GLOMERULONEPHRITIS

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GLOMERULO-NEPHRITIS

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SERIES EDITOR'S FOREWORD

Glomerulonephritis has always been regarded as a complex subject. Different forms of the disease can cause death in a matter of weeks, nephrotic syndrome which might or might not prove responsive to steroid therapy, or no symptoms at all. Improved pathological techniques and criteria have permitted a more accurate diagnosis and prognosis to be established for many patients. With increased understanding of the immunological mechanisms involved it has become apparent that many patients presenting with a variety of symptoms and signs may have glomerulonephritis as their primary pathological process.

This book examines the clinical, pathological and aetiological factors involved in the common forms of glomerulonephritis. Each chapter has been written by a recognized expert in the field and provides information of relevance and practical importance to the average clinician. The developments of the last decade have emphasized that glomerulonephritis is no longer a matter only for the nephrologist but a subject on which all clinicians should be well informed.

ABOUT THE EDITOR

Professor Graeme R.D. Catto is Professor in Medicine and Therapeutics at the University of Aberdeen and Honorary Consultant Physician/Nephrologist to the Grampian Health Board. His current interest in transplant immunology was stimulated as a Harkness Fellow at Harvard Medical School and the Peter Bent Brighton Hospital, Boston, USA. He is a member of many medical societies including the Association of Physicians of Great Britain and Ireland, the Renal Association and the Transplantation Society. He has published widely on transplant and reproductive immunology, calcium metabolism and general nephrology.

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MINIMAL CHANGE NEPHROPATHY*

J.M. BOULTON-JONES

This diagnosis has pleasant associations, particularly for adult nephrologists who can be reasonably certain that their treatment will cure the patient made miserable by the nephrotic syndrome. The treatment is effective but entirely empirical. Some tantalizing clues are there for all to see, but no-one has yet identified the cause for the dramatic change in the permeability of the glomerular basement membrane which characterizes this condition or why it should respond so regularly to steroids. There are remarkably few clues in the glomerulus itself: as with the dog which did not bark in the night, the main finding is the absence of histological change. This was first documented in 1905 by Frederick Muller who coined the term 'nephrosis' to describe the kidneys of patients who had been nephrotic but whose kidneys showed no evidence of 'nephritis'; In 1916, Munk described lipoid droplets in the urine of these patients and invented the term 'Lipoid Nephrosis' which is still used today. The introduction of the technique of renal biopsy in 1954 enabled clinicians to examine the tissue of patients much earlier in the natural history of their illness and in the 1960s the present histological classification of the glomerulopathies was constructed and subsequently elaborated by electron and immunofluorescence microscopy. At about the same time as this was happening, clinical trials of prednisolone were undertaken and it was quickly appreciated that some patients, particularly children,

* This chapter was received for publication in January 1988 and therefore all publications made after that have not been considered.

responded dramatically. These observations led to the identification of the diagnostic criteria of MCN.

DEFINITION

Minimal change nephropathy (MCN) is primarily an histological diagnosis. The glomeruli are normal on light microscopy. The only change on electron microscopy is of 'fusion' or more properly 'retraction' of the epithelial foot processes. Glomerular deposits of immunoglobulin or complement are conspicuous by their absence. C3 is often found in small amounts in the afferent arterioles.

Patients present with the nephrotic syndrome and this is included in the definition. Some also require that there should be a prompt response to treatment with steroids. However, many patients with some mesangial cell proliferation will also respond quickly to a course of prednisolone and it is important that they should not be denied this treatment because they do not have true histological minimal change. Therefore all patients with endocapillary proliferation should be included provided that there is no evidence of immune deposits on immunofluorescent examination of the biopsy.

CLINICAL FEATURES

MCN occurs at all ages and in both sexes, but most commonly presents before the age of 10 with a peak incidence during the third and fourth years. Its incidence is between 20 and 70 children per million per year and about 3 adults per million per year. 76% of nephrotic children[1] and 25% of nephrotic adults[2] have MCN. There is an interesting change in the sex ratio with age. Boys outnumber girls by two to one, but among adults the sexes are equally affected. MCN occurs among people of European and Chinese descent but is rarer among those of African origin.

By definition, MCN presents with the nephrotic syndrome which usually comes on acutely without any warning but may follow a few days after an upper respiratory tract infection, particularly in males. Rarely, the patient may present, and subsequently relapse, during the

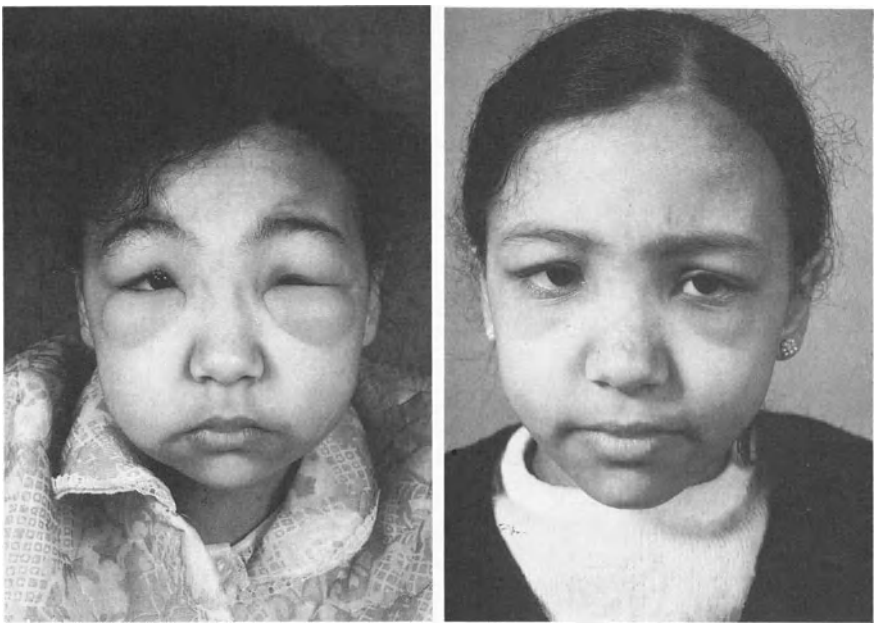

FIGURE 1.1 Photograph of nephrotic girl before and after 'conservative' measures

early summer when the pollen count is high[3,4]. Nearly 40% of children with MCN are atopic[5], but no increase in the incidence of atopy has been found among adults[6].

The oedema of this syndrome is often massive, affecting the face and abdomen of children and the ankles and sacral areas of adults (Figure 1.1). Ascites and pleural effusions may be detected. The skin may become so stretched over the swollen areas that striae develop, particularly on the abdomen and ankles (Figure 1.2). The patient may become breathless because the diaphragm is splinted and because of the increased effort required to remain mobile with water-logged legs. Gastro-intestinal symptoms are common. Patients are anorexic, some have abdominal discomfort and a few vomit frequently. As a result, there is often a loss of muscle mass (Table 1.1).

The physical findings are dominated by the presence of pitting oedema in dependent areas, ascites and pleural effusions. The jugular venous pressure is not raised. Mild hypertension is present in about

TABLE 1.1 Symptoms other than oedema reported in a series of 34 patients with MCN

Malaise	6
Abdominal pain	5
Loin pain	3
Chest pain	1
Nausea	3
Anorexia	2
Vomiting	2
Diarrhoea	2
Breathlessness	3

10% of children and 30% of adults. Macroscopic haematuria is rare but microscopic haematuria is found in about 25% of children and 35% of adults.

INVESTIGATIONS

The features of the nephrotic syndrome are present in all. Proteinuria in excess of 50 mg/kg/day is usual in children. In adults the loss is more than 3.5 g/24 hours. If the serum albumin is very low, the quantity of proteinuria will also drop even though the clearance of albumin remains high. About a quarter of children with MCN present with an albumin of less than 10 g/L and half have a value of between 10 and 20 g/L. The same is true for adults in whom the mean value of serum albumin at presentation is 20 g/L[6].

There is a marked rise in serum cholesterol in all but 5% of patients whether adults[2] or children[1]. This rise affects VLDL and LDL cholesterol. HDL cholesterol is reported variously as normal or low[7]. The serum creatinine is usually within normal limits but is raised in about one third of children, when corrected for age and sex[1], and about 40% of adults[2]. Table 1.2 shows the average values for some of these parameters in relapse and remission for a group of adults.

Selectivity index

This is a measure of the size of molecules crossing the GBM. The index most commonly used is the ratio of the clearance of IgG/the

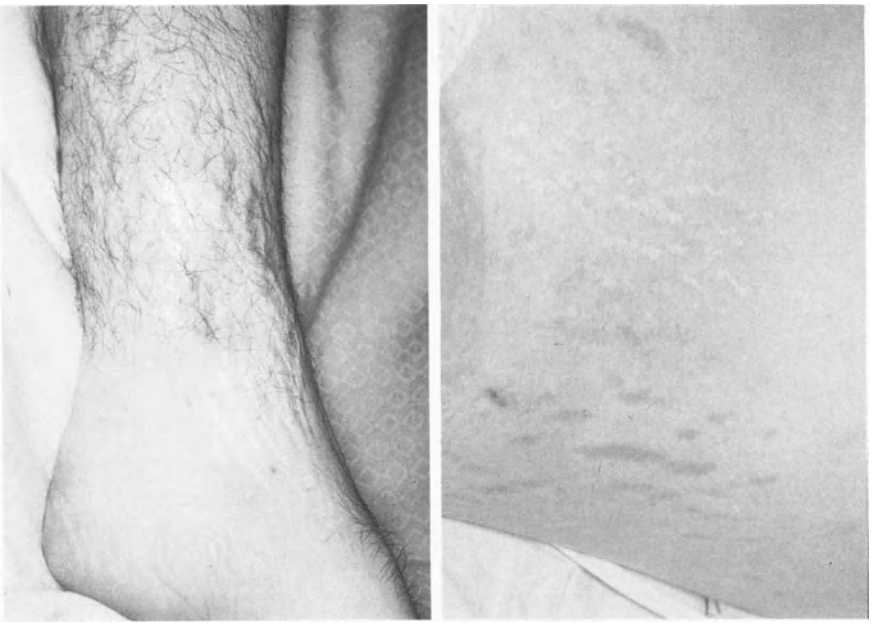

FIGURE 1.2 Photographs of ankle and abdomen of nephrotic patients showing striae caused by oedema

clearance of transferrin. Therefore the smaller the index the greater is the restriction to the passage of large molecules compared with small. MCN is usually associated with a 'selective' proteinuria, which is indicated by a ratio of less than 0.1. This measure is particularly useful in children with MCN, among whom 53% have highly selective proteinuria (ratio <0.1) and only 15% have non-selective proteinuria (ratio >0.15)[1,8]. It is of little use in adults with MCN because half have a ratio of more than 0.15[2].

Renal Biopsy

Renal biopsy is an unnecessary investigation in children under the age of 16 with selective proteinuria and a normal C3 because the probability of the diagnosis being MCN is more than 95% and thus

a trial of steroids is justified without further investigation[1]. In adults, on the other hand, a biopsy should be performed in all who present with the nephrotic syndrome.

TABLE 1.2 Some measurements in relapse and remission

	Relapse	*Remission*
Proteinuria (g/24 hours)	12.1 ± 6.4	0.1 ± 0.3
Serum albumin (g/L)	20.6 ± 4.8	43.0 ± 4.2
Creatinine clearance (ml/min)	89 ± 38	116 ± 34
Haemoglobin (g/dl)	15.0 ± 2.0	14.1 ± 1.5
Cholesterol (mmol/L)	13.0 ± 5.0	6.2 ± 1.1
Weight (kg)	68.4 ± 13.0	64.5 ± 11.9
Systolic	136 ± 22	125 ± 16
Diastolic	87 ± 14	77 ± 14
IgG (g/L)	5.3 ± 3.1	9.9 ± 2.9
IgA (g/L)	2.1 ± 1.1	2.0 ± 0.8
IgM (g/L)	1.9 ± 1.0	1.4 ± 0.7

Every biopsy should be examined by light microscopy, electron microscopy and immunofluorescence techniques. At present, this requires different fixation procedures for the three methods and the biopsy material has to be divided into three parts. It sometimes happens that one of the three portions does not contain glomeruli and the diagnosis has to be made on the evidence available.

Light microscopy

The appearances of the glomeruli, arterioles and tubules should be normal (Figure 1.3). However a substantial minority of patients who subsequently prove to be steroid sensitive have some focal or diffuse mesangial cell proliferation or increase in mesangial matrix (Figure 1.4). Tubular loss is unusual in young patients in whom it may be a sign of focal glomerulosclerosis, but may not be significant in older patients in whom some nephron loss is normal[6].

Electron Microscopy

Electron photomicrographs of the glomeruli are normal except in one respect, which is that the delicate pallisade of foot processes of the epithelial cell on the outside of the GBM is lost together with the slit pores which connect the foot processes (Figure 1.5). This is a non-specific change seen in all glomeruli of patients with proteinuria. Indeed, the number of slit pores per unit length of GBM is inversely proportional to the degree of proteinuria[9]. The finding of electron dense deposits makes response to steroids unlikely[9a].

Immunofluorescence studies

No diffuse deposits of immunoglobulin or complement components are found within the glomeruli. C3 may be present in the walls of some arterioles.

TREATMENT

The purposes of the investigations are to define those patients who will respond to a course of steroids. The reason for including some patients with histological changes as defined above is that their response to prednisolone cannot be distinguished from that of patients with true histological minimal change except perhaps that those with diffuse proliferation may respond more slowly[10]. The results of the immunofluorescence studies are important because it is unlikely that patients whose biopsies contain deposits of immunoglobulin or complement will respond to steroids, except a small group with diffuse mesangial deposits of IgM[11].

Symptomatic measures

The patient's main symptom is oedema, and this can be considerably eased by the use of diuretics. Care must be taken because oedema is not only the patient's symptom but is also the body's defence in

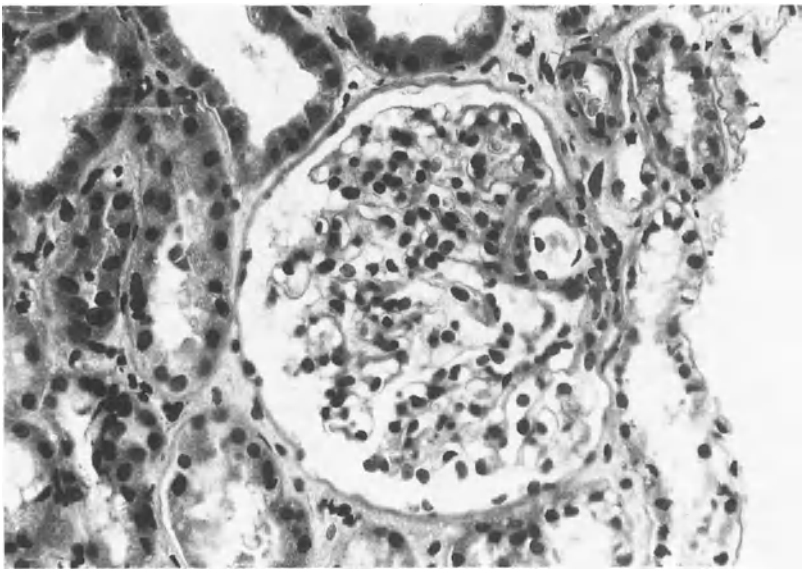

FIGURE 1.3 Normal glomerulus

maintaining the circulating volume. Overtreatment may result in a reduction in circulating volume and renal blood flow and may therefore precipitate or aggravate renal failure. The dose of a diuretic such as frusemide should therefore be titrated against the amount of oedema, standing blood pressure and renal function. The patient's weight can also be a useful guide. Sometimes very large doses of frusemide, such as 500 mg bd are required. This frequently causes severe thirst and the patient should be warned to restrict fluid intake to 1.5 L/day. Using these measures, it is usually possible to restrict the oedema to bearable proportions until prednisolone has secured a remission. The average weight lost between relapse managed by diuretics and remission in adults was 3.9 kg in the Glasgow series[6].

Sometimes more radical measures are required when the patient presents with a nephrotic crisis. These patients look ill and feel dreadful. As well as being oedematous, they are anorexic and vomit continuously, so that their nitrogen balance is negative and they quickly

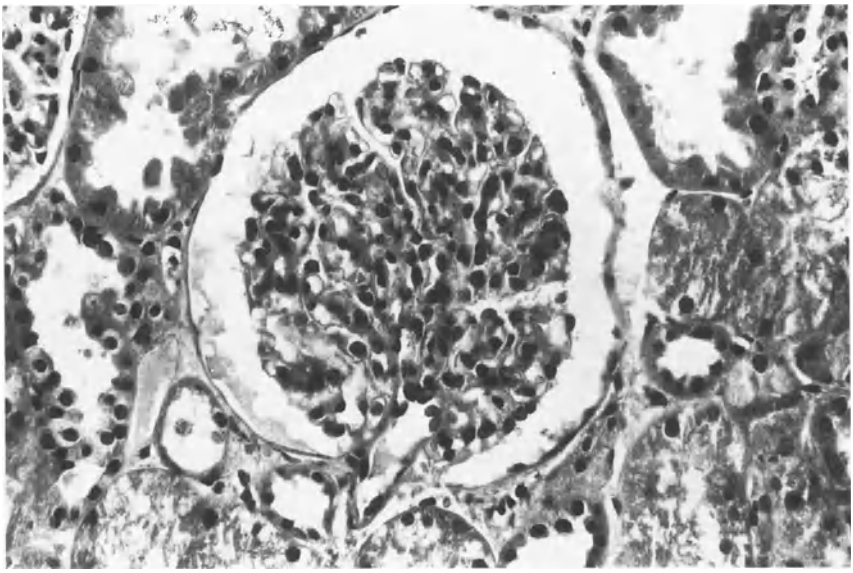

FIGURE 1.4 Mesangial proliferation in glomerulus of patient with steroid responsive nephrotic syndrome

lose muscle mass. Renal failure may develop. Intravenous salt-poor albumin combined with intravenous frusemide should be given in an attempt to reduce the oedema, which also affects the bowel wall, without causing contraction of their circulating volume. Once they can eat again, the crisis is over and routine measures can be introduced. Although this treatment is logical and appears to work well, its logic is at variance with recent observations which show that albumin infusion into a patient with a normal circulating volume increases glomerular permeability and leads to huge increases in proteinuria[11a]. Thus most of the infused protein is excreted within 24–48 hours. It is likely, therefore that patients in nephrotic crisis are hypovolaemic as a result of excessive use of diuretics.

Diet

The traditional response to heavy proteinuria is to increase the protein content of the diet in the hope of increasing hepatic synthesis of

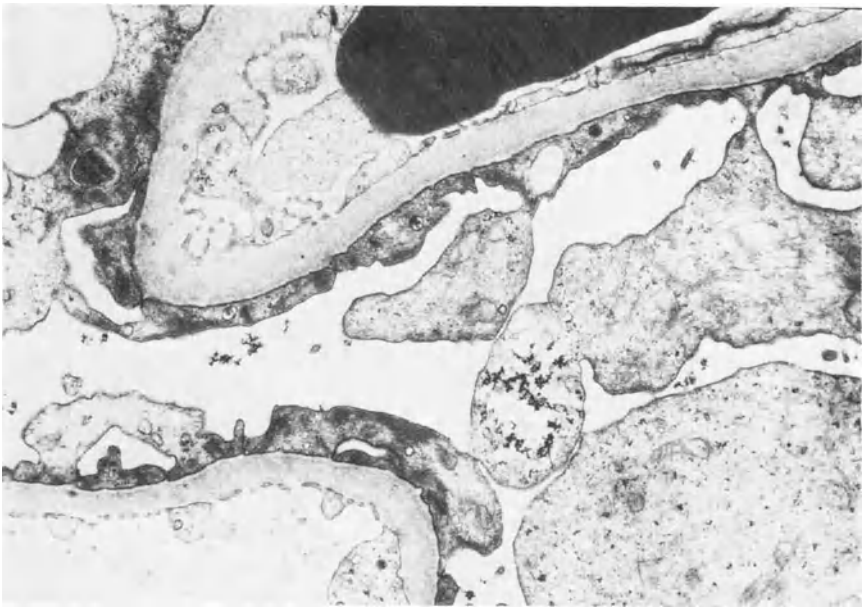

FIGURE 1.5 Electron photomicrograph of glomerulus showing retraction of epithelial foot processes

albumin. The hypoalbuminaemia of the nephrotic syndrome has other causes than simple loss in the urine. Increased catabolism and decreased synthesis also contribute. The rate of synthesis increases with increased dietary protein intake up to a level which is poorly defined. Thereafter, further increases only increase catabolism and may theoretically increase glomerular capillary pressure and thus also increase proteinuria[12]. A recent study of adriamycin nephropathy in rats showed that the proteinuria which is characteristic of this model could be almost completely eliminated by a very low protein diet[13], but this does not apply in other animal models.

Patients should be advised to ensure an adequate protein intake of about 1 g/kg/day. Salt restriction is desirable but should not be too severe or the food becomes unpalatable and the nitrogen balance goes into further deficit. Therefore patients should be advised to avoid salty foods and to refrain from adding salt to the food on their plate.

Steroids

The efficacy of steroids in inducing a remission in patients with MCN has been established for 20 years. In the ISKDC study, 93.1% of 363 children responded to a course of prednisolone given in a starting dose of 60 mg/m^2/day for four weeks, followed by 40 mg/m^2/day on three consecutive days out of seven for a further four weeks[14]. A similar response rate has been reported in adults: in the Guy's series 36 out of 44 patients treated went into remission during a course of prednisolone starting at 60 mg/day and tapering to 20 mg/day at four weeks, maintaining that dose for a further four weeks and then quickly tailing off[2]. The remission rate of untreated patients is approximately 50% at the end of the first year, so steroids clearly hasten remission. The mean time to remission in children is 11 days and nearly 90% of those who are going to respond do so by the end of the third week[14]. The time to response in adults is the same[2,6].

Relapses occur frequently. In the ISKDS study 36% remained in remission, 18% relapsed occasionally and 39% relapsed frequently. 7% did not respond to the first course of steroids but only 2% had persistent nephrotic syndrome[15]. Adults are less likely to suffer regular relapses. About 40% remain in long term remission and 60% have at least one relapse. Thereafter about 50% relapse after each subsequent course of prednisolone[6]. The mean time to relapse after each course of steroids is 21 weeks (Table 1.3)[6]. Once remission has lasted for more than 3 years, a further relapse is uncommon[16], but does rarely occur even after a decade or more.

TABLE 1.3 Number of relapses in a group of 34 patients with MCN. Three went into remission spontaneously and 30 were treated with at least one course of prednisolone

No. relapses remission	*No. patients*	*Duration (months)*
0	16	—
1	7	22
2	6	15
3	2	21
4	2	23
7	1	—

Treatment of frequent relapsers

Many children and some adults who relapse several times require numerous courses of prednisolone or a maintenance dose to prevent a further relapse. They are thus liable to develop the unpleasant side effects of this drug. Some patients develop post-medication hypocortisolism as detected by a subnormal cortisol response following the administration of ACTH. These patients are more likely to relapse earlier than patients with a normal response and should be treated by a more prolonged tapering of the dose of prednisolone[17].

Effective alternative regimens to repeated courses of prednisolone have been established

Cyclophosphamide 3 mg/kg/day for 8 weeks has been shown to prolong remission in this group of patients without causing major early side effects (Figure 1.6)[18,19]. Longer term toxicity, such as sterility, has not yet been completely ruled out but is unlikely. The suppressive effect on T-helper cells is not measurable 3 months after the end of the course[20]. A trial of cyclophosphamide lasting 2 weeks did not confer any advantage over steroids alone. Cyclophosphamide given with prednisolone causes a longer remission than cyclophosphamide given alone.

Chlorambucil has a similar effect which has also been upheld in a controlled trial. The protocol of the trial was slightly different. Chlorambucil was given in a dose known to cause leucopenia and it was stopped when this was achieved. Ten patients who had suffered several relapses received a total dose of 16.9 mg/kg over 9.7 weeks. No relapse occurred in the follow up period of between 12 and 34 months whereas the 11 patients treated with prednisolone alone continued to relapse. No side effects except leucopenia were recorded[21]. However, there is not much information about long term consequences and it is possible that cumulative doses of more than 8 mg/kg may be toxic to male gonads.

Nitrogen mustard has also been shown to induce prolonged remission in this group of patients in an uncontrolled study. 12 children were given 0.1 mg/kg intravenously within 7 days of a steroid-induced

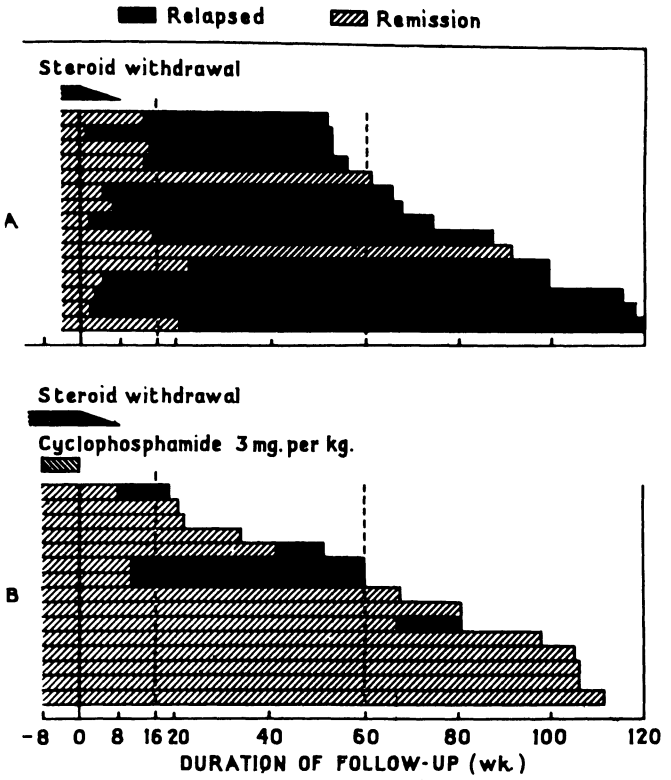

FIGURE 1.6 Results of controlled trial of steroids v. steroids+ cyclophosphamide 3 mg/kg/day for 8 weeks in children with multiple relapses

remission. Some of them had previously received other cytotoxic drugs. 46% of the children had no further relapse during a follow up period of up to 27 months. Six suffered a relapse within 9 months[22].

Azathioprine appears to offer no advantage over prednisolone alone and should not be used[23].

Pulses of intravenous methyl prednisolone promote remission but not as reliably as courses of oral prednisolone. On the other hand, they may not cause as many side-effects[24].

A reasonable strategy for the treatment of frequent relapsers is to give prednisolone for the initial episode and the first three relapses. The fourth relapse should be treated with prednisolone and an 8 week

course of cyclophosphamide 3 mg/kg day. The white blood count should be monitored weekly. If more relapses occur, which is very rare in adults but more common in children, then a further course of prednisolone should be given and remission maintained by prednisolone given on alternate days for a period of 6 months. A second course of cyclophosphamide or a course of chlorambucil may be necessary to spare patients from the toxic effects of steroids.

Other measures

Cyclosporin: Some case reports and short uncontrolled series suggest that cyclosporin may become a useful additional treatment but its place in the protocol is at present confined to the treatment of the small number of patients who continue to relapse despite receiving the regimen outlined above[25].

Patients with seasonal relapses due to pollen sensitivity may be protected by desensitization[4]. One intriguing report treated 6 children with numerous relapses by an elemental diet thus excluding all milk products. Five of the 6 had a history of atopy and positive intradermal skin tests to cows milk. All became protein free during the period on the diet and relapsed when milk was reintroduced[26].

COMPLICATIONS OF MINIMAL CHANGE NEPHROPATHY

The nephrotic state is associated with several complications although they are not as common in MCN because the patients do not remain nephrotic for as long as with other nephropathies and tend to be younger and therefore less prone to vascular complications.

(a) Infections

In the era before antibiotics, infections contributed to the high mortality, estimated at 67% at 5 years[27]. Several factors may contribute to an increased susceptibility to infection. The patients may become protein deficient; the increased fluid content of all tissues may reduce the effectiveness of the inflammatory response; reduced levels of IgG and IgA are common and the function of leucocytes and lymphocytes is impaired. Pneumonia, peritonitis and cellulitis were common and the causative organisms were often pneumococci and Gram negative bacilli. Staphylococcal infections were surprisingly rare[28]. Infections are now rarely a major problem; there were none in the Glasgow series and two in the Guy's series of 49 adults. Early recognition and prompt treatment with antibiotics are responsible for the elimination of this threat even in patients being treated with immunosuppressive agents.

(b) Hypercoagulability[29]

The combination of increased production of acute phase coagulation factors, urinary loss of fibrinolytic factors, increased plasma viscosity, haemoconcentration as a result of diuretic therapy and hyperlipidaemia predispose patients with MCN to the formation of thrombus. Arterial thrombosis is rare compared with other causes of the nephrotic syndrome such as membranous nephropathy. No adult in the Guy's[2] or Glasgow[6] series suffered an arterial occlusion of clinical significance. However, femoral arterial puncture to obtain blood should be avoided as it may lead to thrombus formation. Venous thrombosis is commoner and may cause pulmonary emboli. Thrombi may form in the veins of the legs or in the renal veins. There have been no systematic studies of renal vein thrombosis in MCN as there have in membranous nephropathy and clinically it does not appear to be a major problem – we have not had to use anticoagulants in any patients with MCN but have anticoagulated 11 patients with membranous nephropathy.

(c) Failure of growth

Children rarely grow normally while nephrotic but undergo catch up growth when in remission. The causes of delayed growth are protein malnutrition and steroid therapy which antagonize the peripheral effect of growth hormone. This is an important consideration in the decision when to introduce cyclophosphamide or chlorambucil into the treatment regimen.

(d) Renal failure

A minor degree of renal failure is common during the nephrotic phase of MCN. In a series of 30 patients, the creatinine clearance increased from 89 ml/min to 116 ml/min when the patients went into remission[6]. Rarely, patients present with severe renal failure requiring dialysis. They are usually over the age of 50, and some, but not all, have had previous diuretic therapy. Recovery is common but may take up to 2 months to occur[30]. Irreversible renal failure has also been described[31]. The cause of renal failure is not clear except in those who are hypovolaemic as a result of diuretics. It is possible that the interstitial pressure in the kidney rises, causing a fall in the filtration pressure across the glomerular capillary[30].

FAILURE TO RESPOND TO TREATMENT

In all published series, a small proportion of patients fail to respond to the initial course of prednisolone. If the definition of MCN includes steroid responsiveness, then these patients do not have MCN. However, some with all the pathological features of the syndrome fall into this category and therefore it is a practical problem. There are several possible reasons for this. It is possible that whatever caused the relapse is still present and nullifies the effect of steroids. One patient with a classical history of several steroid sensitive relapses became resistant for a 2 year period during which he had varying doses of prednisolone and a course of cyclophosphamide. It was then

discovered that he had tuberculous osteitis. When this was treated, he quickly responded to a course of prednisolone and did not relapse again[31a]. Therefore, failure to respond should provoke a search for the cause. However, it will rarely be found.

More commonly, a review of the biopsy or a repeat biopsy will reveal histological features of another glomerulopathy. Routine use of immunofluorescence techniques makes it unlikely that early membranous nephropathy will be missed. It is more difficult to be sure that the patient does not have focal and segmental glomerulosclerosis and hyalinosis (FGS). This appearance was originally described by Rich and the juxta-medullary glomeruli are involved first. Affected glomeruli may not be present on the biopsy, and unaffected glomeruli look normal on light microscopy and on immunofluorescence. FGS is found in about 7% of both children and adults who present with the nephrotic syndrome. It is uncertain whether it is a separate entity to MCN or whether a small proportion of patients with MCN go on to develop FGS. McGovern first drew attention to this possibility in 1964[32]. He reported that nine of the 39 patients whose first biopsy was consistent with MCN developed changes of FGS when rebiopsied up to 6 years later. Others have published similar results[33]. The prognosis of FGS is, of course very different from MCN; it is often resistant to both prednisolone and cyclophosphamide and commonly progresses to terminal renal failure. Since no large series of patients with MCN has a significant minority who develop terminal renal failure, the transformation must be rarer than suggested. At present there is insufficient data to reach a conclusion.

A recently described syndrome, the glomerular tip lesion, may also be confused with MCN[34]. It is characterized by the presence of a segmental lesion in the glomerular tuft at the opposite pole to the hilum. It is much rarer than MCN and does not respond as regularly to steroids. It does not progress to renal failure but is sometimes associated with minor elevations of the serum creatinine[34].

OUTCOME

Most adults will be in remission and off all treatment with normal or near normal renal function within 5 years of presentation[6,35]. Relapses after 3 years of remission are extremely rare. Rarely, a patient may die of a pulmonary embolus in the acute phase. The mortality in the Guy's report was 18% and the average age at death was 70 years which is very similar to that of the general population. Only one died of uraemia with continuing nephrotic syndrome[2]. Only one patient in the Glasgow series died and the cause of death was the carcinoma of the bronchus which was the probable cause of the renal lesion[6].

The prognosis in children is similar, although deaths from unrelated causes are obviously rarer. A cross-sectional review undertaken 1–15 years after presentation found that 62% were in remission without treatment, 9% were in remission on treatment, 22% had active disease and 7% were dead of whom 1.4% died of a renal cause[36].

PATHOGENESIS OF MCN

Aetiology of oedema

The traditional explanation of the cause of oedema in nephrotic syndrome is based on Starling's hypothesis (Figure 1.7). This stated that a loss of albumin from the circulation leads to a reduction in plasma oncotic pressure and therefore a reduction in the volume of interstitial fluid drawn back into the venous end of the capillary. The consequent reduction in blood volume is made good by salt and water retention mediated by various mechanisms which include increased release of renin, angiotensin II and aldosterone. Remarkably little evidence for this hypothesis has accrued. Although the plasma oncotic pressure is undoubtedly reduced, direct measurement of the oncotic pressure of interstitial fluid, obtained by implanting a nylon wick subcutaneously, shows a similar reduction so that the difference between the two is only slightly less. It was measured as 6.2 mmHg in relapse and 8.7 mmHg in remission[37]. The plasma volume measured during the phase of sodium retention has been found to be normal, high or low, although most studies have shown it to be normal or

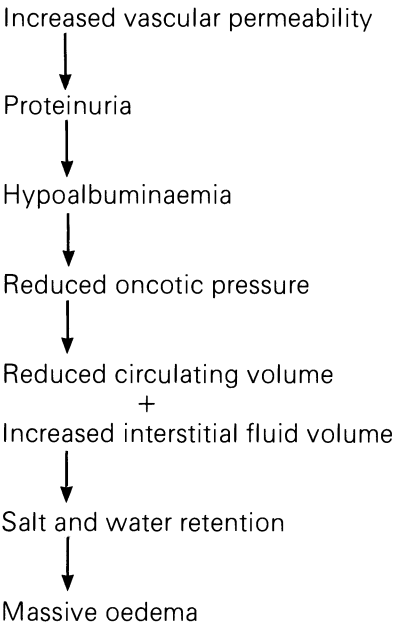

FIGURE 1.7 Traditional explanation for the formation of oedema in the nephrotic syndrome

slightly increased[38,39]. Similarly, plasma renin activity and plasma aldosterone concentrations are not consistently elevated[38,39] and inhibition of this pathway by inhibitors of angiotensin-converting enzyme does not lead to a diuresis.

If hypovolaemia does not occur in nephrotic patients, then it is not the trigger for salt and water retention. Another possible explanation is that the glomerular disease results in both proteinuria and salt retention. There is some experimental evidence for this. Proteinuria induced in one kidney by the infusion of puromycin aminonucleoside into one renal artery leads to salt retention by that kidney but not by the healthy contralateral kidney even though they share a common blood supply[40]. The mechanism by which this is mediated is not understood. Patients with MCN have a normal renal blood flow but a reduced glomerular filtration rate. Thus the filtration fraction is reduced and urinary flow through the distal tubule is also reduced.

This may be a signal for increased salt reabsorption in the proximal tubule. The possibility that antidiuretic hormone contributes to water retention has not been completely excluded[41]; certainly, patients in relapse cannot excrete a water load as completely as when in remission[41]. Whatever the mechanism, primary impairment of water and salt excretion by the kidney appears to be a major cause of the oedema of the nephrotic syndrome (Figure 1.8).

