Cytomegalovirus

Biology and Infection

SECOND EDITION

Monto Ho, M.D.

Cytomegalovirus

Biology and Infection

SECOND EDITION

Cytomegalovirus

Biology and Infection

SECOND EDITION

Monto Ho, M.D.

Graduate School of Public Health and School of Medicine
University of Pittsburgh
and
Presbyterian-University Hospital
Pittsburgh, Pennsylvania

Springer Science+Business Media, LLC

Library of Congress Cataloging-in-Publication Data

Ho, Monto.
Cytomegalovirus : biology and infection / Monto Ho. -- 2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-4757-9944-6 ISBN 978-1-4757-9942-2 (eBook)
DOI 10.1007/978-1-4757-9942-2
1. Cytomegalovirus infections. 2. Cytomegaloviruses. I. Title.
[DNLM: 1. Cytomegalic Inclusion Disease. 2. Cytomegalovirus.
QW 165.5.H3 H678c]
RC136.8.H6 1991
616.9'25--dc20
DNLM/DLC
for Library of Congress 90-14345
CIP

Originally published by Plenum Publishing Corporation New York in 1991
Softcover reprint of the hardcover 2nd edition 1991

Preface to the First Edition

Although there are a number of excellent current reviews on one or another aspect of cytomegalovirus, the last comprehensive treatment of this subject was that of Krech *et al.* (1971a). In view of the amazing advances in the virological, epidemiologic, and clinical knowledge of cytomegaloviruses, an up-to-date book is needed. Such a work should cover many areas of expertise and a voluminous technical literature. Each area might have been reviewed and analyzed by workers more expert than myself. However, I have embarked on the entire venture alone in order to attain unity and continuity in this book, characteristics that are not easily achieved in the more popular multiauthored works.

I have tried to review the literature and provide a critical summary for each area discussed. To do this, I provide as much of the primary data of the relevant works as needed and not just the qualitative conclusions. Inevitably, the flow of the narrative may be interrupted by dry facts and figures. However, such information is essential to make this a meaningful reference work. But for those not interested in such details, I have provided at what I hope are crucial points critiques and summaries.

This book is not an exhaustive review of all the literature. This is probably no longer possible or even desirable. By selection, however, one runs the risk of having missed or ignored important papers. I am keenly aware of this, and I wish to apologize for such oversight, if that is possible.

I visualize this work as being useful for physicians, scientists, and students interested in the biology of and infection by human cytomegalovirus. I feel that to understand these, the basic virology of this virus and related herpesviruses must be included. Topics that have not advanced a great deal and that are adequately described in available works, such as the anatomic pathology, have not been reviewed in detail. The emphasis has been placed on newer insights in the virology, immunology, serology, epidemiology, and especially clinical aspects of human cytomegalovirus. I wish to bring out the interactions of knowledge in these areas. Part II is a much shorter treatment of the nonhuman cytomegaloviruses, reviewed primarily from the point of view of their contribution to the understanding of the human virus.

Finally, I wish to thank Drs. Charles R. Rinaldo, Jr., John A. Armstrong, and Donald N. Medearis for their critical review of the manuscript. Their suggestions were invaluable. Dr. John N. Dowling's thorough review of CMV infection in patients with malignancies was the basis of Section 13.4. I also acknowledge the indispensable and patient secretarial assistance of Ms. Betty Edwards and Hannah Grace.

Monto Ho, M.D.

Pittsburgh, Pennsylvania (1981)

Preface to the Second Edition

The remarkable medical events and advances in knowledge that have taken place since publication of the first edition in 1982 have made a thorough revision of this book necessary. Opportunistic cytomegalovirus (CMV) infections have multiplied in numbers and complexity with the explosion of AIDS on the medical scene and the increase in other iatrogenic and natural deficiency states. At the same time, advances in modern virology and immunology have been such that all our previous assumptions have had to be scrutinized or revised in their light. Thus, although the original structure of the book has been maintained, almost every page or section of the text has been edited, revised, or expanded. In addition, we are indeed fortunate to have Drs. Mark Stinski and Lucy Rasmussen each write a new chapter, one on the molecular biology and the other on the gene products of CMV. They provide authoritative treatments of these critical subjects, which I have tried to incorporate and fit into the unity and continuity of the book that were hallmarks of the uni-authored first edition.

This work is still designed to be a critical, comprehensive, but not exhaustive review. It is intended to be useful for physicians, scientists, and students interested in the biology and infection by human cytomegalovirus. I would like to thank Ms. Peg Corbett, Christine Hallahan, and Betty Edwards for their patient secretarial assistance.

Monto Ho, M.D.

Pittsburgh, Pennsylvania

Contents

List of Abbreviations

Ab	Antibody
ACIF	Anticomplement immunofluorescence
AIDS	Acquired Immunodeficiency Syndrome
ara-A	Adenine arabinoside
ara-C	Cytosine arabinoside
BCNU	1,3-Bis-(2-chlorethy-)-1-nitrosourea
BUdR	Bromodeoxyuridine
C1, 2, 3, etc.	Complement 1, 2, 3, etc.
CF	Complement fixation
C.I.D.	Cytomegalic inclusion disease
CIEP	Counterimmunoelectrophoresis
CMI	Cell-mediated immunity
CMV	Cytomegalovirus
Con A	Concanavalin A
CPE	Cytopathic effect
DEAE-cellulose	Diethylaminoethyl-cellulose
DI	Defective interfering
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
EA	Early antigen
EBNA	Epstein–Barr virus nuclear antigen
EBV	Epstein–Barr virus
EID	Electroimmunodiffusion
ELISA	Enzyme-linked immunosorbent assay
FA	Fluorescent assay
Fc	C-terminal fragment
FCA	Fluorescing cell assay
FT	Freeze-thawed (antigen)
FUdR	Fluorodeoxyuridine

