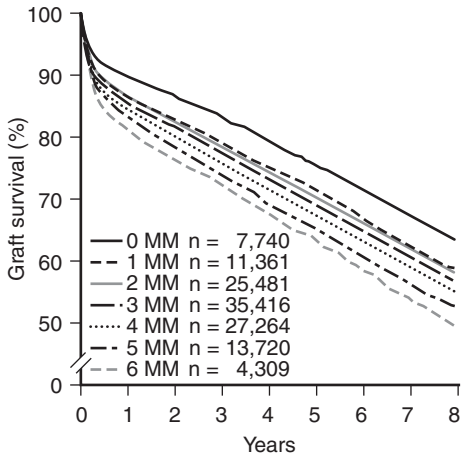

Figure 37–5 HLA-A, HLA-B, and HLA-DR mismatches and first cadaver kidney transplants, 1985 to 2005. MM, HLA mismatches. (Data from Collaborative Transplant Study, available at www.ctstransplant.org.)

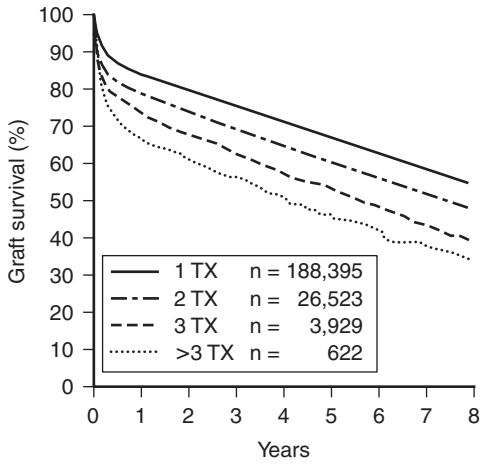

Figure 37–6 Number of cadaver kidney transplants and graft survival, 1985 to 2005. TX, transplant. (Data from Collaborative Transplant Study, available at www.ctstransplant.org.)

at least three of six matches or less than four mismatches. An analysis of UNOS data in 2004 suggested that the impact of HLA compatibility on graft outcome has diminished in recent years with the advent of more potent immunosuppression.[112] Opelz and Dohler[80] have analyzed CTS data in 2 decades, 1995 to 1994 and 1995 to 2004, and in more recent years, however, and have found that the influence of HLA on graft survival remains strong.

Between 1996 and 2005, the number of deceased donor, non-ECD kidney transplants into recipients with a panel-reactive antibody frequency of 80% or greater at the time of transplant more than doubled to 445 in 2005. Highly sensitized patients, as measured by a high panel-reactive antibody percentage, are receiving transplants much more frequently, perhaps owing to the better definition of antibodies and the development of immunosuppressive strategies, such as plasmapheresis and rituximab, to aid in such cases in the United States. Nevertheless, in 2005, more than 6000 patients with panel-reactive antibody greater than 80% were waiting for a kidney transplant. National data for success of these strategies are still lacking. In Europe, the acceptable mismatch strategy, which is based on the precise definition of antibodies in the recipient, is used more often (see Chapter 10).

The available data continue to support the benefit of more HLA matches compared with less, although it also can be argued that even a poorly matched kidney transplant is preferable to dialysis when measured by outcome analysis. Primary renal transplants have better outcomes than retransplants overall, again well illustrated by the CTS registry data (Fig. 37-6). Living donor transplants that are HLA-identical continue to have better outcomes, followed by haploidentical living donor transplants and deceased donor grafts (Fig. 37-7).

Cold Ischemic Time

The percentage of kidney transplants completed with cold ischemic times of less than 12 hours in the United States is shown in Table 37-3 (see also Chapter 9). The shifts in overall percentages of kidneys transplanted with shorter cold ischemic times reflect the value of short preservation times. Most kidneys are now transplanted in less than 31 hours of the time of procurement. Regardless of the choice of preservation solution or cold storage versus machine perfusion, shorter preservation tends to be an advantage in graft function and survival; this is well illustrated by the CTS data (Fig. 37-8). The University of Wisconsin preservation solution is the dominant choice worldwide for kidney preservation and, at least in the CTS European data, is associated with the best graft outcome (Fig. 37-9).[80]

Blood Transfusions before Transplantation

The transfusion effect probably was the most significant factor in the improved graft survival seen in living related and deceased donor transplantation in the azathioprine era before the advent of cyclosporine therapy, as described in the earlier editions of this book. The transfusion effect was thought to have possibly disappeared, as shown in earlier analyses from the UCLA and UNOS registries and the CTS.[2,38,82] Later, a prospective study of the effect of transfusions before transplantation in nontransfused recipients,[81]

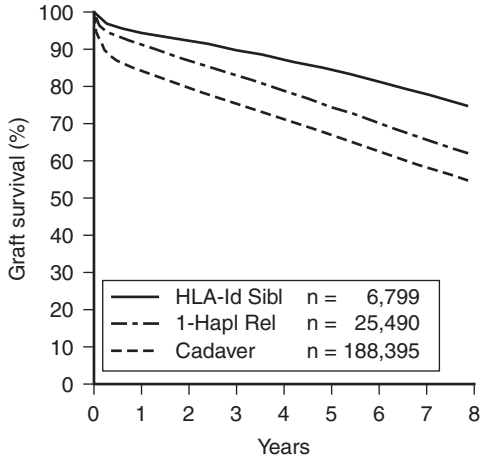

Figure 37–7 Relationship of donors and recipients of first kidney transplants and graft survival, 1985 to 2005. HLA-Id Sibl, HLA-identical sibling; 1-Hapl Rel, first-degree haploidentical relative. (Data from Collaborative Transplant Study, available at http://www.ctstransplant.org.)

Table 37–3 **Transplant Recipient Characteristics, 1996 to 2005: Recipients of Deceased Donor, Non–Expanded Criteria Donor Kidneys in the United States**

	Year of Transplant									
Cold Ischemic Time	***1996***	***1997***	***1998***	***1999***	***2000***	***2001***	***2002***	***2003***	***2004***	***2005***
Total	6640	6630	6807	6807	6939	7037	7282	7270	7918	8231
0-11 hr	733	810	840	925	961	1126	1250	1398	1466	1752
12-21 hr	2751	2903	2933	2675	2719	2678	2989	2879	3235	3184
22-31 hr	2252	2070	1988	1837	1782	1676	1647	1607	1728	1820
32-41 hr	567	501	514	415	376	325	319	282	296	302
≥42 hr	105	84	76	74	53	68	59	52	44	78
Unknown	232	262	456	881	1048	1164	1018	1052	1149	1095
Total (%)	100	100	100	100	100	100	100	100	100	100
0-11 hr (%)	11	12.2	12.3	13.6	13.8	16	17.2	19.2	18.5	21.3
12-21 hr (%)	41.4	43.8	43.1	39.3	39.2	38.1	41	39.6	40.9	38.7
22-31 hr (%)	33.9	31.2	29.2%	27	25.7	23.8	22.6	22.1	21.8	22.1
32-41 hr (%)	8.5	7.6	7.6	6.1	5.4	4.6	4.4	3.9	3.7	3.7
≥42 hr (%)	1.6	1.3	1.1	1.1	0.8	1	0.8	0.7	0.6	0.9
Unknown (%)	3.5	4	6.7	12.9	15.1	16.5	14	14.5	14.5	13.3

Data from OPTN/SRTR Data, as of May 1, 2006.

all of whom were receiving cyclosporine therapy, did show improved graft outcome, however, in the patients who were deliberately transfused. Data from the UNOS also suggest a modest transfusion effect in the cyclosporine era in white recipients.[18]

There is a place still for careful and large, randomized prospective trials of transfusions before deceased donor and living donor transplantation in nontransfused recipients. One trial has been performed in the United States in non–HLA-identical living donor transplants in which donor-specific transfusion was given 24 hours before transplantation, but no effect was seen.[4] A similar small trial in living related recipients of donor-specific transfusions suggested a better outcome in transfused recipients.[62] It would seem that the title of one of the first articles on transfusions in renal transplantation, "The Paradox of Blood Transfusions in Renal Transplantation,"[76] remains apt today.

Expanded Criteria Donor Kidney Recipients

ECD kidneys tend to be transplanted into older recipients with 81% of ECD recipients older than 50 years old compared with 50% of non-ECD kidney recipients (Table 37-4). ECD kidneys also were less likely than non-ECD kidneys to be transplanted into recipients of repeat kidney transplants. The distribution of cold ischemic times for ECD transplanted recipients is the same as the distribution for non-ECD recipients with cold ischemic time of less than 31 hours for approximately 80%.

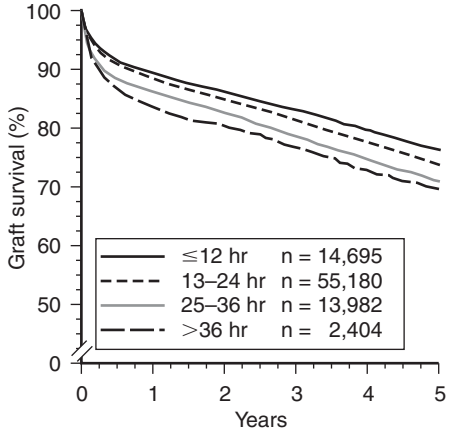

Figure 37–8 Cold ischemia time and graft survival of first cadaver kidney transplants, 1990 to 2005, in Europe. (Data from Collaborative Transplant Study, available at http://www.ctstransplant.org.)

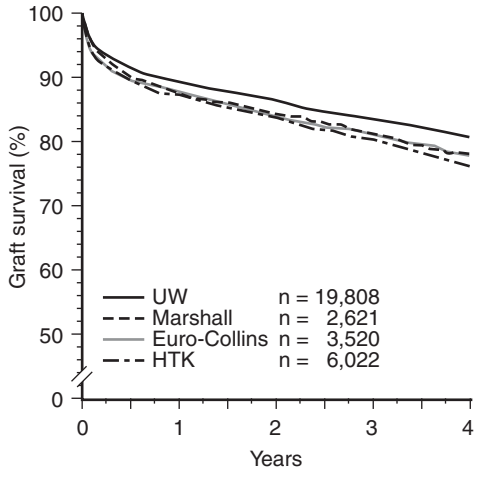

Figure 37–9 Preservation solution and graft survival of first cadaver kidney transplants, 1997 to 2005, in Europe. HTK, histidine-tryptophan-ketoglutarate solution; UW, University of Wisconsin. (Data from Collaborative Transplant Study, available at http://www.ctstransplant.org.)

Table 37–4 **Transplant Recipient Characteristics in the United States, 1996 to 2005**

Recipients of Deceased Donor Expanded Criteria Donor Kidneys

	Year of Transplant									
Age at Transplant	***1996***	***1997***	***1998***	***1999***	***2000***	***2001***	***2002***	***2003***	***2004***	***2005***
Total	1089	1144	1225	1235	1184	1193	1256	1396	1439	1683
1-5 yr	2	—	2	—	—	—	—	—	—	—
6-10 yr	3	2	2	3	1	1	—	—	—	—
11-17 yr	8	4	13	5	3	2	1	1	3	—
18-34 yr	132	112	132	116	107	80	90	59	64	55
35-49 yr	344	332	351	317	304	273	275	254	257	261
50-64 yr	467	532	544	591	539	589	602	687	682	781
≥65 yr	133	162	181	203	230	248	288	395	433	586
Total (%)	100	100	100	100	100	100	100	100	100	100
1-5 yr (%)	0.2	—	0.2	—	—	—	—	—	—	—
6-10 yr (%)	0.3	0.2	0.2	0.2	0.1	0.1	—	—	—	—
11-17 yr (%)	0.7	0.3	1.1	0.4	0.3	0.2	0.1	0.1	0.2	—
18-34 yr (%)	12.1	9.8	10.8	9.4	9	6.7	7.2	4.2	4.4	3.3
35-49 yr (%)	31.6	29	28.7	25.7	25.7	22.9	21.9	18.2	17.9	15.5
50-64 yr (%)	42.9	46.5	44.4	47.9	45.5	49.4	47.9	49.2	47.4	46.4
≥65 yr (%)	12.2	14.2	14.8	16.4	19.4	20.8	22.9	28.3	30.1	34.8

Recipients of Deceased Donor Non–Expanded Criteria Donor Kidneys

	Year of Transplant									
Age at Transplant	***1996***	***1997***	***1998***	***1999***	***2000***	***2001***	***2002***	***2003***	***2004***	***2005***
Total	6640	6630	6807	6807	6939	7037	7282	7270	7918	8231
<1 yr	1	—	—	2	—	—	—	—	1	2
1-5 yr	56	44	40	50	37	42	49	53	48	59
6-10 yr	62	71	70	62	54	61	64	72	65	81
11-17 yr	198	184	162	231	187	183	211	255	264	326
18-34 yr	1301	1238	1235	1199	1203	1167	1135	1045	1175	1109
35-49 yr	2540	2523	2484	2374	2386	2392	2377	2294	2496	2473
50-64 yr	2062	2161	2277	2365	2434	2539	2722	2774	3020	3199
≥65 yr	420	409	539	524	638	653	724	777	849	982
Total (%)	100	100	100	100	100	100	100	100	100	100
<1 yr (%)	0	—	—	0	—	—	—	—	0	0
1-5 yr (%)	0.8	0.7	0.6	0.7	0.5	0.6	0.7	0.7	0.6	0.7
6-10 yr (%)	0.9	1.1	1	0.9	0.8	0.9	0.9	1	0.8	1
11-17 yr (%)	3	2.8	2.4	3.4	2.7	2.6	2.9	3.5	3.3	4
18-34 yr (%)	19.6	18.7	18.1	17.6	17.3	16.6	15.6	14.4	14.8	13.5
35-49 yr (%)	38.3	38.1	36.5	34.9	34.4	34	32.6	31.6	31.5	30
50-64 yr (%)	31.1	32.6	33.5	34.7	35.1	36.1	37.4	38.2	38.1	38.9
≥65 yr (%)	6.3	6.2	7.9	7.7	9.2	9.3	9.9	10.7	10.7	11.9

Data from OPTN/SRTR Data, as of May 1, 2006.

Living Donor Kidney Recipients

Living donor kidney recipients were predominantly white (66%) in 2005. Between 1996 and 2005, the proportion of parental donors decreased from 21% to 12%, and sibling donation decreased from 42% to 26%. Spousal donation increased from 10% to 12%, and the proportion of living unrelated donors increased from 6% to 22% between 1996 and 2005.

Preemptive kidney transplants in patients not yet on dialysis more than doubled over 10 years, and living donor transplants accounted for all of this increase. This trend is in accordance with data showing elevated creatinine to be a significant cardiovascular risk factor and a risk factor for mortality.[34] Data also show enhanced patient and graft survival for patients undergoing preemptive renal transplantation compared with patients transplanted while on dialysis.[67] The number of living donor renal transplants increased by 79% in the United States between 1996 and 2005 with the largest increase in recipients 50 years old or older.

Immunosuppression

In the United States, induction immunosuppression with an antibody at the time of transplantation was used for 76% of kidney recipients in 2005 compared with 27% of recipients in 1995. Antithymocyte globulin was used for 39% of kidney transplants, anti-CD25 antibodies were used for 28% of kidney transplants, and alemtuzumab was used for 9% of kidney transplants in 2005 (Fig. 37-10). Maintenance steroid use decreased from 94% of recipients in 2001 to 74% in 2005. Tacrolimus was used in 79% of recipients and cyclosporine in 15% at the time of discharge; this represents a substantial shift from cyclosporine to tacrolimus. Mycophenolate mofetil was used in 87% of cases. Nine percent of patients received sirolimus at the time of discharge, and 18% received sirolimus during the first year. For patients transplanted in 2004, 12% were treated for rejection in the first year after transplant—a low percentage that suggests improved treatment or

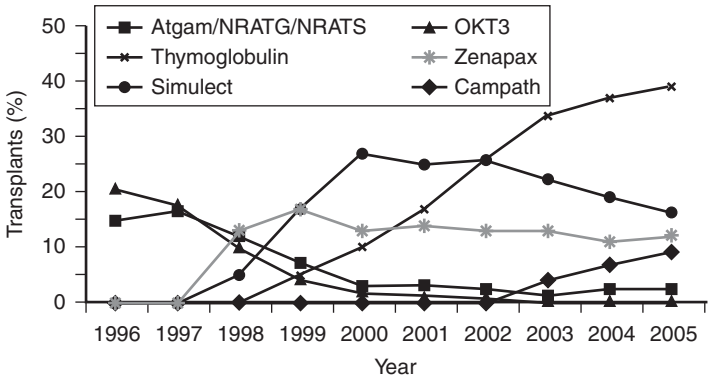

Figure 37–10 Immunosuppression agents used for induction in kidney transplantation, 1996 to 2005. (From 2006 OPTN/SRTR Annual Report, available at http://www.optn.org/ar2006/Chapter IV.)

underreporting to the SRTR, or both. Figure 37-11 summarizes U.S. trends in maintenance immunosuppression for kidney transplants.

In contrast, in Europe, the use of antibody induction is less prevalent than in the United States, although the use of an interleukin-2 receptor antibody for induction is becoming more common. In the CTS European database between 1998 and 2005, 36% of patients had induction with only an antibody (Fig. 37-12). Similarly, there has been a swing toward tacrolimus from cyclosporine for primary maintenance therapy, but not to the same extent as in the United States. There has been a marked change, however, from azathioprine to mycophenolate mofetil.

Compliance (Adherence) with Immunosuppressive Treatment

The importance of noncompliance with immunosuppression often resulting in rejection and graft loss began to attract attention in the 1980s[103,107] and was reviewed extensively by Colon and coworkers in 1991.[19] More recently, Butler and colleagues[13] performed a systematic review of the frequency and impact of nonadherence to immunosuppressive drugs after renal transplantation and pointed out that nonadherence is common, and that the odds of graft loss are sevenfold greater in nonadherent patients than in adherent patients. It is a problem that is probably much greater than most clinicians realize. Santiago-Delpin and colleagues[103] described noncompliance as the "most important problem with which they are currently involved" in Puerto Rico. Compliance rates in reports range from 5% to 43%.[11,27] Raiz and associates[95] suggested that compliance with medication after transplantation is associated with subjective rather than objective variables (e.g., patients' positive feelings for their physicians and the experience of transplantation).

Noncompliance or nonadherence is a factor that cannot be evaluated accurately at present, but it is an important determinant of graft outcome and an important factor determining the outcome of clinical trials.[63] Nevins and Matas[78] stressed the importance of determining nonadherence and concluded that "... successful interventions will significantly reduce adverse events. What is more important, such improvements are available today and do not require the development of a single new drug, rather though only require patients to consistently take the drugs available."

GRAFT SURVIVAL

Graft survival rates for recipients of deceased donor, non-ECD kidneys were 91% at 1 year and 69% at 5 years (Fig. 37-13 and Table 37-5). Three-year survival is based on transplants performed during 2000 to 2003 and 5-year results are based on transplants performed from 1998 to 2003. The best 5-year survival rate of 78% for deceased donor kidneys was seen in Asians with non-ECD kidneys. One-year and 5-year deceased donor, non-ECD kidney survival rates were superior in patients with polycystic kidney disease, with poorer 5-year survival in patients with diabetes, hypertension, nephrosclerosis, and vascular diseases. Since the 1990s, 1-year, 3-year, and 5-year unadjusted deceased donor, non-ECD graft survival rates have improved only 2%. Rates of return to dialysis according to age, gender, and race are shown in Figure 37-14.

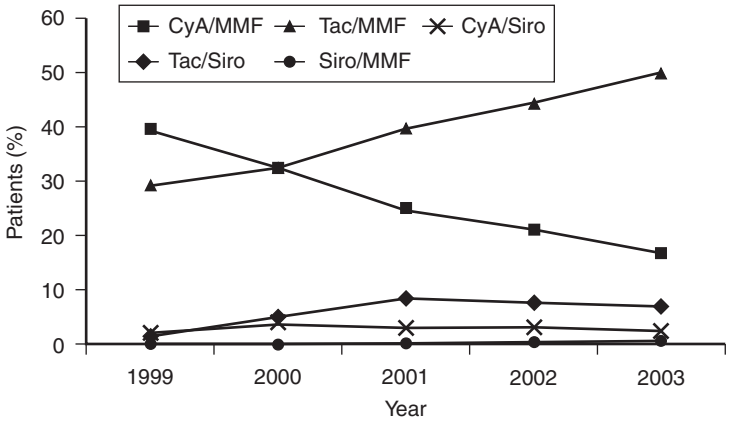

Figure 37–11 Trends in immunosuppression maintenance regimens, 1-year post-transplant for kidney transplantation, 1999 to 2003. CyA, cyclosporine; MMF, mycophenolate mofetil; Siro, sirolimus; Tac, tacrolimus. (From 2005 OPTN/SRTR Annual Report, available at http://www.optn.org/ar2006/Chapter V.)

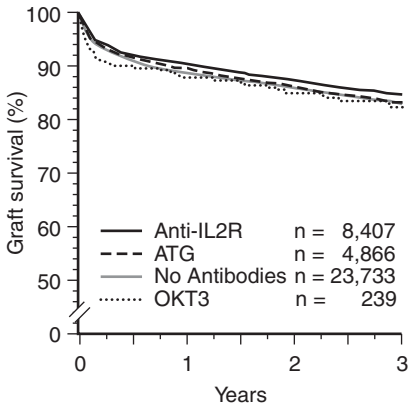

Figure 37–12 Prophylactic antibody induction with OKT3, antithymocyte globulin, and anti–interleukin-2 receptor and graft survival of first cadaver kidney transplants, 1998 to 2005, in Europe. (Data from Collaborative Transplant Study, available at http://www.ctstransplant.org.)

Patients with delayed graft function and requiring dialysis within the first post-transplant week had worse 5-year graft survival. In 2004, graft survival rate at 5 years for non-ECD kidneys was 54% if dialysis was needed in the first week versus 74% if dialysis was not needed.

Chronic rejection and death with a functioning graft are the main causes of late graft loss (see Chapters 25 and 28).[87] Diabetic recipients of deceased donor grafts had a higher incidence of death with a functioning graft (5% in the first year and 10% between years 2 and 5) than recipients with other diseases (see Chapter 34).[94]

Analysis of renal transplant half-lives based on Kaplan-Meier analysis using the U.S. SRTR data showed that half-lives improved overall by 2 years between 1988 and 1995.[69] Most of this improvement was due to better outcomes for retransplants because primary transplant half-lives improved by only 6 months. Figure 37-15 shows the graft years gained per patient up to 8 years of follow-up.[69] In Europe, there has been a dramatic increase in the half-life of first deceased donor transplants from 1982 (7.9 years) to 2005 (21.8 years) but, similar to the data from the United States, the increase since 1997 to 2005 has been less than 2 years (Fig. 37-16). These results suggest the importance of future efforts to focus on improving long-term renal allograft outcomes. It seems that the armamentarium of new immunosuppressive agents available has led to less acute rejection, but this is not reflected in any striking change in long-term graft survival.

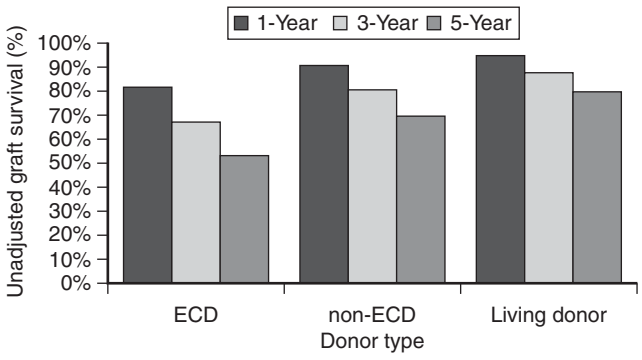

Figure 37–13 Unadjusted 1-year, 3-year, and 5-year kidney graft survival, by donor type, for transplants received 1999 to 2004. ECD, expanded criteria donor. (From 2006 OPTN/SRTR Annual Report, available at http://www.optn.org/ar2006/chapter IV.)