Aetiology of proteinuria

The glomerular basement (GBM) is normally impermeable to albumin. The exact nature of the barrier has been the cause of controversy but is now better understood. The GBM consists of a matrix of Type IV collagen, laminin, nidogen, entactin and glycoaminoglycans of which heparan sulphate is the most important[42]. The structural rigidity of the GBM is conferred by Type IV collagen and the negative charge with which it is covered is largely due to heparan sulphate[42]. This is concentrated in the laminae rarae (Figure 1.9). It is this negative charge, called the glomerular polyanion, which prevents the passage of negatively-charged molecules of the size of albumin. Most animal and human diseases causing proteinuria are associated with loss or masking of the glomerular polyanion. Infusions of a polycation such as hexadimethrine cause proteinuria to develop within 40 minutes[43]. Certain changes in the GBM also occur; the polyanion is diminished as judged by a reduction in staining by cationic dyes such as alcian blue and the structure of the foot processes of the epithelial cells is lost as it is whenever proteinuria is present. The morphology of the foot processes can be restored within minutes by the infusion of a polyanion such as heparin[44]. The importance of heparan sulphate as the proteoglycan which confers the majority of the negative charges was confirmed by infusion of heparanase into the renal artery. Loss of the anionic sites resulted, whereas infusions of neuraminidase, hyaluronidase and chondroitinase had no such effect[45].

The importance of the polyanion to the permeability characteristics of the GBM was confirmed by another set of experiments. Brenner *et al.* studied the clearance of dextrans of varying sizes and charges in

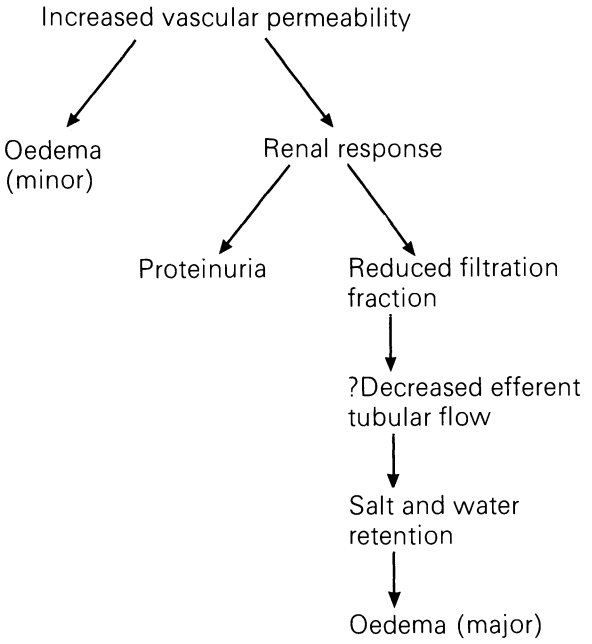

FIGURE 1.8 Alternative explanation for oedema formation in the nephrotic syndrome

normal rats and in rats with nephrotoxic serum nephritis (NSN). They found that over a narrow size range of 20–40 nm, the clearance of cationic dextrans was greater than that of neutral which in turn was greater again than that of anionic dextrans of a given size. In kidneys affected by NSN, in which the GBM charge is lost, the clearance of negatively-charged dextrans was increased as would be expected in a disease associated with proteinuria but the clearance of neutral dextrans was actually reduced compared with the normal kidney[46]. Earlier, Robson *et al.*[47] had performed the same type of experiment in patients with MCN using polyvinylpyrrolidone (PVP) of different sizes. PVP is another neutral molecule. They had intended to show an increase in clearance of larger molecules of PVP during relapse, but their results were inexplicably inverted and showed a reduced clearance of PVP molecules with a radius of less than 40 nm (Figure 1.10). It was not until Brenner's experiments in rats, described above, were

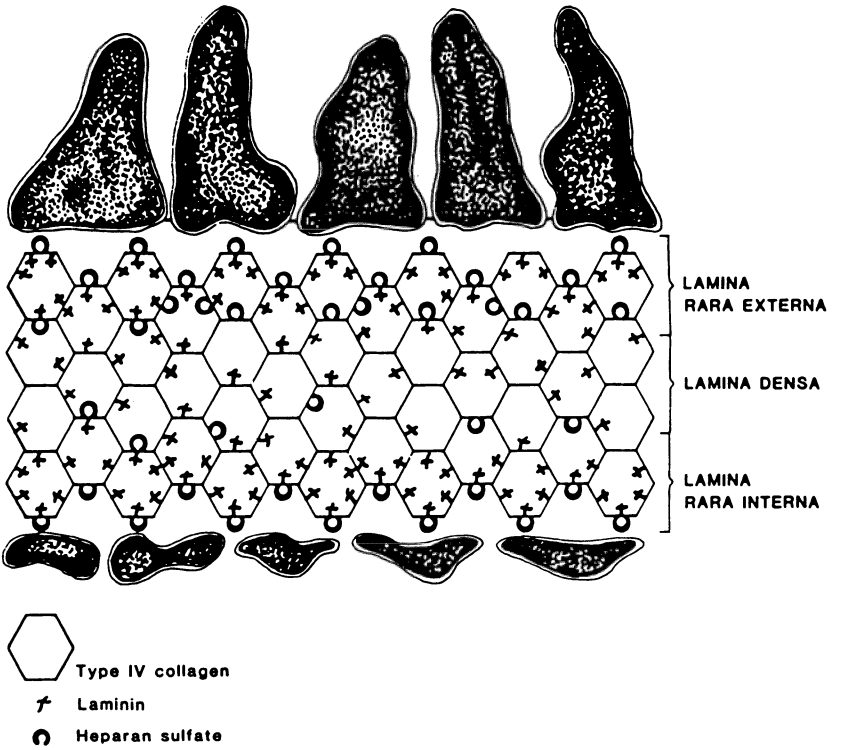

FIGURE 1.9 Diagrammatic representation of the glomerular basement membrane, taken from Martinez-Hernandez and Amenta, Lab Invest, 1983, 48, 656–677. Type IV collagen is distributed throughout the GBM, heparan sulphate and laminin are concentrated in the laminae rarae. Entactin (not shown) is probably concentrated in the lamina rara interna

published that the importance of molecular charge was understood. The abnormality of the GBM in MCN is due to changes in the glomerular polyanion which fails in its function of repelling negatively-charged molecules of a narrow size range that includes albumin thus causing a 'selective' proteinuria.

Aetiology of reduction in the charge of GBM in MCN

In 1974, Shalhoub proposed that MCN was caused by a toxin released by T lymphocytes which was directly or indirectly toxic to the GBM[48].

His reasons for formulating this hypothesis included the observation that children with MCN often went into remission when they contracted measles. The measles virus inhibits many functions of T lymphocytes including their ability to secrete lymphokines of which the normal function is to facilitate delayed hypersensitivity reactions. Secondly, there is a well-recognized but rare association between Hodgkin's lymphoma and MCN. Although Hodgkin's is usually associated with anergy, it was argued that this was because the cells were already committed to maximal function which in a few patients included the production of the factor toxic to the GBM. Thirdly, both

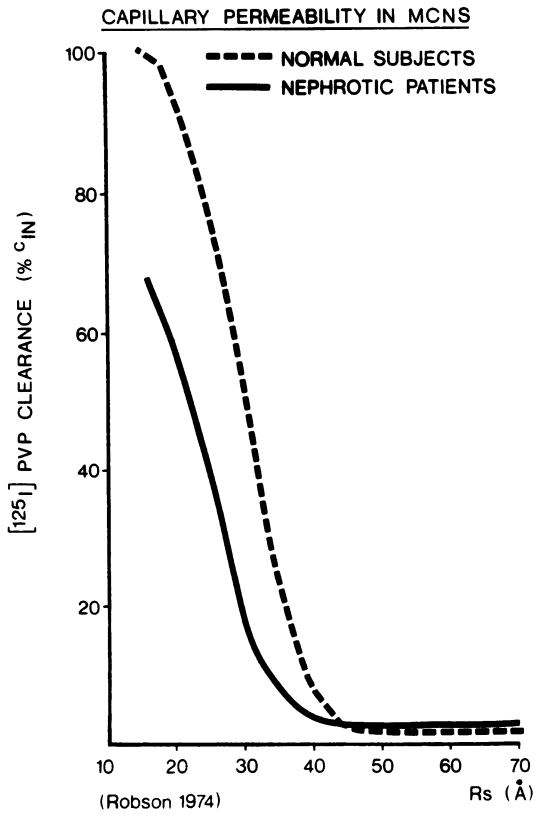

FIGURE 1.10 Clearance of polyvinylpyrrolidone (expressed as a percentage of the clearance of inulin) of differing sizes in children with MCN during relapse and remission[47]

prednisolone and cyclophosphamide act against long-lived T cells, whereas azathioprine does not. This reflects their effectiveness in the treatment of MCN. Other evidence of T cell dysfunction has been inferred from the finding that serum concentrations of IgG and IgA are low even in remission but IgM levels remain high[49]. Since it is a function of T cells to convert from IgM to IgG production, these abnormalities may be due to malfunction of T lymphocytes in patients with MCN.

All this evidence is inferential, but it was not long before more direct support for the hypothesis appeared. Lagrue and his colleagues[50] demonstrated that a soluble factor which increased vascular permeability was released by the lymphocytes of patients with MCN when cultured with a non-specific mitogen. This factor (VPF) was measured by a bioassay, in which the supernatants of lymphocyte cultures were injected intradermally into the skin of a guinea-pig. Evans blue was then given intravenously and the area of blueing around the injection sites was measured. The assumption was that if the factor caused an increase in the permeability of skin capillaries, it was likely to cause proteinuria in the high pressure glomerular capillaries. Lagrue's results showed that the lymphocytes of patients with MCN released much more VPF than normal lymphocytes, but not more than the lymphocytes of patients with membranous nephropathy or membrano-proliferative glomerulonephritis, both of which are associated with heavy proteinuria. Subsequent studies have shown that the lymphocytes taken from patients during relapse release VPF whether stimulated by a mitogen or not, whereas production of VPF by lymphocytes from control subjects was increased by phytohaemagglutinin, but not to the level produced by patients' cells[51] (Figure 1.11). This suggests that the lymphocytes are already maximally activated *in vivo*. Fractionation of lymphocytes into sub-sets led to the identification of T cells as the major producer of VPF[52]. Lymphocytes taken in remission do not release excess VPF when cultured with a mitogen.

The effect of the supernatants of these cultures containing VPF on the glomeruli has been studied. Infusion of the material into the renal arteries of rats led to a reduction of the glomerular polyanion as detected by reduced binding of the cationic dye, alcian blue (Figure 1.12), and to changes in the structure of the foot processes of the

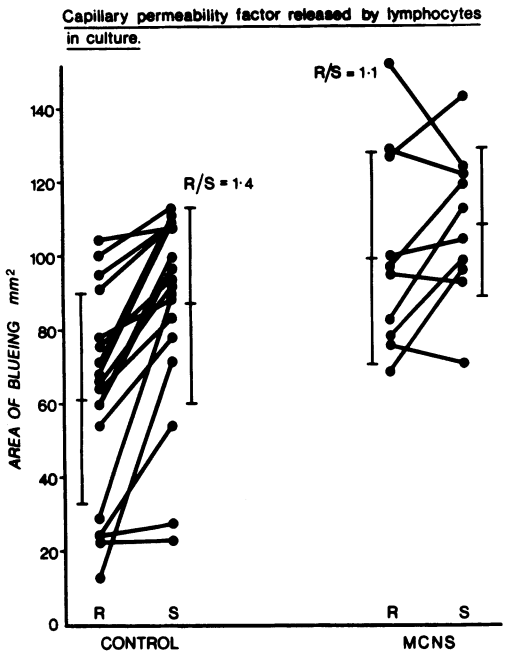

FIGURE 1.11 Bioassay of VPF released by lymphocytes of controls and patients during relapse of MCN before the start of steroid therapy. The results are expressed as the area of blueing following an intradermal injection of the lymphocyte supernatant into the skin of a guinea pig and an intravenous injection of Evans blue. The results from both unstimulated (R) and mitogen stimulated (S) are shown. Mitogen stimulation of control lymphocytes caused production of VPF but did not increase the production by patients' cells. Patients' lymphocytes produced significantly more VPF than those of controls[51]

epithelial cells (Figure 1.13) consistent with development of proteinuria[53]. However, the infused supernatant contained both lymphocyte products and autologous serum which itself may cause proteinuria. Another report described similar reductions in the glomerular polyanion when slices of rat renal cortex were incubated in the supernatants of lymphocyte cultures which did not contain autologous serum[54]. The results of these two experiments suggest that VPF is released by the lymphocytes of nephrotic patients, that its release is

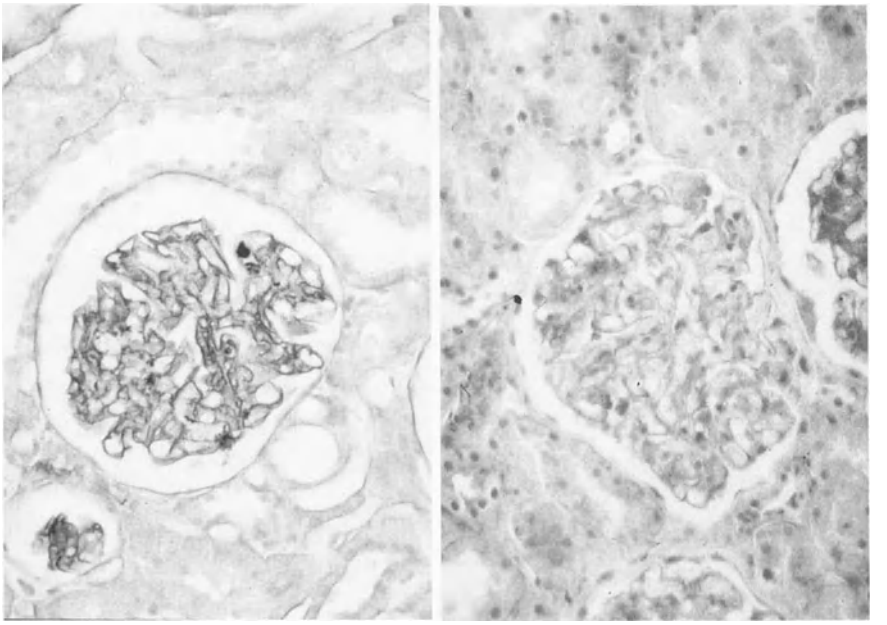

FIGURE 1.12 The effect of perfusion of VPF + nephrotic serum contained in lymphocyte culture supernatants into the renal artery of normal rats on the glomerular polyanion which has been stained by alcian blue. The glomerulus on the left has been perfused by supernatant of control lymphocytes and the GBM is clearly outlined. The glomerulus on the right shows the result of perfusion by a patient's lymphocyte supernatant and the staining of the GBM is much less marked indicating reduction of the glomerular polyanion[53]

not inhibited by autologous serum, and that it can cause glomerular changes compatible with the development of proteinuria.

Clinical events known to exacerbate MCN are also known to cause the release of lymphokines. Pollen is a cause of relapse in a minority of patients[3,4] and has been shown to stimulate the lymphocytes of sensitive patients to release another lymphokine called macrophage migration inhibitory factor. Circulating immune complexes have been detected in the serum of patients early in relapse and disappear as the patients go into remission[55]. Complexes also stimulate lymphokine production. Tuberculosis, which is a classical inducer of delayed hypersensitivity reactions, also provokes and sustains relapse[31a].

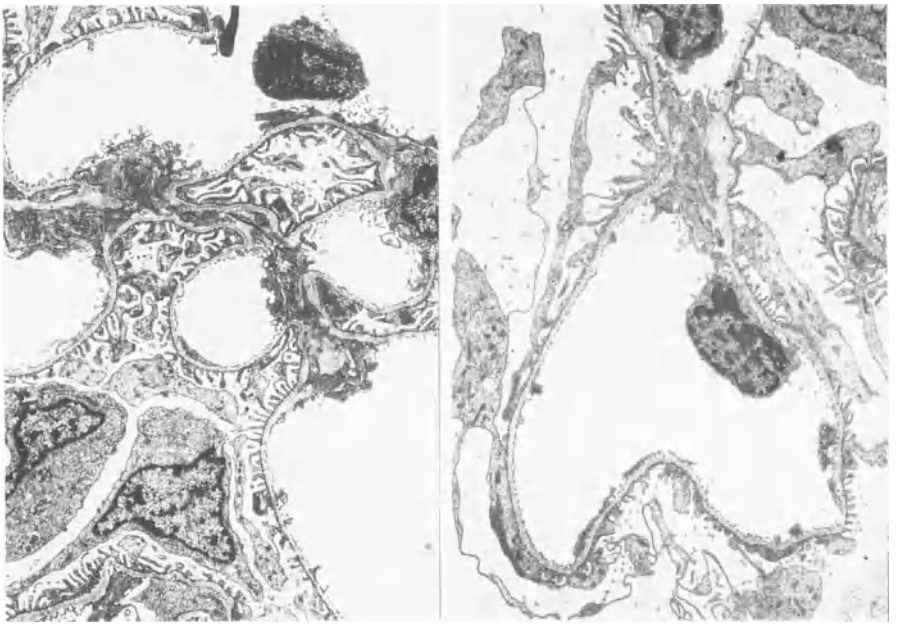

FIGURE 1.13 The effect of perfusion of VPF + nephrotic serum on the ultrastructure of the epithelial foot processes. The control is on the left and the patient on the right, showing considerable loss of structure of the processes[53]

Therefore, this mechanism is consistent with clinical observations other than those already identified by Shaloub.

Identification of the factor has proved difficult. It has a molecular weight of 12 000 daltons and an isoelectric point of 6.4[56]. It is unclear whether it is released systematically or locally within the kidney. Since infusion of nephrotic serum causes proteinuria when infused into the renal artery of animals[57,58] and since proteinuria may follow within hours of transplantation of a patient whose original disease was FGS[57], it is more likely that it is released systemically. However, lymphokines usually act locally and have a very short half life. Failure to find lymphocytes in the kidney by light microscopic examination is not convincing evidence that they are not there as the kidneys of patients with MCN take up gallium avidly but neutrophils are not easily seen on routine microscopy either[59]. It is possible that lymphocytes accumulate in the kidney as a result of renal antigens to which *in*

vitro sensitivity has been demonstrated by a direct lymphocytotoxicity test[60].

One other series of observations deserves mention. The serum of patients with MCN inhibits many *in vitro* functions of lymphocytes, such as phytohaemagglutinin induced lymphocyte transformation[61], production of macrophage migration inhibitory factor (another lymphokine)[53] and mixed lymphocyte reactions[61]. This factor has been found in the serum of all nephrotic patients including diabetics and patients with amyloid. It is likely therefore to be a consequence of proteinuria, especially as the degree of inhibition is proportional to the proteinuria. The phenomenon is not restricted to *in vitro* reactions; patients with MCN proved very difficult to immunise successfully with an antigen which normally induces cell-mediated immunity[62]. The factor responsible for this profound effect on T cells has a molecular weight of between 60 and 160 kilodaltons, is heat stable and has a strong affinity for the surface of lymphocytes. Its inhibitory effect could be reversed by the addition of 10% normal human serum to the cultures[63].

Therefore, it is reasoned that another counteractive factor, present in normal serum, is lost in the nephrotic state. Since its molecular weight is 60 000 daltons, it may well be filtered across the GBM of nephrotic patients, and thus be lost as a consequence of the nephrotic state[63].

Proteinuria may result from the release of VPF in many conditions including MCN. The lymphocytes of normal subjects also release VPF, and a minor degree of proteinuria is sometimes seen in febrile patients. However, the gross degree of proteinuria found in nephrotic patients is never seen. This may be because of inherited differences in the glomerular polyanion. The smaller the charge on the GBM, the easier it is to reverse it and so cause proteinuria. There is some indirect evidence that patients with MCN and idiopathic membranous nephropathy may have a reduced glomerular polyanion both in remission[64] and relapse[65]. The evidence rests on the similarities between the kidneys of patients with membranous nephropathy and Lewis rats, which are susceptible to Heymann's nephritis – a good animal model of membranous nephropathy. Lewis rats have a lesser charge on their GBM than DA rats which are resistant to this form of nephropathy[66]. Infusion of a polycation causes proteinuria in Lewis rats but not in

DA rats[67]. The difference in charge of the GBM of these two strains of rats is also found on their red cells. Examination of the red blood cell charge in patients who were in remission after an episode of nephrotic syndrome due to either MCN or membranous nephropathy showed that the charge was at the lower end of the normal range, and in contrast the charge of the red cells of patients with IgA nephropathy was at the higher end of the normal range and was significantly greater than the charge of the other two patient groups (Figure 1.14)[64]. IgA nephropathy is rarely associated with heavy proteinuria but the lymphocytes of these patients release as much VPF as do the lymphocytes of patients with MCN[68]. It is likely, therefore, that the degree of proteinuria which develops in an individual is a result of the amount of VPF produced and the concentration of negative charges on the GBM. The role of the inhibitory factor in the pathogenesis of proteinuria remains unknown but is probably a non-specific consequence of the glomerular changes[63].

An alternative and perhaps additional explanation has recently been advanced. Soluble immune response suppressor (SIRS) has been found in the urine and serum of patients with steroid-responsive nephrotic syndromes of which MCN was the predominant type, but not in FGS which was not responsive to prednisolone. SIRS is a protein with a MW of 15 000 daltons which inhibits antibody production *in vitro* and *in vivo* and delays hypersensitivity reactions in mice. It is therefore capable of effecting the immunosuppression found in the nephrotic syndrome. SIRS disappears from urine and serum rapidly following the administration of steroids before proteinuria is reduced. It is produced by suppressor T cells and the cells of nephrotic subjects do so *in vitro* without requiring activation by mitogen, whereas lymphocytes of normal subjects require the presence of concanavallin A to stimulate production[69]. Thus there are several similarities between SIRS and VPF (Table 1.4) although the effect of SIRS on vascular permeability has not yet been tested. If they were the same substance, it would offer an explanation for the production of a lymphocyte product causing an increase of vascular permeability when all other lymphocyte activities are suppressed.

SIRS alone cannot account fully for the immunosuppression of the nephrotic syndrome because of the results of the study described above and because absorbing SIRS with an anti-SIRS antibody only partially

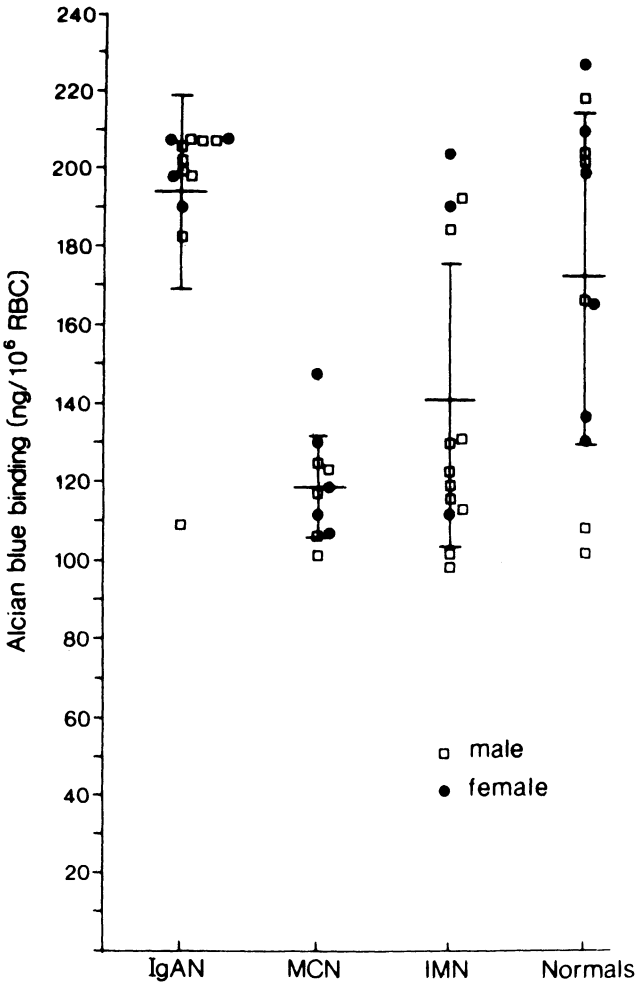

FIGURE 1.14 Red cell charge of patients with IgA nephropathy, MCN and membranous nephropathy compared with controls. There was no significant differences in the proteinuria of the 3 patient groups at the time of study so that the difference in charge is not a result of the disease processes. All fell within the normal range[64]

reduces the inhibitory activity of nephrotic serum[69]. Therefore, the immunosuppression of the nephrotic syndrome is multifactorial.

TABLE 1.4 Comparison of VPF and SIRS

	VPF	*SIRS*
Molecular weight (daltons)	12 000	15 000
Produced by	T-cells	T-cells Suppressor
Requires mitogen in culture	No	No
Present in serum	?	Yes
Demonstrated in MCN	Yes	Yes
Membranous	Yes	Yes
MPGN	Yes	Yes
IgA nephropathy	Yes	?

MPGN = Membranoproliferative glomerulonephritis.
Cultures refer to cultures of lymphocytes from patients with MCN in relapse before starting steroids.

WHO GETS MCN?

MCN is a comparatively rare disease. It occurs in children more than adults, and is more common in boys than girls. Associations with various HLA types have been described. HLA-B12 is more common in atopic children with MCN than in the general population or in patients with MCN who are not atopic. Atopic patients who are HLA-B12 are likely to relapse earlier after a course of cyclophosphamide[70]. These observations have not all been confirmed by subsequent studies[71]. An association between MCN and HLA-DR7 has been reported in two series with a relative risk factor of 4.4 and 5.5[72,73]. A study of the nephrotic syndrome in European families showed that 3.35% of nephrotic children had an affected sibling and that MCN was the glomerular lesion in 50% of those over one year of age. However the response to steroids was not quite as good in these children as in the non-familial cases[74].

Some of the clinical events which can trigger a nephrotic episode in a susceptible individual have been mentioned already: an upper

respiratory infection is a particularly common antecedent, but the cause of the sore throat is unknown. All other recognized causes are very rare; in particular the classical association with Hodgkin's disease is uncommon[75]. Other malignancies, such as carcinoma of the bronchus, are also occasional associations[76]. There are case reports linking MCN to bee sting[77], poison oak[78] and even decompression[79]. Grass pollen and cow's milk allergies are proven causes in a handful of patients. High serum IgE concentrations found in childhood nephrotics[80] and the high incidence of atopy suggest that reagenic reactions may be common initiating events. Tuberculosis, and presumably other infections, may precipitate or sustain a relapse in a susceptible individual. However, it may be difficult to be sure of the association – for example, hepatitis B infection has been linked with MCN but in a study from Hong Kong, it was shown that the incidence of HBV infection was no higher in patients with MCN than in the general population[81].

Regrettably, the cause is rarely identified.

SUMMARY AND HYPOTHESIS (Figure 1.15)

MCN occurs in patients with an inherited predisposition, which may be:

1. an unusually large amount of VPF produced by their lymphocytes in response to a variety of stimuli;
2. failure to switch off production of VPF due to the insensitivity of their lymphocytes to the inhibitory signal;
3. the low density of anionic sites on their GBM.

The reasons why children are more susceptible than adults or why patients seem to grow out of their proneness to the disease are unknown.

The release of VPF, either locally or systemically, leads to an increase in vascular permeability as a result of loss or masking of the anionic sites. Proteinuria is the dominant clinical event because of the high pressure of the glomerular capillaries. It is associated with retraction of the epithelial cell foot processes.

The major degree of immunosuppression found in these patients is due to two causes. A counteractive factor is lost in the urine which leads to full expression of an immune inhibitory substance. Immunosuppression also results from the production of SIRS by T cells as a direct consequence of the original trigger. The possibility that VPF

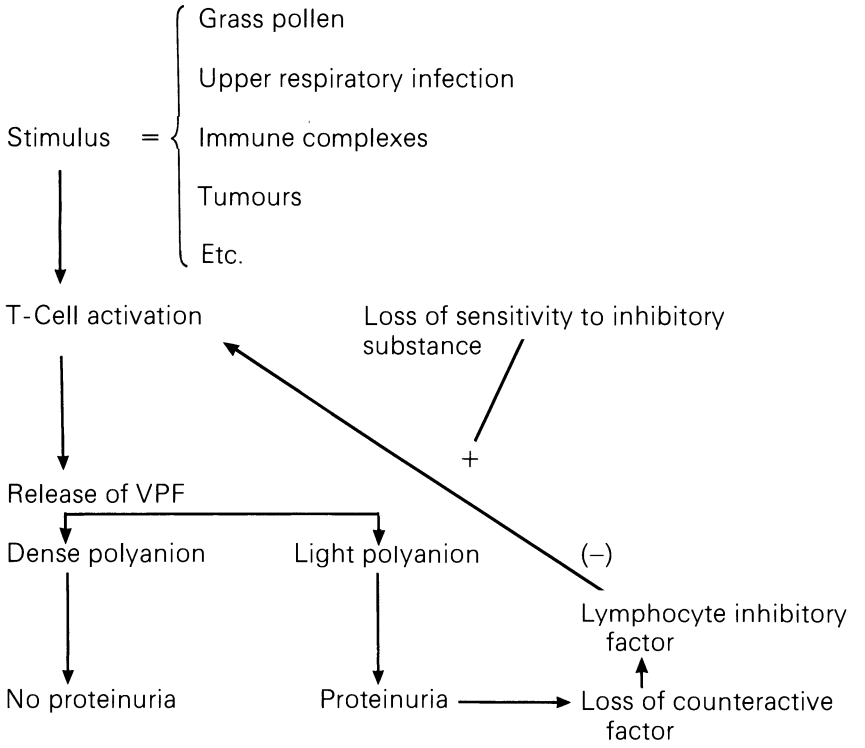

FIGURE 1.15 Hypothetical diagram of the pathogenesis of MCN

and SIRS are the same substance is very attractive but the effect of SIRS on the glomerular polyanion has not yet been tested. They have many properties in common but whereas VPF is released by the lymphocytes of patients with many different glomerulopathies, it appears that SIRS is only found in patients with steroid-sensitive

nephrotic syndrome; interestingly, it is not released in patients with FGS who do not respond to steroids[69].

Only patients with an inherited or acquired reduction of glomerular capillary anionic sites respond by developing proteinuria. Although loss of albumin causes a reduction in plasma oncotic pressure, the main cause of oedema is an unidentified renal signal to retain sodium and water. It is possible that the signal is reduced rate of tubular flow as a result of a lower filtration fraction which occurs in patients with MCN.

Therapy with prednisolone or cyclophosphamide prevents further production of VPF by lymphocytes and allows recovery of the polyanion. Future research will identify VPF and lead to more specific and less toxic treatments.

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2

MEMBRANOUS NEPHROPATHY

N.P. MALLICK and C.D. SHORT

INTRODUCTION

The term 'membranous nephropathy' (MN) describes a histopathological pattern, in which electron microscopy (EM) initially reveals immune aggregates along the outer (sub-epithelial) aspect of the glomerular basement membrane (GBM) in apposition to the epithelial foot processes. Subsequently, the GBM becomes interposed between the aggregates and may later envelop them completely. The appearance is of a thickened GBM; there is usually little increase in glomerular cellularity[1].

It is now clear that a variety of clinical and experimental conditions may have as their end-point the development of this glomerular pathology (Table 2.1)[2]. 'Membranous nephropathy' describes a histopathology, not an aetiologically defined disease. It is necessary to understand, as far as possible, the underlying pathogenesis in a given individual to obtain an assessment of prognosis, and of the benefit which particular therapeutic approaches may confer. For example, if the condition arises in association with a localized carcinoma, or following exposure to a particular drug, cure of the carcinoma or withdrawal of the drug may abort the nephropathy.

In the Western World most cases are 'idiopathic' but increasingly it is clear that this does not imply a single pathogenesis and that any particular treatment such as corticosteroids is unlikely to influence the course of the lesion in every case[3].

TABLE 2.1 Membranous nephropathy may develop in association with the following conditions

Systemic disorders	Systemic lupus erythematosus Rheumatoid arthritis Sjögren's syndrome Mixed connective tissue disease Dermatomyositis Dermatitis herpetiformis Sarcoidosis Diabetes mellitus Temporal arteritis Sickle cell disease Thyroid disease Ankylosing spondylitis Systemic sclerosis Weber Christian panniculitis Bullous pemphigoid Primary biliary cirrhosis Gout
Infections	Hepatitis B Malaria Schistosomiasis Filariasis Leprosy Syphilis (congenital and secondary) Streptococcal infection Enterococcal endocarditis Hydatid disease
Drug or chemical exposure	Mercury compounds Gold Penicillamine Captopril Probenecid Trimethadione (?Hydrocarbons)
Tumours	Carcinoma (colon, stomach, lung, breast, cervix, kidney, prostate, rectum, pharynx, pancreas) Lymphoma (including Hodgkin's) Leukaemia Phaeochromocytoma Melanoma Angiolymphatic hyperplasia

PATHOLOGY

On light microscopy there is a uniform thickening of the capillary basement membrane without significant hypercellularity of the glomerular tuft. GBM thickening is seen by periodic-acid Schiff (PAS)

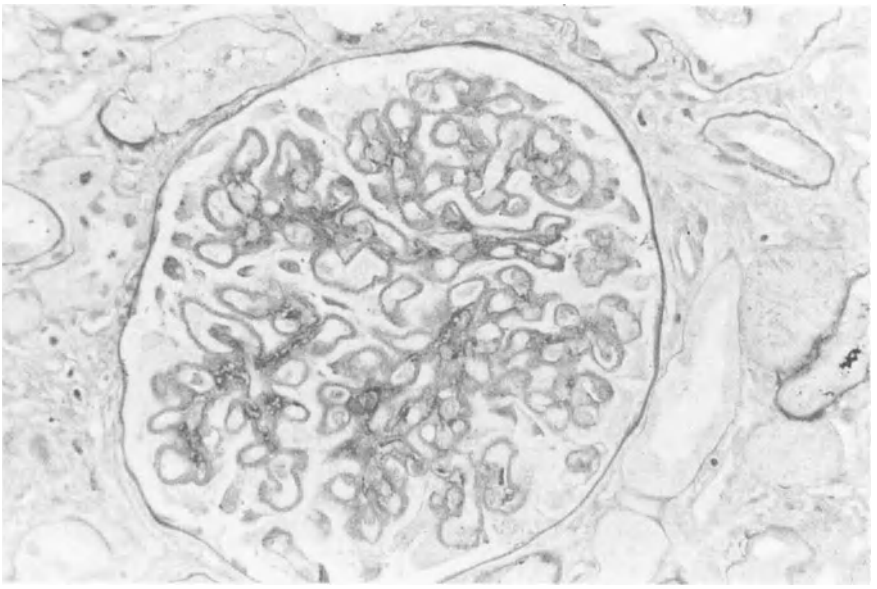

FIGURE 2.1 Light microscopy: PAS staining reveals uniform thickening of the peripheral capillary loops

staining (Figure 2.1). With the periodic-acid silver methanamine technique, argyrophilic extensions of the GBM are demonstrated as black 'spikes' due to lack of light transmission; the interposing immune aggregates appear as lucent areas (Figure 2.2). With Masson's trichome, these deposits have the appearance of red sub-epithelial globules. Global glomerular sclerosis, tubular atrophy, and interstitial fibrosis occur and reflect the degree of nephron destruction; the histopathological features of hypertension may also be present[4]. Rarely, epithelial crescents are seen: this observation requires investigation for anti-GBM disease which may develop on top of underlying MN[5]. Rarely too, both typical MN and mesangial proliferation may be

noted: this is highly suggestive of underlying SLE[6], although co-incident membranous nephropathy and IgA nephropathy has been reported[7,8].

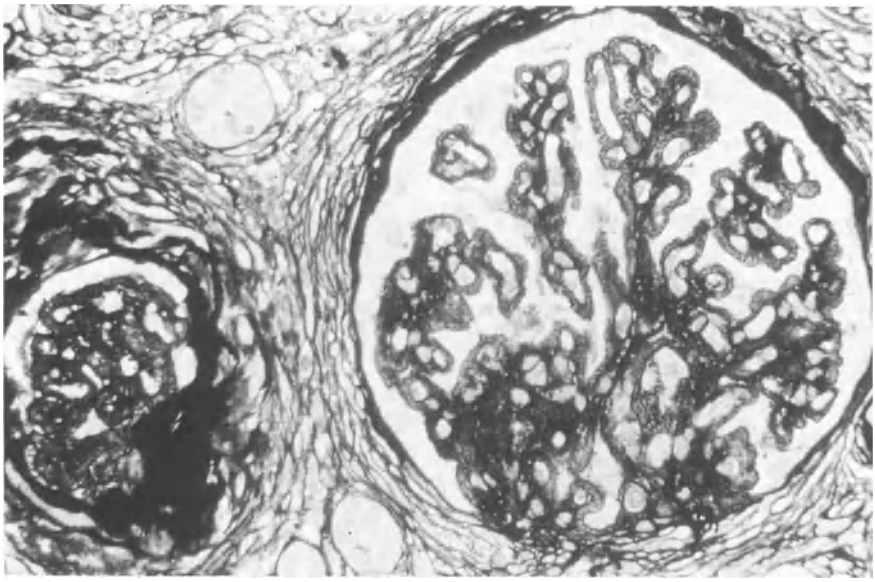

FIGURE 2.2 PASM stain showing the 'spikes' of basement membrane material extending between the immune deposits

Immunofluorescent techniques reveal the deposition of IgG (rarely IgA or IgM) along the GBM with a beaded or granular appearance (Figure 2.3). C3 is found, characteristically, in a similar distribution, but complement components may be less prominent in early disease. Neither complement nor immunoglobulin may be detected in the very late stages[9].