GE	Glycine-extracted (antigen)
GI	Gastrointestinal
GvH	Graft-versus-host
GVHD	Graft-versus-host disease
HCMV	Human cytomegalovirus
HSV-1, 2	Herpes simplex virus type 1 or 2
HvG	Host-versus-graft
IAHA	Immune adherent hemagglutination assay
ICNV	International Committee for the Nomenclature of Viruses
ICSP	Infected cell-specific proteins
IEA	Immediate early antigen
IFA	Indirect fluorescent assay
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect hemagglutination assay
IPA	Immune peroxidase assay
I.Q.	Intelligence quotient
IUdR	Idoxuridine (iodouridinedeoxyriboside)
LA	Late antigen
LD_{50}	50% Lethal dose
LPS	Lipopolysaccharide
LTR	Long terminal repeats
MA	Membrane antigen
MCMV	Murine cytomegalovirus
MGH	Massachusetts General Hospital
MLC	Mixed lymphocyte culture
m.o.i.	Multiplicity of infection
mRNA	Messenger RNA
m.w.	Molecular weight
NK	Natural killer
PAGE	Polyacrylamide gel electrophoresis
PENA	Preearly nuclear antigen
PFC	Plaque-forming center
PFU	Plaque-forming unit
PHA	Phytohemagglutinin
PQ	Phenanthrenequinone
PWM	Pokeweed mitogen
RIA	Radioimmunoassay
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
S.I.	Stimulation index

TCD_{50}	50% tissue culture infective dose
TF	Transfer factor
TI	Transformation index
TM	Transport medium
VP	Virion protein
VZ	Varicella–zoster

1

History of Cytomegalovirus

The early history of cytomegalovirus (CMV) may be divided into two periods. During the first period, the characteristic cytopathology caused by this virus was recognized and described. It may be called the "period of cytopathology." The second period was initiated by the isolation of the virus and may be called the "virological period." Both periods were essential for the elucidation of the expanding spectrum of diseases associated with CMV.

1.1. PERIOD OF CYTOPATHOLOGY (1905–1956)

In 1904, Ribbert (1904) wrote that in 1881 he saw large "protozoan like" cells in the sections of a kidney of an alleged leutic stillborn. He was unable to interpret his observation until he saw the report of Jesionek and Kiolemenoglou (1904), who had noted for the first time the presence of protozoanlike cells in the lungs, kidneys, and liver of an 8-month leutic fetus (Fig. 1.1). These protozoanlike cells were 20 to 30 μm in diameter. The nuclei were large and eccentrically placed, and each contained a "central nuclear body" surrounded by an outer clear zone. Löwenstein (1907), working in Ribbert's laboratory in 1907, found such inclusion in the parotid glands of four out of 30 infants examined. He described cytoplasmic as well as intranuclear inclusions, which these early authors did not believe were normal or altered cellular structures. After submitting their findings to various zoologists for opinions, they concluded that the inclusions represented protozoa, i.e., coccidia, sporozoa (gregarines), or amoebas. Pisano (1910) thought they represented a cellular response to syphilitic infection. Inclusions were described in the thyroid for the first time in a 10-day-old premature infant in 1911 (Pettavel, 1911).

Smith and Weidman (1910, 1914) saw the same kind of inclusion bodies in cells in an infant and thought these were infected by an "amebiform protozoan, *Entameba mortinatalium.*" They were in the area surrounded by a definite inflammatory reaction of lymphoid and polymorphonuclear cells. The child was 2 months

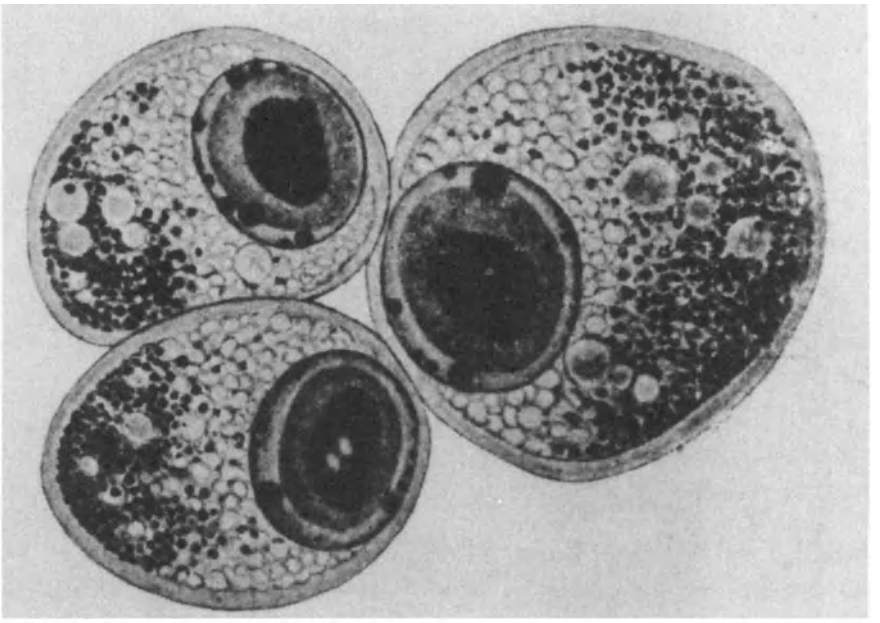

Figure 1.1. "Protozoan"-like cells seen in the kidney, lung, and liver of an 8-month-old leutic fetus by Jesionek and Kiolemenglou (1904). (By permission of *Münchener Medizinische Wochenschrift.*)

old and had ulcers about the mouth, scaly skin lesions, and fibrosis of the thymus and pancreas, and died of an organizing pneumonia. Syphilis was suspected, but the Wasserman test was negative.

Goodpasture and Talbot (1921) provided an excellent case report of a 6-week-old child with eosinophilic intranuclear inclusions in the lungs, kidney, and liver. The rare occurrence of these cells in the liver and kidney without inflammatory changes appeared to indicate, according to the authors, transport of these elements by the bloodstream. The term "cytomegalia" was introduced to describe this condition. Goodpasture and Talbot thought they were dealing with large mononuclear cells that reminded them of the intranuclear inclusions described by Tyzzer (1906) in varicella. They did not believe that the inclusions were protozoa.