Graft Survival for Expanded Criteria Donor Kidneys

Adjusted 1-year graft survival rate is 80% to 84% for all age recipients of ECD kidneys. African Americans experienced the worse overall ECD graft survival rates at 44% at 5 years. Asians had the best 5-year graft survival for ECD kidneys at 66%. These outcomes may reflect compliance with immunosuppression, immunological responsiveness, or genetically determined differences in immunological and nonimmunological parameters as already discussed earlier.

Graft Survival among Living Donor Recipients

Monozygotic Twins

Monozygotic twins are the ideal donor and recipient because of their genetic identity for major and minor histocompatibility antigens. Transplantation between identical twins has not been uniformly successful, however, because failures occur as a result of technical problems or recurrent glomerulonephritis. Tilney and coworkers[118] reviewed the results of 28 identical twin transplants at the Peter Bent Brigham Hospital, where the first successful pioneering transplant between identical twins was performed in 1954. Two deaths occurred within 2 weeks of transplantation—one from infarction of the kidney and one from septicemia secondary to a perinephric infection. Seven other patients developed recurrent nephritis at 6 months to 10 years after transplantation; five patients died of the recurrent disease because of lack of maintenance dialysis to which these patients could be returned. An analysis of the Brigham experience of 30 identical twin transplants,[117] in which follow-up lasted 27 years, showed a 25-year patient survival rate of around 65% and a graft survival rate of around 55%. Eight of the 11 graft failures were due to recurrent nephritis, occurring 3 months to 20 years after transplantation. Generally, the recipients remained in excellent health; cardiovascular disease took its toll as time progressed, primarily in the more elderly recipients.

The European Dialysis and Transplantation Association registry has reported 41 renal transplants between monozygotic twins. Glomerulonephritis was the original cause of renal failure in 24 of these patients. Of 41 patients, 36 were alive with functioning grafts 12 to 174 months after transplantation. Two grafts failed from recurrent disease, two grafts failed from de novo glomerulonephritis, and one recipient died in a traffic accident.[56] One donor developed renal failure secondary to the same glomerulonephritis as in the original recipient. There seems to be a case for using some immunosuppression in identical twin recipients when the original disease is a type of glomerulonephritis with a high recurrence rate (see Chapter 4), but how much and what type of immunosuppression should be used are uncertain. There are no data concerning outcome of renal transplants in monozygotic twins in this situation in the cyclosporine era.

Table 37–5 **Adjusted Graft Survival, Deceased Donor Non–Expanded Criteria Donor Kidney Transplants: Survival at 3 Months, 1 Year, 3 Years, and 5 Years in the United States**

	3 Months*			1 Year*			3 Years†			5 Years‡		
	No.	*%*	*SE*	*No.*	*%*	*SE*	*No.*	*%*	*SE*	*No.*	*%*	*SE*
Age Transplant												
All	14,647	94.9%	0.2%	14,647	91%	0.2%	28,578	80.5%	0.3%	42,055	69.3%	0.3%
<1 yr	1	+	+	1	+	+	1	+	+	2	+	+
1-5 yr	98	94.9%	2.2%	98	90.7%	2.9%	184	81.4%	3.7%	267	75.5%	3.5%
6-10 yr	134	95.5%	1.8%	134	92.5%	2.3%	253	77.9%	3.4%	360	73.1%	3.2%
11-17 yr	503	96.5%	0.8%	503	92.6%	1.2%	890	78.5%	1.8%	1,298	64.7%	2%
18-34 yr	2,181	95%	0.5%	2,181	91.5%	0.6%	4,457	81.1%	0.7%	6,841	69%	0.8%
35-49 yr	4,649	95.5%	0.3%	4,649	91.9%	0.4%	9,312	82.8%	0.5%	13,986	73.2%	0.5%
50-64 yr	5,501	94.5%	0.3%	5,501	90.4%	0.4%	10,541	80.2%	0.5%	15,216	69%	0.5%
≥65 yr	1,580	94%	0.6%	1,580	88.8%	0.8%	2,940	74.1%	1%	4,085	59%	1.2%
Primary Diagnosis												
Glomerular diseases	3,830	94.8%	0.4%	3,830	91%	0.5%	7,596	81.8%	0.5%	11,405	70.7%	0.6%
Diabetes	3,225	94.3%	0.4%	3,225	89.8%	0.5%	6,142	78.3%	0.6%	8,815	65.7%	0.7%
Hypertensive nephrosclerosis	2,904	95%	0.4%	2,904	90.8%	0.5%	5,479	78.3%	0.7%	7,746	66%	0.8%
Polycystic kidneys	1,211	96.8%	0.5%	1,211	94.1%	0.7%	2,435	87.8%	0.8%	3,720	80.2%	0.9%
Tubular and interstitial diseases	839	95%	0.8%	839	91.6%	1%	1,604	81.2%	1.2%	2,428	72.2%	1.3%
Renovascular and other vascular diseases	784	94.9%	0.8%	784	91.4%	1%	1,580	79.4%	1.2%	2,336	66.2%	1.4%
Congenital, rare familial, and metabolic disorders	453	93.5%	1.3%	453	90.5%	1.6%	807	80.6%	1.9%	1,225	70.6%	1.9%
Neoplasms	46	93.6%	3.5%	46	89.4%	4.5%	86	65.9%	6.8%	127	52.6%	6.3%
Other	1,036	95.9%	0.6%	1,036	91.3%	0.9%	2,057	80.9%	1.1%	3,038	70.1%	1.2%
Unknown	319	94.1%	1.4%	319	88.7%	1.8%	792	78.7%	1.7%	1,215	69.2%	1.8%
Recipient Gender												
Female	5,864	94.8%	0.3%	5,864	91.2%	0.4%	11,503	81.2%	0.4%	16,868	70.4%	0.5%
Male	8,783	95%	0.2%	8,783	90.8%	0.3%	17,075	80%	0.4%	25,187	68.6%	0.4%
Recipient Ethnicity/Race												
White	7,135	95.2%	0.3%	7,135	91.3%	0.3%	14,317	82%	0.4%	21,648	72.1%	0.4%
African American	4,401	93.7%	0.4%	4,401	88.8%	0.5%	8,549	75.1%	0.6%	12,353	61%	0.6%
Hispanic/Latino	2,058	95.7%	0.4%	2,058	92.7%	0.6%	3,849	84.6%	0.7%	5,393	74.2%	0.9%
Asian	856	96.7%	0.6%	856	94.2%	0.8%	1,536	85.9%	1.1%	2,181	77.5%	1.3%
Other/Multirace	196	98.1%	1%	196	95.6%	1.4%	325	84.2%	2.5%	478	74.3%	2.7%
Unknown	1	+	+	1	+	+	2	+	+	2	+	+

*Transplanted 2003-2004.
†Transplanted 2001-2004.
‡Transplanted 1999-2004.
SE, standard error.
Data from OPTN/SRTR Data, as of May 1, 2006.

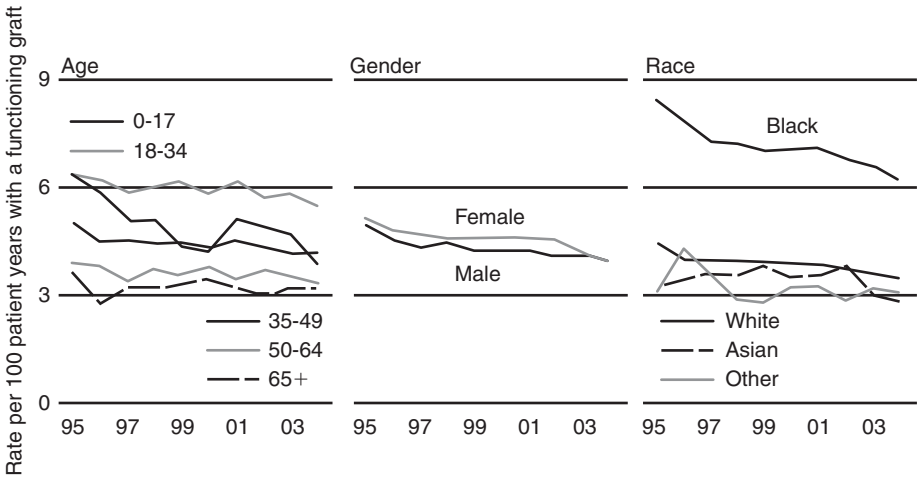

Figure 37–14 Rates of return to dialysis or preemptive retransplant, by age, gender, and race. Adjusted for age, gender, and race. Preemptive retransplantations are counted as a return to dialysis. (From 2006 ADR USRDS, available at http://www.usrds.org/adr.htm.)

Family Donors

One-year graft survival in the United States is 93% among patients 65 years old and older and 95% among recipients in the 1- to 5-year-old age group. As with deceased donor recipients, the best 5-year graft survival rates among living donor recipients occur in patients whose end-stage renal disease was secondary to polycystic kidney disease, and the worst outcomes are noted in patients with diabetes, hypertension, nephrosclerosis, and vascular disease (Table 37-6). These data underscore that the ideal donor is a living donor owing to superior recipient outcomes.

Graft survival was 5% lower in patients with a panel-reactive antibody of 80% or greater. Graft survival in patients requiring dialysis within the first post-transplant week for recipients of living donors was 65% compared with 97% for patients who did not require dialysis. This finding reflects the fact that technical problems with the transplant are usually responsible for the need for dialysis in the first week after a living donor renal transplant and portend a poor outcome.

KIDNEY-ALONE VERSUS KIDNEY-PANCREAS TRANSPLANTATION FOR DIABETES

OPTN data have documented superior graft survival for simultaneous kidney-pancreas (SPK) recipients with type 1 diabetes mellitus compared with patients receiving a kidney transplant alone (see Chapter 34).[61] Half-life of kidneys in SPK patients was 9.6 years compared with 6.3 years in patients with kidneys alone. These results may be related to selection of more ideal donors for patients receiving SPK. Graft survival for living donor kidneys in type 1 diabetes mellitus was nearly equivalent to SPK transplants in the Wisconsin experience.[109] Recipient selection also may favor the better outcome of SPK patients because stricter criteria (healthier patients) are generally selected for SPK transplantation compared with kidney-alone transplantation. Nevertheless, successful pancreas transplantation may prevent recurrence of diabetic nephropathy. These findings apply to SPK transplants and have not been shown for sequential kidney and pancreas transplants.

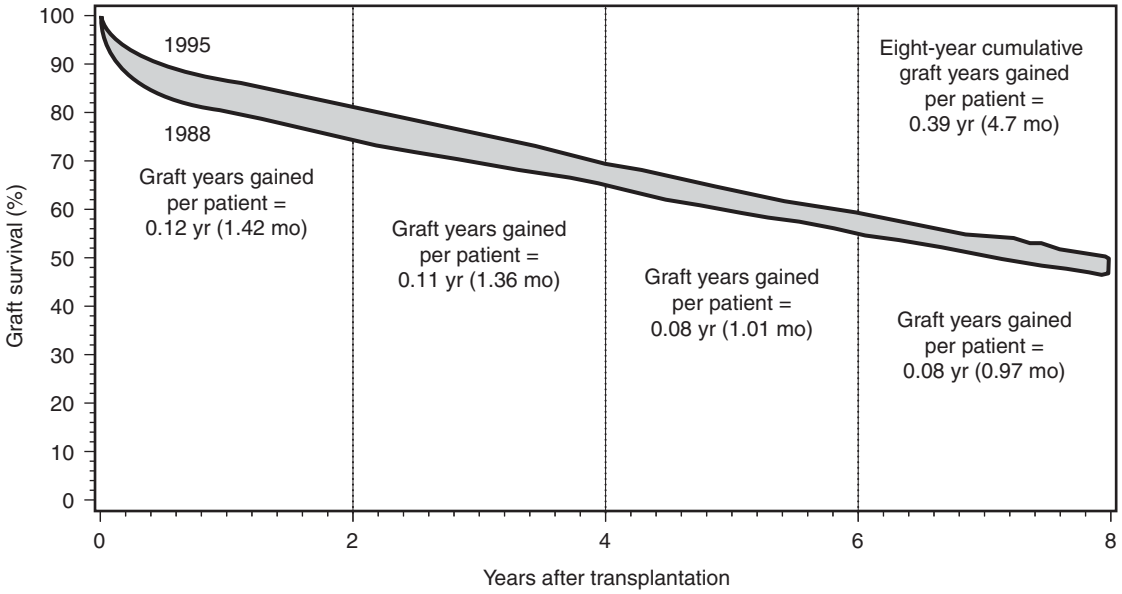

Figure 37–15 Increased cumulative graft years per patient in deceased donor transplant recipients. Kaplan-Meier survival curves from transplants in 1988 and 1995. (Data from Meier-Kriesche HU, Schold JD, Kaplan B: Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? Am J Transplant 4:1289, 2004.)

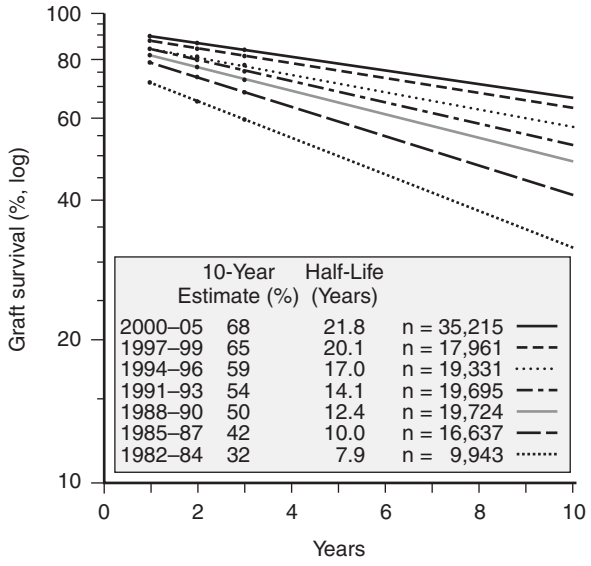

Figure 37–16 Graft survival of first cadaver kidney transplants in Europe according to transplant year. (Data from Collaborative Transplant Study, available at http://www.ctstransplant.org.)

TRANSPLANTATION FOR PATIENTS WITH METABOLIC AND CONGENITAL DISORDERS

Of the other metabolic and congenital disorders causing end-stage renal failure, information is available about many patients with end-stage renal failure resulting from Alport's syndrome, amyloidosis, cystinosis, Fabry's disease, familial nephritis, gout, medullary cystic disease, oxalosis, and systemic lupus erythematosus.[1,16,41,71] The results of renal transplantation are similar to those of the more common causes of end-stage renal failure, with the exception of Fabry's disease and oxalosis (see Chapter 4), but UNOS data show a much improved outcome in these conditions.[16] Cats and Galton[15] found a consistently lower graft survival rate in patients with systemic lupus erythematosus in an analysis of the UCLA Registry data. A previous analysis of UNOS data confirmed the poorer graft survival rates in patients with systemic lupus erythematosus,[71] but a more recent analysis showed similar survival at 5 years after transplantation to most other causes of end-stage renal failure.[16] Nephrosclerosis as a cause of renal failure in transplant recipients was associated with poorer graft survival in African Americans but not in whites.

In patients with Fabry's disease, the high failure rate was not due to recurrent renal damage, so it is not necessary to exclude such patients from renal transplantation. More recent UNOS data show much improved survival. Oxalosis has been considered an unsuitable condition for transplantation because recurrence of oxalosis is common and early, and little palliation is achieved in such cases. There has been a considerable improvement in outcome in more recent years, however (see Chapter 4).[16]

CANCER RISK

Cancer risk is discussed at length in Chapters 32 and 33 and is one of the major long-term complications of renal transplantation. U.S. and Australia–New Zealand databases show an increased risk of malignancies after kidney transplantation[51,113] with the greatest increase in cancers caused by viruses. The risk is highest for nonmelanoma skin cancers and post-transplant lymphoproliferative disease, with the latter linked to Epstein-Barr virus infection and induction with ATG/OKT3. Two antiproliferative agents, sirolimus and mycophenolate mofetil, may be associated with a lower incidence of post-transplant lymphoproliferative disease (Table 37-7), but follow-up of the relevant studies is no longer than 1 year. Robson and colleagues[98] conducted an observational cohort study of mycophenolate mofetil using data from the OPTN/UNOS and CTS database with a follow-up of 3 years. This study showed no increased risk of post-transplant lymphoproliferative disease in patients receiving mycophenolate mofetil, and suggested that there may be a lower risk in some populations.

PREGNANCY AFTER RENAL TRANSPLANTATION

A well-functioning renal transplant usually reverses infertility associated with end-stage renal disease and permits reproductive function to recover.[25] The most important prognostic factor for a good outcome to pregnancy in renal transplant patients is good renal function and absent or well-managed hypertension.[108,116] As in women with normal native kidneys, during pregnancy, glomerular filtration rate may increase even in transplant recipients with a single kidney.[24] Pregnancy generally does not have an adverse effect on renal transplant function or outcomes.[10,21] A case-control study from Germany comparing cyclosporine versus azathioprine immunosuppression during pregnancy concluded that pregnancy does not adversely affect graft or patient survival, independent of immunosuppressive regimens.[33] Because calcineurin inhibitors have significantly improved graft survival, it is most attractive to continue calcineurin inhibitor therapy during pregnancy, albeit with close monitoring of drug levels and renal function.[75]

Data from combined U.S., European, and United Kingdom transplant registries on pregnancies in kidney transplant recipients (Fig. 37-17) show a marked increase over 15 years in pregnancies, including pregnancies beyond the first trimester.[64] This increase is attributed by the authors to reversal of gonadal dysfunction by renal transplantation, return of female fertility, and increased possibility of conception. The combined databases report more than 2000 live births to women with organ transplants (of all types) as of 2006. A consensus conference in 2003 advised that conception is safe after the first post-transplant year if the graft is functioning well and no rejection episodes have occurred in the year before conception.[65] Pregnancies after transplantation should be handled as high risk, however, with close prenatal monitoring. Cesarean delivery is indicated only for obstetrical reasons.[25] Renal transplant patients with a serum creatinine greater than 1.5 mg/dL have an increased risk of allograft loss during and after pregnancy, but the risk is minimal if the creatinine is less than 1.5 mg/dL at the time of conception.

Few data exist on the impact of immunosuppressive drug therapy on the fetus and newborn. Despite reduction of T and B cell counts in newborns, these counts have been reported to normalize within a few months, and there is no reported increase in incidence of infection or

Table 37–6 **Adjusted Graft Survival, Living Donor Kidney Transplants: Survival at 3 Months, 1 Year, 3 Years, and 5 Years in the United States**

	3 Months*			1 Year*			3 Years†			5 Years‡		
	No.	*%*	*SE*	*No.*	*%*	*SE*	*No.*	*%*	*SE*	*No.*	*%*	*SE*
Age at Transplant												
All	13,096	97.3%	0.1%	13,096	95.2%	0.2%	25,336	88.4%	0.2%	35,491	80.2%	0.3%
<1 yr	2	+	+	2	+	+	13	92.1%	7.3%	24	95.7%	4.1%
1-5 yr	206	97.7%	1%	206	95.2%	1.5%	408	92.4%	1.5%	564	90.3%	1.5%
6-10 yr	144	96.6%	1.5%	144	95.9%	1.6%	316	91.3%	1.9%	464	85.6%	2.4%
11-17 yr	459	97%	0.8%	459	94.4%	1.1%	951	88.1%	1.3%	1,380	77.3%	1.7%
18-34 yr	2,836	97.5%	0.3%	2,836	95.5%	0.4%	5,607	87.3%	0.6%	8,135	79.3%	0.7%
35-49 yr	4,163	97.4%	0.2%	4,163	95.5%	0.3%	8,157	89.7%	0.4%	11,675	82.4%	0.5%
50-64 yr	4,199	97.3%	0.3%	4,199	95.3%	0.3%	7,898	88.7%	0.4%	10,699	80.7%	0.6%
≥65 yr	1,087	96.7%	0.5%	1,087	93.3%	0.8%	1,986	84.1%	1%	2,550	70.3%	1.5%
Primary Diagnosis												
Glomerular diseases	3,972	97.6%	0.2%	3,972	95.5%	0.3%	7,698	88.9%	0.4%	10,933	81.6%	0.5%
Diabetes	2,752	96.8%	0.3%	2,752	94.3%	0.5%	5,388	86.2%	0.6%	7,435	75.8%	0.8%
Hypertensive nephrosclerosis	1,695	97.7%	0.4%	1,695	95.4%	0.5%	3,040	87.2%	0.8%	4,120	77.3%	1%
Polycystic kidneys	1,321	98.3%	0.4%	1,321	97.5%	0.4%	2,448	93.5%	0.6%	3,284	88.2%	0.9%
Tubular and interstitial diseases	886	97.1%	0.6%	886	94.6%	0.8%	1,729	87.1%	1%	2,508	79.7%	1.1%
Renovascular and other vascular diseases	435	97.2%	0.8%	435	95.4%	1%	959	89.4%	1.2%	1,391	75.8%	1.7%
Congenital, rare familial, and metabolic disorders	592	96.7%	0.8%	592	94.8%	1%	1,120	88.2%	1.3%	1,529	83.1%	1.6%
Neoplasms	56	98.3%	1.7%	56	98.3%	1.7%	116	93.8%	2.5%	157	83.8%	4.6%
Other	1,163	95.7%	0.6%	1,163	93.7%	0.7%	2,260	88.2%	0.8%	3,270	82.5%	0.9%
Unknown	224	97.3%	1.1%	224	94.5%	1.5%	578	86.4%	1.7%	864	76.6%	2%
Recipient Gender												
Female	5,410	96.6%	0.2%	5,410	94.4%	0.3%	10,481	87.7%	0.4%	14,739	79.8%	0.5%
Male	7,686	97.7%	0.2%	7,686	95.7%	0.2%	14,855	88.8%	0.3%	20,752	80.4%	0.4%
Recipient Ethnicity/Race												
White	8,580	97.2%	0.2%	8,580	95.1%	0.2%	16,942	88.8%	0.3%	23,888	81.1%	0.4%
African American	1,921	97.2%	0.4%	1,921	94.3%	0.5%	3,734	83.3%	0.8%	5,220	71.5%	0.9%
Hispanic/Latino	1,614	97.4%	0.4%	1,614	96%	0.5%	3,050	90.8%	0.6%	4,254	83.8%	0.8%
Asian	485	98.6%	0.5%	485	98.1%	0.6%	953	93.3%	1%	1,345	86.6%	1.5%
Other/Multirace	144	96%	1.6%	144	92.6%	2.1%	260	88.3%	2.2%	341	84.5%	2.5%
Unknown	352	96.9%	0.9%	352	96%	1.1%	397	94.8%	1.5%	443	83.3%	4.8%

*Transplanted 2003-2004.
†Transplanted 2001-2004.
‡Transplanted 1999-2004.
SE, standard error.
Data from OPTN/SRTR Data, as of May 1, 2006.

autoimmune disease in these children.[26,114] The long-term consequences of in utero exposure to immunosuppression are unknown.[6,8,36,85] Of 48 children of recipients of solid organ transplants followed for a mean of 5.2 years, no structural or developmental abnormalities were noted, despite a premature birth rate of 56%.[125]

RENAL TRANSPLANTATION IN HUMAN IMMUNODEFICIENCY VIRUS–POSITIVE PATIENTS

Human immunodeficiency virus (HIV) seropositivity is no longer considered a contraindication to renal transplantation at some centers, based on encouraging results in a small, but growing, number of patients transplanted to date. In the era of highly active antiretroviral therapy, patients have markedly extended survival, and graft survival rates comparable to the rates of HIV-negative patients have been reported (Table 37-8).[88,110] Patients treated with protease inhibitors require far less calcineurin inhibitor therapy to achieve target blood levels, as reported by Stock and colleagues.[111] Despite low CD4 counts, HIV-positive recipients may be prone to acute rejection and particularly antibody-mediated rejection.[99] Nevertheless, few opportunistic infections were reported.