Early in MN, electron microscope studies reveal small discrete electron-dense deposits along the sub-epithelial border of the GBM. Initially these deposits have a diameter similar to the width of the normal GBM (stage I). As the deposits enlarge, the glomerular cells produce more GBM matrix, initially deposited between the immune aggregates, so that on EM section the GBM extends outwards in a 'spiky' fashion (stage II, Figure 2.4). By stage III, GBM material

completely encloses the now large deposits, which may have a diameter of 15 000 Å. (The width of normal GBM is approximately 3 500 Å.) Finally, stage IV is reached when electron-lucent areas appear in place of electron-dense deposits in a distorted and grossly thickened GBM. A stage V lesion, when more than 10% of the glomeruli sampled show global sclerosis, is sometimes noted; this may be particularly pronounced in the juxtamedullary glomeruli. Some authorities use a

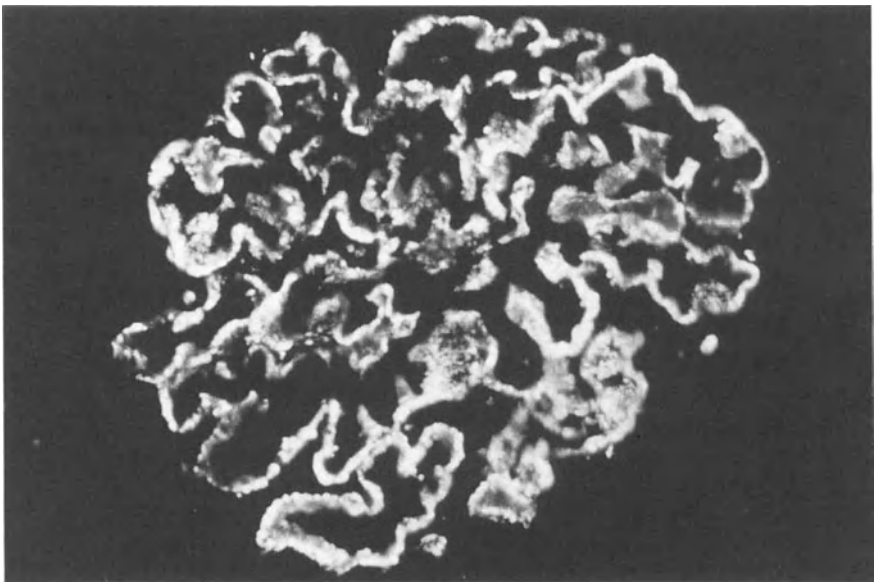

FIGURE 2.3 Immunofluorescence: the FITC-labelled anti-human IgG demonstrates the granular appearance of the immunoglobulin

different staging classification, such that stage V implies resolution towards a normal GBM architecture[10,11].

Although prospective sequential biopsy studies have been performed infrequently, and would be difficult to justify, especially once clinical remission has ensued, sufficient evidence has accumulated from reports where substantial variation in the timing from onset of symptoms to biopsy (and rebiopsy) has elapsed for there to be good grounds for supposing that these stages develop sequentially[12].

However, the rate of progress is very variable and in any one individual progression is not predictable from the onset.

While staging is a broad indication of the time course of the disorder, its clinical relevance remains uncertain. Patients may go into full clinical remission from any stage and without sudden change in ultrastructural appearances. There is probably a tendency for stage I and II lesions to have a better ultimate prognosis[10,13] and some evidence that remission at these earlier stages is more likely to be accompanied over succeeding months by restoration to normal of GBM structure[11]. Whatever the stage at which proteinuria remits completely, GFR will almost certainly remain stable thereafter.

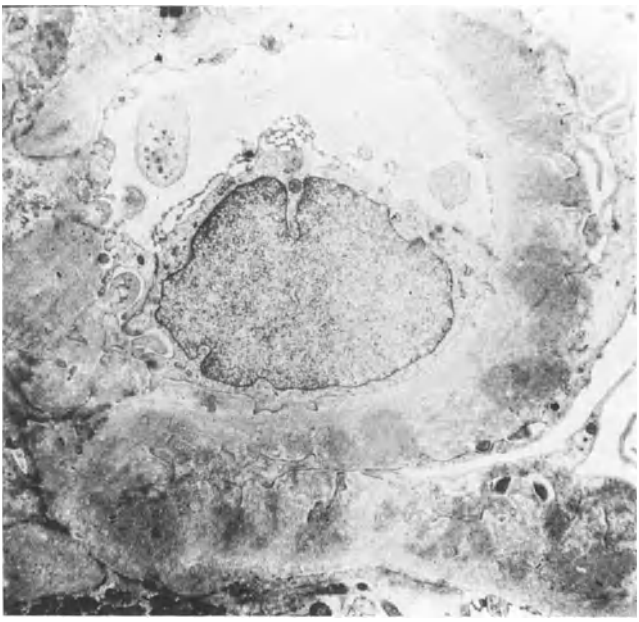

FIGURE 2.4 Electron microscopy: this section of a complete capillary lumen reveals the discrete electron-dense immune deposits, with a markedly thickened GBM which appears to be protruding between these aggregates

The detection of electron-dense deposits subendothelially and in the mesangium, as well as subepithelially, or the presence of mesangial

hypercellularity and the demonstration of a variety of immunoglobulin and complement component deposition, raises the suspicion that the lesion is one in which deposits have formed from different antigen–antibody combinations from an infection or in association with SLE.

PATHOGENESIS

Membranous nephropathy has been produced in the experimental animal. The best-studied model is 'Heymann nephritis'[14,15], in which a crude preparation of tubular brush border, termed FxIA, is injected into allogeneic rats. The active antigen is almost certainly a glycoprotein of molecular weight 330 000 (gp 330)[16]. While other glycoproteins have since been implicated in the genesis of Heymann nephritis, these may be sub-units or multimers of gp 330, or larger molecules of which gp 330 is a part[17]. Other glycoproteins have been proposed[18] but not validated. In rats, gp 330 is present not only in the tubular brush border but also in 'coated pits' on the glomerular aspect of epithelial foot processes[19]. The injection of FxIA in sufficient amounts stimulates the production of antibody; this reacts *'in situ'* with gp 330 in the coated pits of the foot processes. Subsequently, these antigen–antibody complexes are shed from the epithelial cell membrane and come to lie on the epithelial surface of the GBM. They eventually become incorporated into it in the typical sequence of changes seen in MN[20].

This is clearly an auto-immune lesion, triggered by experimental exposure to an endogenous antigen with identical structure to, or sharing epitopes with, a fixed glomerular antigen. In the experimental model, resulting proteinuria is complement dependent, yet neutrophil independent, and does not occur unless the C5–9 membrane attack complex is formed[21].

Two aspects of this model are particularly relevant. First, there is an autoantibody involved, stimulated by an endogenous antigen. Secondly the antigen–antibody complexes which engender the nephropathy are formed *'in situ'* from the fixed antigen and circulating antibody, rather than from the lodgement of circulating, pre-formed complexes in the sub-epithelial space. This sequence of events is confirmed by a modified protocol which utilizes the injection of preformed

homologous or heterologous anti-FxIA antibody into the animal and results in the accelerated development of an identical lesion[22–25].

There is a third consideration. Since the GBM is anionic, or negatively charged at physiological pH, it retards the passage to the sub-epithelial site of similarly charged molecules, while otherwise identical, but positively charged (cationic) molecules pass more readily. Cationic antibodies to gp 330 follow this principle and localize more rapidly

TABLE 2.2 Pathogenetic scenarios

Exogenous antigen	from	an infectious agent
Endogenous antigen	from	malignant tissue
Acquired disordered immunity	in	Hodgkin's disease
Inherent disordered immunity	as with	insulin dependent diabetes

than do their anionic counterparts in the sub-epithelial space[26].

Each of these elements of the Heymann model has been explored experimentally. In Heymann nephritis, the antigen is a fixed constituent of the glomerulus. An exogenous molecule which binds to GBM may act in like fashion to the *'in situ'* antigen in MN. To achieve this the antigen must be cationic, e.g. cationized bovine serum albumen[27]. In another model, an *antibody* raised to a 'third party' antigen has affinity for, and localizes in, the outer GBM. Once bound, it acts as the 'planted antigen' and an anti-idiotypic antibody attaches to it as the antibody moiety of the immune complex[28].

In some experimental models, pre-formed circulating complexes do appear to localize sub-epithelially. However, it seems likely that initially these dissociate within the circulation and then reform along the sub-epithelial border after traversing the GBM independently. This experimental approach extends the range of pathogenetic mechanisms causing MN further than those encompassed simply by Heymann nephritis (Tables 2.2 and 2.3).

While it is unproven that human MN can result from a fixed gp 330 like antigen–antibody reaction, it seems probable that the experimental variants described will be shown to have counterparts in the spectrum of human disorder. For example it has been suggested recently that a glycoprotein of molecular weight 440 kilodaltons in the human kidney may prove to be the counterpart of gp 330 in the rat[29].

TABLE 2.3 Possible pathogenetic mechanisms

(a)	Deposition of circulating immune complexes
	(i) directly
	(ii) after dissociation
(b)	In-situ complex formation
	(i) against intrinsic (Heymann-like) antigen
	(ii) against planted, extrinsic antigen
	– endogenous
	– exogenous

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Both idiopathic and secondary MN probably arise from heterogeneous pathways – an important concept in developing effective treatment. If the pathogenesis varies, so may the therapy, which must be aimed at one or other element critical to the pathogenesis. This might involve the elimination of a circulating antigen or antibody, or of circulating pre-formed complexes, or alternatively may be directed at preventing the incorporation of complement in the immune complexes.

Couser[20] has emphasized that, while such measures may prevent the development or progression of MN in experimental animals, they are much less effective when their use is delayed until the lesion is well established. Since it appears that human MN does not become apparent clinically until it is well established, experimental approaches to treatment may not transfer readily to human disease.

EPIDEMIOLOGY

In most series from European countries, the United States and from Japan, the male:female ratio is about 2:1 for idiopathic lesions[30–33]; probably secondary nephropathies too are commoner in males. In reports from different countries, the incidence of idiopathic lesions is unimodel with a peak incidence between 35 and 40 years of age. Fewer cases of idiopathic membranous nephropathy occur in childhood or

in old age; when the lesion is seen in these age groups an underlying disease should be suspected. In children, glomerular, hepatitis B-derived, antigens are documented[34], while in the elderly, glomerular deposition of antigens from a neoplasm may occur[35] or immunity may have been disturbed by lymphoma or leukaemia, so 'permitting' the development of the nephropathy.

Data from the Glomerulonephritis Registry of the Medical Research Council[36] suggests that in the United Kingdom membranous nephropathy accounts for about 10% of renal biopsies performed for proteinuria and a similar figure holds for other, predominantly Caucasian, series. Neither the true incidence, nor the prevalence can be inferred from these figures however, since they address mainly the findings in patients presenting with nephrotic syndrome and so seeking medical attention. The submerged and asymptomatic majority of patients are never documented and in virtually all these cases the glomerular lesion presumably heals uneventfully[3].

The incidence of secondary MN (Table 2.1) is heavily weighted to children and to those over the age of 55 years. Although there are no certain figures, perhaps one quarter of these cases will have infection or neoplasm-related lesions. Infection and the nephropathy occur together: usually the neoplasm is observed by the time the nephropathy develops but MN may be the first feature of an otherwise occult malignancy which should then become manifest within 2 years.

Repeated, careful clinical assessment is mandatory during the course of MN, particularly in the elderly, so that any underlying, but initially occult, lesion can be detected and dealt with promptly.

Drug-associated MN (Table 2.1) may occur at any age, usually within a short time of the introduction, or re-introduction, of the agent. These nephropathies are of particular interest. Those associated with gold or penicillamine treatment for rheumatoid arthritis are well studied[37,38]. Only patients who are carrying the HLA antigens DR3 or DR2 appear to be at risk[39]. Once the precipitating agent has been withdrawn, the lesions heal clinically and histopathologically, but take months to do so[40]. Such 'secondary nephropathies' represent one exemplar for the common, and eventually healing, asymptomatic idiopathic lesions.

There are a number of other diseases linked to HLA B8-DR3 with which MN is associated, but in which there is no direct link such as a

disease-derived antigen. These are listed in Table 2.4. These lesions, too, are instructive, since they demonstrate the heterogeneous pathogenetic background in which disease-associated MN arises and by inference the term 'idiopathic' must also subsume a similar heterogeneity.

TABLE 2.4 Disorders associated with HLA DR3 in which membranous nephropathy has occurred

Dermatitis herpetiformis
Sjögren's syndrome
Graves' disease
Type I diabetes mellitus

IMMUNOGENETIC ASSOCIATIONS

In any defined population, idiopathic MN (IMN) usually arises in an immunogenetically distinct group. In Caucasian populations the association is with the HLA class I antigens B8 and B18 and the class II antigen DR3[41]. The antigens are common in the general population. However, in the North-west of England, while approximately 20% of the population are DR3 positive, 70% of MN patients express this class II antigen. The relative risk attached to each of the antigens described above is 2.1, 5.0 and 12.0 respectively. There is abundant evidence that these antigens also carry a higher relative risk for other immunologically determined diseases (Table 2.4).

While the *relative* risks hold good the *absolute* risk must be related more to genes located close to, or between the class I and class II sites on the short arm of chromosome 6, and not to the HLA markers directly. One such cluster is of the complement components C4 and factor B which are polymorphic. In different studies the haplotypes B8 (or 18)-BfF1 (fast moving and rarest of the 4 alleles of Factor B in Caucasian populations)-DR3 and also B8-BfSS-DR3 have been statistically more frequent in patients than in controls[42,43]. However the suggestion that the haplotype B18(8)-BfF1-DR3 carries a worse prognosis[44] has not been confirmed so far.

The genetic predisposition may vary from community to com-

munity: for example, in Japanese studies it is the class II antigen DR2 which is associated with MN[45]. Whether this observation implies that the underlying, presumed environmental trigger in these geographically differing communities is different remains speculative.

A family history of IMN has been reported only occasionally[46]. We have observed it in three sets of brothers, not usually living in the same house and not developing the disease simultaneously – persuasive support for the contention that IMN may arise in immunogenetically vulnerable subjects[47].

PRESENTATION AND ITS SIGNIFICANCE

It is not possible to give an authoritative account which will hold good in every country, for most reports have dealt with apparently idiopathic disease in Europe[48,49] and the United States[50,51] and more recently in Japan[33,52]. In IMN in these countries, less than 10% have a history of overt infection preceding the observed onset. The greater prevalence of infection-related disease in other areas may affect the observed course of the prevailing membranous nephropathies. 'Idiopathic' lesions do occur everywhere and on the evidence available so far, an immunogenetically distinct subgroup may be identifiable in any stable, indigenous population.

The observed presentation is usually the nephrotic syndrome accounting for over 70% of cases in some series[30–33,48–52]. This does not mean that it is the common onset of MN, indeed nephrotic subjects may represent a minority of MN lesions in the community. Most affected individuals probably develop asymptomatic proteinuria, and a tiny minority of those, only discovered by chance at medical examination for another purpose, effectively account for the residual, non-nephrotic 15–20% of biopsy-proven cases in reported series. Provided proteinuria remains below 5 g/day, the nephrotic syndrome should not develop and healing occurs over 12–48 months, with a well maintained glomerular filtration rate (GFR). If proteinuria exceeds this level about half subsequently develop a nephrotic syndrome and their prognosis is as already described[3]. The remainder do not, and over 90% of these heal. Therefore the presence of nephrotic oedema is probably not the rule in MN, nor does its appearance necessarily

indicate the onset of the glomerular lesion. It seems clear that MN usually heals silently after having persisted for some months or years. Thus, its prevalence greatly exceeds that suggested by nephrologists' practice and the prognosis is very much better than observation of only nephrotic subjects would suggest.

Unusually, patients with IMN may present with hypertension or with established renal impairment having followed a silent but progressive course. Hypertension is uncommon early in the course of the disease. Uraemic features are distinctly uncommon at presentation. Macroscopic haematuria is rare and suggests a super-added pathology.

INVESTIGATION AND DIAGNOSIS

Urine microscopy reveals red cells in up to 30% of patients; red cell casts are uncommon. GFR is usually well maintained at presentation.

TABLE 2.5 Suggested investigations in suspected membranous nephropathy (these would of course be modified in the light of the clinical setting)

Plasma creatinine
Plasma albumin
Creatinine clearance
24 hour urinary protein
Fasting and post-prandial blood sugar
Serum and urine (immuno)electrophoresis
Scat and Latex tests
ANF
Anti-DNA antibodies
Hepatitis B serology
WR
Urine microscopy
Complement profile
Immunoglobulin profile
Selectivity index
Chest X ray
Renal biopsy

Daily protein excretion rates vary from 1 to 20 g or more. The dynamics of proteinuria in IMN are consistent with the theory of loss of size selectivity of the GBM but there is experimental evidence of concomitant loss of charge selectivity. The IgG:transferrin clearance ratio is occasionally 'highly selective'; less selective proteinuria (0.2–>0.4) is common and the index may become even less selective as the GFR deteriorates[53].

It is our practice to undertake certain investigations routinely (Table 2.5). Genetic markers, complement, immunoglobulin and lipoprotein profiles do not generally assist in practical management, except that abnormally low levels of the complement cascade components C3 and C4 may suggest active immunological disease such as SLE, or hepatitis B-associated cryoglobulinaemia. Exhaustive tests for neoplasia, such as a complete barium series of the gastro-intestinal tract or whole body CT scanning, are not indicated at any age since these associations are rare, especially in the under sixties, but sequential clinical evaluation and a high degree of suspicion in older patients is needed[54].

COURSE AND PROGNOSIS

Once the diagnosis is established, the subsequent clinical course is highly variable. A number of patients proceed to end stage renal failure (ESRF), defined as actual death due to renal insufficiency or a requirement for dialysis and/or transplantation. For most patients, a complete and lasting remission (proteinuria <0.3 g/day) will ensue, usually with normal GFR, but occasionally with (mild) impairment of renal function. Death in such patients is from unrelated causes. In up to 30% of patients one or more relapses of proteinuria develop[55]. The remaining patients have persisting (if varying) proteinuria, with normal, or with stable but impaired, renal function: they too die of some other cause.

The proportion of patients which fall into each possible outcome, and the clinical, biochemical and histological features which may determine the eventual prognosis has been the subject of much controversy over the years[3,30–33,48–52,56–59]. Certainly most of the more recently

published series suggest a better overall prognosis than was previously thought[33,49].

Before further consideration of course and prognosis, it is vitally important to stress the necessity of determining the presence of underlying disease, since appropriate treatment of infection, even with impaired renal function can result in complete clinical resolution and return to normal function[60].

Furthermore, withdrawal of any offending drug generally has a good prognosis[61,62], again even with impaired GFR at presentation, and successful therapy of an implicated malignancy is also associated with a good prognosis[63].

Once untreated patients with idiopathic disease show a progressive decline in renal function the continuation to ESRF may be inevitable, but it is necessary to seek for the presence of super-added, remediable conditions such as the development of an interstitial nephritis[64], anti-GBM disease[65], renal vein thrombosis[66] or hypovolaemic pre-renal failure[67], in any patient who shows an abrupt decline in GFR. Disease-related progressive decline in renal function occurs at different rates in different individuals, usually in a predictable fashion (best seen as a linear change in a graph of reciprocal serum creatinine values) in any one patient[57] and deviation from the projected rate should alert the clinician to the possibility of one of the conditions described above. Uncontrolled hypertension may cause or aggravate the decline in function and requires aggressive intervention.

Most authorities document a better prognosis if presentation is with asymptomatic proteinuria rather than with a nephrotic syndrome[3]; while this has not been shown invariably[2], it seems probable that, provided proteinuria remains below 5 g/day, the outcome is benign. We observed that such initial proteinuria frequently (up to 50% of cases) rose to higher levels on sequential analysis and this may explain discrepant findings when sequential results are not utilized for analysis. In some[57,58,68] but not all[69] reports, the outcome for women appears better than for men. Children rarely appear to progress to terminal renal failure[70]. In adults heavy proteinuria portends a worse prognosis but once in adult life increasing age does not appear to affect outcome materially.

The relevance of persisting proteinuria is debated. It may represent continuing immunological insult, or be merely a reflection of unhealed

glomerular damage. The finding of complement breakdown products in the urine, particularly in patients with declining renal function[71] or the documentation of proteinuria which becomes progressively less selective[53,72] suggests the former explanation. In other patients, the hypothesis that persisting proteinuria is only a residual defect could be supported by the observation that, in patients who eventually remit completely, the loss of proteinuria is usually a gradual rather than abrupt phenomenon. Of course, neither explanation is mutually exclusive. In a given case proteinuria may reflect either, or both, mechanisms.

One recently analysed series shows that, on univariate analysis, the following were related to a poor prognosis: male sex, a low serum albumin, high serum creatinine (and low creatinine clearance), heavy proteinuria and a high systolic blood pressure. On multivariate analysis, only the first two of these variables were shown to be independently associated with a poor prognosis[51]. However, non-treatment with prednisolone was also an independent poor risk factor in the multivariate, but not univariate, analysis. Age was not found to be a valid predictor.

The relevance of HLA status in the progression of disease has been discussed. There is evidence that geographic (and so perhaps immunogenetic) factors may influence prognosis[44,52]. A recent Japanese series included patients with asymptomatic proteinuria and showed 90% survival at 10 years[33]. European series[48,49] report figures of 76 and 83% 10-year survival respectively, but the proportion of patients with heavy proteinuria and NS must be considered, as it should in other reports that suggest up to 50% of patients may eventually develop ESRF[30]. It is also important to consider the contribution to survival of non-renal deaths: hyperlipidaemia and hypertension may contribute significantly to mortality from ischaemic heart disease and cerebrovascular disease, particularly in patients with prolonged heavy proteinuria[73]. While it may be reasonable to describe such mortality as 'renal associated', it is impossible to dissect out the relevant contribution of each factor. An assessment of all current information suggests that probably 25% of nephrotic patients with IMN will develop ESRF: this figure may be as low as 6% in patients presenting with asymptomatic proteinuria. Patients destined to do so will usually progress to ESRF within 5 years of onset. It is unusual to

do so thereafter. Within this time frame however, a decline in renal function usually runs a slowly progressive course, and it has been suggested that this may be sufficiently clear by 2 years from onset for decisions to be made on which patients to treat in order to attempt to prevent ESRF developing.

Variations in published results will require further scrutiny once aetiological and pathogenetic mechanisms are defined, since patients with IMN are unlikely to be homogeneous in these respects. These factors alone may help in resolving the problems encountered by the differences observed between series, particularly in concert with the clinical variables generally thought associated with a good prognosis (female, asymptomatic proteinuric onset, well maintained GFR, highly selective proteinuria). The effect of transience or persistence, or indeed exposure dose, of the triggering insult must await delineation of the aetiological factor. Potential perturbations of the immune response requires delineation of the pathogenetic mechanisms. The effect or otherwise of treatment is dealt with in a subsequent section.

The value of staging the degree of histopathological change at the GBM is uncertain. Milder degrees of damage tend to be associated with a better prognosis, but more severe abnormalities are not inconsistent with a good outcome[13]. Moreover, the classical view on the evolution of membranous changes has recently been challenged. Is it the length of time over which new material is deposited along the GBM that determines its structure and clinical progress?[74]

The value of fine needle aspiration studies in IMN will need confirmation[75].

Until recently the interaction between women with IMN and their pregnancies was thought generally benign. A recent substantive report[76] suggests a more guarded prognosis with regard to foetal loss, premature delivery and to worsening of renal function, development of hypertension and exacerbation of proteinuria in the mother.

COMPLICATIONS

Proteinuria, particularly if heavy and prolonged, causes physiological and metabolic derangements in MN. Most abnormalities related to heavy proteinuria are the same whatever the cause. Some may have a

particular association with certain glomerulopathies, possibly as a result of the absolute value of protein loss in association with the effective pore size (as judged by selectivity) which will play a substantial part in determining which particular plasma proteins are lost in the urine. Although some patients, despite heavy proteinuria, are able to compensate and maintain a normal plasma albumin concentration, the usual result of prolonged urinary protein loss is hypoalbuminaemia and eventually oedema (a nephrotic syndrome). The classical explanation was that hypoalbuminaemia resulted in intravascular volume depletion, which in turn led to salt and water retention. This sequence does not explain fully the phenomenon of oedema. Only about one third of nephrotic subjects are demonstrably hypovolaemic[77]. It appears that intrinsic renal mechanisms may be involved and lymphatic flows are disturbed but the question as to how nephrotic oedema forms remains unresolved at present[78].

Hypovolaemia does occur and is particularly likely in association with the aggressive use of diuretic agents, which exacerbates any incipient or actual hypovolaemia and causes impaired renal function due to inadequate renal perfusion, or even the development of hypovolaemic acute renal failure[67].

Hypoalbuminaemia also affects the metabolism of drugs that are highly albumin bound. Abnormalities in thyroid function tests may be due to the loss of thyroid binding globulin in the urine and consequently low circulating levels. Free thyroxine levels are usually normal, however[79], and treatment with thyroxine replacement is inappropriate. Much of the serum calcium is protein bound, and low total values of total serum calcium are observed. Urinary loss of vitamin D binding globulin, and putatively a direct toxic effect on the renal tubular cells of excess protein resorption have been implicated in the impaired production or release of 1,25-dihydroxycholecalciferol which augments the tendency to hypocalcaemia[80,81].

Changes in serum albumin concentration may be paralleled by alterations in serum immunoglobulins. A profound fall in IgG is not an uncommon finding[82]. It appears to be secondary to urinary losses and compounded by impaired production. Serum IgM levels are moderately raised but the IgA level is usually normal[83]. The nephrotic syndrome is said to result in a hypercoagulable state and this seems to be particularly evident in MN[84]. Plasma levels of fibrinogen and of

high molecular weight zymogens are increased because of increased production, while levels of plasminogen and of anti-thrombin III (which is lost in the urine) are low. Volume depletion, erythrocytosis and platelet hyperaggregability are other factors which may contribute to a significant increase in both venous and arterial occlusion in MN[60].

Renal vein and inferior vena caval thrombosis, and associated pulmonary embolism, are reported proportionally more commonly in patients with MN than in those with any other histologically defined glomerulopathy[85]. We do not know why the incidence of renal vein thrombosis (RVT) varies considerably in reported series, but as it may be a clinically silent event this will be a reflection of the diligence with which it is sought. Most authorities now reject the role of RVT in causation of IMN believing it to be a secondary phenomenon.

Hyperlipoproteinaemia is associated with heavy proteinuria as the result of a variety of mechanisms – increased hepatic production of apolipoprotein moieties, impaired intravascular lipolysis and increased loss in the urine of small molecular weight components which influence lipid metabolism[86]. Safe effective methods for lowering serum lipids in the presence of heavy proteinuria are not readily available; the value of such intervention remains contentious.

The role remains undissected of hyperlipoproteinaemia, hypercoagulability and hypertension (which commonly develops in association with progressive IMN) in the genesis of atheromatous coronary or other arterial disease. We, and others, have noted what appears to be a significant incidence of cardiac ischaemic episodes in patients with IMN[3,60]. Since the age matched 'control' population without glomerular disorder also has a relatively high proportion of vasculopathic disease, a true increase in incidence is difficult to prove. Nevertheless, we think that the evidence points to such an increase, directly attributable to the metabolic changes (particularly in lipoprotein metabolism) in patients with prolonged, unselective proteinuria, a group into which some IMN patients fall.

Rarely, patients with nephrotic syndrome and/or IMN have significant urinary losses of trace metals such as copper and zinc[87,88]. Only occasionally do these appear to contribute to clinically important conditions such as iron deficiency anaemia or hypogeusia. Patients with heavy proteinuria, yet well maintained renal function, complain

of 'tiredness'. It remains to be seen whether this could be attributed to urinary loss of essential trace elements.

The increased susceptibility to bacterial infections of all patients with a nephrotic syndrome has long been recognized. Both neutrophil bacteriocidal activity and defective opsonization have been invoked as causes, as have low circulating IgG levels. Since the advent of effective antibiotics this is not such a sinister complication but it can still be a serious problem and vigilance should be maintained.

TREATMENT

It is essential to exclude all predisposing factors. Diuretic- or antibiotic-induced interstitial nephritis, even the very rare super-added anti-GBM disease, should be considered in patients whose functional decline appears to be accelerating. Treatment of these complications should be on accepted lines.

Treatment is concentrated on two fronts. First, it is directed at modifying the consequences of the membranous lesion itself. The aim is to stabilize or to reverse any decline in GFR, and to decrease the amount of proteinuria. Secondly, it may aim to modify the secondary metabolic effects of the lesion which are essentially those of any heavy proteinuric state.

If significantly impaired function ensues measures should be taken appropriate to that pre-terminal state. These problems will not be dealt with here.

Minor degrees of proteinuria are unlikely to have significant metabolic sequelae. Even with proteinuria in excess of 10 g per 24 h, plasma albumin levels can be maintained at, or near to, normal levels by adequate protein and calorie intake (see below). Nevertheless, oedema usually occurs with such heavy proteinuria and a nephrotic syndrome is frequently observed in MN, more so perhaps than any other glomerular disorder seen in nephrological practice with the exception of minimal change nephropathy. Unless gross, nephrotic oedema itself is largely a cosmetic problem, although skin trauma with subsequent poor wound healing, may be a troublesome complication.

In patients with mild degrees of dependent oedema, salt restriction may be all that is required. With more substantial fluid retention, or

with transudation into serous cavities, diuretic therapy is indicated. Some authorities recommend starting with a thiazide diuretic. Our practice is to initiate therapy with a loop diuretic such as frusemide. If the patient is hospitalized, doubling of the dose every 2–3 days, even up to 1 g per day can be considered. Occasionally, the addition of an aldosterone antagonist (spironolactone) is indicated, but especial care must be taken to monitor serum potassium levels.

In resistant oedema the addition of metolazone is almost invariably successful. Very rarely, the infusion of salt-poor albumin is considered in conjunction with intravenous diuretic administration in an attempt to 'mobilize' the oedema and promote diuresis[89]. Such an infusion may be particularly applicable in patients who are hypovolaemic since further reduction in circulating volume may precipitate acute renal failure. Generally, weight loss of not more than 1 kg per day is desirable. The value of recumbancy must not be forgotten – bed rest appears to favour diuresis. Advocates of water immersion to the neck or even haemofiltration are not yet in the ascendency[90]. Once oedema is lessened, and particularly if plasma protein levels have risen, smaller doses of diuretic may well be sufficient to establish an oedema-free state.

The circulating albumin pool, and with it the plasma albumin, level can frequently be improved by prescription of an adequate protein and calorie diet but this takes some weeks. Our current practice is to give high biological value protein at 1 g/kg body weight, plus 1 g per g of urine protein loss per day, combined with sufficient calories (around 200 kcal/g nitrogen) over a 24 hour period. Severe hypoalbuminaemia is associated with anorexia and nasogastric feeding is occasionally employed, at least in the early stages. The current consensus view is that maintenance of a normal or near normal plasma albumin is desirable in order to minimize the problems associated with hypoalbuminaemia. This is the rationale behind high protein diets in severely hypoproteinaemic or heavily proteinuric patients. Such a view has recently been questioned, and it has been suggested that even with normal renal function, dietary protein restriction may lead to lessening of proteinuria and even a better prognosis of GFR in the long term[91]. These provocative approaches need to be substantiated.

The clinician must be alert to educate the patient when the proteinuria falls substantially or remits, that a gradual return to a normal

diet can follow. Hypertension may develop. It should be treated appropriately, and probably fairly aggressively. Uncontrolled, it contributes to progressive impairment of renal function. Such antihypertensive therapy must include attempts to alter the patient's lifestyle, particularly in respect of stopping smoking as MN patients may be at particular risk of arterial occlusive disease.

There is no generally accepted therapy for the hyperlipoproteinaemia. Manipulation of the quantity and quality of fat in the necessarily high calorie diet is difficult. While it seems prudent to encourage heavily proteinuric MN patients to decrease their intake of saturated fats in favour of the polyunsaturated variety, the benefits in terms of atheroma production are medium to long term, and of putative importance only in prolonged or progressive disease.

There are advocates of vitamin D and zinc supplementation to the diet but this is not a universal practice. Despite the relatively high incidence of venous and arterial thromboses particularly in MN patients, prophylactic anticoagulation is not encouraged, except where particular risk is involved such as enforced immobilization following trauma. Once venous thrombosis (deep vein, renal vein or inferior vena caval) is identified, however, long term anticoagulation is indicated.

The acquired immunodeficiency of very low levels of IgG, in association with defective opsonization, would appear to be responsible for the increased risk of bacterial infection, particularly by encapsulated organisms, that is a feature of nephrotic patients. Neither the administration of immunoglobulin or prophylactic antibiotics appears warranted, but a high degree of suspicion of bacterial infection is indicated and surveillance and relevant investigation suggested, such that prompt and appropriate antibiotic therapy can be administered as necessary. Overwhelming viral and opportunist infection in MN would appear to be attributable to the use of immunosuppressive agents rather than MN per se.

Specific measures

Is it possible to influence IMN? With modern experimental studies, insights into the pathogenetic mechanisms involved are becoming clearer. It is likely that a number of pathogenetic mechanisms will be

described in IMN, so it is hardly surprising that no single therapeutic intervention has resulted in a uniform response. Nor do we have at present indications of disease activity more sensitive than measurements of proteinuria and of serum creatinine or other measurements of GFR.

The rationale for attempting to reduce proteinuria is the perception that once urinary protein loss falls permanently below 5 g, and especially below 2 g, per day a favourable outcome is to be expected. Heavy proteinuria may itself be associated with subsequent glomerular scarring (at least in animals). Furthermore, a reduction in proteinuria lessens such undesirable consequences of the nephrotic state as oedema, hypoalbuminaemia, hyperlipidaemia, hypercoagulability and hypogammaglobulinaemia.

Therapeutic trials have concentrated more on the effect on proteinuria than on its nephrotic consequences, and on the role of treatment on the subsequent GFR. Over the last twenty years there have been numerous reports on retrospective, prospective, controlled and uncontrolled trials[58,69,92–98]. Most have suffered from the statistical problems associated with small numbers of patients. Few have dealt specifically with the problem of treatment for patients with declining function, having concentrated more on the outcome in patients with well maintained GFR at the start of the trial.

Not surprisingly the published results of small trials are variable. Some suggest long term benefit from prednisolone, with or without the addition of such cytotoxic agents as cyclophosphamide or chlorambucil; others do not.

There are only two recent reports on substantive numbers of patients entered into prospective, well controlled trials. A third report from the MRC is expected soon. The first report, from the US Collaborative study in 1979[58], showed a beneficial effect of a 2-month course of alternate day high dose oral steroids (followed by a tapering dose) on patients with IMN and well maintained GFR. There was a short term reduction in proteinuria in the treated group but this did not persist. However, there was a sustained and statistically significant reduction in the number of patients in the treated group going on to progressive (and eventually end stage) renal failure. The major criticism of this study was the relatively high percentage of untreated patients who reached 'creatinine stop points' compared with some other series of

untreated patients. Nevertheless, the results of this trial have remained the most convincing that steroid therapy alone is beneficial in the long term, and many clinicians, particularly in the USA, are committed to treat patients with this regimen.

The other major trial, from Italy, has shown that a regimen of intravenous pulse methyl prednisolone, followed by alternating monthly courses of oral prednisolone and chlorambucil, is also followed by a much improved prognosis in the treated group[98].

Early reports suggested a substantial morbidity and some mortality associated with steroid/cytotoxic treatment, particularly in trials with protracted courses of therapy. This has not been so in recent studies. Nevertheless, there is a growing body of opinion that to expose a patient with mild proteinuria, in whom the prognosis appears to be excellent, to aggressive therapeutic intervention may be ill advised.

It is now apparent that probably 75–80% of patients who have a renal biopsy diagnosis of IMN are not going to die of renal failure. The pressing need is to identify those patients who *are* going to progress to renal failure and concentrate our treatment on them. It would seem reasonable to suppose that in this cohort of patients early treatment would be justified.