In 1922, Lange (1922) and Mueller (1922) reported seeing cytomegalic inclusions in the kidneys of infants. Mueller also dismissed the possibility of the inclusions being protozoal in origin. He pointed out that since they could be observed in stillbirths, they were probably present before birth. Protozoa presumably could not have passed the placental barrier, an assumption we now know to be incorrect.

Von Glahn and Pappenheimer (1925) described for the first time a case in an adult, an unusual 36-year-old white male with a liver abscess that contained cocci, amoebas, and large cells with prominent intranuclear inclusions. Similar cells were

found in the intestines and the lung. These authors pointed out that Lipscheutz (1921) had discovered similar inclusions that were constantly and characteristically associated with lesions produced by herpesvirus, both in man and rabbits. He studied these inclusions in herpes zoster and herpes genitalis and interpreted them as an expression of intranuclear infection by virus, which he classed with "chlamydozoastrongyloplasma." To a subgroup of intranuclear agents called the "karyooikon" group belonged the causative agents of herpes simplex, herpes zoster, "Borna's disease of horses" (epidemic encephalomyelitis), varicella, and fowlpox. The group that produced cytoplasmic inclusions, the "cytooikon" group, included agents that produced the trachoma bodies, Guarnieri bodies (smallpox), and Negri bodies (rabies). On the basis of this background, von Glahn and Pappenheimer concluded that the inclusions in their case were caused by a virus identical to or closely related to the herpes group.

The experimental relationship between herpes encephalitis infection in the rabbit and intranuclear inclusions was demonstrated by Goodpasture (1925).

Although the protozoa theory was kept alive in Germany until 1930 (Wagner, 1930), the viral theory based on Lipscheutz' concepts gradually prevailed. Acceptance was accelerated by the work of Cole and Kuttner (1926) on the transmissibility of guinea pig salivary gland virus (see chapter 15) and the experimental and pathological works of Cowdry (1934), who definitively described the relationship between inclusion bodies and certain virus infections.

By 1932, 25 cases of apparent cytomegalic inclusion disease (C.I.D.) had been described. It was not realized how extensive inapparent infection is. Farber and Wolbach (1932), in a series of 183 postmortem examinations, removed and examined the submaxillary glands of infants who had died of various causes such as tuberculosis and pneumococcal, streptococcal, and other forms of sepsis. Twenty-six (14%) of this heterogeneous group had intranuclear and cytoplasmic inclusions in their glands. Two also had inclusions in the epithelium-lined spaces of the liver, lungs, kidneys, pancreas, and thyroid. No clear clinical correlations were made with diseases ante mortem.

Wyatt *et al.* (1950) suggested the term "generalized cytomegalic inclusion disease" on morphological grounds to describe the congenital lethal infection. He suggested that since inclusion-bearing cells were invariably present in the renal tubules, it might be possible to identify them in the urine of afflicted infants during life.

From 0.5 ml of urine obtained from a 3-day-old premature infant admitted to the Children's Hospital in Pittsburgh with jaundice, purpura, hepatosplenomegaly, and intracerebral calcifications, Fetterman (1952) made a cytologic preparation and found several enormously hypertrophied cells with large intranuclear inclusions. The patient died at 4 days of age, and typical inclusions were found in the brain, pituitary, thyroid, lungs, liver, pancreas, in addition to the kidney, confirming the diagnosis of C.I.D. This was the first time C.I.D. was diagnosed during life. Prior to specific virological diagnosis, cytologic examination of the urine was the most

sensitive and specific method of laboratory diagnosis. Subsequently, Mercer *et al.* (1953) and Margileth (1955) also showed the C.I.D. could be diagnosed during life by demonstration of inclusion-bearing cells in the urine.

Margileth's report (1955) is of interest because his case survived. It was a full-term baby boy who was followed from birth to 15 months of age. He had microcephaly, hemolytic anemia, thrombocytopenia, and hepatosplenomegaly at birth. Large inclusion-bearing cells were found in the urine. Petechiae and episodes of intracranial and gastrointestinal hemorrhage were recorded. His condition later stabilized, but he had retarded motor development, spasticity of the upper limbs, and microcephaly. Numerous attempts were made to isolate a virus from his urine, spinal fluid, serum, and saliva by methods available at the time, including inoculation in HeLa cells.

A more direct morphological demonstration of the virus etiology of C.I.D. was first provided by Minder (1953). Cytomegalic inclusion cells in the pancreas of a 14-day-old infant were examined by electron microscopy. Particles measuring 100 mm were seen at a magnification of ×25,000 in both the cytoplasm and the clear halo about the intranuclear inclusion. In retrospect, this was probably the first time human CMV was seen.

1.2. VIROLOGICAL PERIOD

The isolation of human CMV was not possible until human cells could be routinely grown in cultures (Enders *et al.*, 1949), since the cytomegaloviruses are largely species specific, and human CMV does not grow in experimental animals. When such culture techniques became commonplace in the early 1950s, it became a question of time until isolation was achieved.

The circumstances surrounding the isolation of this virus independently in three different centers, i.e., by M. Smith in St. Louis (Smith, 1956), Weller *et al.* (1957) in Boston, and Rowe *et al.* (1956) in Bethesda, are well described by Weller (1970). I acknowledge his description for the following account (Weller, 1970).

By 1948, the laboratories of Enders and Weller in Boston had become a mecca for virologists eager to learn the new tissue culture methods after these workers had successfully isolated the polioviruses (Enders *et al.*, 1949). Margaret Smith was one of those who went there to learn the methodology and apply it to the mouse salivary gland virus in 1951. In 1954, M. Smith (1954) isolated mouse CMV in roller cultures of mouse embryo fragments embedded in clotted chicken plasma (see Section 15.2). It is not so well known that at around the same time she had already obtained an agent from human uterine cell culture inoculated with salivary gland material of an infant and carried it for more than a dozen passages. The lesions produced were similar to those produced by the mouse salivary gland virus, but the human agent did not grow in mouse tissue, and the mouse CMV did not grow in human tissue. Dr. Smith's paper describing her findings were rejected by a journal on the incorrect ground that she might have been growing the mouse agent in human

tissues. It was not until 1954, after Dr. Smith reisolated human CMV from the salivary gland of one dead infant and from the kidney of another infant who had died of cytomegalic inclusion disease, that her paper was accepted (M. Smith, 1956).