Inclusion criteria for HIV-positive patients being considered for renal transplantation have included (1) a CD4 count greater than 200 cells/mL for the previous 6 months,

Table 37–7 **Incidence of Post-Transplant Lymphoproliferative Disease from Registration Trials (Phase 3) of Commonly Used Immunosuppressive Agents**

Study	Follow-up (yr)	Antibody Induction	Concurrent Immunosuppression	Arms	Patients (No.)	PTLD (%)
TAC						
U.S., Pirsch, 1999	3	ATG/OKT3	Aza/Pred	TAC	205	2.4
				CsA	207	2.9
U.S., Vincenti, 2002	5	ATG/OKT3	Aza/Pred	TAC	205	3.4
				CsA	207	2.9
MMF						
U.S., Sollinger, 1995	0.5	ATG	CsA/Pred	Aza	164	0
				MMF 2 g	165	0.6
				MMF 3 g	166	1.2
Tricontinental,* 1998	3	NA	CsA/Pred	Aza	162	0.6
				MMF 2 g	171	1.2
				MMF 3 g	164	1.8
SRL						
U.S., Kahan, 2000	1	None	CsA/Pred	Aza	159	0.6
				SRL 2 mg	281	0.4
				SRL 5 mg	269	0.7
Europe,* Groth, 1999†	1	None	Aza/Pred	CsA	41	0
				SRL	42	0
Europe,* Kreis, 2000†	1	None	MMF/Pred	CsA	38	0
				SRL	40	0
Dac						
Vincenti, 1998	1	NA	CsA/Aza/Pred	Dac	126	1.6
				PBO	134	0.7
Nashan, 1999	0.5	None	CsA/Pred	Dac	140	0
				PBO	133	0.8
Bas						
Nashan, 1997	1	None	CsA/Pred	Bas	193	0.5
				PBO	187	0.5

ATG, antithymocyte globulin; Aza, azathioprine; Bas, basiliximab; CsA, cyclosporine; Dac, daclizumab; MMF, mycophenolate mofetil; NA, not available; OKT3, muromonab-CD3 antilymphocyte antibody preparations; PBO, placebo; Pred, prednisone; PTLD, post-transplant lymphoproliferative disease; SRL, sirolimus; TAC, tacrolimus.

*North America, Europe, and Australia.

†Phase 2 studies.

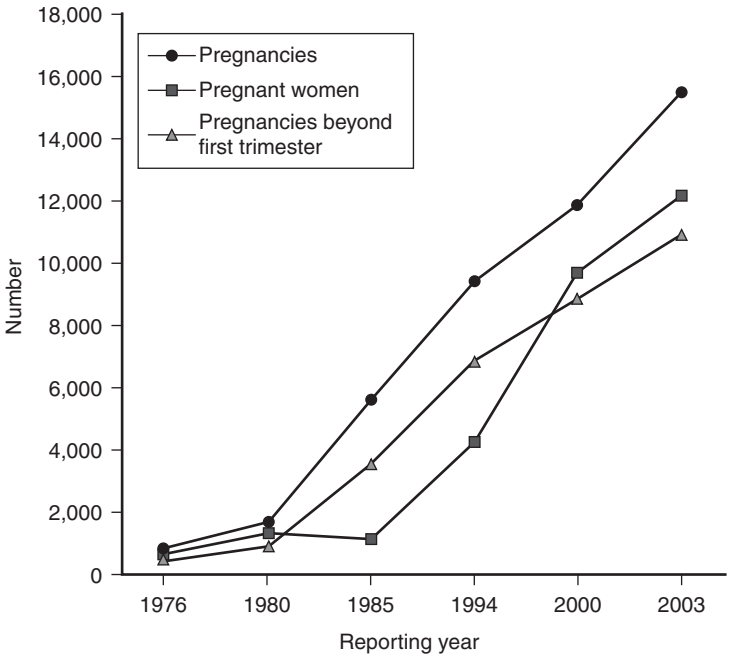

Figure 37–17 Pregnancies in kidney transplant recipients reported worldwide. The circles represent the numbers of pregnancies reported worldwide in kidney transplant recipients during the indicated year. The numbers include therapeutic terminations, spontaneous abortions, ectopic pregnancies, and stillbirths. The *squares* represent the numbers of female transplant recipients reported to have been pregnant during that year, again including all outcomes. The *triangles* represent the numbers of pregnancies beyond the first trimester reported in the literature during the indicated year. (Data from the National Transplantation Pregnancy Registry in the United States, the European Dialysis and Transplant Association Registry, and the United Kingdom Transplant Pregnancy Registry.)

Table 37–8 **Comparison of Graft Survival after Kidney Transplantation from Living and Deceased Donors before and during the HAART Era by HIV Status**

	Deceased Donor						Living Donor					
	HIV Negative			HIV Positive			HIV Negative			HIV Positive		
	No.	*1 yr (%)*	*3 yr (%)*	*No.*	*1 yr (%)*	*3 yr (%)*	*No.*	*1 yr*	*3 yr (%)*	*No. (%)*	*1 yr (%)*	*3 yr (%)*
Overall	87,894	85	74	92	81	67	42,509	93	86	46	89	70
Pre-HAART	45,980	82	71	29	75	50	15,766	92	84	9	78	56
HAART	41,914	88	77	63	84	84	26,743	94	87	37	92	74

HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

Data from Pelletier SJ, Norman SP, Christensen LL, et al: Review of transplantation in HIV patients during the HAART era. In Cecka JM, Terasaki PI (eds): Clinical Transplants 2004. Los Angeles, UCLA Immunogenetics Center, 2005, pp 63-82.

(2) undetectable HIV RNA for 3 to 6 months, (3) no prior opportunistic infections or neoplasm except for drug-sensitive esophageal conditions, (4) antiretroviral therapy stable for at least 3 months or off therapy and able to maintain undetectable HIV RNA, and (5) no signs of significant wasting. Although the published experience is small, good outcomes have been achieved in such patients undergoing renal transplantation. Pharmacological immunosuppression has been similar to that used in HIV-negative recipients except that calcineurin inhibitor doses are much lower.

PREVALENCE OF PEOPLE LIVING WITH A FUNCTIONING KIDNEY TRANSPLANT

The number of people in the United States living with a functioning kidney transplant doubled between 1995 and 2004. At the end of 1995, there were 50,529 people with a functioning kidney transplant and 3156 people living with a functioning kidney-pancreas transplant. By the beginning of 2005, there were 101,440 people with a functioning kidney transplant and 7213 people with a functioning kidney-pancreas transplant. Based on 2004 data from the USRDS, kidney and kidney-pancreas recipients living with a functioning kidney transplant represented 18% of all end-stage renal failure patients in 1995 and 21% of all end-stage renal failure patients in 2002.[119]

The longest surviving recipients of a kidney transplant with a functioning graft are nine patients with more than 40 years' graft survival from the University of Colorado, Denver. More than 100 patients have been reported with greater than 25 years' graft survival.[17] At least eight patients with living donor kidney transplants have experienced greater than 20 years' graft survival without continuing immunosuppression long term.[17] In other words, such patients have clinically shown immunological tolerance for a prolonged period.

LONG-TERM OUTCOMES OF RENAL TRANSPLANTATION

Analysis of long-term kidney allograft survival has been reported by Hariharan and coworkers[43] based on estimated half-lives using USRDS data. The authors concluded that between 1988 and 1995, half-life of kidney transplants nearly doubled. These data were challenged by Meier-Kriesche and associates,[69] who used real half-lives rather than estimated half-lives and found instead that first transplant survival improved only marginally during this era, and greater improvement was achieved for retransplants. The Meier-Kriesche analysis showed that despite improvements in early (1 year) graft survival that occurred during the study period, long-term graft survival (≥8 years) was not significantly altered or improved. The CTS European data (see Fig. 37-16) is compatible, however, with the report from Hariharan and coworkers[43] of the USRDS data, and graft survival at 3 years of deceased donor grafts has steadily increased from 1982 to 2000.

Nevertheless, no one would dispute the suggestion that the availability of more potent immunosuppression over the last 10 years is not yet reflected in improved long-term outcomes. There continues to be a dire need for therapy and diagnostics that translate into better long-term success. This goal depends on (1) better ways to improve patient survival, perhaps through better cardiovascular health management and reduced risk of malignancy; (2) immunosuppressive strategies that better preserve renal function and reduce chronic rejection; and (3) better monitoring and early diagnosis of renal transplant dysfunction.

QUALITY OF LIFE

Although, traditionally, outcomes have been measured in terms of graft and patient survivals because the goal of kidney transplantation is to restore normal kidney function and prolong life, measurements of the quality of life after renal transplantation focus in more detail on the impact of a successful kidney transplant on parameters such as physical function, physical pain, general health, vitality, social functioning, and mental health. Because the immunosuppression associated with renal transplantation has an extensive list of associated side effects, how these affect overall quality of life can be measured.

Neipp and colleagues[77] reported on the quality of life in adult renal transplant recipients more than 15 years after transplantation. This single-center study of 139 patients found that 29% were employed, 7% were seeking employment, 58% were retired, and 5% were homemakers. Using a 36-item health survey, a validated quality-of-life survey, and

Table 37–9 **Prevalence of Sexual Problems in Renal Replacement Therapy Patients Compared with a Control Group**

Treatment Group	Prevalence in Men (%)	Prevalence in Women (%)
Control	8.7	14.9
Hemodialysis	62.9	75
Peritoneal dialysis	69.8	66.7
Kidney transplant	48.3	44.4

From Habwe VQ: Posttransplantation quality of life: more than graft function. Am J Kidney Dis 47:S98, 2006. Data from Diemont WL, Vruggink PA, Meuleman EJ, et al: Sexual dysfunction after renal replacement therapy. Am J Kidney Dis 35:845, 2000.

a kidney transplant questionnaire, the authors reported on eight aspects of the health of these patients. In contrast to retired and unemployed patients, employed recipients reported a significantly improved health-related quality of life, including physical functioning, physical pain, general health, vitality, social functioning, mental health, physical symptoms, fatigue, uncertainty and fear, and emotional health. All of these parameters were improved in employed recipients compared with their counterparts ($P < .05$). The authors concluded that vocational rehabilitation after renal transplantation is crucial and is associated best with improved health care quality of life.

Studies have shown that immunosuppression-related side effects can compromise quality of life. These side effects include hirsutism, gingival hyperplasia, weight gain, cushingoid facies, hand tremors, alopecia, and skin disorders.[42] A cross-sectional study of 350 kidney transplant patients by Moons and associates[73] showed that steroid-free patients experienced better social functioning, fewer psychiatric symptoms, lower symptom occurrences, and lower levels of distress ($P < .03$) for all of the aforementioned side effects.

A Dutch study of sexual dysfunction in kidney transplant recipients compared with dialysis patients and control subjects from the general Dutch population showed significantly less sexual dysfunction in men and women with a successful kidney transplant compared with either hemodialysis or peritoneal dialysis, yet substantially more difficulties compared with control subjects ($P < .001$) (Table 37-9).[28,42] Strategies for improving quality of life include effective management of drug side effects, improved immunosuppressive regimens, psychotherapy, social support, exercise, and vocational assistance.

CONCLUSION

Dialysis and transplantation are costly treatments, and every Western country, faced with rapidly increasing medical costs, has reflected on the cost-effectiveness of expensive therapies. Inevitably, the spotlight falls on dialysis and transplantation: Is this cost justified? Unquestionably, the treatments are expensive, and costs vary from nation to nation. Assuming that one considers treatment of patients with end-stage renal failure justified, transplantation is the cheaper option available. In developing countries, renal transplantation is almost the only available option because often long-term dialysis is unavailable. Of patients with the potential for full-time work, most are restored to full-time work after living donor and deceased donor transplantations. In such situations, a productive member of society is re-established, with the consequent saving in pensions or benefits to surviving family members. The demonstration that survival is enhanced by transplantation compared with dialysis in nearly all patient groups, as discussed earlier, provides more objective evidence of the key role that transplantation should play in the management of end-stage renal failure.

The justification for the treatment of end-stage renal failure by an integrated program of dialysis and transplantation seems self-evident. The primary aim is to achieve a successful transplant, using dialysis to maintain patients while awaiting a transplant, or to treat patients who are unsuitable for transplant for medical or immunological reasons. Because a large proportion of patients with end-stage renal failure who are suitable for transplantation are relatively young, achievement of a successful renal transplant in these patients is one of the more satisfying areas of medical practice today. No one would have predicted at the time of the first successful renal transplant in 1954 that so much would have been achieved over the subsequent 50 years.[74]

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Chapter 38

Psychological Aspects of Kidney Transplantation and Organ Donation

Patricia M. Franklin

End-stage renal disease is a psychologically debilitating illness with emotional morbidity. End-stage renal disease can have a major impact on patient and family lifestyles, blocking future life goals and resulting in a cycle of anger, mood swings, depression, and unfulfilled hopes. All forms of renal replacement therapy have been studied to elicit the psychological impacts of treatments and the particular stressors encountered by patients and their caregivers. These studies show that the treatment of renal failure through dialysis or transplantation creates stress and psychological difficulties for patients. The negative themes reported from study groups include loss of freedom, loss of personal control, loss of independence, blocking of hope and future dreams, and loss of normality.[29,38]

Kidney transplantation is the treatment of choice for patients with end-stage renal disease. Studies have shown that renal transplant recipients are surviving longer and have a better quality of life than patients receiving other renal replacement therapies. A successful renal transplant does not render the patient free of the chronic illness or subsequent psychological problems, however. The transplant enables recipients to enjoy an improved quality of life with freedom from a machine or a dialysis exchange, but it often presents a different set of psychological stressors and challenges to overcome. Understanding of the psychological aspects of transplantation has increased in recent years, and this increased understanding has resulted in the opportunity to offer informed psychological support as an integral part of transplantation care. This chapter discusses the major psychological studies and their findings and provides brief personal experiences reported by patients to the author during 30 years of experience as a nurse psychologist working in transplant centers.

QUALITY OF LIFE AND PSYCHOLOGICAL WELL-BEING FOR RENAL TRANSPLANT RECIPIENTS

Individual quality of life is difficult to assess because it is affected by a wide range of independent and personal variables. A large study by Evans and colleagues[19] in the 1980s,

which comprised 800 patients in all treatment modalities in 11 treatment centers, concluded that "Transplant recipients generally have a higher level of functional ability, are more likely to return to work, are in better health, and have higher levels of well being, life satisfaction, psychological affect, and happiness than do patients on any form of dialysis." Since this study was reported, there have been major advances in dialysis treatments and in renal transplant immunosuppression regimens. There also have been advances in scientific techniques to evaluate issues of life satisfaction and quality of life.

More recent studies incorporating these new treatments and research techniques continue to support the work of Evans and colleagues[19] by showing that transplant patients report a superior quality of life compared with patients on dialysis therapies. A study by Kieninger and Keller,[35] which examined quality of life in kidney transplant patients compared with patients with glomerulonephritis, patients with other chronic diseases, and a group of healthy blood donors, concluded that "Renal transplant recipients estimated their quality of life to be on a higher level in comparison with those of patients suffering from other chronic diseases and that renal transplant recipients estimated global self assessment of quality of life as even better than the healthy volunteers."

A large study by Tomasz-Wesolowski and Piotr-Szyber[70] also concluded that the quality of life of transplant patients is better than patients on hemodialysis in certain domains, including physical, social relationships, pain and discomfort, energy and fatigue, positive feelings, personal relationships, and sexual activity. In the area of body image and appearance, quality of life of transplant patients was reported as worse, however, than hemodialysis patients.

Although quality of life is improved dramatically in various aspects of life satisfaction after transplantation, even life with the best-functioning transplanted kidney can be negatively affected by perpetual uncertainty with the possibility of rejection and failure. Also, continuous immunosuppressive therapy can create psychological difficulties, such as bodily changes and other major challenges, which need to be negotiated successfully by transplant recipients.

RENAL DISEASE—DIALYSIS AND PREOPERATIVE ADJUSTMENTS

Although kidney transplantation is the treatment of choice for most end-stage renal disease patients, with demand for grafts far exceeding supply, many have to wait months or years before this treatment is available to them. A few patients may receive a transplant during the predialysis stage, but for most, the waiting period involves emotional adjustments to the physical, psychological, marital, and dialysis-related changes imposed by the disease. The shock of the initial diagnosis, sexual dysfunction, marital friction, changes in body image, and subsequent lower self-esteem and dependence on a machine, fluid bag, or partner can produce profound stress, adjustment anxiety, and depression. Various psychological coping strategies may be used during this time to help the patient and family members negotiate this period of disease and dialysis adjustment.

Coping strategies are psychological patterns that individuals use to manage thoughts, feelings, and actions encountered during various stages of ill health and treatments. The fundamental need to have an overall sense of control over one's life is paramount throughout chronic sickness, and research has shown that interventions designed to increase an individual's perception of control are likely to have a positive impact on patient well-being. Presenting treatment options with information so that realistic choices can be made helps patients maintain a sense of control.

During the early phase of ill health, denial and suppression are the most frequent avoidance strategies used. Denial as a psychological defense has been prominent in hemodialysis patients. Later, more positive coping strategies include problem solving, actively seeking information, enhancement of spiritual life, and hope of a transplant. These coping strategies are similar to ones used by end-stage cardiac disease patients.

Many younger patients find such adjustment extremely difficult. Research conducted between Oxford and Manchester[2,3] found that "the younger patient, particularly the young male, found dependence on dialysis particularly frustrating, perhaps because society expects the male to be more active, aggressive and ambitious in forging a role in life." Young male patients may express more dissatisfaction and are more likely to show this dissatisfaction in noncompliant, self-destructive, and despairing behavior. One young male hemodialysis patient described his life as a series of frustrating "can't do's": "can't drink beer with friends, can't enjoy meals with friends, can't go on vacation with friends, can't work, and can't make love to a girlfriend."

Reasonably fit elderly patients are in some ways the most satisfied dialysis group. Many elderly patients feel satisfied with their lives and welcome the chance of a further few years resulting from treatment. Even though older patients are more satisfied with their dialysis lifestyle than younger patients, many older patients seek the better quality of life that a transplant can provide.[2,3] A large study involving 2746 kidney transplants[32] examined the medical and psychosocial outcomes in an older group (≥65 years old) and a younger group (18 to 64 years old). This study concluded that older recipients enjoy significant benefits to quality of life after a transplant, similar to benefits seen in younger recipients. A similar study that examined the differences in pretransplant and post-transplant quality of life in kidney recipients in five age groups (range 18 to >60 years old) reported that quality of life outcomes did not seem to favor one age group over another.[12]

The initial dialysis adjustment phases are difficult. For some patients, these adjustment phases can be traumatic. Professional, practical, and psychological support is desirable at this time.

Health beliefs and attitudes toward illness and treatments differ between individuals and among cultures, as do responses to pain and reactions to a new graft. Many white patients find that peritoneal dialysis offers a moderate to good quality of life, but at Oxford a Muslim patient with a strict religious hygiene code found this treatment impossible because she felt "unclean—like a dustbin, always filling up with rubbish." Such feelings made prayer difficult and life unbearable for her. Cultural attitudes may influence the recipient's response to transplantation. A young female Asian patient at this transplant center refused the idea of a cadaver transplant because she was reluctant to accept a kidney from an anonymous donor who might be male.

Many authors have discussed health and illness beliefs and have outlined the need for staff to consider the meaning

that patients attach to their illness and treatment therapies. It is important for staff members to be aware of individual perceptions and beliefs with regard to transplantation. Each belief must be recognized and validated, and, if required, appropriate support must be provided.

Many units provide predialysis information sessions for patients and family members. Bradley and McGee[6] suggest that the "most effective sessions seem to be run on a multidisciplinary basis, with input from Medical, Nursing, Dietetic and Social Work staff, and include information from dialysis and transplant patients themselves." In this unit, such sessions are valuable because they provide an opportunity to give information concerning all treatment options. The sessions also provide a forum to encourage active patient and caregiver participation with regard to treatment issues and initial treatment anxieties.

Meeting other patients who have negotiated various forms of treatment successfully offers a positive image and role model and gives greater credibility to information given. Honesty is an important part of such sessions, and information givers strive to present a realistic assessment of experiences without being overprotective regarding problems or overly optimistic. Such sessions help to develop a close supportive relationship between staff members and patients at the predialysis stage.

HOPE OF A TRANSPLANT

Peretz[49] defined hope "as the capacity to anticipate that even though one feels uncomfortable now, one may feel better in the future." When a transplant is suggested, many patients make an immediate decision to proceed, whereas others agonize over the decision. Some patients may deny the possibility of post-transplant difficulties and may have unrealistic expectations for their future quality of life. Such denial may predispose patients to depression if major complications occur after the transplant. It is essential that in-depth realistic and honest information is given at this stage so that patients may proceed in an informed manner.

In the past, renal programs often required formal pretransplant psychiatric assessment. These assessments are no longer considered necessary, but the experience at Oxford suggests that it is valuable to have a pretransplant meeting at which specific medical, social, and psychological issues are explored with the patient and family members. Individual fears raised at such a meeting include fear of changes in body image resulting from immunosuppression, fear of loss of identity when accepting a foreign organ, and fear of surgery, particularly for older patients. These concerns are similar to concerns reported by other authors. A pretransplant meeting is an opportunity to dispel myths or hearsay that may have been gleaned from other patients. Issues raised in this unit that have required careful explanation include the idea that dialysis is only a short-term treatment, and that the patient may die unless he or she receives a transplant; that it is possible to be infected with venereal and other diseases from a cadaver organ; that a male receiving a female kidney may become feminized, and vice versa; and that the donor persona may be implanted with the transplant, and that the recipient "will become a different person."

The pretransplant meeting offers the opportunity to explore, examine, and resolve individual fears and helps to initiate a trusting and supportive relationship with a member of the transplant team. The meeting is a time to offer specific information and advice concerning coping skills and responses to the profound and conflicting emotions that may be experienced. The knowledge gained by staff members during these meetings concerning individual fears and difficulties alerts the professionals to vulnerabilities that may require help postoperatively. A brief period of counseling may be in order for patients who experience the most difficulty with the decision regarding transplant.

Many patients express concerns relating to immunosuppression regimens and their side effects, and it is important to discuss these fears and, if possible, to offer recipients a regimen that they believe would have the least impact on their post-transplant lifestyle and would present the least difficulties for them with regard to body image issues. A study by Sharkey and Gourishanker[60] supported the view that patients require complete information before renal transplantation to make an informed decision and to enhance the overall transplant experience.

Renal patients must temper their hope for a transplant and subsequent enhanced quality of life with the knowledge that there can be no guarantee that a suitable graft will become available or that the transplant will be successful. These uncertainties increase ambivalence toward transplantation and increase psychological stress. Most patients and family members describe the waiting time as the most difficult phase.

Patient fears that they have been forgotten, that they may miss the call, or that their chance may never come are reported frequently. Ongoing contact with the transplant center is helpful and is vital at times of additional stress when a fellow dialysis patient receives or rejects a kidney, when an abortive call occurs, or when the waiting period becomes particularly lengthy. These instances may upset the usual coping strategies, and psychological stress and depression may result. In our center, a transplant nurse specialist is assigned to each patient at the pretransplant meeting so that a supportive bond can develop, and the nurse can offer information and support during the waiting time and after transplantation.

IMMEDIATE POSTOPERATIVE PSYCHOLOGICAL ISSUES

Many kidney transplant recipients report an immediate feeling of rebirth after the transplant; such feelings are linked to a perceived promise of extended and enhanced quality of life. Studies suggest that psychological stress persists throughout the initial recovery period and during the early rehabilitation process. Many recipients report that although the renal transplant is an opportunity for renewed health, it did not eliminate health-related stress from their lives. The major causes of psychological stress during the early postoperative phase include possibility of rejection and lack of control regarding the body's acceptance or rejection of the kidney, fear of infection, uncertainty about the future and concern about long-term side effects of immunosuppressive therapy.

The fear of graft rejection is the most frequently reported, and anxiety has been shown to precede the first rejection experience. Such anticipatory anxiety is lessened if the rejection is treated successfully. Although recipients are more at ease if faced with future rejection episodes, uncertainty about future health persists for many months.