It is difficult to gain much information from the literature regarding the potential benefit of treating patients with already declining and – untreated – probably inexorably progressive renal function. This problem is compounded by the realization that the pathogenetic mechanisms involved in the formation of the membranous lesion, in the development of proteinuria and in any progression of impaired renal function are not necessarily the same. We have evidence, however, that immunological mechanisms are responsible for declining renal function in some patients[99]. We, and others[100,101], have reported recently that a progressive decline in renal function can be halted, or even reversed, using prolonged high doses of corticosteroids with or without the addition of azathioprine.

Thus, there is no consensus yet for the treatment of the membranous lesion[102]. Some authorities feel compelled to use the US Collaborative study regimen, others have implemented the Italian (Ponticelli) approach. In the absence of the results from the recent MRC trial our approach is to withhold treatment until there is demonstrable decline in function, to ensure there is no other remediable course for such an

event, and then treat aggressively. Yet others may feel that even this approach is not justifiable when the current results of dialysis and transplantation can be so good.

What is certain is that an optimal approach will depend on careful categorization and study of all patients and the determination of the factors responsible for the production, continuation and progression of the condition.

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3

INFECTION-ASSOCIATED GLOMERULONEPHRITIS

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INTRODUCTION

The renal effects of infections are variable and depend on a number of different factors. In undergraduate teaching the relationship between an infection with a group 'A' β-haemolytic-Streptococcus of certain strains and the subsequent development of an acute nephritic illness is used to explain important immunological principles. However, in the United Kingdom, post-streptococcal glomerulonephritis is now relatively uncommon although it has become apparent that infections with many other organisms may produce glomerular lesions. It has been postulated that during an infection there is the introduction of a specific antigen with a subsequent antibody response resulting in immune complex formation either circulating or in situ. There is consequent activation of the complement and coagulation systems to result in an inflammatory response which, if it occurs in the glomerulus, leads to glomerulonephritis.

In addition however, infections may produce other lesions depending on the nature and the severity of the illness. In septicaemia there may be an almost overwhelming infection with significant toxin production resulting in an acute tubular necrosis[1]. In other infections however, the main renal lesion may appear within the interstitium e.g. Hantavirus infection and acute interstitial nephritis. Furthermore, in chronic infections particularly bronchiectasis, osteomyelitis and tuberculosis there may be a chronic stimulation of the immune system

resulting in amyloid deposition. Some infections, particularly viral, may precipitate or cause a relapse in anti-GBM nephritis (Goodpasture's syndrome) or may result in an haemolytic uraemic syndrome with renal involvement. In recent years to this list must be added the complications of antibiotic therapy[2] which may result in the development of allergic type eosinophilic interstitial nephritis. This review concentrates on the glomerular consequences of infections (Table 3.1).

TABLE 3.1 Renal consequences of infection

Vasculitis
Glomerulonephritis
Amyloidosis
Acute tubular necrosis
Interstitial nephritis
Haemolytic uraemic syndrome
Drug-induced (antibiotic) interstitial nephritis

It is interesting to note that the same organism may produce different morphological appearances in different patients. For instance, in patients with persistent hepatitis 'B' antigenaemia, membranous glomerulonephritis, mesangiocapillary glomerulonephritis and IgA nephropathy have all been described. Furthermore, different infections may produce the same morphological appearances within the glomerulus in different patients[3]. In addition to this, it is surprising that reports of similar infections from different geographical areas may present completely different clinical features. It would appear, therefore, that the glomerular response following an infection is dependent on patient factors and characteristics of the infecting organism. In the patient the immune response to infection is clearly of importance and the resulting glomerular damage is likely to be affected by the type, quantity and duration of the antibody response. This to some extent will determine the localization of the antigen–antibody complexes in a sub-endothelial, sub-epithelial and/or mesangial position. It is likely that this immune response is in some way controlled by genetic factors and this may in part explain the geographical differences which have been reported for certain infections. It must also be remembered that organisms have different virulence and this may be affected by passage

resulting in either increased virulence or attenuation. The consequences, therefore, of an infection in any given population are dependent on a number of variable factors and this may well explain why in epidemic infections some patients are affected more than others, while other patients are apparently unaffected.

The situation is further complicated by a number of socio-economic factors. With increasing affluence the general health of the community improves. The associated improved nutrition is a significant factor in the patient's ability to deal with infection adequately; for example at the end of the last century, measles was a significant cause of mortality in children living in socially deprived areas. Now with improved housing, nutrition and health care, it is a rare cause of serious infection. Although improvement in the general nutrition of the population is undoubtedly important, there is probably also a reduction in the virulence of certain organisms, such as the streptococcus. It is interesting to note that in a recent UK study of infection as a cause of renal disease leading to renal biopsy, some 21% of infection-associated renal disease occurred in patients who were not of British origin[3]. Furthermore, some infections such as smallpox have been eradicated from the world, whereas others such as malaria have been eradicated from certain geographical areas. To this must be added improved diagnosis and the development of effective drugs to eradicate infections. As an example, it is now relatively uncommon in the United Kingdom to encounter patients with renal amyloidosis secondary to tuberculosis or chronic oesteomyelitis; indeed in this community primary amyloidosis is now almost as common as secondary amyloidosis. These socio-economic and health factors, therefore, are also significant in explaining the changing clinical pattern of glomerular involvement during infections.

A causal relationship between infection and glomerulopathy has been established by epidemiological studies in which epidemic or endemic infections have been associated with specific glomerular abnormalities. Furthermore there have been numerous animal studies in which glomerular lesions have been induced by infections with viral, bacterial or parasitic infections. A direct causal relationship is determined by the detection within the glomerulus of antigen originating from the infective organism and from elution studies demonstrating antibody to the specific antigen. Involvement of the immune

mechanism is suggested by the demonstration by immunofluorescence of immunoglobulins and complement within the glomerular deposits of infection-associated glomerulonephritis. Many early reports are of doubtful significance due to the poor specificity of the antisera used. Recently, however, there have been significant improvements in the production of antisera and as a consequence specificity has significantly increased. There is now no doubt that the causal relationship between infections and glomerular changes is established using specific antisera and elution studies.

Early reports in the pre-antibiotic era associating infection with renal disease were frequently related to chronic sepsis such as tuberculosis. It is interesting to note that a recent re-examination of the kidneys which formed the original report by Richard Bright in the early 19th century revealed that one had undoubted amyloidosis possibly on the basis of an underlying chronic tuberculosis[4]. At about the same time the association between an infectious illness, scarlatina and renal disease was reported[5]. Detailed studies, of course, could not be undertaken until the nature of the infecting organisms had been elucidated. Furthermore, early investigators relied entirely on postmortem material and it was not until the development of percutaneous renal biopsy that an understanding of renal pathology, particularly with respect to acute illnesses became possible. It is sometimes difficult to remember that the first reports of percutaneous renal biopsy were published only 35 years ago[6]. Since then numerous studies have been published detailing the association between glomerulopathy and bacterial, parasitic and viral infections.

Infections do not always produce an adverse effect on the glomerulus. It has been noted that in children with minimal change nephrotic syndrome, measles may produce a remission of the proteinuria and indeed primary measles vaccination has been used therapeutically[7]. It is possible that the measles infection has an inhibitory effect on T cells and a suppression of lymphokine secretion resulting in capillary wall changes with consequent diminution in proteinuria. Other infections such as varicella, typhoid, malaria and staphylococcal, streptococcal and pneumococcal infections have been reported as having a similar effect. These infections may modify the immune response by the activation of macrophages.

This review will examine the consequences of infection with specific

organisms. Some associations are common whereas others are rare and the available literature is confined to specific reports (Table 3.2).

TABLE 3.2 Infection-associated glomerulonephritis

	Common	*Rare*
Viral	Hepatitis B Cytomegalovirus HIV	EB virus Echo Coxsackie Varicella-zoster Mumps Measles Influenza A
Bacterial	Streptococcus Staphylococcus *Treponema pallidum* *Mycobacterium leprae*	Enterococcus Pneumococcus *Salmonella typhi* *Klebsiella pneumoniae* Mycoplasma Brucella Leptospira Salmonella *Corynebacterium bovis*
Parasitic	*Plasmodium malariae* *Schistosoma mansoni*	*Plasmodium falciparum* *Schistosoma japanocum* *Leishmania donovani* *Loa loa* *Onchocerca volvulus* Toxoplasma Trichinosis Histoplasmosis

VIRAL INFECTIONS

Viral infections resulting in immune-complex formation affecting the kidney are well recognized in naturally-occurring and experimentally-induced diseases in animals: Aleutian disease virus in mink, spontaneous Gross leukemia virus, lymphocytic choriomeningitis virus etc. The affected animal may develop glomerulonephritis. In humans

hepatitis B virus, cytomegalovirus and human immunodeficiency virus have been reported as causing glomerulopathy. Only a few patients with acute viral infections however, seem to develop evidence of renal disease. Sporadic case reports of glomerulonephritis following viral infections have been documented: varicella[8], measles[9], mumps[10], subacute sclerosing panencephalitis[11] and Echo virus[12] although this latter case must be treated with caution as it was reported in a child with immune deficiency. In a few patients with infectious mononucleosis a mild short-lived nephritic illness has been reported associated with a mesangial proliferative glomerulonephritis[13]. Haemolytic uraemic syndrome, particularly in children, may be precipitated by viral infections, often with a clustering of patients. Chronic viral infections, however, do lead to glomerular changes. Hepatitis B virus has been reported as causing a membranous nephropathy[14] and also polyarteritis nodosa[15]. However, many patients with serological evidence of chronic hepatitis B antigenaemia never seem to develop either glomerulonephritis or polyarteritis nodosa. Cytomegalovirus infection may rarely be implicated in glomerulonephritis although in the largest groups studied, that is following transplantation, there is doubt whether the lesion is specific or represents an unusual form of rejection. The human immunodeficiency virus may also produce glomerular lesions but as patients infected with this virus frequently have other opportunistic infections a direct causal relationship is difficult to establish.

Hepatitis B

Renal involvement in hepatitis B infection may take the form of polyarteritis nodosa, membranous glomerulonephritis, type I mesangiocapillary glomerulonephritis or essential mixed cryoglobulinaemia. The first described association was with polyarteritis nodosa[16] and in the following year the associated membranous lesion was reported[17]. Since then there have been numerous reports confirming these associations and while the glomerulopathy is well recognised, the arteritis is considered uncommon. There is, however, widespread geographical variation in the incidence of hepatitis B infection and this may account for the varying reports in the literature.

In the clinical presentation of the glomerulopathy there appear to be marked differences between adults and children. In children the disease most frequently presents as a nephrotic syndrome accompanied by microscopic haematuria and only rarely is there evidence of renal functional impairment. There is usually only minor evidence of hepatic disease and the proteinuria frequently remits within one year. In adults, however, usually the major manifestation is of hepatic disease and this is the most common cause of death. Evidence of hepatic disease may have been present for many years prior to the development of proteinuria.

It is difficult to explain these differences but most reported cases in children come from areas where hepatitis B is endemic and the effects of infection acquired in childhood may be considerably different from those in adults. Only very rarely is an unsuspected hepatitis B antigenaemia detected in adult patients undergoing renal biopsy for suspected glomerular disease although there is evidence that the incidence of antigenaemia in patients undergoing renal biopsy is greater than in the general community[18]. Most commonly the renal biopsy reveals a membranous glomerulonephritis, particularly in children where the idiopathic form is rare[19]. Indeed hepatitis B antigenaemia was found in 14 of 33 children with a biopsy diagnosis of membranous glomerulonephritis whereas it was only detected in 3 of 170 children with other glomerular diseases[20]. On immunofluorescence IgG, IgA, IgM and complement (C3) have been detected. Detailed studies have also shown evidence of HBs, HBc and HBe antigens in glomeruli although not all authors have found all three antigens. There is general agreement that HBsAg and HBcAg are present in the subepithelial deposits although the role of HBcAg is still unclear.

In patients with hepatitis Be antigen-mediated membranous glomerulonephritis it would appear that proteinuria may remit and membranous lesion regress when they seroconvert with the production of hepatitis Be antibody[21,22]. Elution studies have revealed HBsAg to be present in the kidney. On electron microscopy there are multiple subepithelial deposits identical to the findings in idiopathic membranous nephropathy.

In a number of patients the renal biopsy findings are of a type 1 mesangiocapillary glomerulonephritis and this may be particularly true for adult patients[23]. These patients seem to progress and may

develop terminal renal failure. Recent reports from Hong Kong, where hepatitis B is endemic, have described an associated IgA nephropathy[24]. The documented patients had high serum titres of hepatitis B core and surface antigen indicating that they were persistent carriers of the virus. In addition HBcAg and HBsAg were present in the mesangium in eight of ten patients suggesting that these antigens may play a part in the pathogenesis of IgA nephropathy in endemic areas. Hepatitis B surface antigen was detected in the serum of 17.2% of patients with IgA nephropathy in Hong Kong, an incidence significantly greater than the prevalence of HBsAg carriers in the general population[25]. All the available evidence suggests that hepatitis B nephropathy is immune complex-mediated but many patients do not have evidence of circulating immune complexes supporting the concept of 'in situ' formation of complexes in the glomerular capillary wall.

Although the initial report of hepatitis B-associated renal disease was of a polyarteritis, this association would seem to be relatively uncommon even in areas where hepatitis B is endemic. Although hepatitis B antigen has been demonstrated in the blood vessels of patients with vasculitis only few patients with chronic antigenaemia seem to develop vasculitis. The reasons for this are not clear but presumably they relate to the size, charge and quantity of complexes formed.

The role of hepatitis in mixed cryoglobulinaemia is not clear although HBs antigen and antibody have been found in the cryoprecipitates of some patients[26].

Cytomegalovirus

The association between cytomegalovirus infection and glomerular effects has been reported in two patient groups: children with severe congenital or neonatal infection and in patients following transplantation. In children with severe disease circulating immune complexes are common but evidence of glomerulopathy rare. Although granular deposits of IgG and complement (C3) have been demonstrated in glomerular capillary walls, no cytomegalovirus antigen has been detected within glomeruli, casting doubt on the pathogenesis of the lesion.

The situation with respect to cytomegalovirus glomerulopathy in adults also remains unclear. Ozawa and Stewart[27] reported a patient who developed proteinuria and haematuria during the course of a fatal cytomegalovirus pneumonitis. The kidneys showed a mesangial proliferative glomerulonephritis with mesangial deposition of IgG, IgA and complement (C3 and C4). Cytomegalovirus antigen was present in a distribution similar to that of the immunoglobulin and antibody was detected by elution studies. This seemed to confirm the causal relationship between cytomegalovirus infection and glomerulopathy.

Cytomegalovirus infection is common following transplantation. Approximately 66% of renal transplant recipients have serological evidence of cytomegalovirus infection and half of these patients will chronically excrete the virus in their urine. Richardson and colleagues[28] concluded that in 14 renal transplant recipients studied there was evidence that the infection could result in acute allograft dysfunction associated with distinctive glomerular changes which were unlike those of rejection. The glomerular capillary loops contained large eosinophilic cells which were considered to be damaged endothelial cells. There was an associated slight mesangial proliferation, no crescents and minor focal interstitial changes. On electron microscopy, there was subendothelial accumulation of amorphous material, no subepithelial deposits and only occasional mesangial deposits. Immunofluorescence demonstrated IgM and complement (C3) and in one case, examined with specific cytomegalovirus antibody, immunofluorescence was detected in glomerular capillaries, thought possibly to be leucocytes. However, in a further study involving autopsy material from children with bone marrow transplants, children with severe disseminated cytomegalovirus infection and renal transplant recipients[29] there was no direct evidence for a specific cytomegalovirus glomerulopathy and it was concluded that the changes were those of an unusual form of rejection involving endothelial cells. In addition, in a study of 298 renal allograft recipients, 33 were shown to have acute transplant glomerulopathy but there was no correlation with cytomegalovirus infection[30]. Thus although there is strong circumstantial evidence for a cytomegalovirus glomerulopathy, it is more likely that rejection and cytomegalovirus infection occur simultaneously and that there is not a direct causal link.

Acquired immune deficiency syndrome (HIV)

Renal involvement in HIV infection is common with approximately 10% of patients presenting with a nephrotic syndrome and 50% having significant proteinuria. The pathogenesis of the glomerular lesion is not clear but is probably related to immune complex formation which may arise from the infection inducing endogenous antigen production with a subsequent relevant antibody response.

The most common finding is of a focal segmental glomerulosclerosis associated with interstitial nephritis of variable severity[31] although both proliferative and mesangiocapillary lesions also have been described. The pathogenesis of HIV-associated nephropathy is not known but may be related to changes in the humoral and cell-mediated immune system; the polyclonal hypergammaglobulinaemia, circulating immune complexes and the reversal of the normal T helper:suppressor cell ratio (OKT4:8 ratio). An interesting finding is the occurrence in some patients of an hypocellular mesangium which may be of importance in view of the known phagocytic role of the mesangium. A reduction of mesangial cells may be mediated by the same mechanism which results in the generalized lymphopenia of acquired immunodeficiency patients.

It is difficult to be certain of the causal link between HIV infection and the glomerular changes as similar appearances have been described in intravenous drug abusers with heroin nephropathy[32] and such patients frequently have opportunistic infections and tumours. However, a case report in a child without evidence of any drug addiction[33] supports the concept of an HIV-associated glomerulopathy. Patients progress rapidly through the nephrotic phase to renal failure, usually within a few months.

BACTERIAL INFECTION

Streptococcus

The relationship between acute glomerulonephritis and epidemics of scarlatina has been recognized for over two hundred years[5]. However, in this century the epidemiology of post-streptococcal glomerulonephritis has changed significantly in that there has been a

significant decline in the incidence in developed countries, such as Europe and the United States. Although sporadic cases continue to be reported, epidemics would appear to occur mainly in developing countries, such as in Africa and the Caribbean. The reasons for the changing epidemiology are probably related to improved nutritional status in the community and the more liberal use of antibiotics, but there may also be changes in the nephritogenic potential of the streptococcus in particular communities.

Post-streptococcal glomerulonephritis usually develops ten days after an upper respiratory tract infection or some three weeks after a skin infection such as pyoderma (impetigo). In tropical countries, streptococcal infection may complicate insect bites and cases of glomerulonephritis have been reported in association with middle ear infections. Furthermore, glomerulonephritis has also been reported in association with endocarditis due to both α-haemolytic and β-haemolytic streptococci as well as *S. viridans, S. mitis* and *S. mutans*.

Post-streptococcal glomerulonephritis is associated with infection with group A β-haemolytic streptococcus of types 1, 2, 4, 12, 18, 25, 49, 55, 57 and 60. The incidence of such nephritis varies with the prevalence of the nephritogenic streptococcus in the community. In addition, not all patients infected with the same type of streptococcus develop glomerular abnormalities. In a prospective study of children only 22% of infected patients developed urinary abnormalities and/or a low complement (C3)[34]. Those patients who do develop nephritis, have a very variable clinical picture. Most commonly nephritis occurs in children aged 3–8 years, although it can occur at any age. The male:female ratio is approximately 2:1. Signs of renal involvement usually become manifest 1–2 weeks after the upper respiratory tract infection. The classical clinical picture is of macroscopic haematuria which may last for up to two weeks and remains microscopic for a considerable period thereafter, oedema which is variable in amount, but may be severe enough to produce pleural effusions and ascites, and hypertension which is usually mild, but may on occasions be severe. In addition, there is frequently a mild impairment of renal function, although acute renal failure may develop in a small number of patients. Some patients manifest cerebral symptoms in the form of headaches, nausea, vomiting and disturbed consciousness, whilst

others may have cardiovascular complications in the form of congestive cardiac failure.

The pathogenesis of the nephritis is immune complex-mediated. The streptococcus produces numerous products, including toxin and enzymes such as streptolysin hyaluronidase and streptokinase. The immune complex pathogenesis is supported by the finding of diminished serum complement (C3), the presence of circulating immune complexes and cryoglobulins, as well as the demonstration of IgG and complement (C3) within glomerular deposits. The precise nature of the nephritogenic antigen is not known, and it may well be more than one substance. It has been suggested that endostreptosin is a streptococcal antigen which localizes in the mesangium and subendothelial space and that subsequently antibody reacts to form in situ complex formation[35]. A similar mechanism has been postulated for the streptococcal M protein. Streptococcal antigens are only found in the glomerulus, primarily in the mesangium, in the early stages of the disease, i.e. within one week of symptoms, and are seldom detected thereafter, perhaps due to the covering of antigenic sites. A further possibility is that streptococcal neuraminidase exposes antigenic sites on host IgG with the subsequent development of an autologous immune reaction resulting in immune complex formation and deposition[36]. In addition, cationic antigen, which may enter the negatively-charged basement membrane, has been isolated from nephritogenic streptococci and it has been suggested that this acts as a 'planted' antigen with the subsequent in situ formation of immune complexes[37].

On light microscopy the most common appearance is that of a proliferative glomerulonephritis involving mesangial and endothelial cells. In the early stages of the illness, glomerular infiltration with polymorphs is common.

On immunofluorescence IgG is most common, although IgM may also be found. Complement (C3 and C1q) deposits are frequent. On electron microscopy, deposits are seen within the mesangium and there are also large subepithelial deposits, which have been described as 'humps'. In a small number of patients other histological appearances (mesangiocapillary glomerulonephritis (type 1), crescentic glomerulonephritis[39,40] and IgA nephropathy[41]) have been reported.

Considerable debate exists with respect to the long term prognosis of post-streptococcal glomerulonephritis. There is general agreement

that the short term prognosis is excellent, particularly in children. Doubt was cast on the long term prognosis by Baldwin[42] who followed up 168 patients for up to 18 years, 95 patients were followed for more than 10 years. In this group of patients proteinuria, hypertension and impaired renal function appeared to resolve within two years of the initial illness, but there was an increasing incidence of these three features in later years. A further long term study, however,[43] concluded that the majority of patients had a very good prognosis. It is most likely that the chances of progressing to end stage renal failure are less than 1%. In the small group of patients with progressive disease the mechanism of progression is unknown, but it may be related to the development of cell-mediated immunity acting against cross-reacting antigens of the streptococcus and glomerular basement membrane, or as a direct consequence of the induced hypertension.

Staphylococcus

Staphylococcal infections have been implicated in the pathogenesis of glomerulonephritis in a number of instances. The most commonly reported association is with endocarditis of both the acute and subacute types. Glomerular involvement may also occur in other staphylococcal infections. In 35% of patients dying from staphylococcal septicaemia a proliferative glomerulonephritis was found at autopsy[44]. Septicaemia may also be associated with intravenous drug abuse and such patients have a high incidence of associated proliferative glomerulonephritis. Ventriculoatrial shunts may become infected and the most common organism is *Staphylococcus epidermidis* although many other organisms have been implicated. In such patients, the development of an associated glomerulonephritis is relatively uncommon, but when it occurs it is usually of a subendothelial mesangiocapillary type. Other staphylococcal infections, such as osteomyelitis[45] and impetigo[46] have been reported as causing an associated glomerulonephritis.

The glomerular changes reported include focal proliferative, diffuse proliferative, crescentic and mesangiocapillary types of glomerulonephritis. Rarely has an IgA nephropathy been associated with staphylococcal infection[47]. The clinical severity of the reported renal

lesions varies from minor proteinuria to acute renal failure and to chronic renal failure in a small number of patients.

In infective endocarditis the most common organism is *Staph. aureus* and more rarely *Staph. albus*; other organisms such as *Strep. viridans, Enterococcus, Gonococcus, Haemophilus, Chlamydia* and even fungi have been reported. When first described[48], the glomerular lesion was thought to be due to direct aseptic embolism from the infective valve. The detection of circulating immune complexes, hypocomplementaemia, glomerular immunoglobulin deposition and the detection of staphylococcal antigens within involved glomeruli has resulted in the general acceptance that the associated glomerular lesion is immune complex-mediated. However, it should not be considered that this is the only mode of pathogenesis as it is highly likely that microemboli could result in small areas of infarction giving rise to the so-called 'flea-bitten kidney'. Furthermore there is a possibility that staphylococcal cell wall antigens may initiate the alternate pathway of complement activation resulting in glomerular damage. This latter possibility may account for the fact that in some patients there is a lack of glomerular immunoglobulin deposition and that glomerulonephritis may occur after a short duration of endocarditis and in the absence of immune complexes.

Glomerulonephritis occurs with equal frequency in acute and subacute endocarditis and although the incidence is not known, it is probably in the region of 50% of patients. It would appear that glomerulonephritis occurs particularly frequently in association with bacterial endocarditis in intravenous drug abusers, particularly when *Staph. aureus* is the infecting organism[49]. The clinical pattern of bacterial endocarditis has changed significantly in recent years, due to the introduction of antibiotic therapy and the falling incidence of rheumatic fever.

Bacterial endocarditis-associated glomerulonephritis is associated with circulating immune complexes and these occur more commonly and in greater titres in infections with less virulent organisms, those following an indolent course and in right heart valve lesions. Cryoglobulinaemia and hypocomplementaemia are frequently present, but it does not appear that there is any direct relation between these findings and the incidence of glomerular involvement. The high incidence of glomerular involvement occurring in patients with

staphylococcal endocarditis associated with intravenous drug abuse raises the possibility that the glomerulonephritis is a further manifestation of an acquired immune deficiency.

On light microscopy a number of changes have been described. In some patients there is a focal and segmental hypercellularity of variable severity associated with sclerosis and capsular adhesions, in others there is a diffuse proliferative glomerulonephritis, whilst rarely a subendothelial type of mesangiocapillary glomerulonephritis has been described. Crescents may occur in any of these types. Interstitial inflammation with subsequent progression to fibrosis may occur.

On immunofluorescence microscopy IgG is the most frequent immunoglobulin detected, although IgA and IgM have also been reported. The distribution of immunoglobulins is usually diffuse even in those patients with a focal and segmental lesion. On electron microscopy in patients with associated acute endocarditis, electron-dense deposits are most commonly sub-epithelial and intramembranous, whilst in patients with sub-acute endocarditis the deposits are frequently sub-endothelial and mesangial. Staphylococcal bacterial antigen has been detected by immunofluorescence in the glomeruli of patients with acute bacterial endocarditis[50].

Clinically, renal involvement may be the first indication of bacterial endocarditis. Microscopic haematuria and minor proteinuria are common and significant proteinuria and hypertension rare. Renal functional impairment is uncommon, but not unknown. With effective antibiotic treatment there is a clearing of immune complexes, a return of complement to normal values and, when impaired, a recovery of renal function. With appropriate antibiotic therapy the response is rapid, occurring in days or weeks.

Ventriculo-atrial shunts are used in the treatment of hydrocephalus and a common complication is infection, most frequently with *Staph. epidermidis*. Patients frequently present with symptoms suggestive of recurrent bacteraemia, such as fever, arthralgia, hepatosplenomegaly and anaemia. These symptoms usually precede any evidence of renal involvement by a variable period ranging from weeks to several years. Renal involvement is manifest by the presence of haematuria, which is frequently macroscopic, and proteinuria which may be sufficient to result in a nephrotic syndrome. Cryoglobulinaemia, hypocomple-

mentaemia and the detection of anti-staphylococcal antibodies are well described. In patients with minor proteinuria, the light microscopy appearances are those of a mesangial proliferative glomerulonephritis, whereas in patients with nephrotic syndrome a mesangiocapillary appearance is more common. Antibiotic therapy is frequently ineffective in controlling the infection and shunt removal is usually advocated. With the eradication of the infection the glomerular lesions usually resolve.

Salmonella

Renal involvement in typhoid fever may present either as a glomerulonephritis or as an acute renal failure due to acute tubular necrosis or from the associated haemolysis and intravascular coagulation which may occur. This haemolytic uraemic syndrome occurs more commonly in patients with glucose-6-phosphate dehydrogenase deficiency. Glomerulonephritis has been reported in 4% of affected children[51], but the incidence in adults is thought to be less. Clinically patients may have had a nephrotic syndrome for some time prior to diagnosis, splenomegaly and reduced plasma complement (C3) which is unusual in typhoid fever uncomplicated by glomerulonephritis[52]. Renal biopsy has demonstrated a mesangial proliferative lesion with deposition of immunoglobulin and complement (C3) in association with salmonella Vi antigen supporting a direct role for salmonella typhi. A case has also been documented in which the glomerular appearances were of an IgA nephropathy but in which salmonella Vi antigen was also present.[53]

Several reports from Egypt and the Sudan have implicated chronic salmonella bacteraemia in the pathogenesis of an acute nephrotic syndrome in patients with hepatosplenic schistosomiasis[54]. Biopsy revealed acute diffuse proliferative glomerulonephritis. Resolution occurred on effective treatment of the typhoid fever suggesting that salmonella rather than the schistosomes was responsible for the nephropathy in these patients.

Leprosy

Renal disease is a recognized complication of leprosy with glomerulonephritis[55], amyloidosis[56] and interstitial nephritis being described. Most commonly renal involvement is found in lepromatous leprosy although it has occasionally been described in the tuberculoid form. Episodes of erythema nodosum leprosum are associated with acute glomerulonephritis while recurrent episodes are associated with amyloidosis.

The incidence of glomerulonephritis in association with leprosy is not known but may be in the region of 10%. Proteinuria and haematuria are minor and renal functional impairment uncommon. There have been occasional reports of a rapidly progressive disease due to a crescentic nephritis.

The glomerular lesions associated with leprosy are thought to be due to deposition of circulating immune complexes. The nature of these complexes is uncertain as patients produce a variety of autoantibodies such as anti-nuclear antibodies and rheumatoid factor. On light microscopy the most common finding is of a mesangial proliferative lesion with occasionally a focal or diffuse proliferative glomerulonephritis. Only very rarely has a crescentic or membranous glomerulonephritis been described. On immunofluorescence deposition of IgG, IgM and complement (C3) is found with only occasionally IgA. On electron microscopy electron-dense deposits have been seen in the mesangium and in the capillary wall, both subendothelial and subepithelial. In some patients subepithelial 'humps' have been a feature.

Renal amyloidosis is also a well-recognized feature of longstanding lepromatous leprosy. Clinically there is proteinuria, frequently sufficient to produce a nephrotic syndrome. Progression to renal failure is common and uraemia is one of the most common causes of death. There is a marked geographical variation in the incidence of amyloidosis complicating leprosy; it commonly occurs in America but is uncommon in India and Japan. The amyloid is typically AA in type, the fibrils being composed of the serum acute phase reactant amyloid-associated protein.

Syphilis

The association between syphilis and 'dropsy' was reported early in the 19th century[57] although it is an uncommon complication. Nephropathy may develop either in congenital syphilis or in the secondary phase of an acquired infection.

In congenital syphilis nephropathy may become evident in the first few months of life and other clinical signs of syphilis are usually present. The most common mode of presentation is with a nephrotic syndrome although some cases presenting as a nephritic illness have been reported. On renal biopsy, the typical findings are of a membranous glomerulonephritis with subepithelial deposition of IgG and complement (Clq and C3). Mesangial proliferation is variable and in some cases small crescents are present. Associated interstitial nephritis is common. Treatment with penicillin is accompanied by a rapid resolution of the proteinuria, usually in 2–6 weeks, and recovery of the glomerular lesion although patients may be left with a significant number of hyalinized glomeruli.

In acquired syphilis nephropathy may become clinically apparent during the secondary phase of the infection. The majority of patients present with a nephrotic syndrome which becomes manifest at about the same time as the typical skin lesions develop. It is rare to have macroscopic haematuria, hypertension or impaired renal function. On light microscopy, there is a membranous glomerulonephritis with slight mesangial proliferation. There are subepithelial electron-dense deposits which by immunofluorescence have been shown to contain IgG and complement (C3 and Clq) associated in some cases with mesangial IgM. Prompt resolution of proteinuria occurs following institution of penicillin therapy, although a significant number of patients will undergo spontaneous resolution[58].

The lesion is considered to be immune complex-mediated as circulating immune complexes have been detected and treponemal antigen has been demonstrated within glomeruli[59]. In addition anti-treponemal antibody has been eluted from renal biopsy material. The lesion however, may only be transient[58], presumably from efficient clearing of complexes from the circulation and deposits.

PARASITIC INFECTIONS

Malaria

The association between the nephrotic syndrome and malaria has been known for the past 70 years[60] although the pathogenesis remains to be elucidated. It is not clear why some patients with *Plasmodium malariae* develop glomerular lesions and although this infection is endemic in large areas of the tropics the associated nephrotic syndrome has only been reported from certain geographical areas. The early reports from West Africa and British Guyana implicated *Plasmodium malariae* although there was a greater incidence of *Plasmodium vivax* and *Plasmodium falciparum* infection in the community. Following the eradication of malaria from British Guyana there has been a marked reduction in the incidence of nephritis[61] tending to support the causal relationship, but it must be remembered that many other changes with respect to nutrition and health also occurred in this time interval.

Recent studies from Nigeria and other West Africa countries have produced conflicting information. In Nigeria 88% of nephrotic children had *Plasmodium malariae* parasitaemia[62], whereas investigations of children in Ghana, where *Plasmodium malariae* is endemic, have not confirmed this finding[63]. This suggests that not all strains of *Plasmodium malariae* may be nephrogenic or that there are other factors modifying this infection such as nutrition and genetic predisposition.

The main glomerular lesion found in association with *Plasmodium malariae* is a focal and segmental glomerular capillary wall thickening involving the sub-endothelial aspect producing in some areas a double contour appearance. This progresses in time to focal, and ultimately global, sclerosis. Cellular proliferation is minimal if present and only occasionally are small crescents found. Immunofluorescence studies have revealed deposition of IgG, IgM,C3 and antigens of *Plasmodium malariae* but not *Plasmodium falciparum*. On microscopy there is irregular glomerular capillary wall thickening due to an increase in basement membrane material, sub-endothelial electron-dense aggregate and small lacunae containing inclusions distributed throughout the basement membrane.

Initially it was considered that this lesion was 'quartan malarial

nephropathy' but similar appearances were described in nephrotic children from Senegal in whom *Plasmodium malariae* could not be implicated[64]. This finding casts doubt that the described lesion is caused by an effect of *Plasmodium malariae*. In addition, the immunofluorescence studies demonstrating *Plasmodium malariae* antigens may be of doubtful significance because of the cross-reactivity of antibodies to different plasmodial strains and because patients with chronic malaria may produce anti-IgG and other antibodies. Thus although the association between *Plasmodium malariae* infection and glomerulonephritis is accepted the pathogenesis is unclear.

The reported glomerular changes may not be specific for *Plasmodium malariae* although it would be reasonable, until further clarification is made, to use the term malarial nephropathy.

In addition to the lesion described above other glomerular lesions such as minimal change nephropathy, minor proliferative or focal lesions and mesangiocapillary glomerulonephritis have been reported. The link with malarial infection is, however, unclear in view of the endemic nature of malaria and thus these reports may be just a chance occurrence.

Clinically nephrotic syndrome occurs most frequently between 5 and 8 years with an equal sex incidence. Hypoalbuminaemia is often marked and ascites is common. Microscopic haematuria is common but hypertension rare.

Anti-malarial treatment is not effective in producing a remission of the nephrotic syndrome. Steroids with or without immunosuppressive drugs are unlikely to be beneficial and may be accompanied by unacceptable complications due to the general poor nutritional state and increased risk of infection in affected children.

Plasmodium falciparum infection may produce an immune complex-mediated glomerulonephritis or acute renal failure from massive haemolysis and haemoglobulinuria. Proteinuria is common during episodes of fever and usually remits as fever subsides. Acute glomerulonephritis and renal failure has been described[65], as has the nephrotic syndrome associated with proliferative glomerulonephritis[66]. In most patients the glomerulonephritis occurring during the course of falciparum malaria is mild and transient. On light microscopy there is a mesangial proliferative lesion with some thickening of the basement membrane. Immunofluorescence reveals IgM

and complement while on electron microscopy there may be mesangial and sub-endothelial deposits. Resolution of the glomerular lesion usually occurs within six weeks of effective antimalarial treatment[67].

Schistosomiasis

Schistosoma infection (Bilharziasis) is widespread throughout the tropics and it has been estimated that at least 2×10^6 people are currently infected. Four species of schistosomes are known to affect man and associated nephropathy has been described for *Schistosoma mansoni, haematobium* and *japonicum*.

Nephropathy commonly complicates hepatosplenic schistosomiasis in which hepatic fibrosis develops. In the majority of patients hepatic fibrosis occurs approximately 10 years after infection and nephropathy appears some 5 years later. Presentation is usually in males in their second or third decade and is with proteinuria, hypertension and progressive renal failure. There is splenomegaly and evidence of portal hypertension. There are marked geographical differences in the incidence of nephropathy and schistosomes of the same species seem to differ with respect to infectivity and pathogenicity. Some evidence suggests a genetic predisposition in that there was an increased incidence of HLA-A28 in patients with hepatosplenic schistosomiasis and nephropathy[68]. The majority of reports have implicated *Schistosoma mansoni* with only a few clinical reports involving *Schistosoma japonicum*. The role of *Schistosoma haematobium* is still unclear. Despite several epidemiological studies reporting a close relationship between proteinuria and *Schistosoma haematobium* infection[69], no glomerular lesions were detected in a large autopsy series in patients with pure infections[70].