In contrast to Dr. Smith's persistent pursuit of cytomegaloviruses of mice and man, the isolations of human CMV in Boston and Bethesda were serendipitous. Neither group was attempting to isolate CMV prior to its success.

Besides research interests in virology, Dr. Weller was interested in protozoal parasites. In 1955, he attempted to isolate *Toxoplasma* in roller cultures of human skin–muscle tissue from a 3-month-old infant suspected of toxoplasmosis. Instead, the Davis strain of CMV was isolated. Subsequently, he and his coworkers isolated CMV from the urines of two other living infants suspected of suffering from C.I.D. These two isolates are now known as the Kerr and Esp. strains (Weller *et al.*, 1957). A comment on the meticulous care of these workers in demonstrating true viral replication is that the Davis strain was propagated from 20 passages during an elapsed period of 494 days before their first report was published.

Rowe and his co-workers in Bethesda were not searching for CMV either but were studying a new group of viruses—the adenoviruses—by culturing human adenoidal tissue. In one instance, culture of adenoids from three children yielded an unusual type of cytopathology (Rowe *et al.*, 1956) more reminiscent of what Weller (1953) had recently described for varicella. The following is Weller's account (1970:

> Therefore, in May 1955, Row brought [his] . . . cultures of AD169 . . . to Boston to study. The cytopathic changes resembled more closely those [Weller] was obtaining [for CMV] than those of varicella. As a result of Dr. Rowe's visit, . . . strains of virus were exchanged, and the similarity of agents recovered in St. Louis, Boston and Bethesda was established in advance of publication.

An excellent and commendable example of collaboration in science!

1.3. RECENT TRENDS

The most recent developments in the area of cytomegalovirus studies have been impelled by two major forces. On the one hand the clinical spectrum of diseases known to be caused by CMV has broadened significantly from the early days when it was considered primarily a cause of rare cases of cytomegalic inclusion disease in neonates. Finnish investigators first described the CMV-induced heterophile-agglutinin-negative mononucleosis syndrome in immunologically competent normal adults (Klemola and Kaariainen, 1965). But it is in immunologically deficient adults and children that CMV disease has come to assume the position of the primary opportunistic virus par excellence. Transplant recipients iatrogenically immunosuppressed by agents such as azathioprine, antilymphocyte serum, and-cyclosporine, which affect T-cell immunity, can develop a host of CMV-caused syndromes depending on the degree of immunosuppression (Ho 1977; Ho, 1982).

These range from self-limited febrile mononucleosis to CMV pneumonitis, hepatitis, gastrointestinal ulceration, retinitis, and encephalopathy to widely disseminated multisystem disease. With the inception of the AIDS epidemic in 1981, where patients are even more immunosuppressed than transplant recipients, this group of CMV syndromes has become even more numerous and important in the practice of medicine.

The other force molding our views of CMV has been the gigantic strides made recently in our biological, molecular, and immunologic understanding. Cytomegalovirus is now recognized as one of six human herpesviruses, each of which is important in human disease and pathology. They share the interesting property of being incurable, remaining latent in tissues, and becoming reactivable during immunosuppression. How latency is maintained and how an active infection arises from a dormant one are still unanswered questions. However, since understanding of the structure and function of CMV and other herpesvirus genes as well as their expression has advanced so remarkably it is a matter of time before these biological mechanisms are elucidated. This together with incipient advances in the prevention and therapy of CMV infection promise to inaugurate yet another era in the history of CMV.

2

Molecular Biology of Cytomegalovirus Replication

Mark Stinski

2.1. INTRODUCTION

Infection of the host by cytomegaloviruses (CMVs) can lead to productive infection that can either persist asymptomatically, be accompanied by disease, or become latent. This chapter focuses on the molecular events associated with CMV gene expression and replication. The CMV classification is based on the biological properties of host specificity, length of the replication cycle, and cytopathic effects. These viruses are referred to as betaherpesviruses (Roizman and Batterson, 1985).

Cytomegaloviruses are species-specific viruses that replicate slowly in cell culture and in the host. Infectious virus may persist for an extended period of time, after which the virus may establish latency. With the exception of the first viral proteins expressed in the infected cell, each stage in the replication cycle requires a protracted period of time relative to the alphaherpesviruses (Roizman and Batterson, 1985). In cells cultured *in vitro,* the replication cycle requires days for maximum production of infectious virus and weeks to produce visible plaques.

The CMVs have more sequence complexity than the other herpesviruses and may represent expanded derivatives of the ancestral type herpesviruses. The CMVs generally have large double-stranded DNA genomes equivalent to approximately 230 kilobase pairs (kbp) (DeMarchi *et al.*, 1978; Geelan *et al.*, 1978; Kilpatrick and Huang, 1977; Lakeman and Osborn, 1979; Stinski *et al.*, 1979a). The human CMVs (human herpesvirus V) have a genome with a long and short component that

Mark Stinski • Department of Microbiology, University of Iowa, Iowa City, Iowa 52242.

can invert during replication. The animal CMVs studied to date have nonisomerizing genomes. Although these have the largest of the herpesvirus genomes and may express as many as 200 different gene products, these viruses exhibit a growth requirement for differentiated cells *in vitro* and a stimulation of host cell macromolecular synthesis.

Although CMVs replicate in many of the same cells as the other herpesviruses, these viruses have a propensity to cross the placental barrier and cause congenital birth defects. Cytomegaloviruses have been isolated from many different vertebrates, including mice, guinea pigs, Old World and New World monkeys, and man. There are some strikingly similar arrangements of regulatory elements and genes between the human and animal CMVs studied to date. Since the animal viruses allow for *in vivo* experiments, which help us understand the pathophysiology and immunology of CMV infection, some comparisons between the molecular biology of human CMV and various animal CMVs will be made.