One of the most difficult aspects for recipients at this stage is the sudden removal of conscious control. The dialysis patient has become conditioned to control of health through adherence to diet and fluid restrictions and regular treatment regimens. After the transplant, the situation changes radically, and recipients are "at the mercy" of factors beyond conscious control—for example, "their own immune response and the effects of the foreign organ which now needs to become accepted as part of the self." Such loss of control can increase anxiety levels, and some patients report panic attacks. It is vital at this stage to discuss progress in detail with the patient and to answer all questions because many recipients seek to regain conscious control by information seeking and by planning daily psychological and activity goals. It is helpful to encourage patient participation in care with self-medication and self-observation so that partial control is achieved. If recipients can be included in discussion regarding medication options, such choice also offers an element of control at this difficult stage.

Some recipients may have difficulty in accepting the new graft as part of self. Castelnuovo-Tedesco[11] wrote that "the graft is not psychologically inert and that the recipient may develop a prominent identification with the donor." One young female patient at Oxford who was depressed post-transplant stated, "before my transplant I had a broken body and a healthy mind—now, after my transplant, I have a healthy body and a broken mind." During gentle exploration, it was discovered that this patient found it profoundly difficult to accept that she had the kidney of a middle-aged man "inside her," fearing that her femininity was at risk. Other reported patient fears include fear of racial change and in some cases obsessive identification with the donor or the donor family.

Bunzel and coworkers[8] relayed that a few heart transplant recipients (6% [three patients]) reported "a distinct change in personality due to their new hearts—with the belief that they were forced to change feelings and reactions and accept those of the donor." Such statements seem to show severe problems regarding graft incorporation that are based on the age-old idea of the heart as a center that houses feelings and forms the personality. Sylvia,[69] a heart-lung recipient, wrote that her attitudes, tastes, and food likes and dislikes changed to mirror the attitudes, tastes, and food likes and dislikes of the donor.

Studies regarding perceived changes in personality were first reported, in the main, by heart transplant recipients and were usually linked to the belief that the heart is seen "as the source of love, emotions and, for some, the focus of personality traits." More recently, there have been several articles in the national and international press in which such "personality changes" have been reported by individual live donor and recipient pairs. These articles have hypothesized that some form of cellular memory may be responsible for these perceived changes. Such publicity has led to patients at our center becoming anxious that the transplanted kidney may result in a personality change for them. In our experience, these anxieties can be resolved by discussion and reassurance that the graft does not carry the persona of the donor and cannot alter the integrity of the recipient's personality.

Feelings of guilt and sadness concerning the donor and donor family are frequent. An adult receiving a pediatric graft may view the death as a special tragedy and experience profound guilt and grief. Recipients and caregivers report dreams in which they may see a distressed family without a father or mother, and they may relate such dreams to the donor family. Some recipients report also the need to offer prayers for the donor and the family and may experience feelings of unworthiness in receiving such a precious, life-enhancing gift. Fox and Swazey[24] discussed the obligations entwined in such a gift, and they quoted the work of Mauss,[43] who stated that "the obligation of worthy return is imperative too. Face is lost forever if it is not made."

The opportunity to discuss such feelings and to give thanks through an anonymous letter usually aids resolution so that the recipient may move forward toward positive rehabilitation. It is becoming more common, however, for recipients or donor families to request a meeting with each other. Several transplantation units are facilitating such meetings, and initial reports suggest successful outcomes for the recipient and the donor family. Reports cited by Fox and Swazey[24] stress the need for caution, however, because the donor family may be "disappointed in the recipient" or may become "intrusive into the recipient's life." The recipient may face the dilemma of wanting to refuse such a meeting, but may fear that they would be seen as ungrateful, and they may be distressed or disturbed at trying to meet the perceived needs of the donor family. It has been stated that it is paternalistic of professionals to discourage such meetings; however, professionals have a duty of care to recipients and donor families. Thorough discussion and planning must precede such meetings, and fully trained professionals must be available to offer debriefing sessions and to help should problems arise.

Depression may occur in the post-transplant period and may be linked to infection because it is especially prevalent among patients with cytomegalovirus infection or cytomegalovirus mononucleosis syndrome. Also, patients who have unrealistically high expectations preoperatively are susceptible to postoperative depressive symptoms. Such patients may have difficulty accepting that transplantation is an alternative treatment rather than a cure for end-stage renal disease. The most appropriate psychiatric diagnosis for many of these patients is an adjustment disorder. Studies report that the degree of distress often is correlated with the severity of physical symptoms and the occurrence of postoperative complications.

Immunosuppression Regimens and Psychiatric and Psychological Reactions

Florid psychiatric responses to immunosuppressive therapies are rarely seen now because the introduction of low-dose steroid therapy combined with the newer calcineurin inhibitor therapies has resulted in fewer psychiatric disorders. Mania still may be observed in some recipients with particular corticosteroid sensitivity.

Studies show, however, that patients still report that low-dose corticosteroids are responsible for mood changes and irritability in the early post-transplant period. Sometimes these emotional responses are less obvious to the patient, but are reported by friends or family members. Transient disruption of sleep, altered perception, and lability of mood often occur in patients receiving pulses of corticosteroids as anti-rejection therapy.

Several studies have investigated symptom distress after renal transplantation and the introduction of immunosuppression; in these studies, patients report sleep problems,

overeating, fatigue, changed body and facial appearance, mood swings, swollen ankles, decreased interest in sex, and headaches.[20] A study by Zarifian[74] found a significant difference with the symptoms of fatigue, changed facial and changed body appearance, skin fragility, fever, and pain, which were reported more frequently by female than by male subjects. Also, there were differences in age with younger subjects (21 to 35 years) reporting fewer sleep problems and pain than middle-aged subjects, but more problems with acne than middle-aged and older subjects.

Recipients often blame the steroid therapy for most of the drug-related side effects that they experience, although the difficulties with excessive hair growth are always attributed to cyclosporine therapy. A study by Prasad and associates[51] examined the attitudes of recipients toward steroid use and other therapies. When asked which drug they would like to discontinue, 65% of patients responded and cited prednisolone. Another study found that "if given a risk free choice, the majority of recipients prefer withdrawal of steroids over other agents."[16]

A second study by Prasad and associates[52] examined renal transplant recipient experiences with and options about calcineurin inhibitors. Results of this study showed that renal transplant recipients experienced fewer and less severe side effects with tacrolimus when cyclosporine and tacrolimus therapies were compared. These researchers stressed that transplant centers should consider patient's opinions and needs and should tailor the immunosuppressive strategies and regimens to take these opinions and needs into account.

Body Image and Self-Esteem

People in renal failure may experience negative reactions toward their bodies because of the invasive nature of the treatment. The cessation of dialysis after renal transplantation does not abolish this stress. Immunosuppression and its side effects present a major problem related to body image after transplantation. Corticosteroids may cause acne and a cushingoid appearance characterized by an abnormally round face and protruding abdomen. Hirsutism, mild tremors, and gingival hyperplasia commonly are exhibited by patients receiving cyclosporine. Such side effects prompted a young Oxford patient to perceive herself as "something from the *Planet of the Apes*."

Body image is a personal matter; what is a problem for one person may be insignificant for another. If body image is perceived unfavorably, however, feelings of inferiority and intense anxiety may be generated. Studies suggest that many renal transplant recipients report body image problems with subsequent lower self-esteem and feelings of inferiority or of "being altered or damaged." Some recipients find the changes of body image after a kidney transplant more distressing than the changes that occurred while on dialysis. The cushingoid puffy look of the face generally creates the biggest obstacle to acceptance. Dissatisfaction with body image is associated with poor psychosocial adjustment and interferes with successful rehabilitation.

Careful preoperative counseling concerning expected side effects, reassurance that such side effects are dose related and lessen as drug dosages are reduced, and practical help and advice in coping with specific problems may reduce the psychological trauma that altered body image causes recipients. At our institution, a trained skin and beauty therapist offers extensive advice, and recipients report that this service greatly reduces the embarrassment and unhappiness experienced by bodily changes. The introduction and use of tacrolimus has greatly reduced this problem, however.

Body image change may be particularly distressing for adolescent recipients. Adolescents are in a period of structural ego alteration with conflict about identity, psychosexual development, dependency, and authority, and the additional stress of a transplant may become a focus of derangement of their defenses. Many adolescent recipients may require additional support and understanding. For some adolescents, the side effects of immunosuppressive therapies and their perceived effects on social interaction are more unacceptable than graft failure and possible death from voluntary discontinuation of medications.

Psychological Distress and Adherence to Immunosuppression Regimens

Adherence has been defined as the extent to which a patient's behavior coincides with the prescribed regimen.[13,30] Poor adherence is a risk factor for morbidity and mortality after transplantation and has been the subject of much research over many years.

Didlake and colleagues[16] reviewed adherence with cyclosporine regimens in 531 kidney transplant recipients. This study reported major nonadherence resulting in graft loss in 2.8% of the sample and minor nonadherence resulting in rejection episodes in 1.9%. Recipients who showed major nonadherence tended to be white female patients. Subclinical degrees of nonadherence were found to be more common. Of 295 transplant recipients who responded to a questionnaire, 13% reported missing more than three doses per month.

Various explanations have been given for nonadherence, including concern about the effects of immunosuppression on physical appearance, inability to accept the lifestyle limitations, and the cost of medication. Surman[68] noted that nonadherence may occur in major depression or as part of an adjustment reaction, especially in adolescent recipients. Adherence may vary across different transplant groups. Beck and coworkers[4] found nonadherence in 43% of pediatric transplant patients; it was most common in adolescent girls, who may have been especially affected by body changes resulting from immunosuppressive therapy. These studies were conducted in the 1980s, and with new treatments it was hoped that adherence would improve.

More recent studies of psychological distress and adherence to medical regimens continue to report unacceptable levels of nonadherence, however, particularly in adolescent recipients. Penkower and associates[48] explored the prevalence of psychological distress, the prevalence of nonadherence, and the association between the recipient's psychological distress and subsequent medical adherence in a group of adolescents (13 to 18 years old). Results showed that 36.4% had symptoms of depression, 36.4% endorsed anxiety, and 18.2% endorsed excessive states of anger. In this study, nonadherence rates were 13.6% for medications. A study from the United Kingdom[9] examined adherence in 58 adult renal transplant recipients (≥18 years old). Results showed that 7 (12%) subjects missed at least 20% of days of medication, and 15 (26%) missed at least 10% of days of medication. Lower belief in the need for medication and

having a transplant from a live donor were major factors associated with nonadherence. Depression also was common, although not strongly associated with nonadherence. Further research is required to examine the beliefs with regard to live donation, but it seems that some recipients may believe that because a familial graft is a good immunological match, there is less need for immunosuppression.

There seems to be a general consensus in the literature that adherence worsens with increasing time after transplantation. A study in heart transplant patients found that nonadherence with medication was related to the belief that the treatment is ineffective or unnecessary with the prevalence of such beliefs increasing over time.[44]

Valid and reliable predictors of nonadherence are unavailable, although a strong history of poor dialysis adherence in patients with nonadherence after transplantation seems to be an important predisposing factor. Nonadherence may develop postoperatively, however, in patients who had adhered with dialysis and pretransplant medical care. Some studies suggest that patients identified as high risk with regard to medication adherence could receive extensive pretransplant psychosocial evaluation and psychological counseling, to facilitate post-transplant follow-up, to strengthen the nurse-patient relationship, and to ensure patient adherence to the immunosuppressive regimen. It is vital to explore and respect the underlying motives and feelings of the recipient and to offer support to enable adherence to medication regimens.[44] Russell and colleagues[55] noted that "the clinical nurse specialist is paramount in assisting both younger and older renal transplant recipients with immunosuppressive medication taking and, consequently, in fostering better adherence and outcomes."

FAMILY INTERACTIONS

End-stage renal disease and its treatments cause shifts within the dynamics of family interactions. Chronic ill health and subsequent medical treatments may have engendered a sense of helplessness in the patient. Family roles change as the patient is placed into a state of chronic illness and treatment-induced dependency. The spouse may have to accept greater family responsibilities and may have to assist with dialysis treatments. Many caregivers report feelings of being "unsupported, invisible and unappreciated." Individuals trying to come to terms with their own feelings find it hard to spare extra energy to cope with the feelings of those close to them.

One of the important post-transplant psychosocial tasks that the patient needs to accomplish is the gradual relinquishing of the sick role and the eventual return to nonpatient status. After transplantation, recipients may be reluctant to give up the security of the patient role, resulting in the spouse resenting the continued dependence. Wilkins and associates[73] reported a study in which targeted education and specific psychosocial supports were given to transplant recipients to aid their return to normalcy. Normalcy is defined as age-appropriate and socially appropriate activities of the patient, such as employment, homemaker, and student. The researchers reported that "a programme of education and psychosocial support that emphasizes return to normalcy and non disability, beginning with the first exposure to transplant and continuing throughout the first six months post-transplant, yielded high rates of return to normality of kidney transplant recipients.[73]

The return to employment can present another hurdle for some transplant recipients, particularly if they have been unable to work for several years. Employers do not always view transplant recipients as reliable and healthy employees. Health care personnel need to create a proactive employment atmosphere and to encourage and assist recipients in their post-transplant quest for work. Carter and coworkers[10] discussed the addition of an employment specialist to the post-transplant team. They stated that "adding an advocate for employment, in our center, facilitated the shift in thinking and the approach to care from the sick role to one of rehabilitation. This change in attitude has assisted in empowering our recipients to feel as if they can truly resume a normal life."[10] Transplant personnel should ensure that they do not unconsciously encourage recipient dependence, but strive to support independence from the beginning of the post-transplant phase.

Marital difficulties may ensue if the transplant recipient is eager to resume his or her preillness position within the family. The partner or children may be disinclined to forfeit any roles that they have assumed during the pretransplant dialysis phase. Such issues usually can be resolved with the help of an empathetic counselor and honest family discussions. Particular difficulties may occur if a child or adolescent who had been chronically ill returns to the family with new mobility and vigor. Families may tend to regard the child or adolescent as fragile and may be excessively restrictive or permissive. Adolescent recipients may not be required to follow the usual family rules, causing disruption and psychological difficulties for the other siblings. These and other family issues can be treated with brief behavioral therapy.

Sexual problems may develop with incompatible sexual desires between partners, erectile dysfunction, or other sexual difficulties. Progress in the field of renal transplantation has considerably improved the quality of life of patients with chronic renal failure; however, quality-of-life studies do not always include assessment of the patient's sex life. The main causes of sexual problems are many and varied and may be psychological, physical, or related to medications; it is important to explore individual difficulties with the recipient and partner to try to elicit the most suitable interventions. Raiz and colleagues[53] reported a study that investigated sexual function for a sample of 347 subjects after renal transplantation; 50 to 55 respondents reported no sexual difficulties. The remaining respondents indicated mild to severe problems. Raiz and colleagues[53] concluded that assessment of and education regarding sexual functioning must be a routine component of psychosocial intervention.

GRAFT FUNCTION

Delayed or Poor Graft Function

In most cases, the new transplant begins working well almost immediately; however, some recipients may have to wait weeks or months for the graft to function. During this time, the recipient must balance hope for a successful outcome with the fear of graft loss. Recipients respond in different ways; some may become overanxious, continually seeking information and reassurance; others may become angry and depressed, continually asking "why me." In contrast, some recipients may seem unconcerned, using denial to cover their underlying feelings of desperation. Staff members may

conclude that such recipients are unaware of the true situation. In reality, the patient is aware of the issues, but is psychologically unable to face the possibility of graft failure. The fantasy that all is and will be well is more bearable during the waiting time. In this instance, denial can be a useful defense mechanism helping to make the period of delayed function sustainable. Perceived personal control is vital during this time, and empowering patients to take control over exercise regimens, health observations, and medications lessens anxiety and increases self-confidence. Offering regular, honest information within an empathetic setting helps aid emotional stability for the recipient.

In some cases, the recipient may endure months or years of unsatisfactory graft function—a level of function that enables the recipient to be free of dialysis, but not able to obtain the desired quality of life or the expected level of rehabilitation. Many patients expect a great deal from their post-transplant lifestyle: a dramatic improvement in physical health; a return to work, study, or parental role; an improvement in self-image; an improvement in family relationships; and freedom from the sick role. Such expectations may be unrealistic and may not be fulfilled. It can be difficult for recipients to admit failure to achieve such ideals and disappointment in their new health status.

If such disappointment is expressed, the recipient may become anxious that he or she appears ungrateful for the gift of life or the medical and nursing care given. Recipients also express guilt that they are not achieving enough or are in some way letting down the donor family or the transplant team. A young Oxford patient felt that she was not "living up to the right standard" and that she "had been given a special opportunity in which she had failed." Her conversation was littered with shoulds and oughts: "I should be making more of a success of my life.... I ought to be more happy and grateful."

These feelings of guilt may be enhanced by family and friends who previously offered sympathy at the rigors of dialysis, but now expect gratitude and full recovery. Partners may find the continuing need to support and care difficult, and marital problems can ensue, especially if there also is sexual friction. Recipients may experience emotional lability and depression, increasing their guilt, and with the additional physical debility resulting from heavy immunosuppression regimens, the psychological impact may be intense, resulting in low mood and clinical depression.

Psychological support should be offered to recipients and caregivers. In our institution, recipients have been found to benefit from therapies aimed at changing individual beliefs, such as cognitive and behavioral therapies. Caregivers found the opportunity to express feelings and recognize and fulfill their own needs beneficial. Marital therapies help in some cases, and if sexual difficulties are present, referral to a specialist team is required.

Graft Failure

Most recipients experience feelings of profound loss if their kidney transplant fails, although some also may feel relief if the graft has had unsatisfactory function over a protracted period. Relief may be linked to the return to dialysis and perceived control. Occasionally, denial may be used in the initial graft failure stage, but as the reality of the situation becomes apparent, sadness, anger, and depression frequently are reported. Hudson and Hiott[31] noted that recipients displayed a variety of behavior and reactions to graft loss, including bereavement reactions: "At this time patients must be helped to understand that the loss of the graft is not the end and there is still hope for the future through subsequent transplants." Akman and associates[1] found that the return to hemodialysis, especially after a short duration of graft function, is associated with depression. There was less depression among married patients, however, which may be due to support of spouses. Akman and associates[1] concluded that "single persons and transplant failure patients who return to dialysis therapy need greater social and psychological support.

Streltzer and colleagues[67] studied 25 patients who experienced graft failure and found that all but 1 patient made a good readjustment to long-term dialysis. Fourteen patients grieved the loss of their kidney openly, and 10 denied any psychological difficulties. In our experience, the return to dialysis is negotiated gradually and successfully by most recipients, and as the disappointment of the graft failure subsides, most quickly request the chance for another transplant.

PSYCHOLOGICAL ASPECTS OF LIVING DONATION

The first successful renal transplants performed were mostly from living related donors. The psychological reactions of donor and recipient were monitored closely in many psychiatric studies and are outlined in this section.

Early Psychological Findings in Living Related Transplantation (1960s to 1970s)

Many of the initial studies conducted in the 1960s and early 1970s questioned the fundamental willingness of relatives to make this type of sacrifice. Donor altruism—the supreme act of unselfishness and of giving freely without thought of reward—was much debated. Some researchers postulated that although donors were "consciously altruistic," there was considerable "unconscious resentment" toward the recipient and toward hospital personnel who requested or encouraged donation.[7] Other studies concluded that donors may be "victims of family blackmail" and donated because of family pressure or integral guilt. Such pressure could be subtle or direct with a fear of family rejection if the prospective donor decided not to donate. Investigators also reported that in some situations "the black sheep" of the family offered to donate in an attempt to win family approval and become reinstated within the family.[22,23,62]

There were reports of postsurgical depression for some donors with a suspected grief reaction linked to the loss of a body part and donor hostility expressed as anger that the recipient had been perceived to receive a greater amount of care and attention. Several studies also reported difficulties in the donor and recipient postsurgical relationship with the donors becoming overprotective and intrusive into the recipients' lifestyle and the recipients having difficulty with the obligation of the gift.[22,23,62] Although many of these early studies involved small numbers of donors and recipients, the negative psychiatric findings were much reported, and some observers suggested that cadaver organs were psychologically preferable because there could be no continuing obligation for the recipient. In contrast, several studies also reported that donors described the act as positive and as one of the most meaningful experiences of their lives.

Later Psychological Studies in Living Related Transplantation (Late 1970s, 1980s, and 1990s)

During the late 1970s and the 1980s, studies began to report more positive psychological findings. Simmons and colleagues[63] interviewed 230 living related donors and reported that "donors view themselves as more worthwhile because of the donation." In this study, only 5% of donors reported negative feelings about the transplant. Smith and coworkers[64] found that 97% of donors reaffirmed their decisions, and less than 15% said that they felt pressured to donate. With regard to recipient reactions, Simmons and colleagues[63] reported that "although recipients did feel guilt about the gift that they could not reciprocate, most recipients and donors reported that there were no major problems in their relationship 1 year post-transplantation."

Following the positive results of the published studies, in particular, the large Simmons study,[61] the late 1980s saw a change in the way that transplant centers viewed living donor kidney transplants. Although some centers continued a strong stance against living donor transplants mainly because of the physical risks to the donor, many other centers increased living donor transplant. A study by Levey and colleagues[36] noted that the physical risks to the donor were minimal, and that the benefits to the donor were considerable with regard to self-esteem and self-worth. Later studies reported that "to deny the donor the right to donate could do psychological harm." Surman[68] wrote that "kidney donation has a favorable outcome for both donor and recipient and the participation of living related donors in kidney transplantation is now widely accepted."

During the early 1990s, studies again reported psychological difficulties for donor and recipient. Russell and Jacob[56] postulated that "results indicate that while psychological side effects have been reported, including depression and family conflict, these risks are generally underemphasized.... health professionals should be aware that merely raising the issue of live organ donation may instigate powerful psychological processes beyond the potential donors' voluntary control and leave little room for refusal without psychological cost." A sibling donor in our own center expressed similar sentiments by saying that she wished that "The topic of live donation had never been thrown into the family circle as it caused enormous friction and sibling conflict which could only be solved by agreeing to donation."

Fox and Swazey[24] examined the concept of the recipients' obligation to repay the "gift of life" and postulated that "in the case of a live kidney transplant, the donor may exhibit a great deal of proprietary interest in the health, work and private life of the close relative who has received his or her organ, on the emotional grounds that, after all, it's my kidney ... that's me in there ...." The great indebtedness recipients may feel to the parent, sibling, or child whose lifesaving kidney they carry may make it difficult for them to maintain a reasonable amount of psychic difference and independence from the donor. These authors reported that it was common for a recipient who needs freedom from the donor but feels too beholden to him or her to negotiate it to take the drastic step of breaking the relationship completely. These authors stressed the need for careful donor selection and ongoing psychological support for donor and recipient as important aspects of care throughout the living donor and recipient experience.

More Recent Studies and Developments in Living Related Donation

The Scandinavian countries incorporated live donation into their transplantation programs in the 1960s, and the level of live donation has increased over the years. In Norway, living donors account for approximately 45% of the total donor pool. Such large numbers of live donors have enabled extensive research to occur.

Jakobsen[34] reported that nearly 500 living donors in Norway were asked: "If you could turn the clock back, would you do the same again?" Eighty-three percent said "definitely yes," and another 11% said "probably yes." Many donors were deeply grateful for having been given the opportunity to become a donor. A study from Stockholm[21] reported follow-up of 370 living kidney donors; this study concluded that less than 1% of donors regretted the donation, although several donors experienced the first few months after the donation as troublesome from a physical perspective.

Centers in the United States also have published results from studies of follow-up in large numbers of living donors. A study by Schover and colleagues[57] from the Cleveland Clinic examined 167 donors with regard to psychological aspects of the decision to donate, impact of donation on family relationships, donor reactions to graft failure, and overall satisfaction of donors. The study findings suggest that "the majority of donors make the decision to donate with little ambivalence, express comfort with the choice at long term follow up and do not experience negative consequences regarding health ... or family relationships." Jacobs and coworkers[33] published a report from the University of Minnesota with follow-up of 529 living donors who had donated in the period 1985 to 1996. Study conclusions were that "donors scored higher than the general population with regard to quality of life issues. The overall donor experience was stressful for 12%, with donors more likely to say experiences were stressful if they had postoperative complications. If given the opportunity, only 4% of the donors said that they would not donate again, and 9% were unsure."