Renal biopsy findings in schistosomal nephropathy are most commonly of a proliferative glomerulonephritis or a focal segmental glomerulosclerosis although mesangiocapillary, membranous and minimal changes have been reported. Amyloidosis has occasionally been described. On immunofluorescence IgG, IgA, IgM and complement (C3) have been observed in the mesangium and capillary walls. Schistosomal antigen and anti-schistosomal antibodies have been demonstrated in the glomerular deposits. On electron micro-

scopy mesangial, subendothelial, intramembranous and subepithelial electron-dense deposits have been documented.

The role of hepatic fibrosis in the pathogenesis of the lesion appears to be of importance although there is no clear correlation between any laboratory marker of hepatic dysfunction and the severity of the nephropathy. It is most likely that the hepatic fibrosis is associated with a diminution of hepatic macrophage function thereby impairing the removal of antigen from the portal system.

Treatment of the underlying schistosomiasis does not appear to have a marked effect on the underlying nephropathy presumably due to its longstanding presence. There is no evidence that corticosteroids or immunosuppression have any effect on the progressive nature of the renal disease.

Leishmaniasis (Kala-Azar)

In a number of patients with visceral Leishmaniasis (*Leishmania donovani*) increased mesangial matrix and cellularity have been described[71]. Paramesangial electron-dense deposits and mesangial IgG and C3 deposition have been reported. The exact relationship between these findings and kala-azar is difficult to determine as the majority of patients have considerable malnutrition and immunosuppression and acute intercurrent infections are common. Clinical renal disease is manifested by microscopic haematuria and minor proteinuria.

Filariases

A number of nematodes of the family *Filariidae* may affect man and there is a recognized association between both loiasis (*Loa loa*) and onchocerciasis (*Onchocerca volvulus*) and glomerulonephritis. In patients with loasis membranous glomerulonephritis and mesangiocapillary glomerulonephritis have been described, and in onchocerciasis, minimal change nephropathy, mesangial proliferative glomerulonephritis and mesangiocapillary glomerulonephritis[72]. In some patients with onchocerciasis, glomerular deposition of oncho-

cercal antigen has been found. A causal relationship, however, is very difficult to establish in view of the frequent finding of coexisting infections with malaria.

CONCLUSION

The association between infections and glomerulopathy has been well recognized for many years, but not surprisingly many questions remain to be answered. A wide variety of glomerular lesions may be produced from an ever-expanding list of infecting agents, varying from viruses to fungi. In the majority of patients, the glomerular changes are induced by an immune mechanism as a consequence of the introduction of a foreign antigen or by the modification of a host protein, rendering it antigenic with a subsequent autologous response. Organisms, even of the same species, vary with respect to their nephritogenic potential and the response obtained is governed by a wide variety of patient characteristics, including genetic and nutritional status. This explains why a uniform glomerular response is seldom obtained following a specific infection.

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4

IgA NEPHROPATHY

D. A. POWER and J. G. SIMPSON

Following initial descriptions in the late 1960s[1,2], it has slowly been recognized that IgA nephropathy is the commonest form of idiopathic glomerulonephritis in most Western countries and a major cause of end-stage renal failure. Perhaps the best thumbnail sketch of IgA nephropathy is of a disease with 'diverse clinical features and uncertain prognosis'[3], characterized by the deposition of IgA within the glomerular mesangium and, clinically, haematuria. The condition was not recognized prior to 1967 because reagents and techniques necessary for detecting IgA within renal biopsy material were not available; it is evident in retrospect, however, that some descriptions of mesangial proliferative nephritis published prior to this time were largely descriptions of IgA nephropathy[4].

EPIDEMIOLOGY

Centres from many countries have reported IgA nephropathy to be the commonest form of primary glomerular disease in their experience. Studies from France[5,6], Italy[7]. Spain[8], Australia[3], Scotland[9] and elsewhere[10] have reported series of renal biopsies where IgA nephropathy accounted for 20–25% of primary glomerular diseases. Population-based studies from France[11] and Holland[12] have suggested an annual incidence of 19–30 new cases/1 000 000 population/year. Estimates of disease prevalence have emerged from routine pathological examination of individuals in Singapore[13] and Germany[14] – approximately

2% had IgA deposits. Routine immunohistology of transplanted kidneys has confirmed a high disease prevalence in Paris[15] and Gothenburg[16].

A lesser frequency has been reported in renal biopsy series from North America[17,18] and Britain[19]. Assessing the reasons for such apparent variations in incidence has been complicated by:

(1) A lack of uniformity in accepted indications for renal biopsy in different centres and countries. While 'hard' data are difficult to obtain, it seems likely that many large centres either do not attract or fail to biopsy young patients presenting with haematuria. Since patients presenting in this way constitute the majority of cases in most series reporting a high incidence, failure to biopsy them would be expected to bias a series, producing a low incidence.

(2) Differential usage of routine urinalysis in different populations. For example, a very high incidence of the condition has been reported in military recruits in Singapore[21], all of whom were subjected to urinalysis and biopsied if abnormal. Studies from Japan[22], where routine urinalysis has been performed on large sections of the population have shown similar results. Clearly, many countries do not routinely test the urine of all young adults or subject large numbers of them to renal biopsy.

(3) Failure to record the indication for renal biopsy in the whole biopsy population from which cases of IgA nephropathy have been drawn. It becomes, therefore, impossible to detect referral bias or differences in indication for renal biopsy.

In Britain the incidence was considered low for some time, based upon a report by Sissons *et al.*[19] from two London centres. This has not been confirmed by subsequent series from Manchester[23] and Scotland[24]; it seems probable that the rarity of the condition in London Teaching Hospitals is due to their function as tertiary referral centres. To what extent the low incidence in the USA and Canada can be ascribed to such ascertainment bias is not clear.

There are some indications, however, that racial variations in incidence do occur. IgA nephropathy seems distinctly uncommon in American blacks[25], whereas American Indians appear to have a high incidence[26]. At present, the relative contributions of race, environment

TABLE 4.1 Clinical and laboratory findings at presentation in five series

	Nicholls et al.	*Mustonen* et al.	*D'Amico* et al.	*Clarkson* et al.	*Droz and co-workers*‡
Reference	28	38	7	3	5, 39, 132
Number	244	143	374	50	244
Sex (M:F)	2.7:1	1.8:1	2.4:1	5.3:1	2.4:1
Mean age at diagnosis (×1 SD)	32	NP†	34	36	NP†
Raised serum IgA (%)	21	NP†	38	50	45
Hypertension* (%)	22	32	36	52	6
Proteinuria >1 g/day (%)	39	NP†	22	NP†	38
Raised serum creatinine (%)	36	15	24	62	12

* Hypertension was variously defined by the authors of the different series
† NP data not presented by authors
‡ Much of the data presented by Droz and her co-workers were obtained after estimating the apparent date of onset of the condition, rather than at the time of diagnosis, as in other series. Where necessary, data were pooled for this analysis from three separate publications with varying patient numbers, although most comes from one[5]

and biopsy policy to the frequency with which IgA nephropathy is diagnosed in various countries cannot be determined.

Sex

Most studies have shown a clear male predominance, whatever the overall incidence. This varies from 15:1 to 1.2:1 – most are around 2:1 (Table 4.1). While a sexual predominance might be explained by the increased likelihood of a male to enter an occupation where routine urinalysis is required, again leading to an error in ascertainment, this is not supported by data from the Aberdeen experience where the sex ratio is 6:1[24]. We found that males presenting with asymptomatic haematuria were much more likely to have IgA nephropathy than females with a similar presentation.

Age at diagnosis

The usual age at diagnosis in most series is early adulthood, although no age is spared (Table 4.1). The absence of dramatic presenting features in many patients and the insidious nature of the disease make it impossible to date accurately the onset in many individuals.

PRESENTING FEATURES

Common forms of presentation are asymptomatic haematuria with variable proteinuria, macroscopic haematuria and chronic renal failure (Table 4.2). Less frequently encountered are the acute nephritic syndrome, nephrotic syndrome, rapidly progressive glomerulonephritis and malignant hypertension.

TABLE 4.2 Major presenting features of patients with IgA nephropathy in five series

	Nicholls et al.	*Mustonen* et al.	*D'Amico* et al.	*Clarkson* et al.	*Droz and co-workers*
Reference	28	38	7	3	5, 39, 132
Number	244	143	374	50	244
Macroscopic haematuria (%)	59	26	56	34	37
Asymptomatic* microscopic haematuria (%)	35	59	44	26	NP†
Nephrotic syndrome (%)	3	5	0	6	6
Chronic renal failure (%)	<1	<1	0	6	NP†
Acute nephritis/acute renal failure (%)	1	1	0	12	NP†

* Includes patients with microscopic haematuria plus proteinuria
† NP data not presented by authors

1. Asymptomatic haematuria

Patients with asymptomatic haematuria detected by dipstix testing are seen increasingly in renal outpatient clinics; many in the younger age groups (<40) have IgA nephropathy. These individuals usually have associated proteinuria (<1 g per day) which is non-selective[27]. In various series 7–14% had no detectable proteinuria despite microscopic haematuria[28,29]. Most patients have initially normal renal function. Urinary erythrocytes show typical glomerular morphology[30].

Microscopic haematuria is very common in the population at large and was observed by Sinniah *et al.* in about 1% of male military recruits[21], a figure similar to that reported by workers in Finland[31]. Approximately half of the individuals in these two studies had IgA nephropathy when subjected to renal biopsy. In Aberdeen, 37% of patients biopsied on account of asymptomatic haematuria have an underlying IgA nephropathy[24]. Indeed, so common is a glomerular cause for haematuria seen in patients under 40 that we do not routinely cystoscope these individuals, but proceed to renal biopsy provided the kidneys are radiologically normal, an approach supported by the low incidence of urological abnormalities in these patients[32]. A laboratory which can reliably distinguish glomerular erythrocyte morphology can be a considerable help in management. Whether renal biopsy is justified simply to identify the origin of microscopic haematuria when no proven therapy is available for the most common diagnosis encountered is the subject of debate.

2. Macroscopic haematuria

Gross haematuria, sometimes so severe as to produce clot colic, clot retention and anaemia, occurs in a significant proportion of affected individuals. The incidence of patients presenting in this way varies widely in different series. In his review[10], d'Amico suggests that this variation is geographical, with 20–30% of patients in Asia and 50–70% in Europe presenting in this fashion. It is far more common for males to present with macroscopic haematuria and most are aged <35. Associated symptoms, such as loin pain, myalgia, fever and malaise are quite common during episodes of haematuria.

Generally, these individuals have microscopic haematuria in intervals between episodes of macroscopic haematuria, usually with proteinuria < 1 g/day[28]. Macroscopic haematuria often occurs in relation to intercurrent infection (24–48 hours after onset – synpharyngitic haematuria), vaccination, or strenuous exercise, but sometimes has no identifiable precipitant.

Episodes of frank haematuria become less frequent the longer these patients are followed and only 19% experienced >2 episodes in the series of d'Amico *et al.*[7]. Occasional patients have up to 15 episodes; most occur in the first one or two years after presentation, although frank haematuria can recur after an interval of many years. Mild renal impairment may be present during an episode of haematuria. Only rarely is there hypertension or a striking elevation in serum creatinine to suggest the acute nephritic syndrome. Acute renal failure is a rare complication, although it has been reported[35,36]. The outlook for full spontaneous recovery of renal function is good.

Patients who experience macroscopic haematuria may be a distinct subgroup compared with others. Most studies have demonstrated a more benign prognosis in the former (see below) and there may be distinct genetic markers in the two groups[37].

3. Chronic renal failure

Patients presenting with chronic renal failure tend to be older, often having hypertension and heavy proteinuria[19]. It is still not clear whether microscopic haematuria always precedes chronic renal failure, although this seems likely. Most patients do not admit to past episodes of frank haematuria.

4. Acute nephritic syndrome

Although unusual, this is a recognized presentation, as noted above.

5. Nephrotic syndrome

Two forms have been distinguished, based upon biopsy appearances and the response to steroids. In earlier series, most nephrotic patients were hypertensive with renal impairment and had severe, chronic lesions on renal biopsy, such as segmental and global sclerosis with major interstitial scaring[35,38,39]. Prognosis in these cases was poor. More recently, several groups have reported features typical of IgA nephropathy, generally of a very minor degree, with foot process fusion[40,41]. This lesion is steroid-responsive, has a marked tendency both to remit spontaneously and to relapse and has not been confined to children. Most authors have considered it minimal change nephritis superimposed on pre-existing IgA nephropathy although it may represent a distinct variant of IgA nephropathy.

Interestingly, Mustonen *et al.*[42] have recently described several nephrotic patients with mild impairment of renal function whose biopsies showed relatively severe sclerotic or proliferative glomerular lesions who responded to steroids. It may be difficult in future to decide who to treat if even patients with renal impairment and severe histology occasionally respond. Woo *et al.*[43] used protein selectivity to distinguish a steroid-responsive group but it is doubtful whether this will prove adequate for all cases in adults.

6. Hypertension and malignant hypertension

Until recently, only four patients were reported to have presented with malignant hypertension. Subias *et al.*[44], however, found that 10 (15%) of their 66 patients presented with accelerated or malignant hypertension. 60% of these were on dialysis 14 months after presentation. The authors suggested that biopsy of more patients with malignant hypertension would unearth further cases of IgA nephropathy. In recent studies from several groups, about 5% of cases have presented with malignant hypertension[45,46].

Hypertension is commonly found at the time of diagnosis, about 20–30% in most series (Table 4.1). Most authors do not, for some reason, consider this to have been the form of presentation when it is associated, as it invariably is, with a urinary abnormality. Excess

endogenous renin activity has been reported in patients with IgA nephropathy[47,48], perhaps explaining their propensity to develop hypertension even when compared with patients who have other forms of glomerulonephritis.

FIGURE 4.1 IgA deposition in mesangium. Immunofluorescence, × 350

A summary of the frequency of common primary presenting features and associated clinical and laboratory findings in patients with IgA nephropathy is shown in Tables 4.1 and 4.2.

PATHOLOGICAL FEATURES

and 35% from Milan[7]. Generally they involve $<10\%$ of glomeruli and are almost never circumferential. They appear to be more common if patients are biopsied during an acute episode of macroscopic haematuria. Healing is by formation of a triangular scar, which can

1. Immunofluorescence

In addition to diffuse granular mesangial deposits of IgA (Figure 4.1) in most or all glomeruli, extension of IgA to some of the peripheral capillary loops is common (Figure 4.2 and Table 4.3). C3 is generally distributed in a pattern similar to that of IgA and has been reported in about 85–90% of cases in most series. Deposition of IgG and IgM is usually less prominent than that of IgA or C3, although the distribution is similar; the incidence varies considerably between series (Table 4.3). IgG was present in 17–100% of biopsies and deposits of IgM in 28–58% in various series. Fibrinogen has been reported in 25–30% of cases, whereas C4 and Clq are uncommon, usually $< 10\%$ of biopsies. C3 may be deposited in arterioles or along tubules but again this is uncommon.

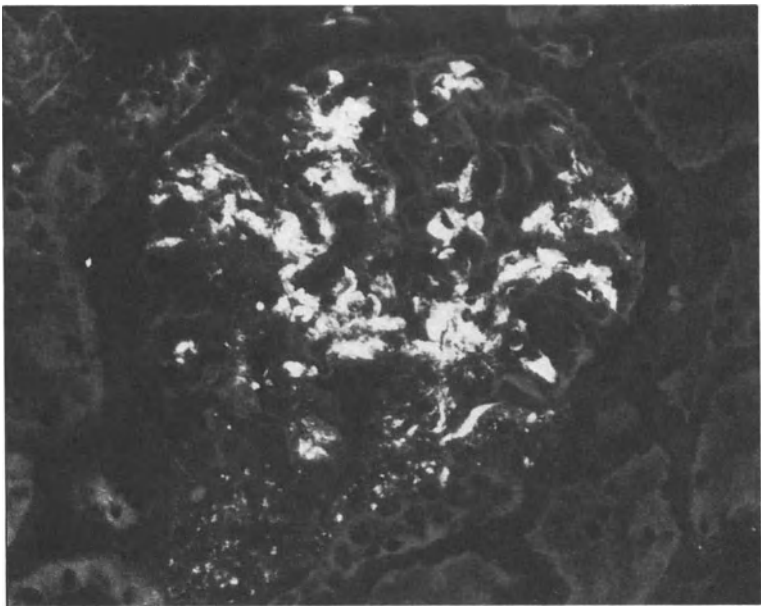

FIGURE 4.2 Large IgA deposits in mesangium with fine granular staining in some peripheral capillary loops. Immunofluorescence, x350

Comprehensive immunogold stained series are not yet available, but are likely to show similar features.

2. Light microscopy

These range from minimal increases in mesangial prominence (Figure 4.3), often difficult to distinguish from normal appearances, through increases in mesangial matrix and mesangial cell hyperplasia of varying degrees. Superimposed on diffuse mesangial enlargement, focal and segmental mesangial proliferation occurs in over 50% of biopsies (Figure 4.4), while areas of capillary collapse and sclerosis are also common in some series. Circumscribed extracapillary proliferation ('crescents') were described in 32% of cases in the Melbourne series[28]

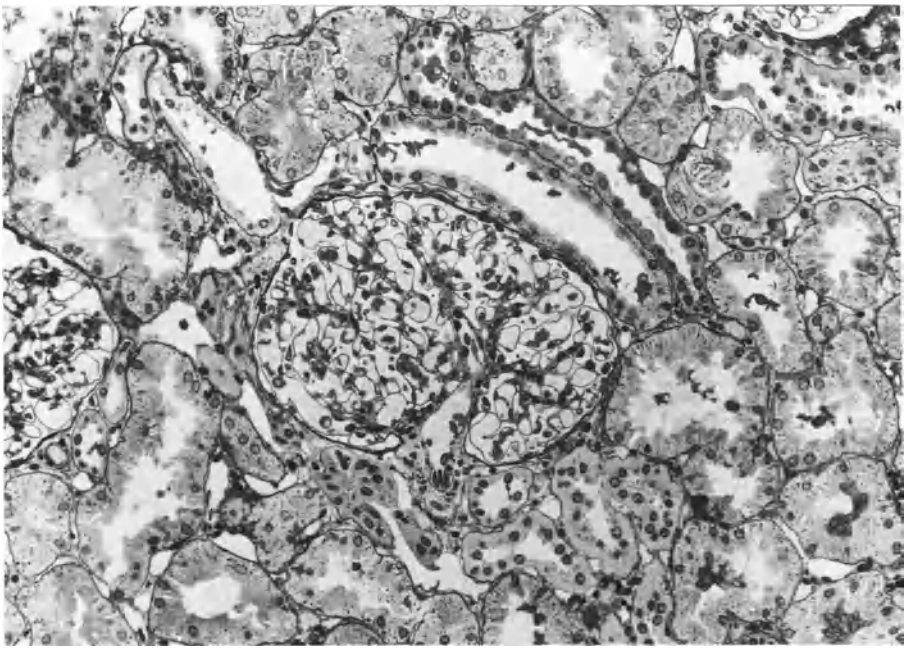

FIGURE 4.3 Minor mesangial prominence. Some of the tubules contain red cells. Silver methenamine, ×110

and 35% from Milan[7]. Generally they involve <10% of glomeruli and are almost never circumferential. They appear to be more common if patients are biopsied during an acute episode of macroscopic haematuria. Healing is by formation of a triangular scar, which can

be difficult to distinguish from focal and segmental hyalinosis and sclerosis, a lesion with a quite different prognosis. Global sclerosis is not uncommon (Figure 4.5), present in 74% of biopsies from the Melbourne series[28] and 70% from Milan[7]. In most cases, $<20\%$ of glomeruli are affected. The basement membrane is, by and large, normal; sometimes areas of mesangial interposition may be seen adjacent to areas of segmental proliferation but the lesions do not resemble membranoproliferative/mesangiocapillary glomerulonephritis. Deposits of hyaline, either paramesangial or within the mesangium are common.

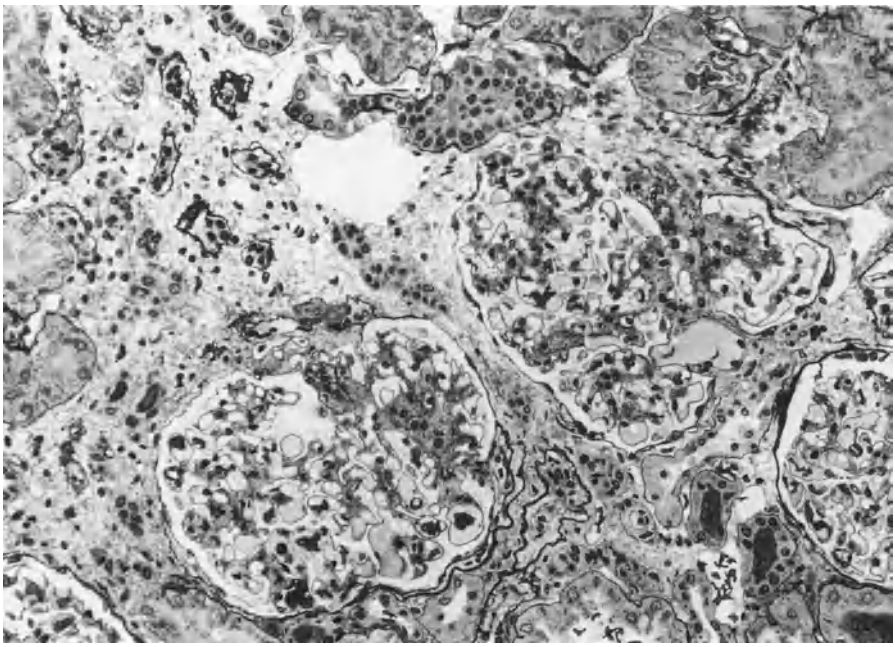

FIGURE 4.4 Two glomeruli showing segmentally-accentuated mesangial proliferation. There is also a focus of tubular atrophy and interstitial fibrosis (top left). Silver methenamine, ×110

Changes in the rest of the kidney (Figures 4.4 and 4.5) reflect the severity and chronicity of the glomerular lesion. They include interstitial fibrosis, a sparse interstitial mononuclear cell infiltrate and arteriolar hyalinosis.

3. Electron microscopy

Electron dense mesangial deposits are almost always present (Figure 4.6). Their position and large size (Figure 4.7) are so distinctive that diagnosis is possible even in the absence of immunohistological confirmation of IgA deposition. Occasionally deposits are also present in the peripheral capillary walls (Figure 4.8). Using electron microscopy, the secondary changes of mesangial hypercellularity (Figure 4.9) and sclerosis (Figure 4.10) can be distinguished. Minor changes in the appearance of the glomerular basement membrane have also been described[40].

TABLE 4.3 Presence of immunoreactants in first renal biopsies from four series

	Nicholls et al.	*Mustonen* et al.	*d'Amico* et al.	*Clarkson* et al.
Reference	28	38	7	3
Number of biopsies	244	143	374	50
IgG (%)	22	29	49	14
IgM (%)	60	50	28	58
C3 (%)	100	92	82	85
Fibrinogen (%)	50	30	24	NP†
C1q (%)	18	17	6	NP†
Extension of IgA onto capillary loops (%)	54	17	40	NP†

† NP data not presented by authors

PROGNOSIS OF IgA NEPHROPATHY

IgA nephropathy is not a benign disease; 10 year survival rates vary from 80–91% and 20 year survival rates decline to 66–84% (Table 4.4). Deterioration in renal function may be rapid in individual patients but the overall pattern is of a slowly progressive renal lesion which produces renal failure in a significant proportion of affected individuals if they are followed long enough. At present, reliable follow-up data are available for periods of up to 20 years; occasional patients

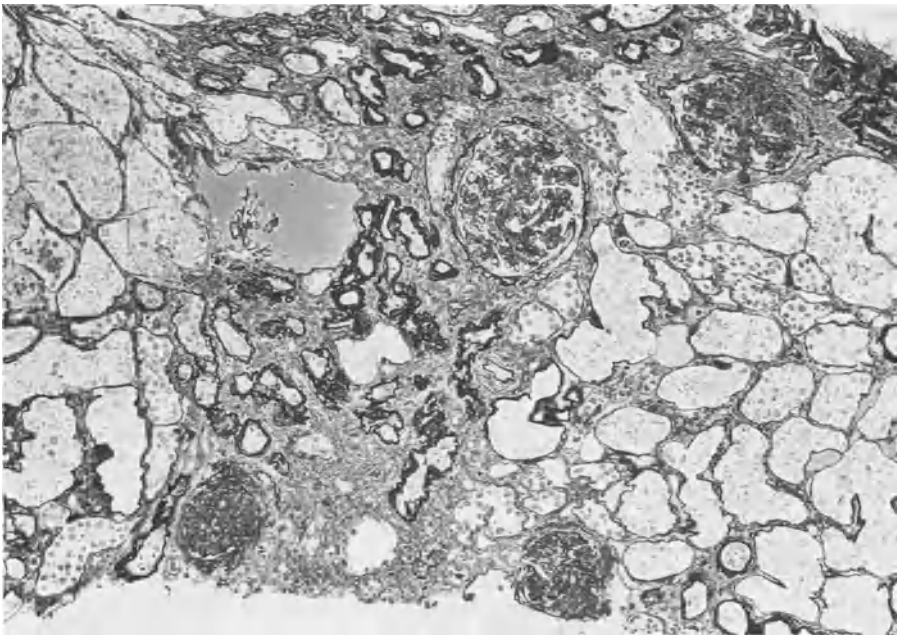

FIGURE 4.5 Severe glomerular damage with two globally sclerosed glomeruli (bottom). A thick band of atrophic tubules and interstitial fibrosis runs down the middle of the picture. Silver methenamine, ×42

have been followed for longer, but their numbers are small. Some individuals appear to lose all clinical evidence of renal disease – none of the small number who have undergone repeat renal biopsy has shown histological clearing of the lesion[28]. Nonetheless, it is apparent that a minority of patients has developed end-stage renal failure during the follow-up periods currently available. It has become important, therefore, to develop criteria which enable prediction of the prognosis in an individual.

Prognostic indicators

These can be conveniently divided into clinical and pathological indicators.

1. Clinical

A variety of factors have been described:

Age
Older age at presentation indicates a poor prognosis[39,50], probably because these patients often have pre-existing renal impairment and may have had asymptomatic disease for many years.

Absence of macroscopic haematuria
Most studies have shown that individuals who experience one or more episodes of frank haematuria have a better prognosis than those who do not[39,50,51]. This is a surprising finding, since patients biopsied during an episode of gross haematuria often have relatively severe histological findings, including non-circumferential crescents. It has been argued, however, that patients with macroscopic haematuria present earlier because they are symptomatic and only appear to have milder disease because of its shorter duration[28]. Certainly, several series have shown that patients with frank haematuria may be, on average, 5–10 years younger than those presenting with microscopic haematuria. However, using multivariate analysis d'Amico *et al.* were unable to confirm that macroscopic haematuria was an independent variable for the development of renal failure[50].

Hypertension
This is very common at onset and even more common during the course of the disease. In two large series[7,28], 8 and 22% of patients developed hypertension *de novo* during follow-up. About 50% of patients are hypertensive at some stage during their course. There is no doubt that hypertension is an adverse prognostic feature[18,28,50,51]. Uncontrolled hypertension in particular is often associated with rapid progression. Rambausek *et al.*[14] were able to correlate deterioration in creatinine clearance over a two-year period with mean arterial blood pressure at diagnosis.

Proteinuria
>1 g/day indicates a poor prognosis[18,28,50,51]. Levels below 1 g appear to have little influence on outcome[52].

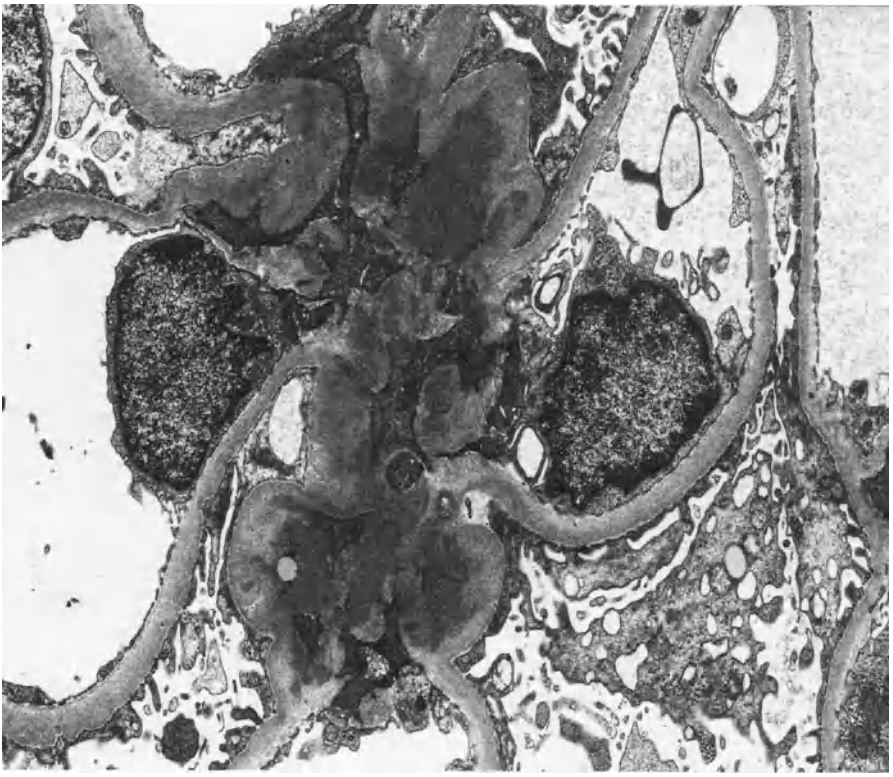

FIGURE 4.6 Large irregular dark grey deposits expand the mesangium. EM, ×4250

Urinary erythrocyte excretion rate

Studies from Melbourne[28] and other centres have reported that persistently high urinary excretion of erythrocytes in urine ($> 10^5$ erythrocytes/ml) correlates with a poorer prognosis, although the levels at diagnosis do not. Unfortunately, many centres do not have the personnel to perform this tedious estimation.

Sex

A few studies have reported that males have a poorer outcome[28,39] but this is disputed[50].

TABLE 4.4 Kidney survival* in patients with IgA nephropathy

Series	*Reference*	*Origin*	*Number*	*10-year survival* (%)	*20-year survival* (%)
Legrain *et al.*	133	France	75	85	—
Noel *et al.*	132	France	260	85	75
Nicholls *et al.*	28	Australia	244	88	—
Wyatt *et al.*	134	U.S.A.	58	78	—
d'Amico *et al.*	7	Italy	365	85	66
Beukhof *et al.*	51	Netherlands	75	80–84	72–75
Woo *et al.*	135	Singapore	151	91	—
Velo *et al.*	136	Spain	97	82	72
Kobayashi *et al.*	137	Japan	40	60	—
Rambausek *et al.*	14	Germany	131	78	—

* Survival from date of renal biopsy or estimated date of disease onset
Table based upon that of d'Amico,[10] updated and enlarged

2. *Histological*

Various histological features have been described which indicate a poor outcome. In general, these are lesions of proliferation and sclerosis[10,28,50,53]. Among them are severe diffuse mesangial proliferation, extracapillary proliferation (crescents), lesions of the glomerular capillary walls, global and segmental glomerular sclerosis, vascular lesions and interstitial sclerosis. Extension of mesangial deposits of IgA or electron-dense deposits into the peripheral capillary loops has also been reported as a poor prognostic indicator[50].

A scoring system for assessment of renal biopsy specimens has been described by d'Amico[10]. This may allow a more uniform assessment of renal biopsy appearances in different laboratories. The system is as follows:

Class I Minimal lesions (normal appearance by light microscopy).
Class II Minor changes, with widening of the mesangium and increased cellularity of groups of up to three cells per area in the periphery of glomeruli.

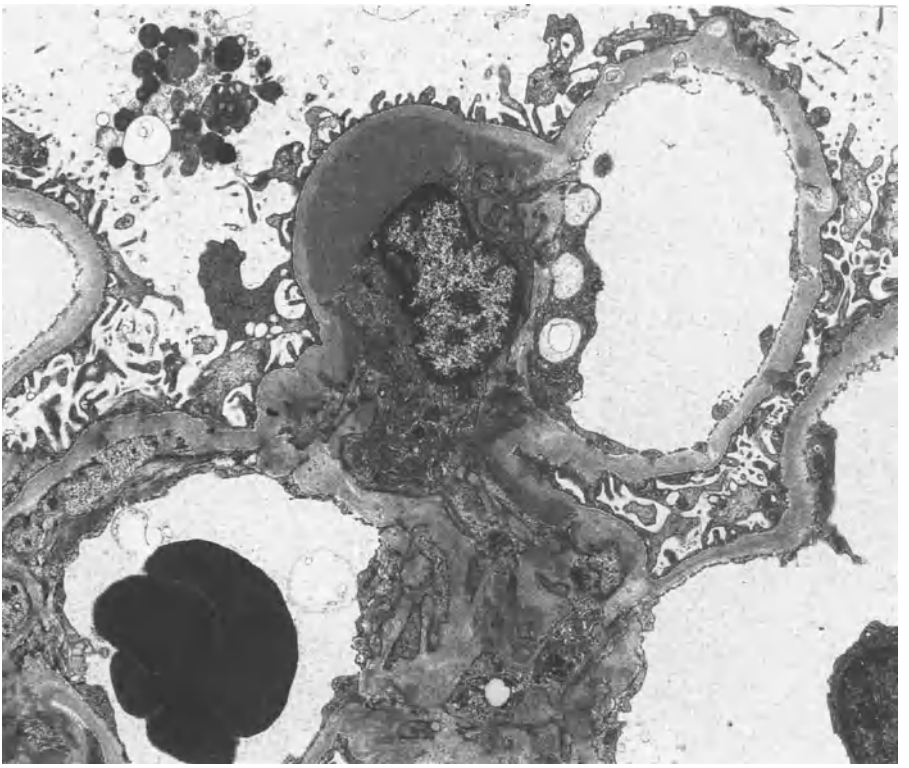

FIGURE 4.7 Massive mesangial expansion adjacent to mesangial nucleus. The loop at top right shows several small deposits. EM, × 4250

Class III Focal and segmental glomerulonephritis, with less than 50% of the glomeruli showing localized or segmental sclerosis and mesangial cell proliferation; the remaining glomeruli showing minor changes. Occasional small crescents and adhesions and mild interstitial fibrosis are seen.

Class IV Marked diffuse mesangial proliferation and sclerosis, with varying degrees of hypercellularity. Obsolescent glomeruli are frequently found, in variable numbers. Focal crescents are present in a variable number of glomeruli. Tubulo-interstitial changes are evident.

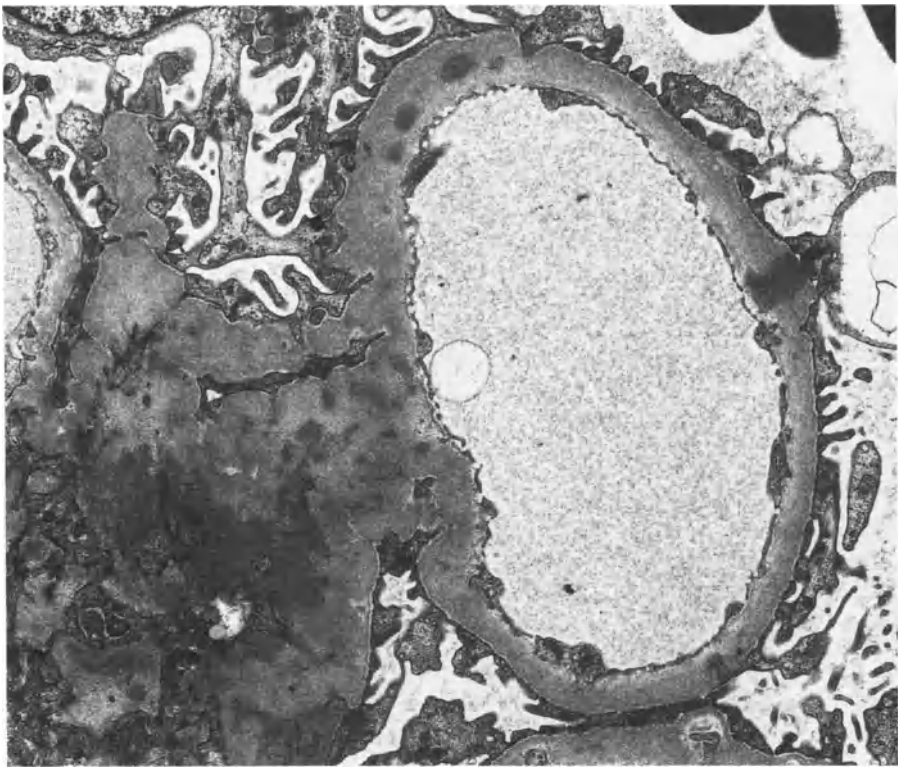

FIGURE 4.8 In addition to mesangial deposits (bottom left), there are deposits in the peripheral glomerular basement membrane. EM, × 6400

Class V Diffuse sclerosing glomerulonephritis, with involvement of 80% of glomeruli. Severe interstitial sclerosis is seen.