2.2. STRUCTURE AND ORGANIZATION OF THE VIRAL GENOMES

2.2.1. Isomerizing and Nonisomerizing Genomes

The human CMV genome represents an isomerizing betaherpesvirus genome. It is extremely large, with an estimated size of 65–68 nm (Geelen *et al.*, 1978), which corresponds to approximately 230 kbp or a relative molecular mass (M_r) of 150–155 $\times$ 10^6 (DeMarchi *et al.*, 1978; Geelan *et al.*, 1978; Kilpatrick and Huang, 1977; Lakeman and Osborn, 1979; Somagyi *et al.*, 1986; Stinski *et al.*, 1979a). Recent DNA sequencing of the AD169 strain determined the genome to be 229,354 base pairs (bp) (Chee *et al.*, 1989c). The human CMV DNA has a density of 1.716–1.717 g/cm^3 (Geelen *et al.*, 1978; Huang *et al.*, 1973), corresponding to a G-C content of 57%. The structure of the human CMV genome (Towne strain) is illustrated in Fig. 2.1. The genome can be divided into two segments referred to as the long (L) and short (S) components, which are capable of isomerization ((Kilpatrick and Huang, 1977). Both the L and S components are flanked by terminal repetitive sequences. Single-stranded circles connected by a double-stranded zone have been detected by electron microscopy of denatured and reannealed DNA. The double-stranded region represents hybridization between the repeat sequences, indicating that the repeat sequences must be inverted one relative to the other (Somagyi *et al.*, 1986). The junction between the L and S components is composed of internal repeat (IR) sequences, which are designated IR_L and IR_S when attached to the L and S components, respectively. The sequences at both ends of the genome are designated terminal repeats (TR). The sequences between the repeat regions are unique (U) sequences and are referred to as the unique long (U_L) and the unique short (U_S) regions. The approximate sizes of each are designated in Fig. 2.1. For strain Towne, the U_L region of 175 kb is encompassed by inverted repetitions of 11 kb. The U_S

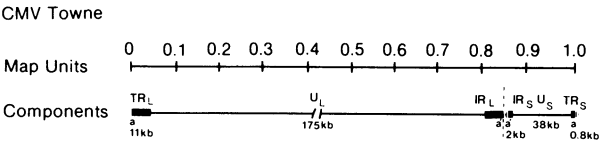

Figure 2.1. Structural arrangement of the human CMV genome. The genome consists of a long (L) and a short (S) component. Each unique (U) sequence is bounded by terminal repeats (TR) and inverted internal repeats (IR). The *a* sequence is at the termini of both the L and S components. The size in kilobases (kb) of each section of the genome for the Towne strain is designated. Heterogeneity at the termini and the joint occurs. The map units for the prototype arrangement of the viral genome are designated. (Data from LeFemina and Hayward, 1980.)

region of 38 kb has inverted repeats of approximately 2 kb (LeFemina and Hayward, 1980). Differences at the termini of various human CMV strains are the result of sequence amplification, which is discussed in more detail below. Recent sequencing of the AD169 strain determined the U_L to be 166,972 bp, the U_S to be 35,478 bp, the TR_L and IR_L to be 11,247 bp, and the TR_S and IR_S to be 2524 bp (Chee *et al.*, 1989c).

Since the L and S components of human CMV can invert during replication, virions contain any one of four isomers of the viral genome (Kilpatrick and Huang, 1977). Therefore, a human CMV is referred to as an isomerizing betaherpesvirus. However, the animal CMVs studied to date do not contain repeat sequences at an internal location. These virions contain only one DNA isomer and are referred to as nonisomerizing betaherpesviruses.

2.2.2. Comparisons between Different Strains of Human CMV

During replication, the *a* sequence (see Fig. 2.1) of CMV may get amplified at both termini as well as at the L–S junction (Geelen and Westrate, 1981; LaFemina and Hayward, 1980; Spaete and Mocarski, 1985). These amplifications represent a duplication of the sequence at the termini. The various strains of human CMVs may differ in both the size and number of additions at the termini. For example, strains AD169 and Davis have smaller repeats at the termini of the L component than the Towne strain (LaFemina and Hayward, 1980). The junction between the U_L and the L repeat is a major site of interstrain sequence variation. DNA probes to this region can be used to differentiate one strain of human CMV from another (Murph *et al.*, 1986). The unique restriction endonuclease digestion pattern between the U_L and L repeat remains stable on passage. With the laboratory strain AD169, reiterations at the termini have occurred as a cluster of direct repeats resulting in a high degree of heterogeneity, which is quite different from the Towne strain (Tamashiro *et al.*, 1984).

The restriction endonuclease profiles of the various strains of human CMV have many similarities, but no two profiles are identical (Geelen *et al.*, 1978; Huang *et al.*, 1976; Kilpatrick *et al.*, 1976; Minson and Darby, 1982; Pritchett, 1980).

Polymorphisms can occur throughout the entire genome. However, this variation arises from the absence or gain of restriction endonuclease sites and not from major rearrangements of the DNA or major sequence duplications. Restriction endonuclease diversity has not been linked to biological differences among the human CMV strains. In general, the human CMV genomes are colinear with at least an 80% sequence homology (Geelen *et al.*, 1978; Huang *et al.*, 1976; Pritchett, 1980).

2.2.3. Animal CMV DNAs

Restriction endonuclease analysis was one of the important methods used to establish the nonisomerizing character of the animal CMV genomes and to confirm that the sizes of these viral genomes are similar to that of human CMV. For example, the molecular mass of the genomes of simian (Jean *et al.*, 1978), murine (Ebeling *et al.*, 1983), guinea pig (Gao and Isom, 1984), and rat (G. Hayward, personal communication) CMVs are approximately 150×10^6. There are some regions of sequence homology between human CMVs and animal CMVs. For example, simian and murine CMVs have regulatory elements upstream of the major immediate early genes in the U_L component that are identical to human CMV (Boshart *et al.*, 1985; Dorsch-Hasler *et al.*, 1985; Jeang, 1984; Jeang *et al.*, 1987; Thomsen *et al.*, 1984). These interesting regulatory elements are discussed in detail below. Simian and guinea pig CMVs have homology with various regions of the human CMV long unique component (Gao and Isom 1984; Jeang, 1984). For example, there is considerable homology between the guinea pig immediate early gene region and that of human CMV (Isom *et al.*, 1984). Some homologies are also likely to exist between genes and encode matrix proteins or envelope glycoproteins. Simian CMVs express many polypeptides with amino acid and antigenic relationships to human CMV (Gibson, 1983).