More recent studies report that most donors enjoy a high quality of life, with a boost in self-esteem and an increased sense of well-being.[14,40,46] The advent of laparoscopic donor surgery has resulted in a shorter hospital stay, a quicker recovery time, and minimal scarring, and these benefits seem to be encouraging more live donors to consent to surgery. In-depth psychological studies suggest, however, that some donors continue to experience covert familial pressure, find it impossible to refuse even though they do no wish to proceed, experience some conflict between the family of birth and the family of marriage, encounter some difficulties in the postoperative relationship with the recipient, and have anxieties concerning their future health. Similarly, some recipients report difficulties in the postsurgery relationship with the donor and with reciprocity and feelings of obligation.[15,25] Research has shown that psychosocial risks are still apparent within the live donation process, that these risks should be recognized within transplant programs, and that professional care should be provided to ensure confidential presurgery donor and recipient advocacy combined with continuing psychosocial support for the family unit after donation.

LIVING UNRELATED DONORS

The successes achieved in living unrelated transplantation have been encouraging, and now most transplantation centers believe that emotionally related living donors represent a valuable option for kidney transplantation. Recipient and graft outcomes have been reported as superior to cadaver kidney transplantation.

A decrease in cadaver organ donation has been reported in recent years in the United Kingdom, Europe, and the United States, and as numbers of patients on the waiting lists have increased, it has become apparent that the full potential of renal transplantation will be realized only if other donor sources are developed. Many units have followed the example of Scandinavia and the United States and increased their living donor programs by using related, unrelated and nondirected donation.

PREEMPTIVE TRANSPLANTATION

Many transplant centers are now reporting the advantages of preemptive transplantation (transplantation before start of dialysis). Several studies have reported that preemptive transplantation can result in better rehabilitation and lower risk of loss of employment.[59,66] Transplantation without prior dialysis resulted in less physical and psychological impact for patients and their spouses.[59,66] Previous anxieties that there would be poorer patient adherence if the transplant is preemptive have not been supported by more recent research.[59,66] Most centers undertaking preemptive transplantation favor the use of living donors because of the shortage of cadaver donors. Given the beneficial effects of preemptive transplantation, the emphasis has fallen again on increasing the donor pool especially from within the live donation arena.

PSYCHOLOGICAL ISSUES AND IMPLICATIONS FOR PRACTICE FOR LIVING DONOR PROGRAMS

The psychological issues cited in this chapter and the results of our own psychological study have formed the basis for the structure of the Live Donor Programme in Oxford. This program offers early concise information to the donor and recipient and preoperative and postoperative psychological evaluation and support. It is hoped that this approach will help the donor and recipient with decision making, avoid adverse psychological outcomes, reduce psychological morbidity, and aid full donor and recipient emotional rehabilitation.

Informed Consent

The decisions confronting the potential donor and recipient generate significant stress because they are considering life-threatening, irreversible, and high-risk surgery. It is imperative that the donor and recipient are informed fully regarding the advantages and risks involved and can make the decision to give or receive freely without overt or covert coercion.

Donor Informed Consent: Anxieties and Fears

Several studies suggest that despite the seriousness of the decision to donate, only a few potential donors deliberated before agreeing to donor assessment. Most donors in these studies regarded their choice as instantaneous and made without conscious evaluation. Conversely, studies in Oxford and London reported together by Franklin and Crombie[15,25] concluded that the mothers in both studies acted altruistically and offered as soon as the possibility of a transplant was suggested. In contrast, some of the fathers in the studies expressed some ambivalence about donation and found the decision making complex. Sibling decision making also was complex and difficult for some subjects in both studies, and within this group, motivational factors involved altruism, manipulation of family dynamics, coercion, and covert pressure. In these studies, the siblings seemed to have the most difficulty with the decision to donate. These sibling responses support the findings of Russell and Jacob,[56] who postulated that "by merely presenting the option, the individual is immediately placed under an unwarranted moral burden, a no win situation." Such a situation is graphically described by a sibling who felt like a "fish on a hook."[56] These results show the need for strict donor confidentiality and for the donor to have a third-party advocate who is outside of the renal and transplant programs. The advocate can support the donor during the decision making process and can give the donor the confidence and support to refuse to donate if that is his or her wish.

Initial information must be detailed, and the initial approach to the donor must come at an early stage to ensure time to deliberate and to make an informed decision. In Norway, the initial approach to the donor often is made in a letter from the recipient's nephrologist. Ideally, recipients should not be asked to make the approach themselves because a refusal can be devastating, and donors may find it is impossible to refuse such a request from an obviously sick relative.

The Norwegian approach of writing to relatives has been rejected in our unit because it was thought that donors may feel unable to refuse a formal medical request. In this center, we believe that information about living kidney donation should be made widely available in predialysis and dialysis outpatient areas through written leaflets and newsletters. Detailed information is given at the predialysis and transplantation seminars for recipients and their families, and in most cases, the donors requested further information without the need for additional approaches.[31] The value of a formal recipient family education program with regard to living donor volunteer rates has been noted by Schweitzer and colleagues.[58]

When a donor expresses an interest in donation, a meeting is arranged with the nurse specialist or counselor to explore in more detail the risks and benefits of live donation. Donors are asked *not* to make a full decision until a further discussion has occurred. The meeting is arranged for the donor, plus donor partner if wished, to explore donation in confidence. We explore other issues as well, such as the preoperative donor-recipient relationship, individual anxieties and fears, and donor partner attitude toward donation. At this stage, the donor is informed that he or she may withdraw consent at any time.

After discussion regarding risks and benefits, we explore the perceived relationship between the donor and recipient. Siblings can be realistic about the relationship, but parents may be unrealistic, presenting an idealized view of their relationship, particularly with an adolescent child. In the Oxford

Study, all parents believed that they had a close relationship with their adolescent child recipient, whereas 30% of the adolescent group believed that the relationship was difficult to poor, with some adolescents suggesting problems of overprotection and inability to make independent decisions.[25] It is necessary to confront such issues before transplantation so that the parent is aware of any difficulties, then problems may not occur after transplantation.

Donor fears and anxieties reported preoperatively involve donor death, fear of rejection and length of life of the graft, fear that the donor kidney may prove unsuitable, and concerns for long-term health. Such issues can be explored throughout the donor preoperative course, and information and appropriate support can be offered.

At this time, we explore donor partner and family attitudes toward the donation. In some situations, the donor partner of a sibling may be unhappy with the donation and may believe that loyalty to the marriage should supersede loyalty to a birth relative.

Donors must be encouraged to make their own informed decisions, but if conflict ensues, appropriate support should be offered. In one case, in our center, a foster mother desperately wished to donate to her foster child, but her husband was adamantly against this decision. The outcome was that the wife withdrew the offer, but conflict within the marriage continued, and marital therapy was offered. In another case, an adult sister offered to donate to her brother, but the sister's husband objected saying "that he would divorce his wife if she went ahead with the donation." The sister decided to proceed, and after the surgery her husband left the marital home. The donor stated that she did not regret the decision to proceed, however.

Some donors may have specific dilemmas to resolve. A partner with a spouse and daughter with polycystic disease decided to donate to the daughter because the tissue match was superior. The spouse joined the cadaver waiting list. Another partner with a spouse and daughter with polycystic disease decided to donate to the spouse, who was unwell and unable to work, with the hope that an unaffected sibling would donate to the daughter at a later date. These and other dilemmas need to be discussed fully and decisions made with further information and psychological support.

Donors who are concerned by the risks involved may delay the decision making. In this center, we respect the need for a delay and resolve the issue by suggesting that the recipient may join the cadaver waiting list and the living donor be held in reserve for a later date. It is important that the donor, recipient, and family members understand that the donation evaluation process may be stopped at any stage, and that the reason for this cancellation would remain confidential between the donor and the medical team. Recipients must not be allowed to pressure or pester the donor, and psychological support must be available to the donor and the recipient. Without this strict understanding, it may be impossible for donors to make a truly honest decision, particularly if they wish to refuse to donate.

Recipient Informed Consent—Anxieties and Fears

Many recipients accept the offer of a transplant with alacrity, but some recipients may wish to refuse. An early meeting with the recipient (plus partner if wished) is arranged with the nurse specialist or counselor. The risks and benefits are discussed, and preoperative relationships, individual fears and anxieties, and partner attitudes are explored.

Recipients may find it hard to refuse such an offer fearing rejection by the donor, but with professional help, it is possible to refuse without conflict by using such reasons as "not wishing to inflict my disease on my family" or deciding to go on the cadaver waiting list with the donor held in reserve until a later date. As discussed earlier, adolescent recipients may find a parental donation difficult, fearing the need for "eternal gratitude" or "lack of independence and intrusion into lifestyle." One adolescent recipient in Oxford became very angry and complained that his father who had donated the kidney to him was continually on the phone telling him to "look after my kidney." It may be possible to resolve such issues with frank discussion facilitated by the nurse counselor, or it may be necessary to help the recipient refuse the donation.

Preoperative specific anxieties and fears reported by recipients in the Oxford study included risks to the donor, fear of rejection, and guilt about asking this of the family member or partner. Such issues can be explored throughout the recipient preoperative course, and appropriate information and support can be offered.

Recipients may find themselves in a particularly difficult situation if parents are divorced and both wish to donate. The decision as to who should be the donor may need to be made with professional advice and appropriate support given to the parents and the recipient. It is hoped that such a structured preoperative program, undertaken through a series of nurse-led living donor clinics, with medical support at designated stages, helps the donor and recipient to make the right decision for them based on full information so that they might proceed to surgery without adverse psychological stress.

The psychological care and information continue into the post-transplant and rehabilitation phases. In our experience, donors and recipients who have close relationships but retain firm boundaries within those relationships achieve the greatest rehabilitation outcomes. Martin and colleagues[41] reported similar results. We advise donors and recipients to celebrate the transplant together on the anniversary, but to continue independent lives at other times. This arrangement facilitates recipient ability to give thanks and donor ability to receive such thanks, but prevents overprotection or intrusion into lifestyle. Any difficulties encountered can be explored with the nurse specialist or counselor, and advice and help can be offered on a continuing basis.

It is rare for a living donor kidney graft to be damaged or to fail at the time of surgery or in the early postoperative phase. It also is rare for there to be donor complications, but if this happens, intensive donor and recipient psychological support must be available. We believe that our comprehensive donor, recipient, and family program helps to reduce psychological morbidity and helps to identify problems so that suitable support and advice may be given to prevent such problems escalating or occurring again in the future.

PSYCHOLOGICAL ASPECTS OF CADAVER ORGAN DONATION

Many potential transplant recipients are denied the chance of a lifesaving or life-enhancing graft because of a shortage of donor organs. Obstacles to cadaver organ donation are

many and varied; however, relative refusal rates remain high in the United Kingdom, some parts of Europe, and the United States. Studies show that some critical care staff still find raising the question of donation with relatives difficult. Often this is because of a fear that they may increase relative's distress and because they lack training in approaching bereaved families to request donation. Such a request may be a rare event in smaller critical care units.

This section outlines grief patterns and discusses aspects of communicating with relatives during the crisis time, informing of death, and requesting organ donation. Personal experience at Oxford with more than 300 donor families suggests that when relatives are approached sensitively, the subject of organ donation does not increase their distress, and organ donation brings comfort and hope through transplantation.

Grief Process

Grief generally is described as a psychological process by which people fill the gap in their lives after a large part of their world has been lost. Engel[18] described this process as grief work: "the work of mourning by which we can become emancipated from bondage to the deceased, readjust to the environment in which the deceased is missing, and begin to form new relationships." Lindermann first described the stages of bereavement in 1944. Other classic texts have supported and expanded this early theory. Most of these writers outlined three stages of grieving: (1) an immediate stage with shock, disbelief, and denial; (2) an intermediary stage with a growing awareness accompanied by anger, anxiety, and depression; and (3) a final stage of resolution, acceptance, and healing.

More recently, theorists have argued that the concept of bereavement in stages is too structured, and that such "classical texts may not entirely reflect how it is to suffer loss." Each individual responds to bereavement in a unique way, and the concept of stages may negate the individual pattern of coping. The grief process is neither universal nor predictable with no two families responding in the same way, and with individual family members reacting with different emotional responses. Generalizations and comparisons at best may be unhelpful and at worse may be damaging, particularly if clinicians try to fit individuals into a fixed model of grief. Phillips[50] stated that "grief is a profoundly idiosyncratic experience that gets over shaped and forced into moulds. There are as many ways of grieving as there are grievers. Putting people under pressure to do it properly is disabling."

Grief now is viewed as an individual experience that may contain common behavior patterns and reactions. The intensity of the reactions may be affected by other factors, such as the nature of the relationship between the patient and the bereaved, the age of the deceased, the type of death (expected or sudden), and the bereaved's responses to previous experiences and relationships. Research and clarification regarding the various individual and familial behavior patterns have been recorded, and it is possible to recognize patterns, and plan and implement appropriate support and care.

Common Behavior Patterns in the Early Phase of the Grief Process

Common behavior patterns in the early phase of the grief process include numbness, panic, shock, denial, inability to concentrate and make decisions, inability to absorb information and use it effectively, demanding and irrational behavior, aggressive and abusive behavior, withdrawal, and passivity. An understanding of these early patterns of behavior is important to clinicians because such behavior may occur soon after the death and at the time the bereaved are meeting with health professionals in the hospital environment.

The phase of stunned numbness is described by a bereaved relative in Speck's book as a "cotton wool time when there seems to be an invisible blanket between you and the world."[65] Others speak of being "frozen in disbelief" and like a "zombie." There is a safety in this numbness in that it denies the more frightening reactions of helplessness, utter despair, and intense fear. Denial can be interpreted as a psychological defense mechanism that prevents too much emotional pain at any one moment. Numbness, denial, shock, and disbelief are increased in cases of sudden and traumatic death in which there has been no preparation for the terrible news and no possibility of anticipatory grieving. Numbness, shock, and disbelief may last for hours, days, or weeks and may damage and impede the exchange of information and all forms of communication. Denial may play a role throughout the grief process, emerging and subsiding at different times. Extended denial lengthens the grief process and may result in the bereaved feeling the reality of the death at a time when others seem to have "forgotten."

Anger, Anxiety, Depression, and Isolation

The gradual awareness of the reality of the situation often is accompanied by anger and anxiety. Such anger may be directed toward God, the deceased, or members of the caring professions, or it may be internalized and used inwardly against the bereaved themselves. Internalized anger often is linked with feelings of guilt and is most apparent after sudden and traumatic death or the death of a child.

Yearning and searching for the deceased may occur and often is accompanied by feelings of emptiness and intense isolation. The loneliness may become extreme with thoughts of not being understood by family and friends. Such intense responses may engender a fear in the bereaved that he or she is going insane and may result in the bereaved becoming absorbed with his or her own feelings to the exclusion of partners and family, increasing feelings of alienation. Sadness, depression, and exhaustion may develop gradually and may continue for many months.

Healing Behaviors to Enable the Bereaved to Continue with Their Lives

Gradual readjustment and reintegration may occur as the intensity of the emotional pain lessens, and the bereaved may start to look forward and find some new purpose in living or new ways of behaving that enable them to continue with their lives. Phrases such as "letting go of the deceased" and "moving on" have been used in the past, but it is widely recognized now that many relatives may wish to find ways of sustaining the bond with the deceased and integrating this bond into future life.

High-Risk Groups—Intense Bereavement Reactions

Several researchers have outlined factors that may indicate a high risk of an intense bereavement reaction requiring additional or specific support as follows: unexpected loss (the deceased was young with no previous history of illness); suicide; sudden loss with no preparation for the death; lack of social support network with the bereaved feeling isolated; the death of a child (parental grief is more severe, complex, protracted, and traumatic than grief following any other bereavement); and a death where the relationship between the deceased and the bereaved is perceived as ambivalent. Research has shown that professional counseling can reduce morbidity significantly in the cases of an intense bereavement reaction. The effect of the counseling is to reduce the risk in high-risk individuals to that of low-risk individuals without counseling.

Needs of Relatives during the Crisis Time

In the 1970s, Molter[45] and Hampe[28] reported similar needs of relatives during the crisis time when the patient is critically ill. The five most important needs were reported as the following: to feel there is hope, to feel that the hospital staff cared about the patient, to know the prognosis, to have questions answered honestly, and to be near the patient. More recent research has supported these earlier findings. Riley and Coolican[54] reported that families need ready access to information; simple, short, repeated explanations to aid sense of participation and control; proximity to their loved one with time to be close and permission for frequent visitation; and sufficient time to accept the reality of the terminal nature of the injury or illness.

It may be difficult to meet all these needs; individual needs should be met as and when they arise. Staff members need to be flexible. Communicating with the family at regular intervals and giving them honest information help the family through the distressing phase when they alternate between hope for recovery and fear of death. Clinicians should focus on the needs of the family and view themselves as a companion, accompanying the family through all aspects of the situation.

Ready Access to Information

The family members need regular information meetings with clinicians, and they need to know the truth of the situation. Truth in itself is not damaging, but its presentation must be planned carefully. Frankness should be diluted with gentleness; relatives need the facts about the clinical condition and a realistic prognosis with its implications for them as a family.

Truth may not be the information that family members are hoping for, but it allows them to take control and to select options and make decisions. Staff members should strive at this time to develop a rapport with relatives so that trust is established, allowing them to inform, support, and offer choices. Clinicians need to listen to the family and hear the concerns that the situation has raised for them. Families should be included in discussions concerning care, and if the family wishes, children should be encouraged to be present and involved. Children and relatives who are excluded may imagine a situation worse than reality.

Proximity to Their Loved One

The family should be allowed to sit with the patient as soon as possible and should be encouraged to help with appropriate aspects of care. Staff members must be aware of cultural differences and religious beliefs; interpreters and religious advisers should be contacted to add comfort and assist in communication.

Support, Comfort, and Cultural and Religious Needs

Relatives in a crisis situation require continual support. A relative who is alone should be comforted by an empathetic caregiver until another family member, friend, or acceptable person can come and support the relative. While waiting, relatives should be kept as comfortable as possible, preferably in a suitably furnished private room near telephone and toilet facilities. They should be offered refreshments.

COMMUNICATING WITH FAMILY MEMBERS

Pelletier[47a] described the importance of providing the family with information that is repeated frequently and is understandable. As stated earlier Riley and Coolican,[54] explanations should be simple, short, and repeated to aid a sense of participation and control.

When communicating with family members, it is helpful to use two people: an informer and a supporter. The clinician often is the informer; the supporter often is a nurse, a religious adviser, or another member of the health care team. The roles of the informer and the supporter should be kept separate. The family members may blame or reject the informer; should this happen, the supporter can offer physical comfort, repeat information, and offer further support. The informer must not take such rejection personally. The family members are not rejecting the informer, but rather the information that he or she has given.

Before communicating with family members, it is important for the informer to prepare himself or herself physically and mentally. Evidence of trauma, such as blood stains, must be removed, as should barriers that impede communication (e.g., surgical masks). A father who was seen in Oxford for postbereavement counseling graphically described the surgeon standing above him, still in his surgical gown and boots, which had fresh blood on them, telling him that his son was fatally injured. He felt that he was in "an abattoir," and he stated that "he lost respect for the surgical team." Haddow[27] cited the case of one mother who was concerned about the attire of the consultant whom she had not previously met: "it was actually a doctor that was in theatre and he came to speak to me in his theatre clothes; his hat on and his mask around here, which I did not like."

The informer should become familiar with the family situation, noting the names of the principal relatives and their relationship with the patient. If there is a large group of relatives, it is helpful to speak directly to the immediate next of kin, using first names as appropriate. Meetings with the relatives should be planned so that there is time for discussion and should take place in a private relatives' room where the family members can express their thoughts and feelings freely.

Verbal and Nonverbal Cues

On meeting the family members, the informer and supporter should introduce themselves, shake hands with the relatives, and sit near to them. It is important to maintain a calm, unhurried approach and to offer relatives the time to ask questions. The informer and supporter should never hover in the doorway as if ready to make a hasty exit.

Nonverbal cues indicating the gravity of the situation should be used so that the relatives receive some preparation for the information. Facial expressions should be serious, as should the tone of voice. The informer should speak to relatives in nontechnical language and give information slowly, gradually sowing the seeds of the seriousness of the situation. The informer should make eye contact and speak softly, with spaces in between words and sentences. Care personnel should never try to overprotect family members from unpleasant reality. If there is a possibility of death, it is essential to inform the family members and help them to prepare. Staff members must be sensitive to relatives' needs and use physical comfort as appropriate, such as holding a hand or placing a comforting arm on the shoulder.

Relatives may try to minimize the seriousness of the situation by misinterpreting information or by hearing only certain parts of the message. Shock and disbelief can block communication and impede understanding; the informer should invite questions to find out what has been understood, then clarify and repeat the information. Distressed relatives can grasp at every word spoken, and it is important to avoid unguarded comments. Relatives may confront different staff members with the same questions about the patient's status hoping for a more positive message. It is necessary to maintain good communication among team members so that the same information is given by all.

Family members must be encouraged to express their thoughts and feelings. Staff members should not tell them how to feel (e.g., "do not upset yourself"). It helps at this time to encourage family members to talk about themselves and their families; insight gained into their world and their feelings can result in greater empathy and understanding from the caregivers. The supporter should arrange further meetings to give family members progress reports, while attempting to resolve any practical problems that arise for them.

Informing of Death

In most cases, relatives wish to be at the bedside at the time of death, and staff members should strive to fulfill this wish, offering them privacy. If it is not possible for the family members to be at the bedside, a member of the staff who has been in continual contact with the relatives during the crisis time should be the individual to inform them of death. The information should be given in a private area by an informer with a supporter present.

Research has suggested that the death of a patient may cause clinicians to experience ill-founded feelings of failure, anger, and guilt at not being able to save the life. It is essential that such feelings are recognized, discussed with colleagues, and resolved before the meeting with the family members. If these emotions persist, they may make the informer defensive and hinder empathetic communication. All staff members approach this task with trepidation at the thought of giving the message and with feelings of helplessness at the thought of trying to ameliorate the relatives' suffering. There are no correct words to use at this time, but it is important to give maximum preparation to the family members with a warning of bad news before the verbal message: "I am afraid that I have bad news for you"—pause, to give the relative the opportunity to say, "Do you mean that he/she is dead?" If this response is not forthcoming, the informer should proceed with, "We did all that we could to save your wife/husband (use the first name if possible)—pause "but I am afraid that he/she has died." The words has died or is dead should be used rather than other ambiguous phrases, such as passed on or left us, because these can be misconstrued.

After the verbal message has been given, the caregivers should anticipate and be prepared for a variety of different emotional reactions. Men and women often have different ways of expressing grief. Men tend to find relief in rage and anger early on and retire to brood alone; women often need to talk about the deceased. When everyone within a family circle is devastated, they are likely to find it particularly difficult to help one another.

Emotional Reactions

Anger

Anger is a frequent reaction to intense feeling and an expression of grief. To express such anger, the relative may shout and rush about the room or kick and punch the air, the wall, or the furniture. It is important that staff members do not do anything to increase this anger. Staff members should not attempt to restrain the relative and not become defensive and enter into an argument. The best response is to remain calm and to wait for the anger to subside. Staff members should show no criticism of this response and should offer support and care.

Hysteria

Regardless of how distressed the relative may be, the outburst ceases after a short time. It is best to remain quiet and calm and to sit and wait for the hysteria to abate. Staff members should not appear judgmental, shocked, or disapproving, but accept that this is an expression of grief. Physical contact and comfort should be offered as the hysteria subsides.

Withdrawal and Isolation

Isolation and withdrawal produce perhaps the strongest feelings of helplessness in caregivers. It is impossible to communicate adequately or to know how the bereaved relative is thinking and feeling. Bereaved fathers may find it particularly difficult to discuss or share their grief, but it is possible to offer a silent yet caring presence in this situation. Eventually, it may become acceptable to ask gentle questions to establish a rapport and elicit a response. It is more helpful to the bereaved to be drawn out and to express reactions, rather than to continue suppressing feelings. The earlier grief is expressed, the healthier the outcome.