Prediction of outcome is important in a disease where most affected individuals suffer no adverse consequences and treatment is likely to carry substantial risks. Unfortunately, many of the predictors described are features of advanced disease rather than true markers of future damage. There is a requirement for better markers of active disease, perhaps based upon a greater knowledge of the pathogenesis of the condition.

Moreover, as is evident, some of the predictive factors noted are

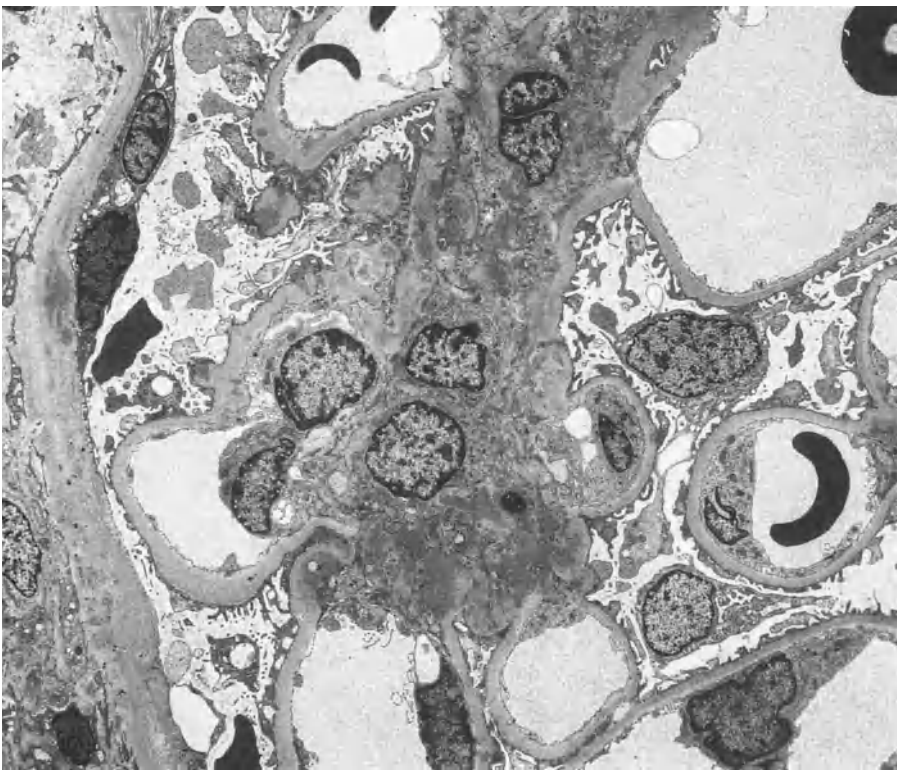

FIGURE 4.9 Glomerular segment (Bowman's capsule on the left). In the middle of the picture is a hyperplastic mesangial stalk. EM, ×2100

not independent variables. For example, patients with proteinuria tend to be older with no history of previous episodes of macroscopic haematuria. Glomerular sclerosis is usually seen in association with interstitial inflammation and vascular lesions. d'Amico *et al.*[50], using Cox's regression analysis, have found that only proteinuria, global glomerular sclerosis, interstitial sclerosis and extension of IgA deposits onto peripheral capillaries were independent predictors of outcome. In an innovative study, they attempted to weigh these independent variables so as to predict the prognosis in patients with normal renal function. Unfortunately, there was considerable overlap in scores between those who deteriorated and those who did not.

Another interesting approach has been that of Kincaid-Smith and co-workers[28] and Droz *et al.*[39], who calculated the relative risk for subsequent progression for each of the factors they defined (Table 4.5). This gives some idea of the practical significance of a particular observation when assessing the likelihood of progression in an individual.

PATHOGENESIS

Aetiology and pathogenesis have been the subject of intense scrutiny and it now appears that steady progress is being made. There is little doubt that IgA nephropathy is due to deposition of circulating IgA, probably together with other molecules. Almost half the patients transplanted because of end-stage renal failure caused by IgA nephropathy develop typical recurrent disease[54], although it is rarely of clinical significance. Moreover, cadaveric kidneys with pre-existing IgA deposits lose them if transplanted into recipients with renal lesions other than IgA nephropathy[55,56].

Biology of IgA

More IgA is synthesized than any other immunoglobulin[57]. It is present in lesser amounts than IgG within serum (50–350 mg/dl and 700–1600 mg/dl respectively) because its half-life within the blood is relatively short (5–6 days compared with 20–23 days for IgG) and the majority of IgA synthesized is secreted into external excretions.

IgA circulates in two forms – a monomer of two light and two heavy chains and as a polymer of two such monomers joined by a J chain[57,58]. About 90–95% of circulating IgA is monomeric, originating from bone marrow, spleen and lymph nodes[59]. Polymeric IgA is typically a product of mucosal surfaces[59]. Polymeric IgA in serum differs, however, from IgA which is secreted because the latter under-

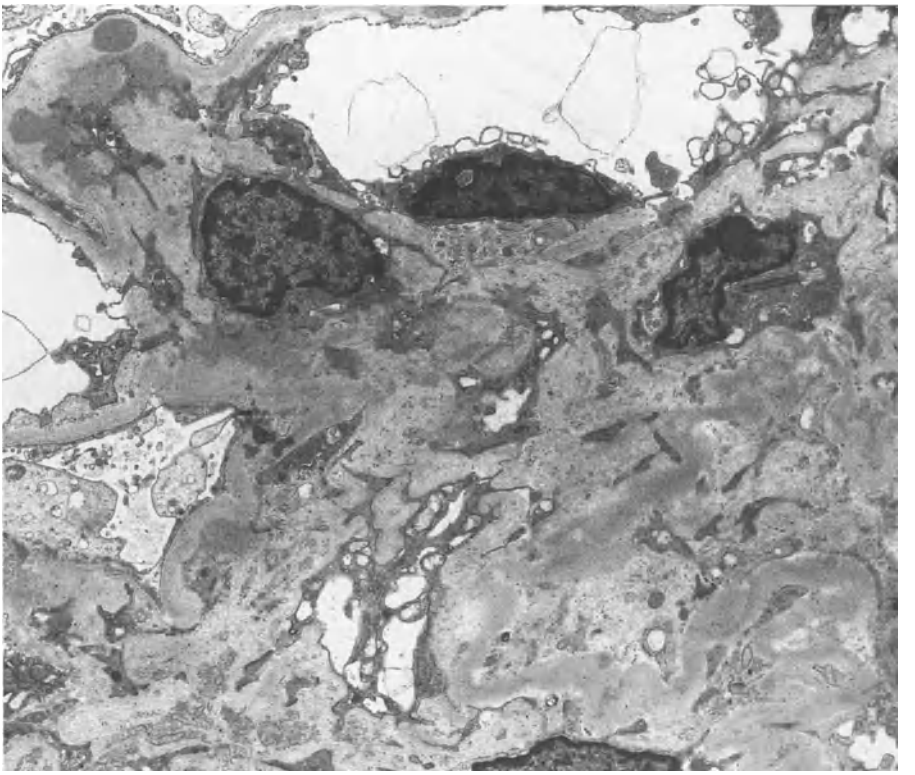

FIGURE 4.10 Mesangial sclerosis. EM, ×4250

goes passage through the cell to the mucosal surface (transcytosis) and during this passage a part of the molecule which acts as a carrier becomes attached as secretory (Sc) piece[60]. IgA exists as two distinct subclasses, IgA1 and IgA2, produced by two heavy chain genes lying side by side at the 3′ end of the row of immunoglobulin heavy chain genes on chromosome 14. IgA1 is the more abundant, especially in serum where it accounts for about 84% of the total; mucosal IgA is also predominantly IgA1 with, however, as much as 40% IgA2[57,58].

These data have assumed much, perhaps too much, importance in studies of the pathogenesis of IgA nephropathy. The general approach

TABLE 4.5 IgA Nephropathy: features associated with subsequent deterioration in two studies[28,39]

	Relative risk	
	Nicholls et al.	*Droz* et al.
At first biopsy		
global sclerosis > 10% glomeruli	3.7	NP†
Mean BP > 110 mmHg	2.8	Incr*
proteinuria > 1 g/day	2.7	1.5/5.2*‡
male sex	2.1	1.3
crescents	1.9	Incr*
age at onset > 35	NP†	2.0
At follow up		
habitual urinary RBC count > 10^5	4.3	NP†

* Incr: increased risk – relative risk not presented
† NP data not presented by authors
‡ relative risk for proteinuria 1 – 2 g/day = 1.5
relative risk for proteinuria > 2 g/day = 5.2

has been that if IgA deposited within the mesangium can be shown to be polymeric by molecular weight, presence of J chain or ability to bind Sc, then it is likely to originate from mucosal surfaces. Similarly, presence of significant amounts of IgA2 would suggest mucosal origin. There are two problems with this approach. Firstly, polymeric IgM also contains J chain and will bind secretory piece so that biopsies which contain this immunoglobulin may be difficult to interpret. Secondly, the low amounts of polymeric IgA noted in serum were found in normal individuals. Patients with IgA nephropathy may possess abnormal quantities of polymeric IgA within their serum which is not necessarily from mucosal surfaces. Indeed, there is some evidence that B lymphocytes obtained from the bone marrow of these individuals secrete more polymeric IgA than normal[61].

Where does deposited IgA originate?

As noted above, one avenue used to address this question has been study of IgA within renal biopsy specimens. Among the techniques applied have been acid elution of renal tissue, ability of IgA in tissue sections to fix secretory piece and stain for J chain, together with labelling of IgA1 and IgA2 with monoclonal and polyclonal reagents.

Initial studies with polyclonal antisera indicated that IgA2 was the predominant immunoglobulin in mesangial deposits[62]. This has not been confirmed by workers using monoclonal reagents of well-defined specificity who have found that they consist entirely of IgA1[63,64]. Several groups have also reported that the IgA will bind labelled secretory piece in renal biopsy specimens and will stain with antibodies to J chain[65,66]. Although it was reported that this staining could be attributed to IgM deposits[67], other groups careful to avoid this criticism have still found anti-J chain and Sc binding suggestive of polymeric IgA[68]. A few reports of IgA eluted from renal tissue have characterized it as polymeric by molecular weight or its ability to bind Sc[69,70].

This pattern is considered by some to be consistent with a mucosal origin for deposited IgA, although the findings with subclass specific antibodies are admitted to be discordant. At present, it seems impossible to determine where mesangial IgA originates – there are groups advocating either mucosal or bone marrow sources with equal vehemence.

Genetics of IgA nephropathy

Evidence suggesting a genetic component in the aetiology of IgA nephropathy includes apparent regional variations in incidence and more definite racial variation, occasional familial occurrence and some linkage with various HLA antigens or complement alleles.

Many studies have attempted to demonstrate a link between disease susceptibility and particular HLA alleles. Most of these have proved to be both weak and difficult to reproduce. HLA Bw35, B12 and its most common 'split' B44, and DR4 are the most frequently associated

antigens, particularly in French and Japanese series. These tenuous associations may have arisen in different centres by chance or reflect true heterogeneity.

Whether splits in known HLA specificities will prove to have a stronger and more consistent association with IgA nephropathy is not known. For example, DR4 now has three serologic splits and it is possible that these may be more powerfully associated. It is also problematic whether the many restriction fragment length polymorphisms (RFLP's) which have been identified for HLA genes using various gene probes and restriction enzymes will establish stronger linkage.

Complement phenotypes in patients with IgA nephropathy have been studied by several groups. Homozygous null C4 alleles were positively associated with IgA nephropathy in Kentucky[71]; a recent extension of the study population to include individuals from other American states has confirmed this[72]. There was no association with C4A or B alleles. Studies from Kentucky and Germany have reported an increased frequency of C3F phenotype[73,74]. Despite associations of this disease with null C4 alleles and some HLA antigens, there has been no evidence of an association with an extended HLA phenotype – i.e. no combination of HLA and complement alleles held in linkage dysequilibrium which together act to confer increased susceptibility to this disease.

Recently, RFLP's involving the switch recombination region for the heavy chain of IgA and IgM have been used to characterize affected individuals[75]. Preliminary reports suggest that a polymorphism of the heavy chain switch region of both IgM and IgA may be common in patients with IgA nephropathy. At present, it is not known whether such polymorphisms lead to preferential switching to IgA during the course of an antibody response. Preliminary attempts to detect structural polymorphisms in the genes encoding IgA have not, so far, proved decisive[76].

Family studies

That there is some sort of genetic factor involved in the pathogenesis of this condition seems likely from the large number of affected families

reported[77–79]. There are now at least 17 separate families in which two or more first degree relatives have biopsy-proven IgA nephropathy[79]. Transmission has been horizontal (siblings) in 10, vertical (parent–child) in 7 and both in one. Clearly, no definite mode of inheritance is indicated.

Study of apparently unaffected family members has revealed that many have subtle abnormalities of IgA production[80]. Increased serum IgA concentrations in apparently normal family members have been noted in similar studies[81].

Overproduction of IgA

Peripheral blood lymphocytes from affected individuals produce more IgA and usually IgG in unstimulated cultures or in response to mitogens than do cells from normals or patients with other forms of glomerulonephritis. Although the data have been a little varied, this conclusion seems inescapable. Williams *et al.*[82], for example, found a significant increase in IgA and IgG production by unstimulated peripheral blood lymphocytes from patients with IgA nephropathy when compared with controls but not when stimulated by pokeweed mitogen. Bannister *et al.*[83] reported increased IgA and IgG in stimulated and unstimulated cultures whereas Egido and co-workers[84] found that IgA alone was increased and then only in stimulated cultures.

Why is IgA overproduced?

A number of separate abnormalities have been detected which might account for overproduction of IgA. Abnormalities of T lymphocyte helper/suppressor ratios, as defined by mouse monoclonal antibodies (e.g. OKT4 and OKT8), have not been consistently confirmed[85,86]. An increase in T lymphocytes programmed to give help to B lymphocytes which secrete IgA has been reported[87], as has an increased number of B lymphocytes in blood and tonsil which possess surface IgA[88] – so

defining them as cells destined to secrete IgA when activated; the latter observation has not, however, been confirmed[89]. At present it is impossible to determine which of these observations, if any, is a primary abnormality.

How is IgA deposited?

Deposition of IgA within the mesangium could occur as preformed immune complexes or by autoantibody binding to mesangial constituents, such as collagen IV, fibronectin, laminin, heparan sulphate proteoglycan or even glomerular mesangial cells.

1. Circulating immune complexes (CIC)

CIC containing IgA have been detected in sera from patients with IgA nephropathy by a large number of assays[90–94]. Many groups have detected CICs using conglutinin binding, C3 binding, Raji and Clq binding assays – there have been few negative studies[90]. About 50% of patients have CICs in their serum at any one time, when assayed by C3 binding methods (conglutinin and C3 binding) or IgA Raji cell assay[91]. Generally, no association with periods of disease activity, presence of proteinuria or renal impairment has been demonstrated which is thought to weaken the argument for a pathogenic role.

CICs have also been detected in 30–45% of patients using the Clq assay[94]; this is more surprising, since only IgG or IgM containing CICs would be expected to contain Clq, because complexed or aggregated IgA activates the alternative – but not the classical – complement pathway. A possible explanation for these findings has recently emerged from studies in which IgA rheumatoid factors were detected in patients with IgA nephropathy[93,95].

Sinico *et al.*[95] found IgA rheumatoid factor in 41% of patients – IgM rheumatoid factor, the usual class detected by rheumatoid factor assays, was present in only 4%. In 5/6 sera analysed IgA rheumatoid factor was predominantly polymeric. Czerkinsky *et al.*[93] reported IgA rheumatoid factor in 30% of their patients, the majority of whom also possessed CICs consisting of IgA and IgG. Most of these rheumatoid factors, whether free or bound to IgG, were of the IgA1 subclass, with

molecular weights consistent with both polymeric and monomeric IgA. Overall, 46% of patients possessed circulating IgA–IgG CICs, so that some patients had such complexes without detectable IgA rheumatoid factor. This leaves open the possibility of alternative interactions between IgG and IgA, such as anti-idiotype binding or binding of both classes to a common antigen.

Interestingly, it has not proved possible to demonstrate rheumatoid factor activity in IgA deposits within the glomerular mesangium[96]. Together with the lack of correlation between clinical disease activity, however assessed, and levels of IgA rheumatoid factor or IgA–IgG CICs, these data do not establish a pathogenetic role for IgA rheumatoid factors.

2. *Autoantibodies*

Those which bind constituents of the mesangial matrix synthesized by mesangial cells, such as type IV collagen and fibronectin, have been reported in sera from patients with IgA nephropathy[97,98]. Most of these findings await independent confirmation. A recent immunoelectron microscopic study showed no physical association of mesangial IgA deposits with type IV collagen or fibronectin[99]. Wilson and coworkers[100] have recently provided suggestive evidence, based largely upon an animal model, that antibodies to glomerular mesangial cells can produce histological lesions in the glomerulus. Ballardie *et al.*[101] have reported IgG anti-mesangial cell autoantibodies in patients with IgA nephropathy. Whether these antibodies are implicated in the pathogenesis of the renal lesion is unknown.

Mediation of glomerular damage

Despite intense interest in substances which mediate glomerular injury in recent years, there is almost no information specifically relating to IgA nephropathy. Mesangial hyperplasia can occur in response to a variety of molecules such as arginine vasopressin, eicosanoids, IL-1, platelet-derived growth factor, fibroblast growth factor and others[102,103]. Both monocyte-macrophage lineage cells present within the mesangium and mesangial cells themselves can synthesize many

of these molecules – whether they stimulate mesangial proliferation and matrix synthesis in IgA nephropathy is an obvious avenue for future research.

The increased mesangial matrix seen in IgA nephropathy is almost certainly synthesized by mesangial cells. Studies using polyclonal and monoclonal antibodies to matrix constituents laminin, fibronectin and collagen type IV have shown that all three accumulate within the hypertrophied matrix[104] – what stimulates the mesangial cell to produce them in such quantity is not known. By analogy with cells whose synthetic capacity has been more fully studied, for example murine fibroblasts and malignant human cells, synthesis is likely to be under complex control and involve mediators such as fibroblast growth factor, tissue growth factor and a range of biologically active molecules.

Role of complement

It has long been known that IgA activates complement by the alternative, but not the classical, pathway. Despite this, levels of complement components, including C3, are usually normal or high in patients with IgA nephropathy, even during periods of disease activity. Recently, more sensitive assays for classical and alternative complement pathway activation, based upon detection of C3 or C4 breakdown products in plasma, have shown that 57% of adult patients have evidence of alternative pathway activation at any one time and 75% if followed serially[105]. Interestingly, 20% also have evidence of classical pathway activation, consistent perhaps with the presence of immunoglobulins other than IgA within the mesangium. Combined with data obtained recently in animal models, these studies suggest a role for complement activation in the pathogenesis of the disease.

Animal models

The aim of these models has been to reproduce the clinical and histological features of human IgA nephropathy. The inability of some models to do so, despite mesangial deposition of IgA, has recently

contributed to our knowledge of the pathogenesis of the condition by emphasizing the importance of other factors.

Rifai *et al.*[106] produced haematuria in 34% of mice together with mesangial deposition of IgA and C3 and mild mesangial expansion by passively administering IgA immune complexes prepared *in vitro*. The mouse myeloma MOPC–315 produces monoclonal IgA to a hapten, dinitrophenol (DNP), so that construction of an IgA immune complex using DNP coupled to bovine serum albumin (BSA) *in vitro* is relatively simple. Because IgA produced by MOPC–315 is 70–80% polymeric and 20–30% monomeric, it is possible to produce immune complexes containing predominantly monomeric or polymeric IgA. It was found that only polymeric IgA immune complexes deposited in the mesangium. C3 appeared to be unnecessary for IgA deposition. Complexes formed *in vivo*, produced by administering DNP-BSA to animals bearing the MOPC-315 myeloma, gave rise to IgA and C3 deposition and more marked histological changes.

Emancipator and co-workers[107] were subsequently able to produce mesangial IgA deposits, without C3, by feeding mice for 14 weeks with oral antigens such as bovine γ-globulin, ovalbumin or horse ferritin dissolved in drinking water. Deposits contained J chain and its presence was correlated with IgA staining, rather than IgM. Although electron-dense deposits were found within the mesangium, histological changes and haematuria were not seen. This model does not establish that IgA deposited in the mesangium of patients with IgA nephropathy originates from mucosal surfaces, but shows that it is possible.

Isaacs and co-workers have described two models of immune complex nephritis in mice using dextran as antigen[108,109]. They were able to produce IgA, IgM and C3 deposits with mesangial proliferation by parenteral administration of neutral or polyanionic dextran of various molecular weights over a 10 week period. Physical characteristics of the dextran preparation appeared to have little influence on the lesion produced. In a passive model of IgA nephropathy, in which soluble immune complexes – consisting of monoclonal anti-dextran IgA and dextrans of various charges and molecular weights – were administered parenterally to mice, IgA, dextran and C3 were deposited predominantly within the mesangium; interestingly, however, some variability was noted dependent upon the physical characteristics of the complex used. For example, 10 kd polyanionic

complexes deposited in a subendothelial location, whereas 10 kd polycationic and 500 kd polyanionic complexes did not deposit well in any site. These data further support the ability of IgA-containing immune complexes to deposit within the glomerular mesangium and suggest that the nature of the complexed antigen plays a minor role in localization.

Recently, Rifai and co-workers[110] have reported that complement fixation is a crucial event in causation of the glomerular lesion and that the nature of the antigen complexed to IgA determines whether this will occur. Following a similar though not identical tack, Emancipator and colleagues[111] have reported that soluble immune complexes consisting of antigen, IgA and IgG elicit haematuria and renal lesions only when the IgG is complement-fixing.

Animal studies emphasize, therefore, the importance of polymeric IgA, the nature of the deposited antigen and complement activation in the causation of the glomerular lesion.

THERAPY

As might be expected from the foregoing discussion of pathogenesis, there is no effective therapy available for the majority of patients with IgA nephropathy. Substantial variation in the prognosis of individual patients and the prolonged periods over which deterioration in renal function may occur have made evaluation of therapies difficult. There is an admitted need for controlled trials of therapies based, perhaps, on a fuller knowledge of the pathogenesis of the condition. The information which is available concerning therapy can be considered by dividing patients into categories depending on their clinical status when treatment is prescribed.

Nephrotic syndrome

As outlined earlier, there are two groups of patients with nephrotic syndrome. Several studies have shown that IgA nephropathy with minimal changes on renal biopsy is a steroid-responsive lesion[40,41].

These patients should be managed as though they had minimal change lesion without IgA nephropathy. More commonly, however, the biopsy shows quite severe histological changes[35,38,39]. Steroids should not be used in these circumstances – the study by Mustonen *et al.*[42], where patients with severe renal lesions responded to steroids does not represent a general experience and requires confirmation.

Macroscopic haematuria

This is a frightening and distressing symptom but does not appear to carry an adverse prognosis in patients with IgA nephropathy. These patients can be reassured and advised to avoid precipitants such as extreme exertion. Episodes of gross haematuria decrease in frequency with time – multiple episodes do not cause progressive renal damage despite the often severe acute histological changes reported in renal biopsies from these individuals. Although both tonsillectomy[112] and therapy with doxycycline[113] have been reported to reduce the frequency of these episodes, we do not prescribe antibiotics routinely and have never advised tonsillectomy.

Acute renal failure

Patients with this rare presentation have been treated aggressively with immunosuppressives, steroids and plasma exchange[114]. At present, there is no strong evidence that such therapy improves outcome because spontaneous improvement is common[36].

Persistent asymptomatic haematuria

Most patients do not have one of the dramatic presentations noted above, but those who do usually become members of this group when, and if, the acute lesion resolves.

Tonsillectomy and antibiotic therapy have been reported to reduce the frequency of episodes of macropscopic haematuria, but it is not

known whether these therapies improve the prognosis in the larger number of patients who do not have frank haematuria; certainly, tonsillectomy and antibiotic therapy cannot be advised for the majority of patients with IgA nephropathy, whether they have macroscopic haematuria or not.

Phenytoin therapy has been reported to decrease serum IgA levels in patients being treated for epilepsy[115]. Two controlled studies have shown, in patients with IgA nephropathy, that levels of polymeric IgA in serum were reduced by this drug, but there was no effect upon clinical, biochemical or histological parameters[116,117]. Its use is not routine.

A prospective controlled trial from Japan[118] of corticosteroids followed by anti-platelet therapy has shown a significant benefit when compared with anti-platelet therapy alone in patients with proteinuria 1–2 g/day. At the end of the study period, which averaged 19 months, patients in the treatment group had less proteinuria and better renal function than those in the control group. It was suggested that those with creatinine clearance >70 ml/min would benefit most from therapy. However, this study conflicts with that of Lai *et al.*[119], in which all patients were nephrotic – resolution of proteinuria occurred in most of those with minimal changes on renal biopsy, but there was no benefit in the majority who had more severe histological lesions over a three-year treatment period. At present, caution seems warranted in the use of steroids as monotherapy.

An impressive trial of combination therapy with steroids, cyclophosphamide and dipyridamole has recently emerged from Singapore[120]. Most of the patients had presented with microscopic haematuria, but all had proteinuria >1 g per day. They were, therefore, a group with a poorer prognosis than the majority of individuals presenting with asymptomatic haematuria. A clear statistical difference in the rate of deterioration in renal function emerged between treated and untreated groups. The results await confirmation by other groups in further controlled studies.

Cyclosporin A has, as usual, been mooted as a possible therapy. It seems unlikely that it will prove effective, as pointed out by Clarkson and Woodroffe[121], because IgA nephropathy recurs regularly in renal transplant recipients treated with cyclosporin A. A short-term controlled study by Lai *et al.*[122] has shown, however, reduced proteinuria

in patients treated with cyclosporin A. Unfortunately, the most likely interpretation is that the reduction in proteinuria noted in the treatment group was simply due to the temporary deterioration in glomerular filtration rate which also occurred. At present, there is no evidence to suggest a beneficial effect of long-term cyclosporin A on renal function in these patients.

Angiotensin-converting enzyme (ACE) inhibitors, such as captopril and enalapril, have not yet been tested extensively. Their beneficial actions in reducing intraglomerular hypertension suggest that controlled trials may be worthwhile in this condition.

Plasma exchange has not been used in any controlled way in this group of patients. Although plasma exchange sometimes stabilizes deteriorating renal function, any benefit is rapidly lost with cessation of therapy[123].

Dietary therapy with fish oils has been reported by Hamazaki *et al.*[124] to have a beneficial effect in a controlled trial. The theoretical basis of this therapy is a reduction in local synthesis of vasoconstrictive eicosanoids within the kidney because of the different fat composition of fish oils. No confirmation of this work is yet available.

As will be evident, there is no established therapy to prevent progression in this disease, apart from control of hypertension. The latter is generally agreed to be of major importance and there is probably a theoretical basis for use of ACE inhibitors early in the course. Perhaps the most promising avenue is that of Woo *et al.*[120] in a selected subgroup of patients – this does, however, require confirmation.

IgA NEPHROPATHY IN CHILDREN

IgA nephropathy appears to be less common in children than in adults[126], constituting about 10% of biopsies. The majority of children have episodes of macroscopic haematuria during their course, even when they have been biopsied on account of microscopic haematuria[127]. Prognosis is said to be better than in adults[128], although the validity of this statement is dubious – they may simply have had the disease for a shorter period. Certainly, some do develop end-stage

renal failure. Histological changes are also said to be less severe[127], although this may have a similar explanation.

Relationship of IgA nephropathy to Henoch–Schönlein purpura

There are two views concerning the question of the similarity of these two diseases[129,130]. Histologically, they are indistinguishable and some have argued that IgA nephropathy is simply Henoch–Schönlein purpura without the rash, joint symptoms and abdominal pain[130]. Families have been described, including a set of identical twins, in which one member has had IgA nephropathy and the other Henoch–Schönlein purpura[131]. A patient whose primary renal disease was Henoch–Schönlein purpura who developed *de novo* IgA nephropathy in a renal allograft, without evidence of systemic disease, has also been reported[130].

Against this evidence is the clearly different disease course described by some authors[129]. Henoch–Schönlein purpura is an acute and largely self-limiting disease which may cause renal damage, but does not generally produce the relentless, slow progression to renal failure frequently seen in patients with IgA nephropathy. At present, the issue is undecided.

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5

PLASMAPHERESIS IN GLOMERULONEPHRITIS

C. D. PUSEY

INTRODUCTION

Therapeutic plasmapheresis is a technique in which whole blood is withdrawn from a patient and pumped into a device that separates cellular elements from plasma; the cells are then returned to the individual together with substitution fluid and the plasma is discarded. When large volumes of blood are processed the substitution fluid must provide oncotic pressure and is commonly a plasma derivative such as 5% albumin; the preferred term for this procedure is plasma exchange (PE).

Although manual plasmapheresis had been used prior to 1970 for the collection of blood products and for the removal of myeloma protein, it was the coincidental development of automated cell separation and the recognition that circulating autoantibodies could be pathogenic which led to the application of PE in autoimmune renal disease in the 1970s. Lockwood *et al.*[1] first reported the successful treatment of a patient with Goodpasture's syndrome, arguing that the rapid removal of antibodies to glomerular basement membrane (GBM) by PE should limit renal injury pending the onset of action of concomitant immunosuppressive drug therapy. Since that report, a a wide range of glomerular diseases has been treated by PE[2–4], in the belief that humoral immune factors are involved in their pathogenesis.

EFFECTS OF PLASMA EXCHANGE IN GLOMERULONEPHRITIS

It must be remembered, however, that the immunopathogenesis of most forms of glomerulonephritis is poorly understood[5]. Anti-GBM disease is the only example in which there is reasonable evidence for the direct pathogenicity of autoantibodies[6], although even in this condition a role for T-cell-mediated injury has not been excluded. Autoantibodies might also be involved in mesangio-capillary nephritis (Type II), which is associated with nephritic factor, an antibody which stabilizes the alternative pathway C3 convertase[7]. There are recent suggestions that autoantibodies to various glomerular components could be involved in membranous nephropathy (by analogy with Heymann nephritis)[8], mesangial proliferative nephritis, lupus nephritis and systemic vasculitis – although in none of these is there yet firm evidence in human disease.

The majority of cases of glomerulonephritis have previously been attributed to immune complex (IC) deposition. Although this may be so in mesangiocapillary nephritis (Type I), mesangial IgA disease, mixed essential cryoglobulinaemia, infective endocarditis and some cases of systemic lupus, the evidence is generally lacking. In other conditions, particularly those with subepithelial deposits such as membranous nephropathy, post-streptococcal nephritis and some cases of systemic lupus, it seems more likely that immune deposits form *in situ* due to the localization of autoantigen or 'planting' of foreign antigen in the glomerulus[9]. It is apparent that the distinction between 'immune complex nephritis' and 'antibody mediated nephritis' is becoming blurred. Finally, there are disorders where the immunopathogenesis is still obscure, the commonest being minimal change disease and focal segmental glomerulosclerosis. The prompt recurrence of the latter in some renal allografts is perhaps the best evidence for the involvement of humoral factors[10].

Whilst PE might have an effect in the above conditions by removing the putative pathogenic molecules, it should be recognized that it will also have a variety of non-specific effects which could modify immunological glomerular injury[11,12]. These include reduction in blood viscosity, removal of complement components and clotting factors (particularly fibrinogen) and improvement in reticulo-endothelial function. Since PE is generally used together with immuno-suppressive

drugs, there is also the possibility that it could potentiate their effects, for example by increasing the sensitivity of B-cells to cytotoxic agents.

CLINICAL APPLICATIONS OF PLASMA EXCHANGE IN GLOMERULONEPHRITIS

Despite the above theoretical considerations, the proof of the effectiveness of PE in glomerulonephritis must come from clinical experience, preferably from properly designed controlled trials. Unfortunately, few such trials have been performed and much of the available information comes from retrospective series or case reports. The rarity of certain types of nephritis, the chronicity of others, and the difficulties inherent in multi-centre studies using technically demanding treatment, have all mediated against adequate clinical trials. Since there is no clear answer as to the benefit of PE in most types of nephritis, I shall review our own experience together with the current literature and attempt to put the use of PE into perspective for each disorder. Details of the clinical features and immunopathology of the various forms of glomerulonephritis will be covered elsewhere in this volume, and I shall refer to them only where appropriate.

ANTI-GBM DISEASE AND GOODPASTURE'S SYNDROME

Goodpasture's syndrome is the eponym applied to the association of glomerulonephritis and lung haemorrhage, but has become almost synonymous (to nephrologists) with anti-GBM disease[13]. About two thirds of patients with anti-GBM antibodies have lung haemorrhage as well as nephritis[14] and it has recently been shown that a single autoantigen present at both sites is involved[15], but that other factors (such as smoking) influence the expression of lung disease[16]. Following the demonstration of the pathogenicity of anti-GBM antibodies[6], and the development of assays allowing their detection in the circulation[17], this was the first autoimmune disorder in which PE was used[1]. The rapid loss of renal function in untreated or conventionally-treated patients led to the notion that removal of the autoantibody might limit damage whilst control of its synthesis was achieved by cytotoxic

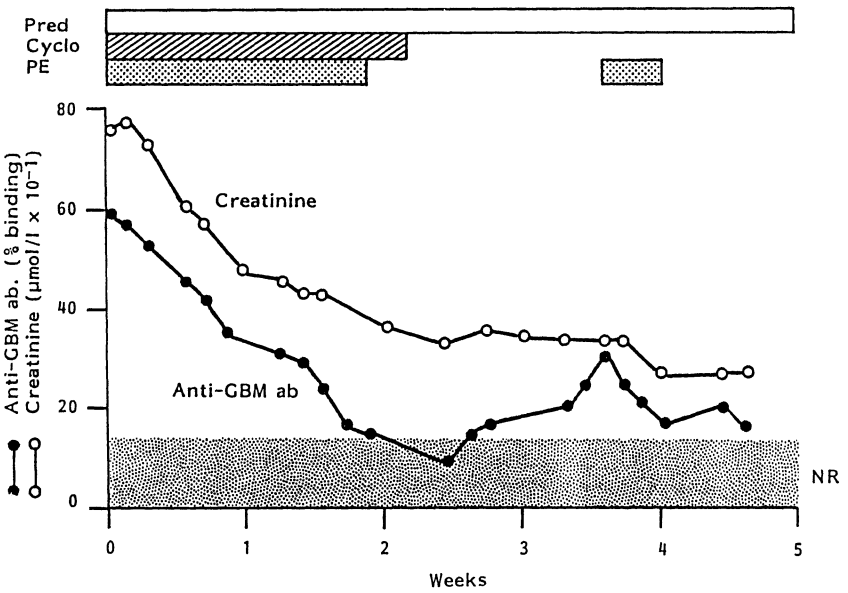

FIGURE 5.1 Effect of treatment with PE and immunosuppressive drugs on anti-GBM antibody levels and serum creatinine concentration in a patient with anti-GBM disease

drugs. The demonstration that the fall in circulating levels of anti-GBM antibodies during PE was associated with clinical improvement in certain cases (see Figure 5.1 for example) provided the impetus to continue with this approach[18].

There is no doubt that rapid removal of anti-GBM antibodies can be achieved in almost all cases, and that circulating antibody becomes undetectable by 8 weeks in adequately treated patients[19]. The rate at which antibody levels fell in our recent cases is shown in Figure 5.2. We have also found that the initial levels of anti-GBM antibody correlate with severity of disease, and that their removal is associated with clinical improvement in both renal and pulmonary lesions[14,18]. What has been surprising is that once antibody levels have fallen to background there is very rarely any recurrence of their production or of the disease. We have seen only one proven case of recurrent antibody (without disease)[20] and one case with recurrent disease (no antibody levels available) in more than 50 patients.