2.2.4. Homology with Cellular DNA

There are certain regions of both human and animal CMV genomes that hybridize with cellular DNA because of either DNA sequence homology or high G-C content. There are regions that hybridize under high-stringency conditions (Shaw *et al.*, 1985) and other regions that hybridize less strongly (Jeang and Hayward, 1983; Ruger *et al.*, 1984a; Spaete and Mocarski, 1985). The latter regions may have given false-positive hybridization of CMV DNA with human tumor DNAs.

2.2.5. Defective Viral DNA

The human CMV genome described above is present in virions produced in the infected host or in tissue culture. However, the viral genomes can be defective when virus is passaged at a high multiplicity of infection in tissue culture. Smaller DNA

molecules become packaged into virions, which leads to a decrease in infectivity and an increase in the particle-to-PFU ratio (Ramirez *et al.*, 1979; Stinski *et al.*, 1979a). With human CMV, the majority of defective DNA molecules had a molecular weight of approximately 100×10^6. However, the development of defective DNA molecules during infection of the host has not been detected.

2.3. CIS-ACTING ELEMENTS ASSOCIATED WITH THE VIRAL GENOMES

Immediate Early (α) Enhancer-Containing Promoter-Regulatory Regions

Cytomegaloviruses have cis-acting DNA elements and other DNA domains that provide a very strong entry site for RNA polymerase II and other components of the transcriptional machinery. There are three independent enhancer elements in different locations of the genome. At least one of the enhancer elements initiates the expression of viral gene products that influence subsequent viral gene expression. The other enhancer elements are upstream of putative glycoprotein genes. These viral gene products might influence the physiology of the infected cell. DNA sequencing demonstrated that regulatory regions of the human and animal CMVs have sets of repetitive elements that are interspersed and highly conserved, but the relative organization of these repeat motifs in the different CMVs varies markedly (Boshart *et al.*, 1985; Henninghausen *et al.*, 1986; Jeang *et al.*, 1987; Nelson and Groudine, 1986; Thomsen *et al.*, 1984). This suggests that the presence of the repeat motif is more important than the organization. Figure 2.2 illustrates the organization of these repeat motifs upstream of the IE1 gene for human CMV. The regulatory region has been divided into the modulator region and region I and II. The modulator region (−750 to −1145) responds differently in various cell types (Lubon *et al.*, 1989). In region I, there are repeat elements with consensus sequences for interacting with known eukaryotic cell DNA-binding proteins such as the nuclear factor 1 (NF1)/CAAT-binding protein (CBP) superfamily. This family consists of a diverse but related group of proteins that are involved in both sequence-specific transcriptional activation and DNA replication.

The NF1/CBP-like proteins have been implicated as both negative and positive regulators. Overlapping the NF1/CBP binding sites in human CMV are 13-bp repeat elements (5′AATCAATATTGGC3′) (Fig. 2.2B). The number of NF1/CBP binding sites varies greatly between CMVs. For example, simian CMV has over 20 adjacent sites, and human CMV only four (Henninghausen *et al.*, 1986; Jeang *et al.*, 1987). NF1/CBP can bind to at least three of these upstream regions in human CMV and protect the DNA from DNase I digestion (Henninghausen *et al.*, 1986; Jeang *et al.*, 1983). Downstream of the NF1/CBP binding sites is the consensus cis binding site for a serum response element (SRE), 5′CCATATATGG3′ (Fig. 2.2B). Region I can have a positive effect on IE1 expression, but the effect is relatively low