Continuing Care

When the initial reaction has subsided, staff members should strive to answer the questions that the family members

may have and to offer them support in the tasks that lie ahead. Staff members never should try to console family members with platitudes or say, "I know how you feel." Grief and its pain are unique to each individual, and it is impossible to feel as another does in such a situation. The bereaved will never again have the opportunity to work through this most difficult time, and the staff member should give him or her the space and freedom to do so. Hodge[30a] stated, "The grief work must be done. There is no healthy escape from this—people have a natural protective tendency to avoid the unpleasantness of the grief work, but it is necessary and the more actively it is done, the shorter will be the period of grief." Simple expressions, such as "I am very sorry," bring the most comfort at this time, and if spoken with warmth and understanding, they impart more than eloquent words or false statements. The knowledge that the death was peaceful or pain-free, and that the deceased was not alone is a comfort to the family members.

Sudden or Traumatic Death

Sudden or traumatic death robs family members of preparatory grieving, and the shock, numbness, and disbelief are more intense in such situations. During the initial period, the bereaved often feel disoriented, powerless, and vulnerable. Breaking bad news in such circumstances requires empathy, clear communication, and support to help the relatives emerge from the acute state of shock.

Difficulties in communication may occur because the clinician and relative may be influenced by their own fears, thoughts, and feelings. The bereaved may misinterpret the message, may pretend not to hear, or may not understand owing to confusion and distress. The clinician may be anxious and unable to put thoughts and feelings into words, speaking too quickly and using language that is too technical. Effective and empathetic communication requires clear nonverbal clues (i.e., serious intonation of voice, serious facial expressions, and caring body posture) combined with simple information using terms that the bereaved can understand.

Following sudden loss, family members are likely to have many questions that need to be answered with honesty because this information can help them to make some sense of meaning from the death. Open-ended questions (e.g., "how can we help you?" and "what other information would you like?") help to develop rapport and trust, ease the conversation, and encourage relatives to seek the answers that they need. Acknowledging the family's feelings and emotions (e.g., "you must be very shocked") helps family members to discuss their feelings and influences the grief process in a positive way. The aim must be to support, inform, and offer choices because helping the bereaved to make decisions themselves also helps them to regain their coping skills. Active decision making stimulates a healthy grief process.

Many relatives benefit from a further meeting with the clinician at a later stage so that unanswered questions may be asked and discussed when the numbness and shock have passed. As mentioned earlier, psychological morbidity can be reduced with early counseling, particularly for relatives who have no supportive social networks or who are unable to support each other.

Brainstem Death

One of the most difficult deaths to understand and accept is the situation in which the patient has had a major brain insult and is subsequently found to be brainstem dead. In the case of brainstem death, it is especially important to consider the content and the timing of the information to be given to the family members. In this situation, the relatives have to understand and accept a new concept of death. Traditional acceptable images of death involve a lifeless body that is cold and asystolic. Brainstem death presents an image of life in a setting of high technology and hope where the victim is warm and has a heart beat and is breathing, albeit on a machine. The situation and setting suggest life and hope to the family, in sharp contrast to the message of death that is given to them by the clinician.

The same preparations and procedures for information giving should apply as mentioned earlier using a dual approach. The informer and supporter must understand and accept the brainstem death concept themselves, and they must use language that the family members can understand. Any hesitation or fudging of the explanation can confuse the relatives and may introduce hope that recovery is possible. The message to be given must stress that irreparable damage to the brain has occurred, and that there is no hope of recovery, that death of the brainstem is evident, and death of the brainstem is death of the person. The family members must be allowed time to assimilate and accept this information. The central facts may need to be repeated at several meetings before the relatives can understand the diagnosis and its implications.

Haddow,[27] who conducted a qualitative study with semi-structured interviews with donor and nondonor families, explored the respondents' understanding of brainstem death. She concluded that "most felt that the explanation given to them was sufficient, however for some, there was an inability to understand the terms. Another study[46] quoted a donor husband: "I was all mixed up, you see, and my head was spinning around." This man later described how he had come to understand: "The best way that one of the doctors said to me was like you've got a jigsaw [puzzle] and one piece of the jigsaw [puzzle] is missing and you take it away and all the rest of the pieces are trying to, but it doesn't work. It's like that with the brain."[46]

OPTION OF ORGAN DONATION

As stated earlier, it is helpful wherever possible to offer hope to the family members. If death has occurred, all hope of recovery for their loved one is lost, but the bereaved can be offered an option of hope and life for others through organ and tissue donation. Tissue donation (i.e., corneal, heart valves and skin) can be offered in most cases of asystolic death. Kidney donation can follow asystolic death in certain circumstances. Clinicians should consider the possibility of donation in every case of death and should seek specific advice from the local transplant coordinator service.

Multiple Organ Donation

Brainstem death can offer the family the option of multiple organ donation. Reports suggest that many clinicians are

reluctant to introduce the option of donation because they fear that such a suggestion may increase the grief of the bereaved. Research studies have shown, however, that families gain enormous comfort from the knowledge that their tragedy has resulted in life for others. A survey in New Zealand found that approximately 72% of individuals questioned had gained some comfort from knowing that others had benefited from their loss. Similar findings were reported in a United Kingdom survey, with 94% of families who had donated believing that they had made the right decision. A Dutch study supported the previous surveys and noted that some families who had refused donation regretted their decision at a later stage. Such research conclusions are supported further by the positive feedback from donor families that is reported by the transplant coordinator teams.

Organ donation can provide something positive in an otherwise negative situation. Offering the choice to donate, if performed with empathy, does not increase the distress of the bereaved. The bereaved should not be denied this choice or this chance of comfort. A letter from a donor mother reads: "It is certainly a source of comfort to me and indeed to all our family to know that our son has been able to touch and enrich the lives of others."

When to Offer the Option of Donation

Several studies have reported that the timing of the approach may be the crucial factor in the potential family's ability to give permission for organ donation.[17,26] These studies suggest that several factors influence the consent process. First, the longer the patient is in the hospital, the more time the family members have to appreciate the fact that the patient is critically ill and will not survive. It seems to follow that family members who have had more time to absorb and accept the prognosis are better able to move beyond the denial phase and become more receptive to options. Second, the timing of the approach for organ donation has significant consequences. Research suggests that if the request for donation is made after notification of death, as opposed to before or simultaneously with the notification of death, the family members are more likely to grant consent for donation, and this trend seems to hold true regardless of whoever makes the request. Ehrle and coworkers[17] stated that one must allow time for the family members to accept death before the approach for organ donation is made.

Who Should Approach Family Members

There is no one person who is ideal to approach the family members because of the enormous variety of individuals and situations. It is most appropriate for the person who has formed a close and trusting relationship with the family members to introduce the option of donation. It is essential that this person has a positive commitment to donation and introduces donation in a positive way.

A United Kingdom study[71] reported that clinicians working in the crisis areas thought that a lack of training and a lack of experience in offering the option of donation inhibited them in making the request. Conversely, a Canadian study showed that each experience of making the donation request built confidence. Every clinician who was experienced in talking to family members about organ donation felt positively about the experience and believed that requesting donation was easier than seeking permission for a postmortem examination.

It is helpful to remember that the family members are being asked to relate the wishes of their relative and whether objections to donation had been expressed, freeing the family members from accepting responsibility for the decision. Many family members may have discussed the idea of organ donation previously, perhaps at a time of national publicity. This knowledge of their loved one's wishes helps them with their response. It is reported widely that bereaved family members strive to fulfill the wishes of their relative at the time of death, and the presence of an organ donor card, registration on a donor registry, or a living will may help the family members toward a positive response. The bereaved may inquire about the possibility of donation before a formal approach is made.

How to Approach Family Members

Staff members often are reluctant to raise the question of donation because they fear that they may increase the family members' distress by saying the wrong thing. There are no right words, however; each situation is unique, and family members have their own individual responses. Requests for organ donation cannot be preplanned, although anxiety can be reduced for the person making the request if suitable phrases are considered before meeting with the family members. Examples follow:

Family member: How could this happen? What a terrible waste of a young life.
Response: This is a terrible time for you, but it need not be a complete waste; John's death could bring hope to others.
Family member: He was a lovely man; he didn't deserve to die.
Response: He sounds like a lovely man; do you think his generosity would extend to helping others through his death?

Family members respond to the option of donation in a variety of ways. Whatever the response, the caregiver should show empathy and understanding. Some family members require time to consider their response and should be offered privacy. Many relatives have additional questions concerning the process of donation and its implications. It is helpful to use open-ended questions, beginning with how, where, or what (i.e., "what further information would you like"), at this time. Such questions offer the bereaved the opportunity to make choices and to gain the information that is important to them.

Research suggests that at this time it may be helpful for the bereaved to meet with a member of the transplant team, usually the transplant coordinator, who can answer specific questions and start to develop a rapport with the bereaved. Family members require reassurance that their loved one will be treated with dignity and respect throughout the donor surgery, that the body will not be mutilated or grossly disfigured, that the surgical wound will be sutured, that they can view the body after surgery, and that the funeral will not be delayed. The transplant coordinator works closely with other health care professionals to answer such questions and

to facilitate the wishes of the family members. It often is comforting for the family members to know that the transplant coordinator will be present throughout the donor surgery and will perform the final care in accordance with their wishes.

There will always be family members, regardless of the manner in which the request is offered, who refuse the option of organ donation, and health care professionals must accept this decision. If the family members seem undecided or if the immediate response is an angry "no," it is acceptable, after a short period of reflection, to explore gently the reasons for such a response. It is found frequently that the family members may have specific concerns or unfounded ideas and fears that can be allayed by further information, removing the barriers to permission.

Research suggests that the most commonly quoted reasons for refusal include the following: the deceased had stated that he or she did not wish to donate, a fear of gross mutilation, a difference of opinion between family members, problems understanding brainstem death, and religious reasons. Regarding the last-mentioned reason, however, all the major religions support the act of donation.

If the family members agree to organ donation, many relatives may wish to spend time alone with their loved one so that they might say goodbye before the scheduled surgery. The opportunity to touch or kiss is especially appreciated. Family members should be offered privacy and should never be hurried.

Information after the donation is provided to the family members, unless they express otherwise. This feedback contains general anonymous information about the recipients and offers further contact and support. Some transplant coordinating teams offer postdonation home visits so that ongoing support is activated and any subsequent anxieties or concerns can be addressed. In some areas, donor family support groups are available.

Most centers facilitate the exchange of letters between recipients and donor families, believing that the bereaved gain comfort from the personal gratitude and well-being of the recipient, and that recipients need to express their thanks to adapt psychologically and to assimilate the new organ into their body and their new life. A few centers help to arrange meetings between the donor family and the recipient; however, such meetings are controversial (see discussion in earlier section).

STAFF SUPPORT

The care of individuals who grieve is an important part of clinical practice; however, dealing with the dying and their family members is stressful for staff, and if this stress is unresolved, the individual staff member may become depressed and burned out. A supportive environment can reduce this stress; such an environment requires that staff members care about each other, listening to each other's problems and offering support across all levels. Health care professionals have individual coping strategies, but also they should have the opportunity to discuss issues of death and dying together formally or informally as requested. Clinicians who do not have this opportunity to replenish their own emotional reserves may find that they do not have anything left to give to future patients and their families.

VIEWING THE BODY AFTER DEATH

All family members should be offered the opportunity to view the patient after death. If they are reluctant, they should be encouraged gently because it is an important step in accepting the reality of the situation. The body should be prepared carefully, and the bereaved should be given privacy and permission to touch, hold, and kiss as desired. The loss of a young child is particularly distressing, and parents may appreciate a lock of hair or a photograph or handprints.

FURTHER CARE

Before family members return home, it is important that they are aware of follow-up arrangements. In most cases, this follow-up involves an appointment with the bereavement officer, who offers help and information concerning the tasks that lie ahead. In some cases, it may be appropriate to arrange a further meeting with medical staff so that additional questions may be answered.

Advice concerning expected grief reactions may be helpful; relatives can be overwhelmed by the enormity and intensity of their distress. It is important that local support is available, and the clinician should alert the family physician or other support person to the needs of the bereaved. Some relatives may request medication, but in most cases the request should be denied gently because sedation dulls reality and response and inhibits the process of grief.

Most families recover from the death through the normal phases of grief. If a family member experiences specific problems, further help should be offered. Information about local bereavement organizations that can offer practical advice and experienced counseling should be made available.

Death and bereavement are an integral part of human life, and the care of individuals who grieve is an important part of clinical practice. All professionals approach the tasks of "breaking bad news" and "informing of death" with trepidation. With a knowledge of grief patterns and appropriate communication skills, it is possible to feel more comfortable with the situation and to offer empathetic and understanding care. Experience suggests that when relatives are approached sensitively, the subject of organ donation does not increase their distress. Many families gain comfort through donation and transplantation—something positive from a totally negative situation.

CONCLUSION

Kidney transplantation is the treatment of choice for most patients with end-stage renal disease. Life with the best-functioning transplanted kidney is a life with uncertainty, however. The fear and possibility of rejection are constant. Immunosuppressive therapy can lead to psychiatric and psychological morbidity, and necessary shifts in family dynamics and readjustment into society can cause emotional difficulties.

Publius Syrus (1st century B.C.) wrote that "pain of mind is worse than pain of body." Understanding of the psychological aspects of transplantation has grown in recent years, and this increased understanding has resulted in the opportunity to offer informed psychological support as an integral part of transplantation care, reducing psychological morbidity and enhancing rehabilitation and quality of life.

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Chapter 39

Ethics in Transplantation: Allotransplantation and Xenotransplantation

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In ethics, the terms used need definitions. To start, we consider the meaning of two words: ethics and morals. The use of these two words is not uniform. For some, ethics is the study of behavior between people in relationships in accordance with their cultural values, whereas morals takes into account some wider principles that govern personal behavior, independently of others but often in relation to transcendental principles or beliefs or concept of deity. In this chapter, we use the two words morals and ethics synonymously. This claim is based on the origins of both words—one from ancient Greek (*ethos*) and the other from classical Latin (*mores*)—both meaning the accepted customs and values to which societies and cultures aspire.

As transplantation becomes increasingly globalized, it is important to consider whether the values that are brought to bear on transplant issues are determined by local cultures or are universal (held by all world cultures). There is a lack of uniformity. We claim that all cultures share some values (e.g., it is wrong to abuse children, it is wrong to torture the innocent, and life is of utmost value to each individual). It also is true, however, that some values are held in a different way in different cultures (e.g., individual autonomy versus interests of family, clan, or tribe; the varying intrinsic value of individual lives to the society or culture, as distinct from value to self and the varying respect for individual persons, their personal dignity, and equality before the law). At this time, only some values are held universally, and there is as yet no universal ethical system. These differences are important to intercultural transplantation debates.

DEFINITIONS

Altruism: Actions that are motivated by concern for the well-being of others, sometimes against personal preferences and self-interest.

Consequentialism: See Utilitarianism, including teleology.

Deontology: Also called duty ethics from *deon* (Greek), a binding duty. This theory stresses the intrinsic value of all individual persons, the duty of individual dignity and respect, the value of self-determination, and the cardinal importance of patient autonomy. In secular philosophy, this theory draws heavily on the writings of Kant (1724-1804), and its essence is captured by the claim that individuals should always be treated as ends in themselves and not as means to other persons' ends.

Resource allocation: It is useful to distinguish between three levels: (1) Microallocation refers to the one-on-one encounter between patient and caregiver and is dominated usually by duty-based or deontological ethics. (2) Mesoallocation refers to allocations by program directors, taking into account the needs of programs and individuals. (3) Macroallocation refers to allocation at the levels of government, taking into account wide-ranging social policies. Mesoallocation and macroallocation tend to reflect utilitarian or consequentialist ethics. (A fourth allocative level—mega-allocation—may be used in reference to policies involving international relations and allocations.)

Risk/Benefit: To the deontologist, this ratio (or calculus) refers to the risk taken and the benefit achieved by a given individual in a given situation. It should be distinguished from the concept of risk to the risk taker balanced against the benefit to another, others or society as a whole, although that calculus may have to be made in some situations using a utilitarian approach. A similar conceptual differentiation applies to burden/benefit analysis.

Utilitarianism: The other well-known tradition in ethics. It contrasts with deontology. This is an outcomes-based or consequentialist theory, based on the ethical objective of maximizing utility, or achieving the greatest good for the greatest number. It may use statistical probabilities applied to groups of individuals. The term *teleology* also is used for outcome-based ethics (*telos* [Greek] = end, or goal).

Xenotransplantation: In the human setting, the use of live cells, tissues, or organs from a nonhuman animal source transplanted or implanted into a human or used for ex vivo contact with human body fluids, cells, tissues, or organs that subsequently are given to a human recipient. Xenografts include live cells, tissues, or organs from a nonhuman animal source used for xenotransplantation.

Xenozoonosis: Infection resulting from xenotransplantation, especially of viable perfused organs, in which the risk of generating new viruses exists (e.g., retroviruses). New forms of bacterial and fungus infection may result from mutations.

ETHICAL PRINCIPLES IN TRANSPLANTATION

In many issues in health care, there is apparent conflict between the two principal ethical theories[8]—deontology and utilitarianism. Neither theory can be exclusively applied; both serve to bring relevant ethical perspectives into debate of difficult issues. In transplantation, because of the severely limited resource of available transplantable organs, transplant teams, while being aware of their deontological obligations to each patient, are forced to draw more on utilitarian considerations in making allocative decisions. Considerable ethical tension is created by this mesolevel obligation to utility (greatest good for the greatest number) because of the tendency for it to override duty owed to each individual as a unique person, at the microallocative level.

Justice comes into play insofar as we try to treat like cases alike (the principle of equity). In organ allocation, the principle of distributive justice also is at play, wherein the sickest (who have the most to gain, i.e., by a lifesaving procedure) are prioritized according to established criteria.

In the final analysis, properly informed and obtained public opinion is the arbiter of practice, and physicians are obliged to explain to the public what they do and to obtain its assent. In this process, the various public media also play an important role in informing and obtaining public opinion.

ORGANS FROM DECEASED DONORS

Ethics Issues in the Determination of Death

Medical, ethical, religious, legal, and political issues influence notions and criteria of death. Different societies accept more easily some definitions of death than others. In Japan, most transplants are from non–heart-beating donors, although the country introduced a law in 1997 enabling organs to be removed from brain-dead donors under strict conditions.[113]

Brain Death by Neurological Criteria

Since the 1970s, there has been a general acceptance that the criteria for death from cerebral causes are valid (see Chapter 6). The process was initiated by a Harvard Medical School consensus in 1968,[9] and there is near-universal acceptance that a person is dead when there is irreversible loss of function of the entire brain, including the brainstem.[109] This definition recognizes that a body may be dead even though the heart is beating and the circulation is maintained with a blood pressure that is adequate for organ perfusion. This definition means that the animate and the vegetative parts of the brain must be irreversibly nonfunctional.[93] This concept can be difficult for families to understand and accept, especially when their recently brain-damaged loved one is warm to touch and has an evident heartbeat and other functions. It is a measure of public trust in the medical profession, in which the media has played an important part, that families can accept the diagnosis of brain death, despite these contextual and conceptual difficulties.

Despite widespread agreement, there are authors who dissent, pointing out that a rigorous definition of loss of all brain and brainstem function implies loss of vasomotor tone, temperature control, and diabetes insipidus. This dissension may be more a legal problem than a medical one, but it is a problem nonetheless.[52,105,111,112]

Death of the Cerebral Cortex Alone

Frequently, individuals experience brain damage that is insufficient to destroy brainstem function, although all cerebral cortical function is lost. By currently accepted legal definitions for brain death, these individuals are not dead. They differ markedly from brain-dead individuals in that they may breathe spontaneously; have a gag reflex, and may undergo apparent sleep-wake brain cycles with opening and closing of the eyes but without seeing, and are unable to exhibit meaningful relations with the outside world. This state, when present for more than 6 months, is termed persistent incognitive vegetative state. Some experts believe that such entities are no longer to be thought of as functioning organisms because they no longer possess "coordinated integration of two types of function: organic and mental. If these two are irretrievably disjoined, then human life no longer exists."[111] For this opinion to prevail, we need to move from a whole-brain–oriented definition of brain death to a higher brain–oriented definition. This definition may come about in the future if the diagnosis of irretrievable loss of all higher brain functions becomes more precise and certain. Presently, most people consider patients in a persistent incognitive vegetative state to be alive.

Although there may be ethically defensible circumstances in which life-supporting systems may be discontinued, this is a separate issue from claiming that patients in a persistent incognitive vegetative state are already dead. Patients in a persistent vegetative state are not deceased donors.

Anencephalic Infants as a Source of Organs

Anencephalic infants resemble patients in the persistent incognitive vegetative state in that they have no higher brain or neocortical function. Some experts hold that anencephalic infants "do not have the minimal biological substrate as the basis for sentience, a necessary condition for being alive as a person" and might be used as donors if law and public policy were framed to recognize that.[17] Others disagree, however, holding that the legally recognized brain death criteria are also the only valid moral criteria.[67,115] Experience is limited. We do not yet have societal

understanding and agreement concerning the moral status of anencephalic infants.[89]

Donation after Cardiac Death (Non–Heart-Beating Donors)

Attention has been drawn, in Europe[37] and in North America,[61,120] to obtaining organs from the original source of transplant organs, before the establishment of brain-dead criteria—bodies after death from cessation of heart beat (>90% of individuals who die in hospitals). In some places, non–heart-beating donors now account for 10% to 40% of all donations.[16] (Preemptively excluded are individuals dying with disseminated cancer or infection.) Long-term results for kidney transplants from this source are comparable to those from brain-dead sources.[77]

According to the Maastricht classification,[60] there are five main categories of non–heart-beating donors. Categories 1 and 2 are termed uncontrolled, referring to donors who die suddenly and unexpectedly. Categories 3 and 4 refer to controlled situations, where death of the donor is expected, usually after the withdrawal of life-sustaining measures.

1. *Dead on arrival*: Individuals who are dead on arrival at emergency departments (e.g., from severe head trauma), some of whom provide viable organs.
2. *Unsuccessful resuscitation*: Individuals who experience cardiac arrest outside the hospital where cardiopulmonary resuscitation is initiated by the ambulance crew. The patient is brought into the hospital, and resuscitation efforts are continued by the hospital team. If unsuccessful, the team initiates the non–heart-beating donor procedure.
3. *Awaiting cardiac arrest*: Individuals dying in intensive care units where a prior decision was made with the patient and with the family that extended life measures, such as life support of various types (e.g., stomach tubes, tracheal tubes, assisted artificial ventilation), would be withdrawn, and that death would be allowed to happen in a natural fashion.
4. *Cardiac arrest while brain dead*: Patients who have been declared brain dead or are in the process of being diagnosed as brain dead in the hospital and experience cardiac arrest.
5. *Cardiac arrest in hospital inpatient*: New category added in 2003.

The debate on non–heart-beating donors has highlighted the difficulty of finding a specific moment to declare death. It may be more appropriate to think of death as a process rather than a finite event. Further debate has focused on the appropriate length of time to elapse after asystole before declaring the death of the potential donor. Different protocols call for durations ranging from 2 to 10 minutes.[25]

Respect for the Dead Body

The act of procuring organs presents particular challenges for health care professionals who are otherwise engaged in the care of living patients (organ recipients). Health care professionals may need help to deal with the emotional challenges surrounding procurement. The normally deeply felt human value of respecting the dead may become eroded in such difficult situations. Nurses feel moral distress about instituting therapies that are for the benefit of another person (the recipient).[86,101] In this situation, the patient's prior consent to donation outweighs the harm associated with organ procurement.

New Duties Owed by Health Care Professionals

Duty Owed by Health Care Professionals' Duty to Provide Organs

Now that organ transplantation is established as a medical treatment for heart, liver, and kidney failure, patients who are selected for transplantation waiting lists have established an expectation to be provided with the organ they need. This expectation places a moral obligation on physicians, nurses, and health care administrators to provide as many organs as possible, although this obligation does not yet seem to be accepted proactively into the codes of professional ethics. Individuals who support transplantation also have an obligation to support measures—a duty shared with the public at large—to encourage everyone to make their wishes known, in advance, with respect to organ donation. These wishes may be recorded in documents such as health cards, advance directives, or living wills. Some jurisdictions use presumed consent, whereas others do not (see later). The important issue is that families are aware of a potential donor's wishes regarding organ donation.