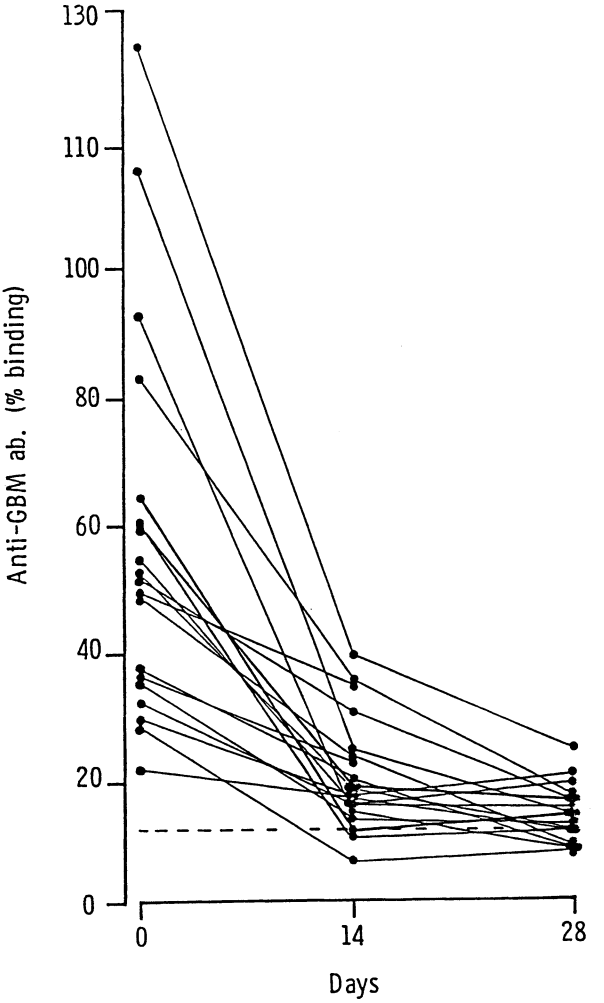

FIGURE 5.2 Anti-GBM antibody levels in 19 cases of anti-GBM disease treated with PE and immunosuppressive drugs

The evidence for the benefit of PE however, rests largely on the improved outcome in treated patients compared with that in previously reported series. Although other factors, particularly advances in the management of renal failure and of infection, will have contributed, the apparent benefit was so striking that we did not consider a controlled trial to be justified. In the series of Benoit *et al.* in 1964[21],

50 of 52 patients died, and of a further 51 cases reviewed in 1970[22], 43 died. In Wilson and Dixon's study[13], in which the diagnosis was confirmed by detection of anti-GBM antibodies, only 4 of 32 cases recovered renal function, including 13% of those not requiring dialysis. A recent analysis of our patients shows recovery in 17 of 49 cases, including 71% of those not requiring dialysis[14]. The outcome of our patients at 8 weeks, related to their renal function at presentation, is shown in Table 5.1 (Pusey CD, unpublished observation).

It is of particular interest that the great majority of patients presenting with a serum creatinine of $<600\,\mu$mol/L recovered renal function, whereas only 3 of 27 dialysis-dependent cases did so. It has been argued that the risks of treating the latter group outweigh the possible benefit, and this remains our view except when dialysis has only just commenced and/or when the renal biopsy shows some normal (or only partially damaged) glomeruli. Our experience is borne out by the rarity of successfully treated oliguric patients reported in the literature[23–25]. Lung haemorrhage also improved in 33 of our 38 cases, providing a separate indication for treatment[14]. Those patients who did not respond generally died of uncontrolled haemorrhage together with infection, and had required artificial ventilation. As the disease has been shown to recur in renal allografts performed in the presence of circulating anti-GBM antibodies, PE should also be considered if early transplantation is desirable. The alternative approach is to wait for the spontaneous disappearance of autoantibody, which usually takes around a year.

TABLE 5.1 Outcome of anti-GBM disease at two months

Renal function	*Patients*	*Improved*	*No response*	*Died*
Creat $<600\,\mu$mol/L	21	18	2	1
Creat $>600\,\mu$mol/L	8	4	3	1
Dialysis-dependent	27	3	17	7

The benefits of PE in anti-GBM disease were soon supported by reports from other centres using a similar approach[26–28]. More recently, Walker *et al.* reported 22 patients of whom 16 showed an improvement following treatment and 9 showed long-term recovery[23]. Of particular interest is that 5 of 11 oligo-anuric or dialysis-dependent patients

showed some improvement (maintained in 3 cases), but of these 11 patients, 5 with anuria did not respond. The authors suggested that anuria and a high percentage of crescents were better predictors of outcome than the requirement for dialysis. There has also been a randomized study of the use of immunosuppressive drugs alone compared with immunosuppression and PE. However, PE was only given once every three days, using FFP as the main replacement solution, and a mean of only 9 exchanges was performed. The authors found that the use of PE led to a significantly more rapid disappearance of anti-GBM antibody and to an apparent improvement in renal function (6/8 not requiring dialysis following PE and drugs, compared with 3/9 following drugs alone). Like ourselves, they found that renal function and crescent score at the start of therapy were important predictors of outcome, but they made the additional observation that patients with early renal disease could recover following drugs alone.

The long-term outcome of successfully treated patients is generally good[19]. A few of our cases, in whom the serum creatinine remained raised after treatment, have shown progressive glomerular scarring without immunological recurrence, but the majority have retained good function on follow-up for up to 12 years. The actuarial survival curves for patient and kidney survival are shown in Figure 5.3, which illustrates a stable situation after the early deaths and loss of kidneys (Pusey CD, unpublished observations).

There has been recent interest in the application of selective or specific removal of anti-GBM antibodies by immunoabsorption, since this would reduce the risks and expense of PE. Protein A columns have been used successfully in a small number of patients[30], and the feasibility of specific antibody removal by GBM-coated columns has been demonstrated in an experimental model of the disease[31]. These approaches can be no more effective than PE in removing antibody however, and are unlikely to improve the outcome. The most important factor in altering the prognosis will be earlier diagnosis, so that patients can be treated before irreversible glomerular damage has occurred. As a separate issue, the development of effective extracorporeal respiratory support systems could also help by reducing early deaths from lung haemorrhage.

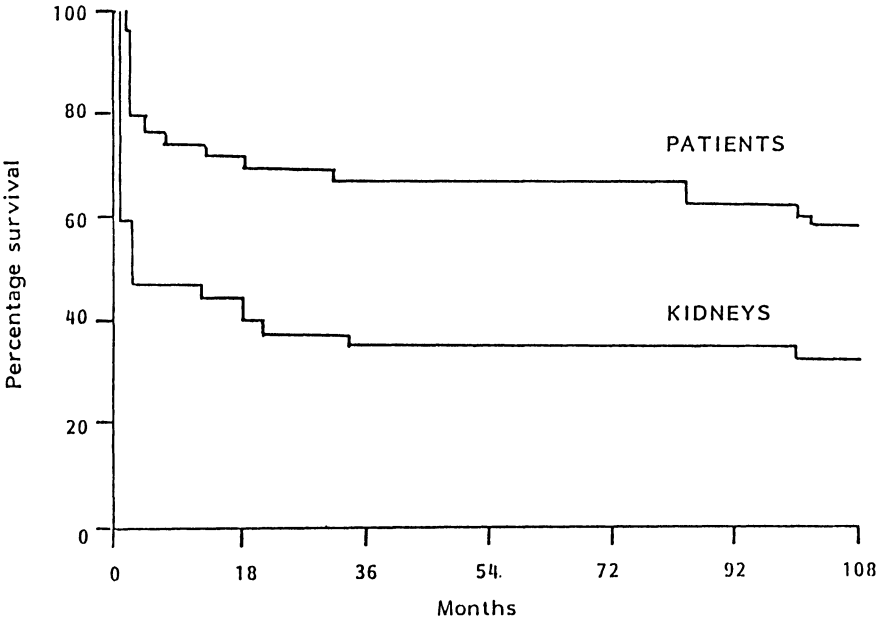

FIGURE 5.3 Actuarial survival curves for patients, and for patients with functioning kidneys, treated for anti-GBM disease at the Hammersmith Hospital

SYSTEMIC VASCULITIS AND RAPIDLY PROGRESSIVE NEPHRITIS

The other major cause of crescentic nephritis in our practice is small vessel vasculitis. In most cases the clinical diagnosis is of Wegener's granulomatosis (WG)[32] or microscopic polyarteritis (MP)[33], although it must be recognized that they form part of the spectrum of systemic vasculitis and that precise 'pigeon-holing' is not always possible. The recent recognition of autoantibodies to the cytoplasm of neutrophils and monocytes (ANCA) in patients with both WG and MP has proved a valuable diagnostic tool, as well as suggesting a common disease mechanism[35,36]. Whether these antibodies are directly pathogenic is not yet known, although they have been shown to react with glomerular endothelial cells in culture (Abbot F E, personal communication). Of further interest is our recent observation that some patients with

'idiopathic' rapidly progressive nephritis (RPGN) also have detectable ANCA. This observation provides further justification for our belief that such cases represent a limited form of systemic vasculitis, and their management will therefore be discussed together with that of WG and MP.

Plasma exchange was first used in such cases because of its success in Goodpasture's syndrome, and in view of the then current notion that immune complexes were involved in the pathogenesis. The initial success of this approach[37] led to its widespread application, and our uncontrolled results suggested a benefit in comparison with previous series[2]. The outcome of these patients at 8 weeks, related to initial renal function, is shown in Table 5.2. It can be seen that there is a striking difference between these results and those obtained in anti-GBM disease – namely that the majority of dialysis-dependent patients improve. Similar results were subsequently reported from other centres[38–41], and a comprehensive review of the literature by Heaf *et al.* in 1983[42] confirmed the impression that advanced cases of RPGN did better when treated with either PE or pulse methyl prednisolone (in addition to oral immunosuppressive drugs) than when treated with conventional drugs alone.

TABLE 5.2 Outcome of rapidly progressive nephritis at two months

Renal function	*Patients*	*Improved*	*No response*	*Died*
Creat $<600\,\mu$mol/L	16	14	1	1
Creat $>600\,\mu$mol/L	10	8	0	2
Dialysis-dependent	23	17	1	5

Note: Includes 25WG, 18MP and 6 idiopathic cases

However, it was apparent that many patients with WG and MP responded very well to standard drug therapy, particularly cyclophosphamide. In Fauci's series of 85 cases of WG[43], which did not include as many patients with severe glomerulonephritis, there was a 93% response to cyclophosphamide and steroids, with a mean duration of remission of 48.2 months. We established therefore a randomized controlled trial of standard drug therapy (prednisolone, azathioprine and cyclophosphamide) compared with the same drugs together with PE (mean of nine 41 exchanges)[2]. We included patients

with WG, MP and idiopathic RPGN, but excluded those with other defined causes of crescentic nephritis, and stratified patients for renal function before randomization. Results of the outcome of the trial at 4 weeks are summarized in Table 5.3 (Pusey CD, unpublished observations). It can be seen that PE confers no additional benefit in patients not requiring dialysis at the start of treatment, almost all of whom improve on either treatment, but that there is a significant benefit in the dialysis-dependent cases. Follow-up of these patients to one year confirms that this improvement is almost always maintained. However, unlike anti-GBM nephritis, this disorder tends to relapse (in around 30% of cases) if treatment is withdrawn. For this reason we have used maintenance therapy of low dose prednisolone and azathioprine for at least one year after any evidence of active disease.

TABLE 5.3 Outcome of controlled trial of PE in RPGN as judged by improvement at one month

	Treatment	*Control*
Creatinine $<500\,\mu$mol/L	9/9	7/8
Creatinine $>500\,\mu$mol/L	5/5	7/7
Dialysis-dependent*	10/11	3/8

* $p=0.024$, Fisher's exact test

Although most uncontrolled series (see above) have also suggested improvement after PE, particularly in dialysis-dependent patients, a multi-centre controlled study from Germany did not confirm any benefit[44]. There were, however, several differences between this trial and our own, including smaller numbers, the lack of stratification for renal function on entry, the diversity of patients included (SLE, scleroderma, etc) and the drug and PE regimens used. The results (at 4 weeks) showed responses in 4/12 of the control group and 7/14 of the PE group, perhaps suggesting a more rapid effect of PE, but by 6 months the figures were 7/11 and 7/12 in control and PE groups respectively. By this time, however, 3 non-responders in the control group had received and responded to PE according to the protocol used.

Immunological monitoring using serial measurements of ANCA is not yet of proven value, although untreated patients with active disease

are generally positive and then rapidly become negative on treatment (see Figure 5.4 for example, courtesy of Dr CM Lockwood). On follow-up however, a proportion of patients again develop the auto-antibody without clinical signs of disease (Pusey CD, unpublished observations); whether they are at particular risk of relapse is not yet known. Measurement of the C-reactive protein, a non-specific acute phase reactant, has been shown to be of value in monitoring the disease, and levels change more rapidly and consistently than the ESR[45]. Relapses may also be triggered by intercurrent infection (as in anti-GBM disease), and aggressive investigation and treatment is necessary.

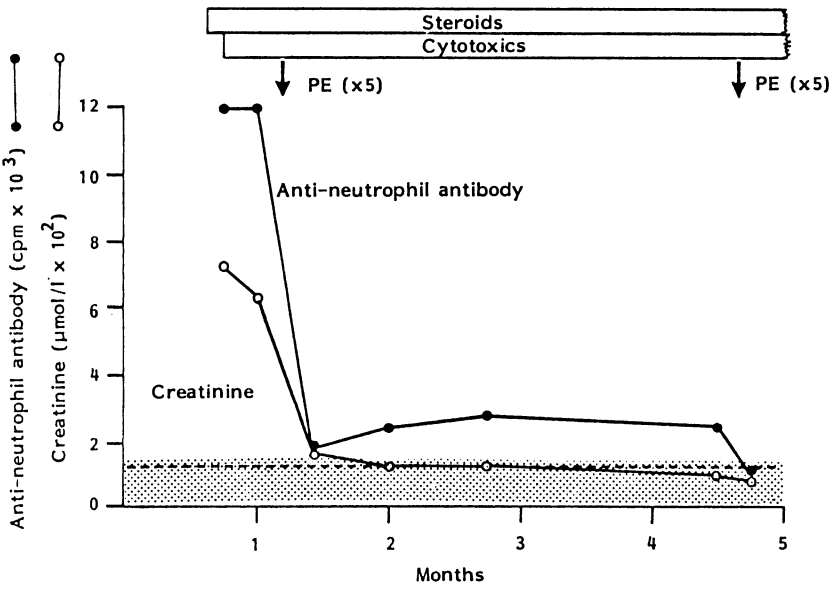

FIGURE 5.4 Effect of treatment with PE and immunosuppressive drugs on ANCA levels and serum creatinine concentration in a patient with Wegener's granulomatosis

Overall, it would seem reasonable to treat patients with WG, MP or idiopathic RPGN in a similar way. Although RPGN is rare in Churg–Strauss syndrome and relapsing polychondritis, responses to PE in these disorders have also been reported[46,47]. Our current induc-

tion therapy comprises: prednisolone 60 mg daily reducing to 20 mg by 6 weeks, cyclophosphamide 3 mg/kg daily (2 mg/kg daily in patients > 55 y) and, for dialysis-dependent cases only, a course of at least five (generally ten) 41 exchanges. Azathioprine does not seem to be an essential component of the induction regimen, since several patients treated without it (since the trial protocol finished) have done equally as well. For maintenance therapy, azathioprine is substituted for cyclophosphamide at 8 weeks, and continued for at least a year with reducing doses of prednisolone. Relapses, unless minor, are treated by re-introduction of the induction regimen, and low dose cyclophosphamide is used for maintenance in patients relapsing on azathioprine.

The use of pulse doses of methyl prednisolone (MP) may also be effective in oliguric patients[39,48,49], although to my knowledge there have been no prospective controlled studies of MP versus PE or conventional therapy. The use of Protein-A columns, as for anti-GBM disease, has recently been reported in a small number of patients with WG (Taube D, personal communication), but the rationale for their use is less clear and experience as yet too limited to draw any conclusions.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) was widely regarded as an 'immune complex' disease, due to the deposition in certain vascular beds of circulating IC. It now seems likely that *in situ* formation of immune aggregates, or direct binding of autoantibodies to their target antigens, is also involved[5]. Whichever mechanism is most important, the evidence for humoral factors in the pathogenesis of lupus is strong enough for PE to be a reasonable approach.

The major cause of mortality in SLE is renal disease, and glomerulonephritis occurs in a large proportion of patients – the precise percentage depending upon diagnostic criteria. The use of corticosteroids had a beneficial effect on the outcome of lupus nephritis, as judged by historical series, and the introduction of immunosuppressive agents such as azathioprine and cyclophosphamide further

improved the prognosis[50]. Of great interest is the large and detailed controlled study from the National Institutes of Health, USA, in which a significant long-term benefit from intravenous cyclophosphamide plus steroids, as opposed to steroids only, was demonstrated[51]. Patients receiving oral cyclophosphamide (±azathioprine) also appeared to do better than those on steroids alone, although this effect did not achieve statistical significance.

TABLE 5.4 Outcome of systemic lupus erythematosus related to indication for PE and initial renal function

	Patients	*Improved*	*No response*	*Died*
Indications				
Failure drugs	15	8	5	2
Vasculitis	12	6	1	5
Toxicity	3	1	2	0
Renal Function				
Creat $<500\,\mu$mol/L	14	11	2	1
Creat $>500\,\mu$mol/L	6	2	3	1
Dialysis-dependent	10	2	3	5

SLE was one of the first diseases in which PE was used[52], and despite many uncontrolled reports of its benefit[53–56] it is disappointing that there are few controlled data to support its use. One review of the early reports[57] showed that 74% of 70 patients showed some signs of improvement, but that a sustained response was seen only in 19% (including 6/7 on cyclophosphamide). One small controlled trial of PE in patients with mild lupus (some of whom were on steroids) failed to show a clinical benefit, despite improvement in IC and anti-DNA antibody levels in the treated group[58] – this is hardly surprising. Other small trials have suggested benefit from PE when used chronically[59], or with bolus doses of cyclophosphamide[60]. Of greater interest are the results from a large multicentre American trial, comparing the outcome of patients with active lupus nephritis treated with prednisolone and cyclophosphamide with those also receiving PE. Although not yet published, and therefore difficult to evaluate, the preliminary data have been presented (Lewis EJ, personal communication) and showed no overall benefit from the addition of PE.

Our approach has been to use PE for certain subgroups of patients

with lupus nephritis, in particular: 1) failure to respond to drugs alone; 2) severe systemic vasculitis; 3) unacceptable drug toxicity[61]. The results are summarized in Table 5.4, which shows outcome related to the indication for PE and the initial renal function. We observed an overall response of around 50%, including 8/15 who had failed to respond to drugs. Of interest was the finding of a higher response rate in cases receiving cyclophosphamide as opposed to other drugs. We were also able to show a significant fall in anti-DNA antibodies and a rise in complement C3 and C4 levels following treatment, which was maintained for at least a month (Figure 5.5). Although our data are uncontrolled, they do suggest that PE may be of value in certain patients with severe lupus nephritis.

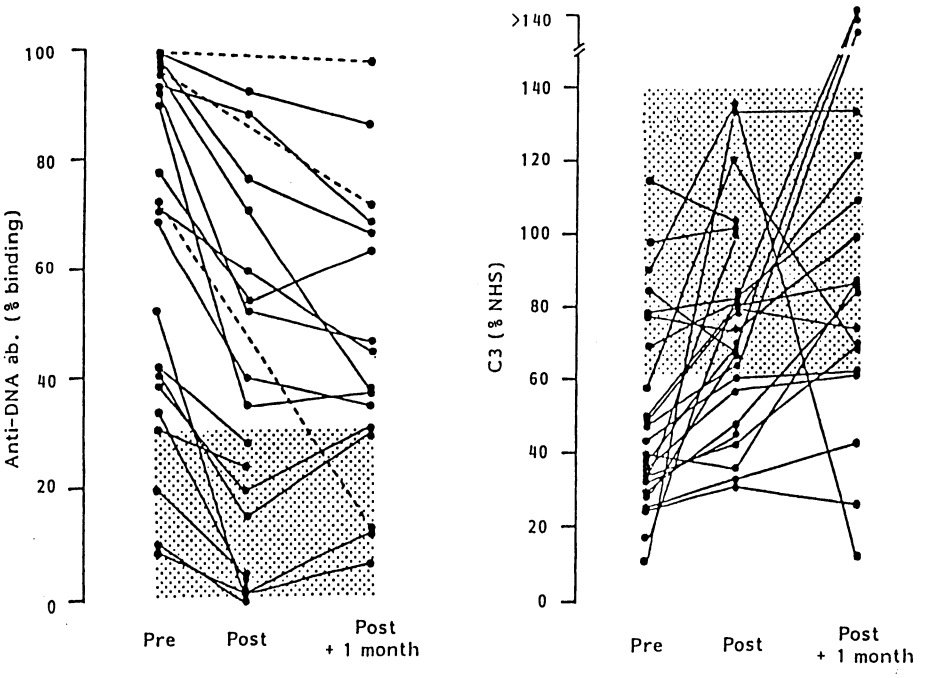

FIGURE 5.5 Levels of anti-DNA antibodies and complement (C3) in patients with SLE treated with PE and immunosuppressive drugs

Similar conclusions have been drawn by other authors with experience of smaller numbers of patients, both adult and paediatric. Leaker

et al.[62] studied 12 adults with severe lupus nephritis and crescentic changes on renal biopsy; these patients had all received steroids, and the majority were also on cytotoxic therapy. An initial course of 3–5 PE daily was followed by less frequent exchanges over the following 6 weeks to 3 months. There was an early improvement in serum creatinine in 6/12 and stabilization within the normal range in 5/12; this was maintained at one year in most cases. The authors suggested that PE should be reserved for patients with crescentic lupus nephritis, and combined with high dose steroids and cytotoxic drugs. A recent paediatric study[63] demonstrated a sustained improvement in 5/6 children with renal lupus (3/3 with renal failure) which had failed to respond to pulse methyl prednisolone and (in 3 cases) cytotoxic drugs.

Although the pathogenicity of anti-DNA antibodies remains unproven in man, there is some interest in the specific removal of these antibodies by immunoabsorption techniques. The feasibility of this approach has been demonstrated in animal models of the disease[64] and preliminary results in patients have been reported[65], although clinical benefit has not been clearly demonstrated.

MIXED ESSENTIAL CRYOGLOBULINAEMIA

Renal involvement is a common feature of mixed essential cryoglobulinaemia (MEC), especially Type II, and most frequently takes the form of mesangiocapillary nephritis[66]. The outcome of these patients is largely dependent upon renal disease and hypertension and the disorder is frequently resistant to conventional drug therapy[67,68]. Although cryoprecipitation is likely to be a factor in the cutaneous features of this disorder, the involvement of internal organs, including the kidney, is presumably largely due to immune complex deposition. The removal of preformed IC and of the monoclonal IgM rheumatoid factor involved provides the rationale for the use of PE in cryoglobulinaemia. Reduction in blood viscosity and improvement in reticuloendothelial system function may also play a role[12].

We have recently reported our experience of 16 cases, of whom 10 had Type II MEC.[69] Six of these had clinically important renal disease, which responded to treatment with PE in all but one advanced case. Our approach has been to use an initial series of around five 41

exchanges, combined with steroids and/or cytotoxic drugs in the more severe cases. Maintenance therapy has been variable, depending upon the rate of recurrence of cryoglobulins and clinical features. In some cases, recurrence is slow enough that intermittent PE alone (generally one or two exchanges per month) is adequate management. In others however, cryoglobulins are produced more rapidly and concomitant treatment with cyclophosphamide is required.

Similar benefits from PE have been reported by other groups, using various treatment regimens[70–73]. In a detailed study of nine cases with severe mesangiocapillary nephritis[73], there was long-term improvement in five patients treated with PE and steroids (but no cytotoxic drugs) who had not responded to drugs alone. One recent review found that renal disease improved in 77% of 52 cases surveyed[74]. There are also isolated reports of rapidly progressive nephritis in MEC which has responded to PE[75].

Attempts have been made to take advantage of the phenomenon of cryoprecipitability to allow selective depletion of cryoglobulins. In this process – known as 'cryopheresis' – separated plasma is cooled extracorporeally to allow precipitation and filtered before return to the patient[76]. Alternatively, because of the high molecular weight of the complexes (and of the IgM paraprotein in Type II), 'cascade filtration' has been employed. Here the plasma is separated from cells by a large pore primary membrane and returned via a smaller pore secondary membrane, which allows albumin to pass but excludes larger molecules. Both of these approaches are feasible, and avoid the need for replacement solutions, but cannot be more efficient than standard PE and are not yet widely used.

PRIMARY GLOMERULONEPHRITIS

Despite the lack of understanding of the underlying immune mechanisms in most types of glomerulonephritis (see above), the circumstantial evidence for the role of humoral factors has led to the use of PE in various conditions. Since there are generally no good serological markers to follow, and since the natural history may be prolonged and variable, it is difficult to draw any firm conclusions.

In mesangial IgA nephritis (Berger's disease) and Henoch-Schonlein

purpura (HSP) nephritis, raised levels of IgA and of immune complexes have been reported[77] although their relationship to disease remains unclear. Although most patients have a benign course, progression occurs in around 10% of adults and crescentic changes are occasionally seen. The use of PE, together with drugs, has been reported to be effective in such cases[78,79]. Coppo *et al.*[80], in a study of 5 patients found that a clinical response was accompanied by improvement in a number of immunological parameters, including IgA–IC levels, mononuclear-phagocytic system function and complement C3 levels; however, only 2 cases with active disease showed long-term improvement. Lai *et al.*[81] reported that short-term improvement followed PE in two rapidly deteriorating patients, but that there was still long-term progression of renal disease. A recent review from France[82] emphasized the importance of treating active disease rather than chronic progression; improvement followed PE in 8/8 cases of HSP and 5/7 of IgA nephritis judged to be active, but in 0/5 of HSP and 2/7 of IgA nephritis showing slow progression.

Mesangiocapillary nephritis (MCGN) may be associated with subendothelial immune complex deposition (Type I), or linear 'dense deposits' along the GBM together with circulating nephritic factor (Type II)[5]. There are isolated case reports of the benefit of PE, used acutely, in both types; in two patients with Type II this was associated with a fall in nephritic factor activity[83]. In an Australian series of eight patients, there was improvement in 4/5 cases with active or crescentic nephritis but no response in 3 with chronic MCGN[84]. A different approach was taken by McGinley *et al.*[85], who used chronic PE (initially weekly then less frequently for up to 44 months) in patients with slowly progressive renal failure due to MCGN and membranous nephropathy. They reported stabilization of renal function in 3/3 with MCGN Type I, 1/3 with MCGN Type II and 0/3 with membranous nephropathy. In focal segmental glomerulosclerosis, the best evidence for humoral mediation is the rapid recurrence sometimes seen in renal allografts[10]. This observation has led to the use of PE in such recurrent cases, with apparent success in some reports[86,87], but with failure in others.

Overall, there is no clear evidence that PE is of benefit in primary glomerulonephritis. However, it may have a role in some cases when crescentic changes are superimposed upon the background pattern –

particularly in IgA associated nephritis and MCGN.

CONCLUSIONS

Plasma exchange is an expensive, time-consuming and potentially hazardous form of therapy[11,88], and any possible benefits must be assessed in this context. Despite its wide application in immunological diseases, including glomerulonephritis, over the last decade there is still little firm evidence for its effectiveness. However, the treatment of severe glomerulonephritis, which would otherwise lead on to the requirement for renal replacement therapy, is a situation in which PE may be of benefit to the individual patient and also cost-effective. Although controlled data are lacking, the treatment of anti-GBM disease is validated by historical comparisons and by the demonstration of the removal of autoantibodies of proven pathogenicity – unfortunately, anuric patients are unlikely to recover renal function although their lung haemorrhage may respond. In RPGN related to small vessel vasculitis, our controlled study demonstrates that dialysis-dependent cases are more likely to improve if treated by PE, whereas those treated at an earlier stage do extremely well on immunosuppressive drugs alone. The relative merits of pulse methyl prednisolone therapy remain unproven, although this may be a satisfactory alternative to PE in advanced cases.

In more chronic systemic disorders affecting the kidney, such as SLE and MEC, there could also be a role for PE. Its use in lupus nephritis is controversial, but is worthy of further assessment in selected cases resistant to drug therapy or with crescentic changes on biopsy. In cryoglobulinaemia, the removal of the cryoglobulin is accompanied by clinical improvement in the majority of patients and few would doubt that PE is having an effect. Assessment of any benefit of PE in the various forms of primary glomerulonephritis is made difficult by the lack of understanding of pathogenetic mechanisms and by scanty clinical data.

The development of specific and selective immunoabsorption techniques should allow safer and cheaper treatment, and may also provide the opportunity to analyse disease mechanisms. However, any major advances in treatment are likely to stem from basic research into auto-

immunity at a cellular and molecular level – hopefully our current crude attempts at immune modulation will be superseded by specific immunotherapy in the not too distant future.

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6

PROLIFERATION OF GLOMERULAR CELLS

A. J. REES

INTRODUCTION

Proliferative nephritis is an old term and one often used loosely to mean glomerular hypercellularity. It has featured prominently in pathological classifications of nephritis since the systematic studies of Volhard and Farr.[1] Individual types of proliferative nephritis are described in detail elsewhere in this series, and it is not the purpose of this chapter to reiterate these discussions; but rather to consider glomerular proliferation more generally. This is especially pertinent now for two reasons: first because recent clinical and experimental studies have defined the cellular composition of glomeruli much more accurately than has previously been possible; and secondly because of the wealth of information about the control of cell proliferation that has emerged from *in vitro* studies over the past 5 years. These emphasize the close links between factors controlling cell proliferation, acute inflammation and synthesis of the extracellular matrix (i.e. scarring).[2,3]

Until relatively recently it was assumed that, with the exception of neutrophil infiltrates, glomerular hypercellularity was purely a reflection of glomerular cell proliferation affecting mesangial, endothelial, or parietal epithelial cells.[4–6] But the demonstration of macrophages within inflamed and later in normal glomeruli[7,8] initiated a vigorous debate between the respective advocates of proliferation and infiltration.[9] The debate was especially fierce over the composition of 'epithelial crescents', but now even this has been largely resolved by

studies using monoclonal antibodies which show the importance of both processes.[10] Perhaps the most important message to emerge is that in most but not all circumstances macrophage infiltrations appears side by side with glomerular cell proliferation.

Macrophages are a rich source of polypeptide growth factors which are released in response to inflammatory stimuli.[11] The importance of these factors has been emphasized by recent studies using tissue culture to investigate cell proliferation in pure cultures of normal cells,[12] including those from glomeruli.[13] However, many cell types release their own growth factors, as well as responding to those from elsewhere; so the situation is extremely complicated. Many growth factors also have direct effects on acute inflammation and on scarring, so one of the hopes for the future is that methods to detect local productions of growth factors *in vivo* will provide better ways to investigate the pathogenesis of glomerulonephritis, as well as providing clinically important information about disease activity and prognosis.

The principal purpose of this chapter is to use recent advances in the understanding about growth factors and inflammation to help with interpreting the meaning of biopsy findings in patients with proliferative glomerulonephritis. The hope is that methods currently being used for studying cell proliferation *in vitro* will, in the future, be useful for studying proliferative activity in renal biopsies.

CLINICAL STUDIES

Proliferative nephritis is characterized by glomerular hypercellularity and increased matrix synthesis, and is often associated with progressive glomerulosclerosis. Since Langhans,[4] and until recently, the prevailing view was that glomerular hypercellularity in nephritis was the result of proliferation of intrinsic glomerular cells. Much of the early work concerned patients with acute post-infectious glomerulonephritis, and the proliferating cells were thought to be endothelial in origin.[5] This was inevitable as the mesangium had not yet been described and only later was its importance recognized. The most important evidence in favour of cell proliferation was the presence of mitoses which were considered unlikely in infiltrating cells. The contrary view was first expressed by Jones and his colleagues,[7,14,15] and was based on careful

glomerular reconstructions of 1 μm sections. These were interpreted as showing an early influx of neutrophils which was followed by a mononuclear cell infiltrate which differentiated into histiocytes. Eventually these were removed to leave a normal glomerulus. Although undoubtedly prescient, one needs to be cautious before attributing a contemporary interpretation of these findings. The principal point Jones was making was that the extra cells were not endothelial in origin, and so he assumed (most probably correctly) that they must have come from the blood, again because the mesangium had yet to be defined.

Better techniques were needed to distinguish infiltrating monocytes from proliferating mesengial cells. Electron microscopy was the first of these to be used effectively,[16] even though there are severe limitations to using the technique quantitatively. More recently glomerular cell culture has been used as an alternative way to demonstrate the presence of increased numbers of glomerular macrophages;[17] but again cannot be used to identify the respective proportions of hypercellularity attributable to proliferation and infiltration. Histochemical techniques have also been used to identify cells; for example using stains for cytoplasmic enzymes, such as non-specific esterase[18–21] and alpha-1-antitrypsin, which are found in macrophages but not in intrinsic mesangial cells. Lastly and most accurately, renal biopsies have been stained with panels of monoclonal antibodies which are specific for particular cell types including monocytes and lymphocytes.[10,18,22–28] Results from studies using these techniques are summarized in Table 6.1.

Taken together these studies establish the presence of large numbers of glomerular macrophages in patients with acute post-streptococcal nephritis, and in focal necrotizing glomerulonephritis. There are smaller increases in patients with mesangiocapillary glomerulonephritis, whereas there is little, if any, increase in glomerular macrophages in patients with focal proliferative glomerulonephritis and so presumably mesangial cell proliferation is responsible for increased cellularity in this situation. Inevitably studies such as these are only descriptive, and provide little information about the cause of monocyte infiltration. However, Magil and Wadsworth[19] and Hooke *et al.*[22] have suggested that increased monocyte counts are associated with subendothelial deposits of immunoglobulin.

TABLE 6.1 Glomerular infiltrates in nephritis

	Hypercellularity	*Neutrophils*	*Monocytes*	*B lymphocytes*	*T lymphocytes*		*Comment*
					CD4	*CD8*	
Minimal change	−	−	−	−	−	−	
Minor glomerular changes	±	−	−	−	−	−	
Membranous nephropathy	−	−	−	−	−	−	
Idiopathic mesangial proliferative glomerulonephritis	+	−	−	−	−	−	
Mesangial IgA disease	+	±	±	−	−	−	
Mesangiocapillary glomerulonephritis	+	±	+	−	±	±	
Post-infection glomerulonephritis	+ +	+ +	+	−	−	−	
SLE focal proliferative	+	±	±	−	−	−	
SLE diffuse proliferative	+ +	+	+	+	+	+	Few glomerular T cells compared to numbers in interstitium
Polyarteritis/Wegener's	+	+	+ +	+	+	+	
Anti-GBM disease	+	+	+ +	+	+	+	

The situation regarding the cellular composition of crescents has been just as confused until recently. Until the middle 1970s it was generally believed that crescents were formed by proliferating glomerular epithelial cells,[6] but more recent studies have stressed the large number of macrophages that they contain,[8,20–27] as well as the presence of lymphocytes.[10,23] The reason for crescent formation is less controversial. Conventional light microscopy, as well as ultrastructural studies,[29,30] have shown repeatedly that crescents are associated with breaks in the glomerular capillary wall through which cells and macromolecules, especially fibrin, appear to be leaking. Detailed studies of the glomerular basement membrane (GBM) with scanning electron microscopy of acellular preparations have shown holes in the GBM from crescentic glomeruli. The holes are roughly $10 \times 5\ \mu m$ in size, and are regularly accompanied by mesangiolysis.[29,30]

The cellular composition of crescents has been studied intensively over the past few years but in many ways this has only added to the confusion. Atkins and his group have invariably reported that crescents contain a substantial number of macrophages[17,22,26] and others have agreed.[20,21,24,25,27,28] Nevertheless, it always seemed likely that a proportion of the cells were epithelial in origin and, in what is perhaps the most detailed study to date, Boucher and her colleagues[10] reported that in many circumstances these predominate. They used a series of monoclonal antibodies which were able to identify separately both parietal and visceral glomerular epithelial cells, monocytes and lymphocytes of various types. The principal findings were that parietal epithelial cells were the most abundant cell type in crescents when Bowman's capsule was still intact, whereas monocytes predominated when the capsule had been disrupted. Perhaps more important still was the observation that the predominant cell type varied from glomerulus to glomerulus within the same biopsy. The conclusion drawn by Boucher and her colleagues was that the integrity of Bowman's capsule was critical to the composition of crescents. However, that is unlikely to be the whole explanation and, both intuitively and from direct observation of the published pictures, it seems much more likely that glomerular epithelial cells predominate in less severe crescents when neither GBM nor Bowman's capsule has been extensively breached, whereas more extensive damage to either structure is associated with a greater proportion of macrophages.