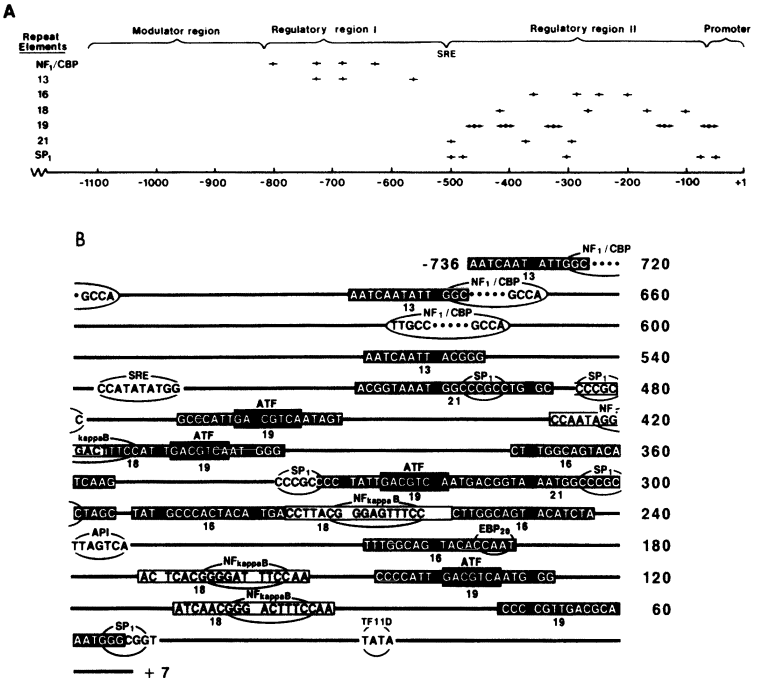

Figure 2.2. The human CMV major IE enhancer containing a promoter–regulatory region. (A) Upstream of the promoter containing a CAAT and TATA box is a series of repeat elements designated according to number of base pairs. The modulator region is described in the text. Regulatory region I contains the 13-bp repeats and the overlapping binding sites for nuclear factor I [NF1:5′TGGC/ANNNNGCCAA-3′/CAAT-binding protein (CBP) superfamily]. In the same region are inducible DNase I sites (not shown; Nelson and Groudine, 1986). Regulatory region I also contains a serum response element (SRE). Regulatory region II contains five GC boxes for SP1 (5′GGGCGG3′ or 5′CCGCCC3′) and four different repeat elements. The consensus sequences of the 16-, 18-, 19-, and 21-bp repeats are 5′CTTGGCAG-TACATGAA3′, 5′CTAAAC*GGGACTTT*CAA3′, 5′CCCCATT*GACGTCA*ATGGG3′, and 5′ACG-GTAAATGG*CCCGCC*TGGC3′, respectively. Italic regions designate important core elements. The 19-n repeat contains the CAAT box located 62 n upstream of the cap site at +1. This promoter–regulatory region contains enhancer elements plus other regions that bind eukaryotic cell transcription factors as described in the text. The promoter–regulatory region drives the transcription of both the IE1 and IE2 viral genes. Both genes have a cap site at +1 but differ because of alternate splicing illustrated in Fig. 2.3. The region upstream of the cap site is expressed as negative base pairs. (B) The distribution of the various repeat elements and the consensus sequence elements for binding various eukaryotic transcription factors are described in the text. (Data from Boshart *et al.*, 1985; Heilbronn *et al.*, 1987; Jeang, 1984; Jeang *et al.*, 1987; Stenberg *et al.*, 1984; Stinski, 1985, 1984; Thomsen *et al.*, 1984).

compared to region II (Nelson *et al.*, 1987). Nevertheless, the modulator region and region I presumably contain cis sites to facilitate the initial binding of eukaryotic nuclear proteins for activation of downstream expression. These factors may act in concert to enhance binding to region II.

In region II, there are several different consensus cis sites within and outside of

the repeat elements that have potential to interact with known eukaryotic transcription factors. Several of the repeat elements have also been found in simian and murine CMV. The 21-bp repeat 5′ACGGTAAATGGCCCGCCTGGC3′ has an SP1 consensus binding site (indicated in Fig.2.2B). The number of functional SP1 binding sites is not known. The SP1 binding site immediately downstream of the CAAT box appears to be nonfunctional based on *in vitro* DNase footprint analysis (P. Ghazal, personal communication). The 19-bp repeat element 5′CCCCATTGACGTCAATGGG3′ has the properties of being palindromic. The core has a cAMP response element (CRE), 5′TGACGTCA3′, and a consensus binding site for the eukaryotic transcription factor ATF, 5′GACGTCA3′ (Jones *et al.*, 1988; Fig. 2.2B). Whether or not the 19-bp repeat actually binds ATF is not known. The 19-bp repeat independently is a major contributor to the strength of this enhancer–containing promoter–regulatory region in the Jurkat T lymphocyte and in the permissive human fibroblast cell (Hunninghake *et al.*, 1989). Agents that increase intracellular cAMP levels also enhance expression when the 19-bp repeat is present and contains a T residue in the core dyad symmetry. However, deletion of this T residue renders the 19-bp repeat element unresponsive to these same agents (Hunninghake *et al.*, 1989). Binding of nuclear proteins to the region at −63, which lacks a T residue in the 19-bp repeat, is weak, whereas binding to the upstream 19-bp repeats that contains a T residue is strong (Ghazal *et al.*, 1988a).

Although the 19-bp repeat has a major role in the IE1 promoter–regulatory region, the proteins that bind to it presumably act synergistically with proteins bound to the other repeat elements. The 18-bp repeat element 5′CTAAAC*GGGACTTTCC*AA3′, which contains a core sequence (italic) that interacts with the eukaryotic transcription factor NF-κ B (Blanar *et al.*, 1989; Nabel and Baltimore, 1987; Parslow *et al.*, 1987; Wirth and Baltimore, 1988), also has independent activity in stimulated T lymphocytes and human fibroblast cells (Hunninghake *et al.*, 1989; Fig. 2.2B). Although the 18-bp repeat is not as active as the 19-bp repeat in Jurkat and human fibroblast cells, the 18-bp repeat element also plays a role in the activation of the IE1 promoter. Deletion studies have demonstrated that the relative level of downstream expression is significantly higher if both the 18- and 19-bp repeat elements are present than for either repeat element alone (Stinski and Roehr, 1985). The core sequence of the 18-bp repeat is also found in the regulatory region of the interleukin-2 receptor gene (Blanar *et al.*, 1989), the human immunodeficiency virus LTR (Blanar *et al.*, 1989; Nabel and Baltimore, 1987), the immunoglobulin κ enhancer (Parslow *et al.*, 1987), plus other genes (Blanar *et al.*, 1989). Whether or not the human CMV 18-bp repeat actually binds NF-κ B is not known. The role of the 18-bp repeat for downstream expression B cells containing significant amounts of NF-κ B also remains to be investigated. Binding of nuclear proteins to the 18-bp repeat is weaker than that to the 19-bp repeat in the cell types investigated to date (Ghazal *et al.*, 1988a).

No activity has been detected with the 16-bp repeats (Hunninghake *et al.*, 1989). The majority of these repeats can be deleted without a negative effect on the strength of the IE1 promoter (Stinski and Roehr, 1985). Although critical cis-acting

sites are associated with several of the repetitive sequences, they are also associated with nonrepetitive sequences. These include the SRE (−539 to −529), an AP1 site (−238 to −232), and several SP1 sites (Fig. 2.2B). Again, whether or not these cis sites are functional is not known.

In the major IE promoter–regulatory region there are at least nine major sites for protein–DNA interactions as defined by crude nuclear extracts and DNase I protection experiments (Ghazal *et al.*, 1987). Binding of nuclear proteins was detected to both repetitive as well as nonrepetitive sequences. A common sequence motif, (T)TGG/AC, was present in many of the binding sites (Ghazal *et al.*, 1988a). It is likely that one repeat element interacts with more than one nuclear factor. It is also possible that the presence of one repeat element influences binding to one or more other repeat elements. The duplication of the regulatory elements and their consensus binding sites for eukaryotic transcription factors presumably makes the CMV promoter–regulatory regions highly efficient in competing for limited transcription factors in the cell. These transcription factors presumably act in concert to enhance IE transcription in a modular fashion (Ghazal *et al.*, 1988b). Factors binding to distal sequences may function ultimately by contacting a factor(s) more proximal to the transcription initiation site such as TF11D (see Fig. 2.2) and RNA polymerase II.