Duty Owed to Declared, Intended Donors and Their Family Members

Individuals who agree to leave their bodies to be used for transplantation or their family members who permit it create responsibilities for health care professionals. These responsibilities include making optimal use of organs procured and distributing them according to just principles of allocation, as outlined subsequently. Society does not extend to donors the right to say to whom the organs should go, unless there are close relatives in need. This limitation of their entitlement recognizes the wider societal principle of not permitting discrimination on the basis of sex, ethnicity, race, or age.

Duty Owed to Donors and Their Families to Preserve Their Option to Donate or Not to Donate

It is recognized that individuals or families have a right to give their organs should death come unexpectedly. The possibility of preserving the option for families to donate is inherent in newly suggested protocols for individuals who die suddenly and unexpectedly—non–heart-beating donors.[2,53] This also is known as donation after cardiac death. It may be acceptable ethically to subject the body of someone who has died recently and unexpectedly to preconditioning agents and techniques (e.g., vascular cannulation for cold perfusion) to preserve for the family members the option to donate organs for transplantation,[68] even though this involves touching the dead body without prior consent from a family member.

Duties Owed by Health Care Administrators and Government Officials to Patients Awaiting Transplantation

Public education by means of publicity programs promoted by government or transplant-related agencies is one measure

for obtaining organs from deceased donors. This measure promotes public altruism. Several studies indicated that despite a high percentage of the public being in favor of using organs from deceased donors for transplantation, low organ availability rates were caused partly by poor collaboration by health care professionals who are *not* involved in transplantation. Required request, required consideration, and required notification policies have been introduced widely, especially in North America, to improve collaboration, although initial improvements in obtaining organs have not always been maintained. Other measures to facilitate the process are organ removal permission statements on driver's licenses, tax returns, or other repeatedly used public documents. These measures also require support by public education for optimal participation.

There is debate on the use of systems of organ procurement referred to as opting-in (consent not assumed but sought at time of death) and opting-out, or presumed consent (consent mandated by law whereby procurement occurs based on an assumed consent, unless the individual has registered that consent is denied). Belgium and Spain are leaders among the countries that successfully practice presumed consent; the United Kingdom, Canada, and the United States have opt-in systems. In Europe, with the support of the Ministers of the Council of Europe, more attention than elsewhere has been turned to convincing the public that organs should be used without permission of next of kin or prior designation by the deceased. Presumed consent legislation permits those who do not accept this assumption to opt out of the scheme by placing their names in a registry, which must be consulted before taking organs.

Evidence suggests that opt-out systems are effective in increasing organ procurement, especially in Austria and Belgium.[58] Since enacting presumed consent legislation in 1986, no more than 2% of the population of Belgium has registered an objection to having organs donated.[51] In France, Spain, and other European countries with presumed consent legislation, physicians often require family permission even when not required by law. It is possible that such legislation is more acceptable in societies that are more homogeneous, although Singapore may be an exception. Since changing to a system of presumed consent, Singapore's rates of donation from deceased donors have increased significantly.[57] In a study of 13 Asian countries, Singapore had the highest rate of kidney donors at 21.4/1 million population.[110]

Spain achieved a 2004 procurement rate of 34.6/1 million population[91] by means of a centralized, coordinated in-hospital system, with individuals specially trained in detecting prospective donors and approaching families to obtain permission.[65] The 2004 rate is consistent with Spain's trend of continued increase in annual procurement. The 2002 donation rate was 33.7/1 million population—a number that far exceeded rates in other parts of Europe, which range from 10.4 to 24.3/1 million population donors.[21] The Spanish success may be partly due to the built-in financial incentives given to the hospitals, physicians, and coordinators involved in organ procurement.[62] Another contributing factor may be that many of the coordinators are themselves hospital intensive care specialists, nephrologists, or anesthesiologists,[18,65] although they do not coordinate for the donors who had been their own patients before death. To some individuals, these issues raise the question of conflict of interest. For these reasons, the model may not be adopted easily by other countries that lack the same level of social cohesiveness and trust.

Other factors may be influencing the Spanish donation rate. Spain accepts a high number of organs from marginal donors. Donors older than 60 years old make up more than 30% of the total donor pool, whereas donors older than 60 years make up 13.3% of the total donor pool in the United States.[18] Part of the Spanish model's success can be attributed to its strategy related to mass media; this includes a 24-hour transplantation hotline where media can obtain information from trained professionals, periodic meetings between journalists and leaders in transplantation, and training in communication for regional and hospital coordinators who deal with controversial issues.[66]

Incentives for Donors and Donor Families

Another controversial area assumes that organ procurement might be increased if incentives were offered to families of individuals whose organs might be procured after death. Suggested incentives fall into two classes: (1) proposals that anticipate death and prepare advance incentives to donate after death and (2) proposals that apply without prior planning to recently bereaved families. The former include creating a futures market,[19] or creating a priority system, such as LifeSharers. Members of LifeSharers agree to give their organs on death to individuals who also agreed to eventual postmortem organ donation. If the organ cannot be matched to a fellow member, it is made available to a nonmember.[107] LifeSharers encourages people to join while healthy by imposing a 180-day waiting period before a new member can be allocated an organ.[11]

The second category includes "ethical incentives," such as reimbursement of funeral expenses,[38] providing postmortem educational grants for bereaved children, or providing other insurance policies that become active only after donation from a deceased donor.[71] This category could include such public acknowledgment of societal indebtedness as the planting of a tree in a park or awarding donor families a medal.[80] All of these incentives have been framed as programs of rewarded gifting.[28] Much more controversial (see later) is the use of cash payments as direct incentives for organ donation. Individuals who oppose all these suggestions believe that they may lead to a lessening of the spirit of altruism in society and a descent into commercialization of organs and usage of the body and lessened societal value in the uniqueness and dignity of the human body. There is widespread repugnance over commercialism in organs from the deceased through sale or purchase, although few oppose compensation for any additional expenses incurred by the family as a result of organ procurement. Efforts to thwart the buying and selling of organs from living donors have been ineffective in many countries, and the practice is increasing.[24]

Duties Owed by Organ Recipients

Poorly defined as yet, the costs and sacrifices involved in providing organs create a moral obligation on the individuals who receive them. In the context of scarcity of organs, how far should issues such as poor adherence to treatment be used in the selection of candidates for transplants?[119] If a recipient needs retransplantation, should his or her failure to comply with antirejection medication or other requirements

preclude their being awarded another organ? Obligations of this type have been formulated poorly for society, but many see it as part of the barely articulated contract that exists between members of society and health care providers when interacting with each other within a publicly funded system.

Issues of Ownership and Authority

Issues in transplantation that seldom are addressed include the following questions: Who owns the organ after it has been procured, before it has been implanted into someone? Who has the authority to establish the rules by which the organs are distributed? What rights do family members have in saying what they want done with their relative's body?

Who Owns the Excised Organ?

The law has not determined who owns a dead body or the organs excised from it. In the Middle Ages in Europe, matters relating to dead bodies were delegated to the ecclesiastical courts (now obsolete) by the civil courts. Inherent in the concept that there is no property value in a dead body, an individual who steals an excised organ from an operating room in one hospital to take and implant it at another hospital could be charged only with trespass. It would be a theft only if that individual had stolen the container for transport purposes. Some experts advocate an end to this extraordinary anomaly[2] when such great value is placed on organs by would-be recipients and the professionals obligated to find them. Apportioning property value and ownership rights to organs from the dead is seen as a big step toward unwanted commercialization, however, which might not be prevented by concomitant legal steps to prohibit market transactions of organs. In the case of *Moore v. Regents of University of California*, a spleen donor initially was refused property rights by the California Supreme Court, but the case was subsequently settled initially by sharing in the profits from the cell line grown from the excised diseased spleen.[39]

Who Should Decide on Allocation from Deceased Donors?

The question of ownership relates to the questions of allocation. At present, although there may be no legislation to support it, it generally is *assumed* that ownership of organs resides in the state, which is *assumed* to have delegated its authority to the institution, and then to the transplantation service. It is widely assumed that the disposition of transplantable organs is not at the whim of the transplantation team simply by virtue of their skill in being able satisfactorily to remove and then implant them.

Principles Used in Organ Allocation in Transplantation

Many principles are used in the just distribution of access opportunities to scarce resources; this includes how deceased donor organs are shared, and how transplant waiting lists are managed.

Ethical Commitment to the Principle of Rescue

Despite possible injustice, we all recognize rescue as an ethical imperative to which we should respond. Sometimes rescue impels action when it is unlikely to provide the optimal outcome. It also brings out the tension created when the consequentialist principle of the greatest good for the greatest number conflicts with the deontological commitment to the quality and dignity of each human life together with the principle of justice that recognizes claims in proportion to need. The seeming imperative to carry out a subsequent organ transplant when the first has failed may present the ethical conflict between rescue and utility.[106] Veatch[113] also recognized that efficiency and equity may conflict in the allocation of organs. Rescue should not be applied to situations that fail to meet the minimal standard of utility, referred to subsequently.

Optimizing the Medical Outcome (Utility Principle)

In transplantation, particularly when setting public policy, actions usually are governed by applying the principle of greatest utility. As decision making moves from the microlevel to the mesolevel or macrolevel, the utilitarian consequentialist ethic increasingly dominates over the deontological ethic. This change explains why ethical conflict seems greater for physicians than administrators because the latter do not have personal relationships with individual patients and hold responsibilities only in the field of public policy. Monaco[70] emphasized that programs should have a minimal threshold for medical utility and make decisions above that threshold. Veatch[113] suggested that the utilitarian's goal should be to allocate the organ to the individual who is likely to gain the greatest number of quality-adjusted life-years from the organ. When all potential recipients meet the minimal threshold of utility, other ethical factors may be used for organ allocative decisions in addition to optimizing medical outcome.

Fiduciary Principle

The fiduciary principle recognizes physicians' duty to care for each patient. Tension often is created between the deontological duty imposed by this principle and some of the other legitimate principles, especially for professionals who may have responsibilities at the microallocative and the mesoallocative levels.

Random Choice (Lottery Principle and Use of First Come, First Served) and Random Factors

The two principles of random choice and random factors have much in common in that the allocative factors are value neutral. Both principles acknowledge that there are factors such as chance, or good or bad luck, that are legitimate in decision making for organ allocation because they affect all people in society in a more or less random, yet equal way. Patients find this randomness acceptable in systems based on an egalitarian principle. In contrast, physicians and transplantation coordinators may be reluctant to place any weight on random choice and random factors because it seems to deny their professional expertise in wielding medical science knowledge. Nevertheless, there are occasions when these principles would be just. Length of time on the waiting list and distance from home to center may be ethically legitimate factors in allocation provided that time of entry to the list is achieved at a comparable time point for each potential recipient, and that distance interferes with ability to accept some opportunities for receiving a graft. In different programs, other value-neutral circumstances may be accepted as weighting factors.

Ability to Pay

Ability to pay has operated largely in health care in previous centuries in all Western countries. Inevitably, it is the dominant principle in most, but not all, developing countries, where transplantation is available mainly for the rich. In a capitalist society based on libertarian principles, such as the United States, ability to pay as a dominant principle would not be unjust provided that a commonly accepted standard of basic care were available to all. Renal dialysis and kidney transplantation in the United States is covered by an egalitarian Act of Congress, which does not extend to other organ transplants. Ability to pay is excluded as a factor in allocation in transplantation in most developed countries, where there is a social commitment to support health care on egalitarian principles.

Social Worth

In an egalitarian system, estimates of social worth are ethically inappropriate and may not be used in estimating good outcomes. One often finds social worth parameters, such as lack of adherence to treatment, lack of family support, undesirable personal habits, or inability to speak the dominant language, masquerading as factors for optimizing medical outcomes, however. In our opinion, these parameters should be recognized for what they are and resisted. These factors may identify areas where patients need support and opportunities for assistance.

Lobbying and Using the Media

Another factor that may be unjust but is difficult to resist is the influence of individuals who advance their cause by obtaining greater publicity of their need through the media or a lobbying process. In a libertarian atmosphere of the marketplace, this activity might be termed a competitive edge. With use of the Internet a part of our daily lives, we need to develop strategies to address this in organ donation.[118] One advantage it offers to recipients is it redresses the imbalance caused by nature of the availability of living donors.

Using the Needs of the Program in Allocation

When a program is starting up, it can be ethical to select patients so that initial results are good enough to ensure continued funding. This selection approach should operate only for a limited time and is ethical only if it is publicized as public policy so that potential recipients and their advisors all know of the policy and its limited duration.

KIDNEYS FROM LIVING DONORS

Benefit/Burden Calculus for Living Donors

There always has been an ethical issue in living donors stemming from the injunction *primum nihil nocere*—above all do no harm.[95] Can it be claimed that removing a sibling or parent's kidney is not doing harm? It usually is argued that the good (benefit) that comes to the donor as a result of restoring his or her family member to well-being and renewed life justifies the possible burden borne by the donor. The donor is acting altruistically (acting for the good of another, without primary regard to self-interests) but has this good result as an added compensation.

Living donor kidney transplantation is not without its risks. Donors face a perioperative mortality rate of 0.03%.[76] A study following up with donors who had given kidneys between 1963 and December 1979 (20 to 37 years after transplantation) revealed a few donors develop renal dysfunction or renal failure at some point.[83] It is unclear if this risk is more than in people who have not donated, and there are studies that have shown a survival benefit in healthy individuals who have donated one kidney.[45]

International consensus statements recommend standards regarding the care of living organ donors. These practice guidelines emphasize the elements of informed consent: capacity, disclosure, understanding, and voluntariness.[5,43] In some places, only an emancipated minor (a minor who has undergone a legal process to attain legal adulthood before reaching the age at which they would usually be considered adults) or an adult can make the assessment meaningfully and give informed consent. Minors are rarely used as living kidney donors, but in such instances many jurisdictions insist that only a family court judge or equivalent can sanction the donation.

It is not deemed ethical to balance the possible harms to the donor against the benefit to the recipient; this is considered to be an unethical way of calculating burden versus benefit. Calculated in that way, the ratio could be used to justify the use of mentally incompetent relatives and the reluctant but competent relative. It is necessary that overall donor benefit is present.[100] One must consider the burden/benefit ratio to the donor against the burden/benefit ratio to the recipient. Included in calculating benefit for the donor is the knowledge that his or her kidney would give a better result than is obtainable from a deceased donor kidney[10,87] and relieving the burden of continued dialysis and (in children) further risk of stunted growth.

Increased demand for kidneys continues to outstrip supply.[44,108] The shortage of organs from deceased donors has led to continued use of living donors and a widening of the donor pool. Living donors now include extended family members, friends, acquaintances, and even strangers.[64] This expansion of the living donor pool has raised further debate on whether the emotional connection between donor and recipient should influence the degree of risk that the living donor undertakes.[88] Research indicates that transplantation is the best treatment for most patients with end-stage kidney disease,[94] and generally the longer a patient is on dialysis, the poorer the outcome after transplantation.[48]

Commerce in Human Kidneys, Especially from Living Strangers

One very controversial area in organ transplantation is the ethical probity of exchanging viable kidneys for money or other forms of payment. Before considering that aspect, there are several less challenging issues, which involve some form of altruism. The key factor seems to be donor (vendor) motivation.

These issues may be analyzed by considering the motivation of donors or vendors of their own kidneys. Other stakeholders in these transactions are recipients of commercially obtained kidneys, entrepreneurs who arrange for kidney transactions, physicians who perform the surgeries, and, most importantly, spokespersons for society as a whole. These individuals all have ethical dilemmas but of lesser dimensions than the vendors.

Spousal Altruism

Earlier reluctance to accept spouses as altruistic kidney donors largely has evaporated. The reluctance was due to spouses having no more probability of being well matched for HLA than any randomly tested individual or deceased donor source, and these grafts were expected to have a poorer survival than an HLA-matched deceased donor kidney. Wives, as recipients of their husband's kidney, might have degrees of prior sensitization against HLA and other systems because of exposure to the husband's antigens on fetal cells during pregnancy, which might not be detected. In some social settings, wives might be seen as prone to coercion by husbands. With improved immunosuppression, however, poorly matched combinations now give much improved outcomes (see Chapters 10 and 37); also, subtle HLA sensitization is detected more easily, and its potentially deleterious effect is overcome more easily. At present, spousal donors are acceptable ethically when the relationship is stable, and coercive obligations are excluded.

Purely Altruistic Motivation

Friendship and acquaintance are accepted more and more by transplant centers as an altruistic basis for a nonrelated living kidney donation. In our experience, kidney donation to a one-time college roommate was described by a 60-year-old woman, 6 years after giving her kidney, as follows: "I look upon giving one of my kidneys to my friend as being the most satisfying single act of my life."

Although altruism sometimes is expressed toward unknown others—as when individuals agree to participate in research that brings them little or no direct benefit—organ donation on this basis occurs most frequently by means of a postmortem donor card. Kidney donation by anonymous living donors is now being performed in some centers.[64] A well-documented example is that of a German professor of transplantation surgery who donated one of his kidneys to a patient (unknown to him) on the Munich waiting list.[35] Kevorkian[59] claimed that most criminals about to die by capital punishment wish to give their organs, but this request has not been taken up by any state legislature in the United States. This claim is used as the basis for transplantation in China with kidneys from executed prisoners. China has been widely criticized for this practice.[40]

Altruism with Compensation

The ethical debate over "rewarded gifting" has not produced clear consensus.[28] Compensation may be divided into financial profit for organ donation, which is illegal in most countries of the world, and compensation for financial costs associated with organ donation. The latter may be seen as an issue of justice (i.e., that is it is unfair for an organ donor to be financially penalized for incidental expenses incurred in organ donation). Compensation of these costs (e.g., loss of income, costs of transportation and accommodation) is increasingly considered reasonable. Compensation that constitutes financial profit resembles a contract for commercial sale and is considered by most experts to be flawed ethically.

There is ongoing debate about payments related to organ transplantation, mainly with respect to living kidney donors. At a conference in Munich in 2002, the following resolution was passed related to this issue: "The well-established position of transplantation societies against commerce in organs has not been effective in stopping the rapid growth of such transplants around the world. Individual countries will need to study alternative, locally relevant models, considered ethical in their societies, which would increase the number of transplants, protect and respect the donor, and reduce the likelihood of rampant, unregulated commerce."[24]

Kidney Selling

Selling kidneys is illegal in most countries where there is legislation related to organ transplantation. Ethical analyses of kidney sales need to consider contextual features, such as availability of dialysis and alternative opportunities for meeting the necessities of life. Opponents of the practice, such as Kahn and Delmonico,[56] warn of the possibility of societally endorsed exploitation of vulnerable individuals. They argue that governments have a duty to provide for the poor, and that commodification of the body could discourage them from providing less risky sources of income for the destitute.

Caplan[15] raised concerns that the practice may erode public trust in transplant medicine. He noted that kidney sales can have poor outcomes for vendors, and that the creation of a market in organs means changes in the nature of the relationship between physicians and their patients in these situations. Physicians, he argued, have a greater duty to "Do no harm" in this context than to assist patients financially through removing their organs.

Murray[75] approaches the matter from a different angle, urging us to recognize the impact that organ selling might have on social relationships. We live in a "community of needs," both biological and cultural, and needs related to transplants and blood transfusions are best met through "gifts of the body." He claimed we can realize important social values through noncommercial donation, such as fostering a sense of connectedness among people, recognizing the universality of human needs, and protecting the dignity of individuals. Two types of kidney selling are definable and are considered separately.

INDIRECT ALTRUISM

Indirect altruism, a concept developed by Dossetor, refers to when donor motivation for organ selling is altruistic toward a third party. Indirect altruism is a term coined to describe the following form of altruism: Person *A* wishes to carry out a good deed for a family member, person *B*, whose needs can be met only through using money. *B*'s needs cannot be met by *A* giving her a kidney because renal failure is not *B*'s problem. *A* does not have the money to meet *B*'s need, and society would not or could not provide it. Person *C* is rich and in need of a kidney. If *A* makes a contract to give a kidney to a third party *D* on the understanding that *D* would then sell that kidney to *C* and use the proceeds to help *B*, *A*'s contract with *D* is implicitly altruistic, but *D*'s contract with *C* is purely commercial. The money *D* obtains from *C* enables *A* indirectly to carry out the altruistic intention toward *B*.

Many would find this scenario compelling. Dossetor has defined, at greater length than here, the context in which indirect altruism would have to occur, using an ethically responsible third-party regulator, *D*, who is trustworthy and respected. Other criteria would need to be in place[41] for such arrangements to meet ethical standards. Examples that seem to meet these criteria are described from India.[84] Daar[24,29] and others[82] also have written extensively about this complex subject.

PERSONAL GAIN

Many people find the thought of vending organs for private gain to be repugnant. Some who had taken this position subsequently changed their minds. Others point out that it has been difficult to articulate convincingly the reasons for banning the practice.[12,46,82] The United States has recently looked at financial incentives to increase donation rates. These include partial reimbursement for funeral expenses, reimbursement for travel, and reimbursement for other expenses.[24]

There has been renewed discussion of organ sales in the West because of numerous factors, including great and continuing shortage of kidneys for transplantation, the number of deaths on the waiting list, the knowledge that early transplantation is the preferred treatment for individuals with end-stage renal disease,[63] and the number of Westerners who travel abroad to purchase organs. Veatch[114] argued that the failure to provide adequate income levels for some members of society supports the legalization of kidney sales.

The subject of payments for organs is complex.[27] We previously published a classification of the various types of living kidney donations, with consideration of their ethical acceptability or otherwise, so as to enable discussion to focus on each individual issue, rather than combining all the considerations at once. Living kidney donors can be grouped into the following five categories[36]:

1. Living related donor transplantation: Donation to a blood relative.
2. Emotionally related living donors: Genetically unrelated donors, including spouses and close friends.
3. Altruistic donation: The donor does not know the recipient, with no expectation of material reward.
4. Rewarded gifting: The donor is reimbursed (at least partially) for costs related to the donation, including lodging, travel, loss of income, and hospitalization.
5. Rampant commercialism: Payment for kidneys often to a broker or middleman, of which the donor may receive an amount.

This classification has evolved into the "gray basket concept"[26]—the gray basket being that category in the classification wherein ideas such as indirect altruism[41] or the donor trust,[98] founded on certain ethical principles but nonetheless still controversial, can be discussed sensibly.

Arguments have been made on both sides of this debate, which has many nuances. Radcliffe-Richards and coworkers[82] concluded that "we are not arguing for the positive conclusion that organ sales must always be acceptable, let alone that there should be unfettered market. Our claim is that none of the familiar arguments against organ selling work, and this allows for the possibility that better arguments may be found." Although there is some validity to the various arguments for organ vending for personal gain, our view is that rampant, unregulated commerce in organs for personal gain is against the best interests of society and should remain prohibited throughout the world. The matter deserves ongoing debate, however.

Dossetor, who has given this matter more thought than perhaps most commentators, approves a practice whereby an altruistic good can be achieved by a method that involves obtaining money from wealthy recipients by vending organs through an ethically reliable third party, under conditions in which the donor makes no profit or personal gain except through the spiritual or psychological benefit inherent in acts of altruism. Whether or not such a system can be or needs to be established in a given country depends on many societal factors. These factors are reviewed by considering situations at both ends of the world prosperity spectrum: (1) from the viewpoint of an affluent society and (2) from the viewpoint of a country where the bulk of the population lives in poverty.

For affluent cultures, such as the West, many factors operate to support individuals with special transplant needs, such as state health care programs, unemployment and health insurance, and resources to support existing altruistically based deceased donor programs and new initiatives to increase organ procurement. The benefit/burden calculus for the would-be kidney donor to a third-party vendor who then obtains money for the donor's intended act of indirect altruism is not compelling. The conditions of abject poverty do not exist. Also, in Western cultures, the benefit to society of allowing kidney transplantation through third parties raising funds from kidney vending to carry out acts of indirect altruism do not seem to outweigh the probable harm to the fabric of society that would stem from commercialization of the body, including lessened respect for others, affront to religiously based convictions, decay of primary or direct altruism, and other risks for social corruption. There are many more opportunities to sustain the lives of individuals with chronic renal failure.