Clinical studies have demonstrated the presence of macrophages in glomeruli showing either endocapillary or extracapillary proliferation. But macrophages do not account for all the extra cells, and in fact hypercellularity is found on occasions without any excess of macrophages. This raises the question of the relation between lymphocyte and macrophage infiltration and glomerular cell proliferation. Currently there are no clinical data that bear on this point, because biopsies merely provide an example of what is happening at a particular time. Further evidence has had to be indirect, and derives first from the study of experimental models of proliferative nephritis, and secondly from *in vitro* studies in which the factors controlling proliferation of glomerular cells in culture (human as well as from other animals) have been investigated.

EXPERIMENTAL PROLIFERATIVE GLOMERULONEPHRITIS

The observations on patients provide definite clues to the pathogenesis of nephritis, and in particular for the involvement of both infiltrating cells and intrinsic glomerular cells in glomerular hypercellularity. Unfortunately they cannot be used to evaluate these processes in detail, so investigators have resorted to the experimental models since the beginning of the twentieth century. Most frequently these models have been used to define the pathogenic consequences of injected proteins; for example heterologous anti-GBM antibodies, or immunization with foreign proteins to produce immune complexes. These and more recent models have been analysed repeatedly to define mediator systems involved and have been excellently reviewed,[31,32] and the only reason for discussing them again here is for the information they provide about glomerular hypercellularity.

The two great advantages for studing experimentally induced glomerulonephritis are: first that it can be induced at a particular moment; and second that studies can be made at defined times thereafter. This is a powerful combination which sets these models apart from spontaneous glomerulonephritis that develops in some strains of animals, and which develops much more slowly. In many respects the difficulties spontaneous models present for investigators are analogous to those of human disease in the difficulties they present for detailed

morphological and functional studies. For this reason most detailed studies of proliferative glomerulonephritis have employed variations of three models of experimental nephritis: (1) nephritis following injection of heterologous anti-GBM antibodies, often in animals pre-immunization with the relevant foreign protein; (2) immune complex nephritis, either with single or repeated immunization of foreign protein or more recently by intrarenal infusions of antigen after systemic immunization; and (3) injection of heterologous antibody to mesangial cell antigens. Most of the information comes from experimental anti-GBM disease, so this will be discussed in more detail.

Renal injury after injection of anti-kidney sera has been studied since 1900, but it was not until the 1930s that Masugi[33] made the important observation that injury in this model could be separated into two phases: the first, or heterologous phase, occurring within the first 24 hours as a direct consequence of heterologous antibody binding to the kidney; and the second, or autologous phase, occurring 10–14 days later, coincident with host 'autologous' antibody response to the foreign IgG (heterologous anti-GBM antibodies) fixed to the GBM. He also noted that the second phase of injury was much more severe, often resembling the crescentic morphology seen in patients with rapidly progressive glomerulonephritis. Unanue and his colleagues[31] increased the consistency and severity of the autologous phase by pre-immunization with foreign IgG, and it is this 'telescoped' model of nephrotoxic nephritis that has been used most frequently to study the role of different cell types in proliferative nephritis experimentally.

Injection of heterologous anti-GBM antibodies is associated with a transient influx of neutrophils into glomeruli which reaches a peak roughly 4 hours after injection and which is largely over by 24 hours. The glomeruli are clearly hypercellular by this stage and ultrastructural studies have shown them to contain numerous monocytes; despite these albuminuria has largely resolved by this stage. The autologous phase of injury is much more severe, and is associated with considerable glomerular hypercellularity before proceeding to extracapillary proliferation and crescent formation, which varies in intensity with different species.

The relative importance of glomerular infiltration with leucocytes and proliferation of endogenous cells has been assessed using increasingly sophisticated markers for infiltrating cells, and also by *in vivo*

cell labelling with tritiated thymidine and autoradiography, which is a method of identifying local cell division. Cattell and her colleagues have provided the most comprehensive studies using a combination of *in vivo* cell labelling, renal transplantation and unilateral renal irradiation.[34,35] These studies show clearly that the diffuse endocapillary hypercellularity was partly due to local proliferation of cells, but also associated with an appreciable influx of circulating mononuclear cells. Studies by Sterzl and his colleagues[36,37] have confirmed these observations, and in addition suggested that over the first 4 days there is change in the cell type that is proliferating. Early in the course there is evidence of endothelial cell proliferation, but later mesangial cells are the dominant cell type that are dividing. Crescent formation in both studies was associated with parietal epithelial cell division without evidence of visceral (podocyte) cell division. Interestingly, unilateral renal irradiation before induction of nephritis did not prevent crescent formation, suggesting that the majority of dividing cells within crescents were extrarenal in origin, i.e. monocytes.

These studies demonstrated very clearly that large numbers of monocytes accumulate during both endocapillary and extracapillary phases of nephrotoxic nephritis, and the crucial question is their exact role in injury. Holdsworth, Neale and Wilson,[38] using anti-macrophage serum to answer this question, showed that autologous phase injury was abrogated in animals in which monocytes had been depleted by anti-macrophage serum. In later experiments, Holdsworth[39] investigated the role of antibody in localizing these macrophages. He used the model of passive autologous phase injury, a modified version of autologous phase injury in which the host's own autologous antibody response is pre-empted by injection of IgG containing high titres of antibody heterologous anti-IgG antibodies. In this way it is possible to produce passive model monocyte-dependent injury and to show that monocyte accumulation was dependent on the Fc piece of the passively administered antibody. However, these experiments should not be over-interpreted, and it may be that there is a direct relation between antibody deposition and monocyte accumulation in the natural autologous phase, but that other cell types are involved and may distort the simple relationship. This is an important caveat as passive autologous phase injury is generally less severe than the natural equivalent.

Two lines of evidence suggest a more complicated situation. First Naish *et al.*,[40] in experiments using anti-neutrophil serum, which was said not to cross-react with monocytes, showed that autologous phase injury could be abrogated by anti-neutrophil serum but not by complement depletion (both were effective in preventing heterologous phase injury). A role for neutrophils is strengthened by the observation that neutrophil numbers increase just before the monocyte influx.[41] Recent careful studies of glomerular leucocyte influx cells have also suggested a role for lymphocytes. Tipping, Neale and Holdsworth[42] showed that T lymphocytes infiltrated the glomeruli just before the monocytes; influx of the T lymphocytes as well as that of monocytes could be prevented by treatment with cyclosporin A. These experiments have been interpreted as providing unequivocal evidence of T cell involvement in this model, but this is going much too far, as it is apparent that the cyclosporin is less specific for T cells than originally thought,[43] and there is evidence that it also influences cytokine release from macrophages.[44] Cytokines are peptides that have important modulating effects on inflammation, and are released in large amounts from activated macrophages. Glomerular macrophages in nephritis have many of the properties of activated macrophages, including the expression of tissue factor of the coagulation pathway. Expression of tissue factor correlates clearly with fibrin deposition within glomeruli,[45,46] which in turn has long been known to be essential for crescent formation. Lymphocytes secrete a number of substances that activate macrophages; these are known, generically, as macrophage activating factors (MAF).

It is known that MAF activity is not a single entity but represents the combined effects of a number of different lymphokines.[47] Interferon-gamma is probably the most important, but others include granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 and interleukin-4 (IL-4). There is no direct evidence that glomerular lymphocytes secrete these lymphokines but they have been reported to release another pro-inflammatory activity, 'migration inhibition factor'.[48] This is a chemotactic activity and provides indirect evidence that the infiltrating lymphocytes are involved in localization of macrophages in the kidney, and a clue to their possible role in differentiation into activated inflammatory cells.

The only direct evidence of active participation of lymphocytes in

the injury of nephrotoxic nephritis comes from studies by Bhan and his colleagues[49] in which T cells from lymph nodes of rats immunized with rabbit IgG were purified and transferred into rats which were then injected with rabbit anti-rat GBM antibodies. Transfer of these 'educated T cells' increased injury in syngeneic rats with nephrotoxic nephritis, whereas transfer of control T cells had no effect. This model is in many respects the lymphocyte equivalent of the 'passive autologous phase' described above, except that, in these studies, Bhan did not provide evidence that the antigen to which the T cells were sensitized had to be present in the glomerulus, or whether its presence in the circulation was just as effective; whether the increased injury was merely a consequence of a systemic allergic reaction. Nevertheless transferred T cells did increase injury, and the most plausible interpretation is that these sensitized lymphocytes released lyphokines on exposure to antigen, and that the lymphokines activated macrophages to increase injury.

There is a great need for studies of the direct effect of T cells that react with glomerular antigens without the complicating effects of antibodies. The only way to achieve this in rodents is by transfer of T cells sensitized to glomerular antigens, but unfortunately this has not yet proved possible, mainly because of the formidable problems of raising autoreactive T cell lines in rats, which appears to be more difficult than in other species. Isolated T cell immunity is much easier to achieve in avians. The bursa of Fabricius is a discrete organ in birds that is essential for maturation of B cells. Surgical removal of the bursa renders birds B cell-deficient and thus incapable of producing antibodies, a fact exploited by Bolton and his colleagues when producing a model of anti-GBM disease in chickens.[50] They were able to show clearly that bursectomized chickens did develop glomerular inflammation when immunized with GBM. Unfortunately, the structure of chicken glomeruli is very different from those of mammals, but they were infiltrated with lymphocytes in the absence of antibody deposition and the disease could be transferred by T cells. Despite T cell infiltration there was no glomerular necrosis in either active or passive form of the disease, and albuminuria could not be measured. Clearly, it is a long way to extrapolate from these experiments to clinical types of glomerulonephritis.

The data on models of proliferative nephritis caused by immune

complexes tend to confirm the findings in nephrotoxic nephritis. First the infiltrating monocytic cells play a crucial role in modulating injury. They are present in large numbers in both acute and chronic serum sickness,[51–54] as well as in autologous immune complex disease,[55,56] and injury in acute serum sickness can be abrogated by anti-macrophage serum.[53] The role of lymphocytes is less clear, but they are certainly within glomeruli in chronic serum sickness.[54] Bhan and his colleagues[57] have done transfer experiments to show that T cells sensitized to mesangiae aggregate of immunoglobulin increased injury, at least as expressed by proteinuria.

Injection of antibodies to mesangial surface antigens also causes a proliferative glomerulonephritis which follows a phase of mesangial cell lysis.[58,59] In this model the role of infiltrating macrophages is less prominent, and it appears as though mesangial cell proliferation is derived in large part by the reduced mesangial cell numbers. It will already be apparent from this discussion that the experimental models have produced considerable additional evidence about the way in which injury is brought about in proliferative glomerulonephritis. This evidence supports the idea of the crucial role for macrophages in injury in these models, and confirms the ideas first raised from the analysis of human renal biopsies. Stimulated macrophages could cause injury by releasing oxygen radicals or proteolytic enzymes, but they are also known to synthesize a wide variety of cytokines and growth factors with powerful effects on the intensity of inflammatory response. These are likely to be just as important, and perhaps more so. Cytokines and growth factors have come under intense study recently.

MACROPHAGES AND THE CONTROL OF INFLAMMATION

The experimental evidence which has been discussed puts enormous emphasis on macrophages in determining the severity of injury in proliferative nephritis. This coincides with current views on the role of macrophages in inflammation and scarring more generally. Monocytes and macrophages are capable of releasing a wide variety of substances that either cause inflammation or influence severity through effects on adjacent cells.[11] These include: enzymes, oxygen radicals and other reactive inorganic species which produce injury directly; pro-

staglandins and leucotrienes which affect inflammatory cell function; and complement components and coagulation factors which are directly involved in the inflammatory response. Macrophages also produce a large number of 'factors' which affect cell function. These have been recovered from supernatants of macrophages cultured *in vitro*. The molecules responsible for many of these activities have now been purified, sequenced, and their genes cloned and re-expressed in bacteria to produce recombinant molecules which can be used for detailed studies. These factors include the interleukins, interferons and growth factors, and are collectively known as cytokines or peptide regulatory factors. It is apparent now that cytokines play a central role in inter-cell communication, and are responsible for controlling the intensity of inflammation, as well as many of the processes necessary for repair and scarring of tissue. Macrophages are the predominant source of these peptides which have autocrine, paracrine and occasionally hormonal function but most (possibly all) other cell types, including those of glomeruli, synthesize a more limited range of cytokines that nevertheless are important.

Macrophages are found in all tissues of the body. They are long-lived cells, derived from bone marrow precursors, and reach the tissue as monocytes. Resident macrophages differentiate in slightly different ways in different tissue. They are numerous in all tissues including the kidney, where they are found in glomeruli and in the interstitium; in fact the majority of renal interstitial cells are probably macrophages. Macrophages have an essential role in host defences and accumulate in inflammatory foci. They are important for killing intracellular parasites, such as listeria and mycobacteria, and also of virally infected or tumour-bearing cells. They also present antigen to T lymphocytes to initiate immunological reactions. Resting macrophages, however, are incapable of many of these functions and have to be 'activated' first. There are multiple steps to macrophage activation and they are being increasingly understood.[44,47,60] Some macrophage functions increase within minutes of exposure to an activating stimulus; such early changes include the increased ability to adhere to surface, to synthesize toxic oxygen radicals and to release proteolytic enzymes; other functions, such as induction of tumour killing, take much longer to develop. Macrophages may be partially activated by exposure to chemotactic factors such as the complement component 5 (C_{5a}) but

full activation involves exposure to bacterial products such as lipopolysaccharide (LPS) or to certain lymphocyte-derived cytokines (lymphokines). Interferon-gamma released from sensitized T cells is the prototype of these macrophage-activating lymphokines but now there are many others (Table 6.2). Obviously macrophage activation has to be tightly regulated, and inhibitory factors are beginning to be defined; for example transforming growth factor-β (TGF-β), which is a polypeptide cytokine, and prostaglandin E2 have powerful inhibitory

TABLE 6.2 Macrophage activation

Factor	*1a expression*	*Tumour killing*
Activating		
Interferon-γ	+	+
Bacterial lipopolysaccharide	+	+
Interferon-α/β	− (except with INF-γ)	+
Interleukin-2	− (except with INF-γ)	+
Interleukin-3	− (except with INF-γ)	+
Interleukin-4	− (except with INF-γ)	+
GM-Colony-stimulating factor	− (except with INF-γ)	+
Inhibitors		
Transforming growth factor β		
Prostaglandin E_2		

effects. Tumour necrosis factor (TNF) and interferons alpha and beta (INF-α/β) activate some functions but not others. Activated macrophages all increase cytokines when stimulated with TNF, interleukin-1 as well as more traditional stimulatory molecules (e.g. LPS, C_{5a}, platelet activity factor – PAF).

Some cytokines relased by macrophages, such as GM-CSF, are capable of causing further macrophage activation, whilst others, like TGF-β, have the opposite effect. Presumably the net effect reflects the balance between these forces, and the result *in vivo* cannot yet be predicted with confidence. An added level of complexity is that cytokines released from macrophages are capable of stimulating adjacent cells to release other cytokines, which in turn are capable of affecting macrophage function.[3] Two examples will suffice: first IL-1 released from macrophages stimulates T helper cells to release IL-2 and INF-

γ, both of which are macrophage-activating factors; and second, TNF released from macrophages stimulates endothelial cells to synthesize GM-CSF,[62] which is also capable of activating macrophages. Clearly the situation is very complicated, and most of the observations come from *in vitro* studies using single cell types stimulating a single cytokine; and the net effect of the release of cytokines *in vivo* is likely to vary in different settings. Even with these reservations TNF and IL-1 have both been shown to have important pro-inflammatory effects *in vitro* which are reflected in increased injury *in vivo*. These effects are directly relevant to proliferative nephritis clinically.

INFLAMMATION

Inflammation involves the local accumulation of phagocytes and lymphocytes, and traditionally this has been assumed to be property of the inflammatory cells alone. These can be attracted by locally produced chemotactic factors, such as C_{5a}. These then bind to specific targets or via Fc receptors binding to tissue-fixed antibody with neutrophils and monocytes. Until recently endothelium had been assumed to have very little more than a passive role, but this is far too simple and it is now clear that endothelial cells also have a very active role in inflammation, and that TNF and IL-1 are important in its regulation.

Tumour necrosis factor[64] was first identified in the serum of rabbits that had been infected with BCG and then injected with LPS, and was identified because it caused necrosis when injected into tumour-bearing mice; hence its name. TNF has a molecular weight of 17 kDa but circulates as a trimer. It binds to specific receptors that are found on many different cells but most attention has been concentrated on its potential role in inflammation. *In vitro* it primes neutrophils and monocytes to release more enzymes and oxygen radical. It increases numbers of Fc receptors that they express, and also the number of adhesion molecules on their surface. These adhesion molecules belong to a family of proteins called integrins and include the molecules LFA-1, CR-3 and P150/95, which bind to ligands on endothelium and other cells. TNF also induces the release of IL-1, GM-CSF, platelet-derived growth factor (PDGF), IL-8 (a neutrophil chemotactic peptide) and TGF-β from monocytes. TNF has analogous effects on endothelium

(at least as reflected in studies *in vitro* using cultured human umbilical vein endothelium).[63] TNF increases endothelial surface pro-coagulant activity transiently. This activity reaches a peak between 4 and 8 hours and is dependent on fresh protein synthesis. Simultaneously surface anticoagulant activity is decreased, though effects on thrombomodulin (acting through protein S and protein C), fibrinolysis is decreased because of decreased plasminogen synthesis and increased synthesis of short-acting plasminogen activator inhibitor (PAI-1). TNF also increases the adhesiveness of endothelium for leucocytes by increased expression of at least two adhesion molecules; ELAM-1 (endothelium leucocyte adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1), which is the ligand for the leucocyte molecule LFA-1. Expression of ELAM-1 is transient with maximum expression between 4 and 8 hours, and gone by 24 hours, whilst expression of ICAM-1 is slower in onset but more persistent. Lastly exposure of endothelium to TNF promotes the synthesis of a number of cytokines including IL-1, IL-6, IL-8, GM-CSF and PDGF.

Interleukin-1 is another predominantly macrophage-derived cytokine that shares many of the properties of TNF.[63] Two genes for interleukin-1 have been identified coding for IL-1α and IL-1β respectively.[61] They have limited homology but bind to the same receptors and appear to have identical functions. The major difference is that IL-1α is a membrane-bound form, whereas IL-1β is released into tissue culture media. IL-1 was originally recognized because of its activity on T cell activation (it increased expression of IL-2 receptors on T cells and promotes T cell IL-2 synthesis), but it has almost the same range of activities on neutrophils, monocytes and endothelial cells as does TNF. Monocytes are the main source of IL-1, but small amounts are produced by many cell types including fibroblasts, epithelial cells and mesangial cells.

The properties of TNF and IL-1 *in vitro* suggested that they might have powerful effects of inflammation *in vivo*, and this turned out to be the case. Injections of both TNF and IL-1 produce inflammation when injected locally either subcutaneously or into a joint space.[63,65] The inflammation is transient and associated with a neutrophil influx, almost certainly through the release of locally produced chemotactic factors such as interleukin-8. The time course of neutrophil accumulation is identical to that of ELAM-1 expression after endothelial cells

are exposed to TNF or IL-1 *in vitro*, which suggests that endothelial expression of ELAM-1 is involved in the phenomenon. This suggestion is further strengthened by recent studies showing increased expression of ELAM-1 *in vivo* in animals injected intradermally with TNF.[66]

The presence of both TNF and IL-1 can be demonstrated in inflammatory foci *in vivo* in inflammatory exudates in joints of patients with arthritis.[67] Cells producing mRNA for IL-1, TNF and a variety of other cytokines have also been identified in synovium of inflamed joints.[68] The effect of TNF on inflammation *in vivo* has been examined experimentally using anti-TNF antibodies in various infective causes of inflammation where tissue injury results from the immunopathological consequences of the infection. Grau and his colleagues were able to abrogate symptoms of cerebral malaria in mice by injection of anti-TNF antibodies,[69] and similar results have been produced in systemic BCG infection[70] and graft-versus-host disease.[71] The same group were also able to inhibit injury in cerebral malaria using antibodies to GM-CSF and IL-3, cytokines that activate macrophages which were presumed to be the source of the TNF.[72] Perhaps the most interesting results of using anti-TNF antibodies to suppress inflammation came from the systemic BCG infection.[70] This model is associated with accumulation of macrophages in the liver and substantially increased hepatic synthesis of TNF mRNA without a detectable increase in circulating TNF concentrations. Administration of anti-TNF antibodies in this model reduced not only hepatic inflammation but also local production of TNF itself (as reflected by the amount of TNF mRNA). Thus they seem to have broken a vicious circle.

These experiments provide convincing evidence of the importance of TNF in a variety of models of inflammation, and raise the question whether cytokines are similarly involved in glomerulonephritis. Rats with heterologous phase nephrotoxic nephritis (Ryan, Koshino and Rees, unpublished) have been reported to release TNF *ex vivo* as have glomeruli harvested from patients with crescentic nephritis release IL-1 when cultured[73]; similar results have been reported in rabbits with autologous phase nephrotoxic nephritis.[74] There is also evidence that TNF modulates injury in experimental nephritis. We have shown that rats with heterologous phase nephrotoxic nephritis have substantially increased injury when pretreated with systemic injections of TNF

using doses that increase the plasma concentration to the same extent as occurs naturally after intraperitoneal injections of a small dose LPS.[75] This increase in injury can be prevented by injections of anti-TNF antibodies (Koshino, Karkar, Cashman and Rees, submitted for publication). In other experiments in the autologous phase of injury Lowry and his colleague have been able to decrease injury by injection of anti-TNF antibodies.[76] Thus it appears that in proliferative models of nephritis acute injury can be exacerbated by injection of the TNF and attenuated by anti-TNF antibodies. Kelley and her colleagues[77,78] have shown increased glomerular production of IL-1 in lupus strain mice with nephritis, and that macrophages are the main but not exclusive source of IL-1; mesangial cells also released small quantities. Similar observations have been made in a model of immune complex injury.[79]

Experimental studies such as these have demonstrated the importance of infiltrating cells for glomerular injury in proliferative glomerulonephritis. They have also suggested an important pathogenetic role for cytokines released from these cells as well, as for polypeptide growth factors. If this is true then it will be very valuable to have detailed knowledge of how cytokines and growth factors influence glomerular cell growth, proliferation and matrix synthesis as a prerequisite for better ways to assess 'activity' in nephritis, and to develop new treatments. This is the premise underlying much of the work to develop reliable techniques for culturing glomerular cells.

GLOMERULAR CELL CULTURE

Cell culture is the only way to study the effects of cytokines and growth factors on glomerular cell biology, but it is important to remember the severe limitations on the inferences that can be drawn from results using these techniques. Cells in culture grow under highly artificial conditions and behave very differently from the way they do *in vivo*; for example, mesangial cells proliferate continuously in culture unless starved of essential nutrients, which is clearly not the case *in vivo*. Nevertheless, it is possible to learn a considerable amount about glomerular cell proliferation and mediator synthesis from *in vitro* studies. These studies have to be seen in the context of recent developments in the understanding of cell growth more generally. These serve

to emphasize two principles: first the very close relation between cytokines that modulate acute inflammation and those that control cell growth and matrix synthesis; and secondly that substances known to control the glomerular filtration rate, such as angiotensin-II, atrial naturetic peptide[80] and prostaglandins, also have effects on glomerular cell growth. These investigations are in their infancy, and it should be stated firmly at this stage that it is not known yet whether either observation will turn out to be relevant *in vivo*.

Over the past decade the techniques for glomerular cell culture have become increasingly standardized and have been well reviewed.[81,82] The first step is to purify glomeruli, and this is usually done by differential sieving with mesh sizes that vary slightly depending on the species.[83] The technique provides populations of glomeruli stripped of their Bowman's capsule and of varying degrees of purity; very pure for glomeruli harvested from human or rat but much less so with those of rabbits. The glomeruli can then be plated directly on to tissue culture flasks, cluster plates or Petri dishes. Alternatively the whole glomeruli can be partially digested with collagenase or with a mixture of collagenase, trypsin and DNAase. Enzyme treatment can be useful either to produce single cell suspensions of individual cell types, or to 'loosen up' the glomeruli. This increases their plating efficiency and shortens the interval before cells begin to grow out of them. Obviously there are great advantages in starting off with pure populations of cells, but the technique is considerably more difficult. Cell losses are formidable and, even using fluorescence activated cell sorters (FACS), it is difficult to avoid contamination with more than one cell type. These difficulties mean that the most reliable way to grow pure cell lines is to start with plated glomerular fragments and to derive the lines by selective pressure of different tissue culture conditions, such as those shown in Table 6.3. Partially digested glomeruli adhere to tissue culture plastics within 2–5 days, and the first cells begin to emerge over the next 5 days. Small round cells tend to predominate in the early days of the outgrowth but later more elongated cells emerge. They grow in a swirling pattern, divide faster than the round cells and overrun the cultures. There are no definitive methods for identifying the different cell types but a wide variety of reagents have been used (Table 6.4). There is little doubt that the elongated cells are mesangial in origin and they are most easily recognized by fluorescent

TABLE 6.3 Conditions for glomerular culture

Favoured cell type	*Medium*	*Serum*	*Supplements*
Mesangial cells	RPMI 1640	10%	
Epithelial cells	RPMI 1640	2.5%	Epidermal growth factor Thyroxine Prostaglandin E_1
Endothelial cells	RPMI 1640	20%	Heparin Acidic fibroblast growth factor

Insulin, transferrin and selenium are added to all the cultures

TABLE 6.4 Glomerular cell characteristics

	Mesangial	*Epithelial*	*Endothelial*
Morphology	Stellate	Round	Round
Vimentian	+	−	−
Desmin	+	+	
Myosin	+	−	−
Actin	+	± (peripheral)	± (peripheral)
Cytokeratin	−	+	−
CALLA*	−	+	−
von Willibrand's/F VIII	−	−	±
Ulex europus binding	−	−	+
1a expression	−	−	±
Acetylated LDL uptake	−	−	+

* Common acute lymphoblastic leukaemia antigen

methods to detect the actin–myosin filaments. Identification of the round cells is much more problematic. Originally they were assumed to be epithelial, but now it appears that some at least are endothelial cells. These, however, tend to proliferate more slowly and survive passaging poorly. Human glomerular endothelium can be identified easily by the uptake of acetylated LDL, which can be purchased already labelled with fluorescence on rhodamine, and is a much more reliable marker than antibodies to von Willebrand's factor or lectins, such as *Ulex europus*, that bind to endothelial cells.[84] Unfortunately endothelial cells from rat glomeruli do not take up acetylated LDL.[85]

Most round cells have epithelial cell markers such as cytokeratin and the human common acute lymphoblastic leukaemia antigen (CALLA), but this presents a paradox. Podocytes in tissue sections do not contain cytokeratin, whereas epithelial cells from those lining Bowman's capsules do. Conversely the CALLA antigen is expressed on podocytes but not on parietal epithelium. So the origin of these epithelial cells remains uncertain.

Currently it is possible to grow pure lines of mesangial cells with comparative ease. They have many of the characteristics of vascular smooth muscle cells in culture and thrive in relatively high concentrations of serum.[86] They grow densely and pile up on top of each other to form what has been described as 'hillocks'. Glomerular epithelial cell lines, regardless of their provenance, can also be grown with comparative ease. They are round, show contact inhibition and thus grow in monolayers. Endothelial cells have proved the most difficult to grow reliably, and successful culture through many passages has only been reported sporadically.[84,87–89] Not all the reports have proved reproducible. Nevertheless Daniel and his colleagues[89,90] have been able to grow what appears to be renal microvascular, possibly glomerular, endothelium. Before discussing factors that influence growth of renal cells it is important to summarize what is known about cell growth more generally.

GROWTH FACTORS AND CELL DIVISION

The past 5 years have seen a massive increase in knowledge of the factors controlling cell differentiation and cell division. Numerous polypeptide growth factors have been identified which bind to specific receptors to induce cell division or differentiation (activation) depending on the circumstances. There are four phases to the cell cycle: G_1 which precedes DNA synthesis; the S phase of DNA synthesis, and which, if followed by G_2 before the cell finally enters the M (mitotic) phase, results in two daughter cells in the G_1 phase. Cells that are not undergoing continuous division (cycling) stop in the G_1 phase and this long-lasting early part of G_1 is often referred to as G_0. Although non-cycling cells can be provoked from G_0 into G_1 by mitogens, there is now evidence that 'growth arrest genes' (gags) actively restrain cells

from entering the cell cycle.[91] Potentially this is very important, as looking for activation of gags might prove useful in monitoring proliferative activity in renal disease.

The growth factors which initiate cell division have been categorized into two classes; competence factors and progression factors. Competence factors (such as platelet-derived growth factors, PDGF) by themselves are incapable of pushing cells into mitosis but induce changes that allow cells to respond to progression factors that induce mitosis. The changes that occur when cells are exposed to competence factors are very complex. PDGF, for example, induces the transient expression of at least 50 genes, some of which have been identified as the normal cellular equivalents of viral oncogenes.[92,93] Oncogenes were first identified in virally transfected cell lines and shown to be directly responsible for unrestrained proliferation of malignant cell lines. Unsurprisingly perhaps, they have been shown subsequently to code for proteins that bypass the normal controls of cell division, and to be homologous to normal genes involved in cell division that they bypass.[94] These normal genes have been called protoncogenes, and are distinguished from their viral counterparts by the prefix C (C-myc as opposed to V-myc, for example). By convention the products of these genes are written in capital letters (myc codes for the protein MYC).

Many of the polypeptides that were identified originally because of the ability to promote cell division have equally potent, and, possibly more important, effects on inflammation and scar formation. As discussed previously, macrophages are the most important source of growth factors, and it is unfortunate that the nomenclature of these polypeptides is thoroughly confused because many of the molecules were identified originally relating to activities on particular systems and were named accordingly. Only since the amino acid sequences of these molecules have been elucidated, and their genes cloned, has it been apparent many substances with different names turn out to be the same molecule; the case of tumour necrosis factor (TNF) has already been discussed, but unfortunately is by no means unique. The interleukin nomenclature is an attempt to improve the situation but has, if anything, aggravated it. Interleukin numbers may be applied to cytokines that have been purified, sequenced, cloned and expressed. Currently eight polypeptides (interleukins 1–8) have been granted an interleukin designation,[95] but the anonymity of the names makes it

difficult to correlate molecule with function. Interleukins is also a misnomer, because leucocytes are not the exclusive source of these molecules, or the target of their functions. Table 6.5 shows some of the properties of these molecules.

TABLE 6.5 Interleukins

Cytokine	*Mr* (kDa)	*Sources*	*Effects*
Interleukin-1α Interleukin-1β	14–17	Macrophages predominantly plus most other types	T cell proliferation B cell proliferation Macrophage activation Endothelial cell activation
Interleukin-2	15	Activated T cells	T cell proliferation Macrophage activation
Interleukin-3	28	Activated T cells	Haemopoietic colony-stimulating activity Macrophage activation
Interleukin-4	15–20	Activated T cells	Growth factor for T cells B cell proliferation Ig class switch
Interleukin-6	26	Macrophages predominantly most other cell types	T cell proliferation B cell proliferation Ig secretion Acute phase reactant
Interleukin-7	17	T cells	T cell proliferation
Interleukin-8	8–10	Macrophages Fibroblasts ? many other cell types	Neutrophil chemotaxis Neutrophil priming T cell chemotaxis

GROWTH FACTORS AND GLOMERULAR CELLS

It is against this background that systemic studies are being made on the effects of growth factors on glomerular cells. Early on it became apparent that, as in other cell types, glomerular cells synthesize as well as growth factors being able to respond to them, and this complicates interpretation of individual studies considerably. The comparative ease with which mesangial cells can be grown, as well as their similarity to vascular smooth muscle cells, which have been studied extensively

by others, has meant that they have received far more attention than either glomerular endothelial or epithelial cells. Nevertheless, studies have been reported for all three cell types.

Mesangial cells from both rats[96,97] and humans (Rees, unpublished) proliferate in response to platelet-derived growth factor (PDGF). PDGF is the main growth-promoting factor in serum and is the archetypal 'competence factor'.[92,93] It is released in large amounts from the alpha granules of platelets, and also from macrophages, and to a lesser degree by vascular smooth muscle cells and endothelial cells. Recently Abboud and his colleagues[98] have shown that rat mesangial cells release PDGF. PDGF is a cationic polypeptide composed of two types of chain, named A and B (the B chain is coded for the gene C-sis which is the normal equivalent of the viral oncogene V-sis). PDGF itself is formed either of homodimers (AA or BB) or of heterodimers (AB). Platelets contain predominantly AB heterodimers, whereas vascular smooth muscle synthesizes homodimers. Provisional studies suggest that mesangial cells proliferate much more vigorously when PDGF (BB) is added to the culture medium than with AB or AA dimers. The effects of PDGF on mesangial cells is likely to be important not only for its effect on cell division but also because it has been shown to synthesize mRNA for IL-1 and to release IL-1-like material into the surrounding medium.[37] Synthesis of IL-1 mRNA is transient, being detectable within 30 minutes and gone by 4 hours of stimulating mesangial cells with a variety of substances including PDGF, IL-1 itself, TNF and LPS. As yet it is not known what role IL-1 plays in mesangial cell differentiation or proliferation, and in particular whether it has an autocoid function and if so what that function is. In vascular smooth muscle it appears that the effects of IL-1 are mediated by PDGF, whose synthesis is increased when IL-1 receptors are stimulated. Mesangial cells express receptors for PDGF *in vitro*, and *in vivo* increased expression of PDGF receptors has been reported in patients with nephritis.[100] Renal microvascular endothelium has been reported to synthesize PDGF in culture, especially when stimulated with thrombin;[89] these cells also synthesize IL-1. Clearly there are very intimate relations between cytokines, and this is exemplified by those of PDGF and IL-1. Irrespective of whether IL-1 is important for glomerular cell proliferation, mesangial cells do express IL-1 receptors and exposure to IL-1 results in activation of 'second messengers'

such as myristalation of proteins,[101] and in increased matrix synthesis.[102] Equally local release of even small amounts of IL-1 transiently, could activate adjacent inflammatory cells.

The effects of IL-1 on mesangial cells have been studied equally extensively. Human mesangial cells were first reported to proliferate in response to IL-1 by Striker *et al.*[103] but only when the cells were plated at very low densities and in the presence of 'competence factors'. Broadly similar observations were made by Lovett *et al.* using rat mesangial cells.[96] These observations have become increasingly controversial, though. All the early studies were performed with IL-1 purified from macrophages, and it is now apparent that these preparations would have been heavily contaminated with a number of other cytokines, IL-6 in particular. More recent experiments using human recombinant IL-1β have usually failed to confirm the proliferative effect of IL-1 on mesangial cells in rats and humans, but it is difficult to know whether these failures are due to minor differences in experimental design or to culture conditions. Experiments such as these can be influenced by how long the cells have been kept in culture (passage number), and whether cell cycles have been synchronized and if so how. Nevertheless, even if IL-1 has no influence on mesangial cell proliferation it does appear to stimulate a variety of other effects.[101,104,105] As yet, it is impossible to construct a coherent picture of how these cytokines interrelate, and which play a critical role, but the necessary experimental tools are just becoming available. These include methods for inactivating individual genes such as IL-1β specifically with anti-sense cDNA to enable functional studies to be performed *in vitro*,[106] and antibodies to growth factors which can be used to inhibit cytokine function *in vivo*.[69–71] The combined use of these techniques should clarify the situation considerably.

Mesangial cells have also been reported to synthesize small amounts of TNF, but this is controversial and we have tried hard but failed to confirm this result (Abbott and Rees, unpublished observations). However, we have found that mesangial cells synthesize mRNA for IL-6, and that the synthesis is increased with exposure to IL-1 or LPS but not by TNF. Epidermal growth factor (EGF),[107] insulin-like growth factor[108,109] and GM-CSF[37] have all been reported to stimulate mesangial cell proliferation and atrial naturetic factor[80] and transforming growth factor β[37] to be inhibitors. PDGF has also been reported to

increase matrix synthesis by vascular smooth muscle cells, and this is also likely to be the case for mesangial cells. Lastly PDGF is chemotatic for fibroblasts and smooth muscle cells, as well as for neutrophils and monocytes, and it also primes neutrophils and monocytes to release more oxygen radicals and enzymes.

CONCLUDING REMARKS

Proliferative nephritis with progressive glomerular and tubulo-interstitial sclerosis is still one of the major causes of chronic renal failure. Better techniques for identifying cells in tissue sections, combined with dynamic studies made possible using experimental models, have focused ideas about the pathogenesis of, but have not provided better, treatments. Furthermore they have yet to produce sensitive ways to assess disease activity, especially in patients with slowly progressive nephritis with proliferation and scarring. Recent developments in molecular and cell biology should prove useful in this. This includes the knowledge of which genes are expressed in dividing cells combined with techniques to assess their activity. The fact is that techniques are already available that are capable of assessing gene expression in tissue samples as small as renal biopsies, and even as small as individual glomeruli. They just need to be used in nephrological research.

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