Affluent countries offer protection against dire need in many ways, and members of society are largely protected against abject poverty, starvation, and lack of shelter through a tax-financed social security net. Affluent societies provide protection against the need for self-imposed acts of heroism, such as those involved in donating a kidney altruistically, which is then sold to obtain money to benefit others.

Nonaffluent cultures differ in striking ways. Not only is there an absence of the general social security net but also of government-funded health care programs for special needs. People die for lack of adequate housing, nutrition, and simple medical needs, including good sanitation and pure drinking water. People in such conditions already are victimized by abject poverty. The context of their whole lives is different from those of citizens of affluent countries. In such situations, although we still deplore kidney commerce for personal gain, it is impossible for us to condemn kidney donation for prearranged vending through a third party to raise money for an act of indirect altruism to a family member. For the donor in the personal no-gain setting of indirect altruism, the burden may be offset by the benefit to the family member, whereas the welfare of society is not at risk because of the underlying altruistic nature of the act, even though an organ has been obtained for money.

Inherent in this support for indirect altruism in nonaffluent cultures is an insistence that the benefit to *B*, the intended beneficiary of this form of altruism, must be ensured. This ensurance necessitates a socially responsible, noncorruptible panel or tribunal of societal and professional peers to approve individual cases and set up a mechanism to collect money from the recipient purchaser and to effect the intended altruistic good of the donor. In our judgment, if this situation cannot be ensured, an institution would be acting unethically in pretending to meet a standard if it knows it cannot.

Lastly, we consider in this section the ethics issues facing recipients who have bought kidneys from living unrelated individuals—the purchasers of kidneys. Purchasers of kidneys in nonaffluent countries, where kidney transactions could be used to raise money for acts of indirect altruism, are disproportionately rich compared with the donors. Purchasers are buying parts of someone else's body, which many see as a manifestation of victimization of the poor by the rich, akin in some ways to prostitution or enslavement. Wealth is accepted in most cultures as giving special privileges to individuals who possess it, but this does not extend to victimization and partial enslavement of others.

Dossetor[41] suggests, because of the good that might result from indirect altruism to the donor's intended beneficiary of the sale, the purchaser of a kidney might be ethically justified if two conditions were met. In addition to giving a fair price for the organ, (1) the purchaser should be obliged morally to give additional funds to support another distressed person, perhaps from the section in society from which the donor comes, and (2) the purchaser should give additional funds toward the ultimate establishment of a deceased donor renal transplant program. These additional funds, which Dossetor[41] termed mandated philanthropy, should not be paid out at the expense of a fair and generous price to the kidney donor, who uses third-party vendors to effect acts of indirect altruism. The purchaser's responsibility in this regard should be in the hands of a tribunal or panel of peers at the transplant institutions.

So far, the only country that has openly and institutionally created mechanisms for paid organ donation is Iran. Implementing and refining the Iranian model while addressing most of the ethical concerns has made Iran perhaps the only country in the world to reduced the waiting list for kidney transplants.[47] However, the Iran model is not without blemish.[52a]

Daar has noted[30] that despite our condemnation of the practice, the number of commercial transplants has increased in recent years. He argues that serious consideration ought to be given to regulating the practice where such practice is rampant, causing harm to donors and recipients (usually only recipients who can afford to pay), and where countries are unable to stop the practice or provide alternatives.

EMERGING ISSUES IN TRANSPLANTATION

Xenografts

Efforts to obtain organs for direct transplantation into humans have had a positive impact on the xenotransplantation field by factors including (1) advancements in immunosuppression, which have led to improved outcomes in interspecies kidney transplants; (2) ability to manipulate the recipient's immune response; and (3) ways of altering some of the foreignness of pig tissue by inserting into the tissue human genes coding for complement regulatory proteins and other genes. Xenotransplantation already is a highly controversial area. Kantian deontologists may see animals as outside the province of human ethical concern because they are not moral agents. Other traditions believe that animals share ethical status with humans in proportion to their ability to have relationships with humans and a social life among themselves and their capacity to suffer pain and anguish and possibly suffer from frustrated self-awareness and thwarted self-interests.

Although animals may not have rights, many people attribute them with varying degrees of ethical status. People who strongly hold this perspective view xenografting as another form of animal exploitation and another excess of medical hubris, especially if directed at species whose behavior more resembles that of humans (as denoted perhaps by the notion of genomic proximity to humans). Transplant teams should try to understand the motivations of such believers in attempts to avoid extreme polarization of emotional viewpoints. Indifference to these concerns leads to angry confrontations, such as characterizes the abortion issue. Efforts to understand the rational and philosophical basis for people who oppose development of this branch of transplantation science are important. It can be assumed that most people who presently find the prospect of xenotransplantation abhorrent value individual human lives much more highly than individual animals. This assumption should be taken as a given in the debate.

Some ethical issues of xenotransplantation and the possible implications for allotransplantation have been explored.[23,32] These and other ethical issues in xenotransplantation stem from the unique combination of perspectives that constitute the debate (Table 39-1). Some of these are expanded on in this section, although they are in the course of rapid change.

Breeding Animals for Xenograft Purposes

The great British reformer Bentham (1748-1832), regarded as a key figure in the development of utilitarian ethics, also was one of the earliest to advocate the humane treatment of animals. In 1780, he asked two fundamental questions: (1) "The question is not can they reason? Nor can they talk? But can they suffer?" (2) "What insuperable line prevents us from extending moral regard to animals?" A modern utilitarian philosopher, Singer, has taken on the mantle of Bentham where animals are concerned.

Table 39–1 Xenotransplantation Debate

Great scientific research
Significant industry involvement
Much greater public awareness of the *existence of a problem (without a sense of the details)*
Public opposition to the exploitation of animals in this way
Lack of consistency of what the public is told about
State of science
Magnitude of risk
Much greater involvement of scientists with industry in terms of contractual obligations and funding of research
Depletion of traditional sources of university-based funding
Difference in assessment by scientists and policy makers of
Scientific base
Risk of infection
Much more active and organized constituency of ethicists, philosophers, concerned citizens, and animal rights activists with a larger capacity to make their (sometimes confused) views known and not all willing to engage in polite discourse
Much stronger constituency of patients' advocacy groups, who cannot understand why important research is being held back by *theoretical* and *academic* fears and risks

Pain is perceived essentially in the same way by all vertebrates, and it is not controversial that vertebrates used in experiments feel pain. There is a growing consensus, however, that animals can suffer, not just feel pain. Suffering implies self-awareness, and many experimenters are not ready to concede this point because it then implies a degree of intelligence and worth that would allocate rights to animals.[99] Regan[85] and others have argued that animals do have many rights, even if these are of a lesser magnitude than those of humans. Ignoring animal rights (a term popularized by Regan) is a form of speciesism, which is equivalent to racism.

We appreciate the tremendous complexity of animal lives. Animals in captivity can experience fear, boredom, isolation, and separation. They may not be able to use language (that we can understand), but they do communicate. The emotional repertoire of nonhuman primates, according to ethologists Goodall and Fossey, apparently includes love, sorrow, and jealousy.[74] These features also explain partly the increasing concern for animal welfare, culminating in the tendency to pass laws recognizing animals as sentient beings with inherent value. If animals are sentient and have value, it could be argued that they *must* have rights. Are animals members of the moral community? Even if we concede that animals are moral subjects and not just objects, they could never be moral agents as far as humans are concerned. There is an inherent problem in the discourse on animal use in that one of the parties being discussed does not participate in the debate, and we are restricted to evaluating moral sensibilities, principles, and values of *Homo sapiens*.

What is it in humans that bestows on them the moral superiority or higher moral value that would justify the killing of an animal to save a human being? Is it language, tool use, rationality, intentionality, consciousness, conscience or empathy?[14,97] Because philosophers disagree, because premises are different, and because rights theories contain elements of arbitrariness, it seems that, short of a complete change in human consciousness, the issue will remain controversial and divisive.

There are laws to protect research animals in many countries, and there are international guiding principles, such as those of the Council for International Organizations of Medical Sciences. Sensible guidelines include the "3R's" of Russel and Burch,[90] which are to reduce, replace and refine, to which could be added reconsider and respect. There is much effort today directed at looking for alternatives to animal use. Ultimately, it will be public, rather than professional, acceptance, acquiescence, or rejection that determines the issue of using animals in xenotransplantation. Today, a stronger case can be made for the use of pig organs but not organs from nonhuman primates, for human xenotransplantation. At this stage of development, it is perhaps more productive to worry about and attend to animal welfare rather than animal rights.

Within the three major monotheistic religions, Judaism, Christianity, and Islam, humans were made in the *imago dei*, and the rest of creation is there to serve humans. God blew His own breath into the body of man, transfiguring him and making him different from the rest of creation. The pig is ritually unclean in Islam (*najs*) and Judaism (not *kosher*), however. We have looked at this issue[31] and concluded that it would not be a barrier to xenotransplantation, based on the theological argument that need and necessity can allow that which is forbidden, and in any case, the prohibition is to eating only. There is a minority opinion, however, that pigs, partly because they are ritually unclean, cannot be used as source animals. From the religious perspective, it would be important that a xenotransplant should not tamper with the human personality, its freedom and its ability and eligibility to bear responsibility. Humans have stewardship responsibilities accepted noncontroversially by almost everyone, making it necessary to reduce the pain and suffering of animals being used for human purposes.[31,55]

The psychosocial aspects of humans adapting to xenotransplanted organs are unclear. Some recipients may experience emotional difficulties or have problems integrating the transplant in their self-image.[4] Although xenotransplantation eventually may eliminate the wait for an organ, it may give rise to other challenges, such as seeing animals as an infinite resource. One study[102] found adolescents to be very accepting of xenotransplantation in the form of porcine islet cells and raised the question of how recipients would deal with nonadherence to treatment if there were a steady supply of organs through xenotransplantation.

Ethics of Consent When Society Is Also at Unknown Risk

The issue of consent in xenotransplantation has not been addressed adequately, and its implications are underestimated. The major issue in xenotransplantation today is whether we are ready to proceed to systematic clinical trials. Our understanding today is that consent for experimental procedures should be informed, unhurried, and voluntary. Informed consent exists for the purpose of protecting the subject from the risks of the experiment. Normally, taking into account societal considerations might prejudice the interests of the individual subject. Generally, consent has nothing to do with protection of contacts or of society. It requires that the subject be made aware of the risks involved, the potential benefits to the subject, and all the alternatives available.

For xenotransplantation, there is a risk (especially from new xenozoonoses) to the public at large. Zoonotic infections such as human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), parvoviruses, and the SARS coronavirus have spread around the world, prompting calls for global international surveillance of xenotransplantation-associated diseases.[33] Trials cannot proceed ethically until there is agreement from society as a whole that it is willing to accept this risk. There are no easy and reliable ways of obtaining such a societal consent. It is a major ethical problem that initially can be addressed only by making every effort to inform and involve all segments of society, using every media outlet. Public policy decisions based on a risk-benefit analysis would likely favor individual patients, rather than the public at large. The "precautionary principle" may place priority on society as a whole.[22] This principle, as formulated in the Wingspread Declaration, states: "When an activity raises threats to human health or the environment, precautionary measures should be taken, even if some cause-and-effect relationships are not established scientifically."[33]

In xenotransplantation clinical trials, particularly for the early patients, many of the normal elements of individual consent would need to be compromised. Subjects would probably be very sick, and voluntariness would be questionable because, especially in the case of liver and heart subjects,

the alternative may be death. The risks of rejection and the potential benefit can be estimated vaguely, but the risk from xenozoonoses cannot, because clinicians do not know which viruses would be more pathogenic in humans or would mutate or recombine in the host. Clinicians would not know if the source animal has any viruses about which nothing is known. The incubation period and latency of some retroviral infections (e.g., HIV) could be several years. There is considerable evidence that HIV jumped species from nonhuman primates to humans.

Clinicians have become aware only more recently that porcine endogenous retroviruses can infect human cells in vitro.[81] The demonstration that 160 patients exposed to live pig tissue[79] did not become infected by porcine endogenous retroviruses is partly reassuring but should not be seen as definitive evidence justifying large-scale clinical trials.[116] Oldmixon and colleagues[78] discovered a unique herd of pigs that do not transmit porcine endogenous retroviruses to humans. Studies suggest it may be possible to produce pigs for xenotransplantation that pose a greatly reduced risk of infection.[7,96,117]

The main foreseeable problem with clinical trials in xenotransplantation is with the question of postoperative monitoring. The recipient would have to agree to the requirement for strict monitoring, which may be intrusive and may result in quarantine, containment, or other physical restrictions if the recipient develops infections likely to endanger contacts, health care workers, or the public. Privacy and confidentiality almost certainly would have to be signed away in this consent procedure, especially because the contacts also would require monitoring. The recipient may be restricted from having sexual relations for perhaps 1 year or more. Contacts themselves would have to consent to postoperative monitoring, which may be intrusive in the case of a major infection difficult to diagnose or treat. There is an implicit need for community consent—not an easy thing to obtain because it normally would require public hearings, advisory bodies, and legislative and executive branch processes.[54]

The fact that the patient is going to be required to comply with postoperative monitoring alters the nature of consent to something more aggressively binding and contractual. There is another normal feature of consent—the subject has the right to withdraw at any time from the experiment. This right would have to be transgressed because the recipient could not opt to withdraw later from the experimental procedure, which must conform to standards such as the Declaration of Helsinki. It would be extremely difficult, for example, for the recipient of a pig heart to withdraw from a study and have the organ removed[20]; another example is when the participant harbors an infection that might jeopardize public health. The consent would need to be enforceable in a direction different from that in the past—this time against the best interests of the subject and in favor of the public. This situation would be a travesty of the concept of consent as it is known today. A type of "Ulysses Contract" could be used to compel the investigation, treatment, or confinement of a xenotransplant recipient, even in the event of rejection of the graft.[22]

Avoidance of Regulation by Xenotourism

Almost all of the influential discussions about the dangers of xenotransplantation and development of guidelines and control frameworks are taking place in Europe and North America (see later). Xenotransplantation may start elsewhere, however, in environments where the regulations are lax, and the scientific base and facilities are inadequate. An example was the case of Baruah,[73] a physician who was arrested in Assam, India, early in 1997 for violation of the Organ Transplantation Act. He had claimed to have transplanted successfully the heart, lungs, and kidneys of a pig into a human recipient at his own hospital, assisted by local colleagues and apparently by a colleague from Hong Kong. The patient died a week later, and the family, feeling suspicious, lodged a complaint with the police. This kind of activity might pose dangers because in the near future clinicians from scientifically advanced countries may start collaborating with colleagues in countries where the regulations may be more permissive. It would be better to consider seriously an international effort to draw up universal guidelines, while hastening to lay the groundwork for national regulatory mechanisms for clinical trials.

Cost and Other Economic Considerations

Xenotransplantation will be expensive for at least a number of years. The biotechnology companies are likely to control the cost of the organs and in the absence of real competition would want to keep this cost as high as the market would tolerate. The cost of rearing source animals under special conditions, monitoring them, developing laboratory tests, training staff, taking extra precautions, monitoring recipients and contacts, and installing infection control measures all would add to the cost. There also is the question of who would pay for expensive new immunosuppression.[69] It is unknown if, in the long run, the cost would decrease sufficiently for this to be one of the justifications for xenotransplantation. When the results achieve sufficient success to be seen as established treatment and not clinical research, countries with ethical commitment to equity in access to established therapies would need to assess carefully how to maintain the principle of distributive justice.

National and International Efforts to Develop Guidelines

One must approve the efforts that have been made to consider the challenging issues of xenotransplantation and be prepared to regulate its development along ethically acceptable lines. Table 39-2 lists some of these efforts. There is great concern about ethics issues, regulatory frameworks, relationship with industry production of source animals, and the risk of xenozoonoses and their detection. In addition to those listed, there are initiatives by other international bodies and by national bodies in France, the Netherlands, Spain, and Switzerland.

In January 1999, the Parliamentary Committee of the Council of Europe decided to call for a moratorium on xenografts. This moratorium has been criticized as inhibiting research funding and investment, but it has been praised by others.

The government of the United Kingdom developed the Advisory Group on the Ethics of Xenotransplantation, which published a report entitled "Animal Tissues into Humans (the Kennedy Report)"[1] in August 1996. It advocated an effective embargo against clinical trials in the United Kingdom until a National Standing Committee could be established to supervise and coordinate the many aspects of accumulation of knowledge and set up mechanisms to

Table 39–2 **National and International Reports in Xenotransplantation and National Regulatory Efforts**

National and International Reports on Xenotransplantation
World Health Organization (WHO) Consultation in Xenotransplantation
Institute of Medicine (U.S.)—Xenotransplantation Science, Ethics, and Public Policy
United Kingdom Advisory Group on Ethics of Xenotransplantation—The Kennedy Report
Nuffield Council on Bioethics—Animal-to-Human Transplants: Ethics of Xenotransplantation
Organization for Economic Cooperation and Development (OECD)—Policy on International Issues in Transplantation Biotechnology
Health Canada—National Forum on Xenotransplantation: Clinical Ethics and Regulatory Issues, November 1997
National Regulatory Efforts
United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA)
Canada: Standards for Xenotransplantation—Canadian Standards Association (CSA)
German Medical Council on Xenotransplantation
Council of Europe Steering Committee on Transplantation—responsible for the moratorium on xenotransplantation of January 1999
Èstablissement Français des Grèffes

protect the public and patients, look after the welfare of animals, and decide when clinical trials could start. It concluded also that it would be ethically acceptable to use pigs and to modify them genetically for xenotransplantation.

The British government responded to the Kennedy Report in January 1997 and announced establishment of the United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA), to be chaired by Lord Habgood of Calverton. The response agreed broadly with the Kennedy Report's conclusions, but it called for more input in regard to (1) the unacceptability of using nonhuman primates for therapy and (2) the conclusion that not enough was known about the immune response, physiology,[72] and risk of xenozoonoses to proceed to clinical human trials.

The Ethics Committee of the International Xenotransplantation Association[103] published a Position Paper in 2003. It stressed the need to minimize the risk of infectious disease transmission and suggested standards for clinical trials. Einsiedel[42] argued that the Position Paper needed to examine the issue of public education more closely. This sentiment was shared by others,[6] who suggested that town hall meetings, referenda, and possibly virtual meetings over the Internet ought to occur when considering public policy that may pose risks.

One attempt was made by the Canadian Public Health Association,[13] which conducted six citizen forums across Canada that featured 107 panelists. The project also sought public opinion by telephone, mail, and website surveys. Although Canadians did not think xenotransplantation should proceed at this time, they wanted to explore alternatives, such as stem cell research, widening the human donor pool, and disease prevention. A similar project led by the Australian National Health and Medical Research Council was initiated, which involved public meetings in several cities.[104] Although attendance was low, the meetings revealed strong support for animal rights. Obtaining such informed societal opinion and agreement is difficult and costly.

Physiological Issues

Less discussed are the hazards inherent in an animal organ, such as the liver synthesizing animal proteins that might (1) be unphysiological for humans, having a dysfunctional effect; (2) induce an immunological response; or (3) interact with human protein homologues in some unforeseen way. There are other physiological incompatibilities for other organs.

Regenerative Medicine

According to Daar and Greenwood[34] and Greenwood and colleagues,[49] regenerative medicine is an interdisciplinary field of research and clinical applications focused on the repair, replacement, or regeneration of cells, tissues, or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma, and aging. It uses a combination of several existing and newly emerging converging technological approaches that moves it beyond traditional transplantation and replacement therapies. The approaches often stimulate and support the body's own self-healing capacity. These approaches may include, but are not limited to, the use of soluble molecules, gene therapy, stem cell transplantation, tissue engineering, and the reprogramming of cell and tissue types.

Developing Countries

Low-income and middle-income nations tend to have high rates of communicable diseases and are experiencing an alarming increase in noncommunicable diseases, such as cancer, diabetes, and cardiovascular diseases (Table 39-3).[49] Many of these countries have developed initiatives related to regenerative medicine. The Nacional University of Cordoba in Argentina has conducted gene therapy experiments in mice to treat rheumatoid arthritis with promising results. The Chaoyung Hospital in Beijing, China, has begun using cells derived from fetal tissue to treat many neurological diseases, such as amyotrophic lateral sclerosis, Parkinson's disease, and spinal cord injuries.[50] Physicians in India have used adult stem cell therapy to repair the eyes of 125 patients who have experienced infections, burns, and trauma.[92]

Regenerative medicine could reduce the financial burden created by many diseases.[49] Bone marrow stem cell transplantation or microencapsulated islet cells could reduce the amount of spending on insulin treatments for diabetics and could lower the incidence of related complications, such as blindness, heart disease, and diabetic ulcers. Autologous cells could be injected into heart muscle to repair tissue damaged by myocardial infarction and cardiomyopathies, saving lives and reducing the cost of treating heart failure. Specially engineered immune cells could help reduce the devastation caused by diseases such as HIV/AIDS, tuberculosis, hepatitis, and malaria.

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Table 39–3 **Top Ten Regenerative Medicine Applications for Improving Health in Developing Countries**

Ranking	Applications of Regenerative Medicine	Examples Identified by Panelists
1	Novel methods of insulin replacement and pancreatic islet regeneration for diabetes	Bone marrow stem cell transplantation for pancreatic regeneration Microencapsulation (e.g., poly-lactide-co-glycolide) for immunoisolation of transplanted islets Cultured insulin-producing cells from embryonic stem cells, pancreatic progenitor cells, or hepatic stem cells Genetically engineered cells to express insulin stably and contain a glucose-sensing mechanism
2	Autologous cells for regeneration of heart muscle	Myocardial patch for cardiac regeneration Direct injection of autologous bone marrow mononuclear cells for cardiac repair Stromal cell injection for myocardial regeneration Localized angiogenic factor therapy through controlled-release systems or gene therapy
3	Immune system enhancement by engineered immune cells and novel vaccination strategies for infectious disease	Genetically engineered immune cells to enhance or repair immune function Single-injection DNA vaccines
4	Tissue-engineered skin substitutes, autologous stem cell progenitor cells, intelligent dressings, and other technologies for skin loss owing to burns, wounds, and diabetic ulcers	Bilayered living skin constructs (e.g., Apligraf) Engineered growth factors (e.g., rhbFGF, rhEGF) applied in conjunction with topical treatments (e.g., SD-Ag-Zn cream) Intelligent dressings composed of a slow-releasing growth hormone polymer Epithelial cell sprays
5	Biocompatible blood substitutes for transfusion requirements	Polyhemoglobin blood substitutes for overcoming blood shortages and contamination issues
6	Umbilical cord blood banking for future cell replacement therapies and other applications	Preserved umbilical cord blood stem cells to provide future cell replacement therapies for diseases such as diabetes, stroke, myocardial ischemia, and Parkinson's disease Pooled cord blood for the treatment of leukemia
7	Tissue-engineered cartilage, modified chondrocytes, and other tissue-engineering technologies for traumatic and degenerative joint disease	Matrix-induced autologous chondrocyte implantation for cartilage repair Tissue-engineered cartilage production
8	Gene therapy and stem cell transplants for inherited blood disorders	Genetically engineered hematopoietic stem cells to restore normal blood production in patients with β-thalassemia
9	Nerve regeneration technologies using growth factors, stem cells, and synthetic nerve guides for spinal cord and peripheral nerve injuries	Synthetic nerve guides to protect regenerating nerves Embryonic stem cell therapy for spinal cord regeneration Growth factor–seeded scaffolds to enhance and direct nerve regeneration
10	Hepatocyte transplants for chronic liver diseases or liver failure	Microencapsulation of hepatocytes to prevent immunological reaction Derivation of hepatocytes for transplantation from bone marrow cells Transdifferentiation of hepatocytes for transplantation from bone marrow cells

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