There is little significant homology between the regulatory regions of beta- and alphaherpesviruses with the exception of the SP1 binding domain and a region in pseudorabies virus with a distant homology to the 18-bp repeat of CMV (Campbell and Preston, 1987). Even though human CMV and HSV have a similar genome organization and replicate in many of the same cells, these viruses have evolved to have unique cognitive and regulatory domains upstream of their IE genes.

The human CMV major IE promoter–regulatory region has many cis-acting sites for eukaryotic cell transcription factors to make it independently very strong during virus infection. However, it is also positively and negatively regulated by virus-specified proteins. The organization of the viral IE genes and their role in regulating expression from the IE1 promoter are discussed below.

2.4. TRANSCRIPTION

2.4.1. Immediate Early (α)

Since the CMV genomes can remain in cells in either a quiescent, persistent, or productive state, there are presumably a number of control features that regulate transcription of the viral genome. In productive infection, there is a sequential expression of the viral genome that is temporally regulated. The viral genes have been placed into three broad categories termed immediate early (IE) (α), early (β) and late (γ) (for review see Stinski *et al.*, 1983). The categories are based on the time of appearance of either viral mRNA or protein in the infected cell. The first

genes transcribed after infection of a permissive cell are the IE genes. Efficient transcription of the IE genes does not require preceding viral protein synthesis, and consequently, these genes are transcribed in the presence of an inhibitor of protein synthesis. *In vitro* transcription from the human CMV major IE gene promoter is inhibited by the addition of α-amanitin (Thomsen *et al.*, 1984), and, therefore, it is probable that this class of CMV genes is transcribed by host cell RNA polymerase II. Human CMV contains several enhancer elements that are located in both the U_L and the U_S components. The major site of IE transcription is the U_L component between approximately 0.66 and 0.77 map units for the prototype genome arrangement (see Fig. 2.1). Approximately 88% of the IE viral RNA hybridized to this region (Wathen and Stinski, 1982). The nonisomerizing animal CMVs studied to date also have a major amount of IE transcription in the same approximate location on the viral genome. In addition, the abundant polyribosome associated poly(A) viral RNAs originated from this region of the viral genome (Wathen and Stinski, 1982). The viral RNA transcribed from the IE genes constitutes approximately 0.6% of the infected total cell RNA when the cells are infected in the presence of an inhibitor of protein synthesis (Stinski *et al.*, 1983).

Two other regions of IE transcription map at approximately 0.24 map units in the U_L and 0.86 map units in the U_S. These regions could code for potential glycoproteins, which are described in more detail in Section 2.7. However, the amount if IE transcription is low relative to the region between 0.66 and 0.77 map units. In the case of human CMV, there are at least two IE transcription units located downstream of the major IE promoter (Stinski *et al.*, 1983). The IE promoter–regulatory region drives the expression of the IE1 and IE2 genes (Fig. 2.3). The IE2 RNAs can have the same 5′ leader sequence as IE1 RNA. These RNAs arise by an alternate splicing mechanism, which is described in Section 2.4.4. The IE gene products are involved in stimulating subsequent viral and possibly host cell gene expression (Chang *et al.*, 1989a; M. Davis *et al.*, 1987; Depto and Sternberg, 1989; Hermiston *et al.*, 1990; Nelson *et al.*, 1987; Tevethia *et al.*, 1987). After the synthesis of the IE gene products, there is a switch from restricted to extensive transcription of the viral genome (DeMarchi, 1981; McDonough and Spector, 1983; Stinski, 1983; Wathen and Stinski, 1982; Wathen *et al.*, 1981). Assuming asymmetric transcription, approximately 20% of the coding capacity of the human CMV genome is transcribed within approximately 4 hr after infection. The level of transcription continues to increase, reaching approximately 40% of the asymmetric coding capacity within 24 to 36 hr after infection (Chua *et al.*, 1981). Likewise, the relative amount of cellular RNA synthesis increases dramatically after the synthesis of the IE gene products (Stinski *et al.*, 1984; Tanaka *et al.*, 1975).

2.4.2. Early (β)

Transcription in the presence of an inhibitor of viral DNA synthesis is the method for detection of early genes. They originate in highest abundance from the

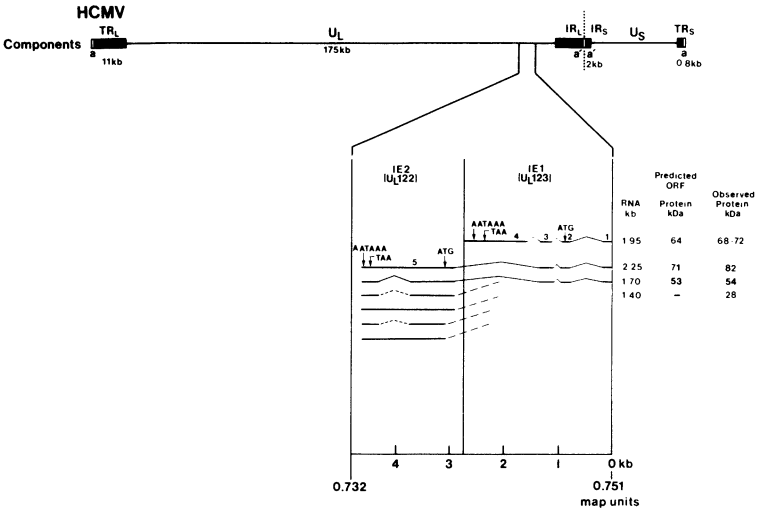

Figure 2.3. Organization of human CMV IE1 and IE2 genes. The mRNA structures, sizes, predicted ORFs, and molecular masses of observed proteins for the IE1 and IE2 genes are shown (Towne strain). Bold and thin lines represent mRNA exons and their introns, respectively. Dashed lines represent potential introns that were not confirmed by either exonuclease VII, primer extension, or cDNA analysis. Transcription signals such as the CAAT, TATA, and AATAAA elements are shown. Translation initiation and termination sites are also shown. The strong promoter–regulatory region upstream of IE1 and the cap site are described in Fig. 2.2. The distance in kilobases (kb) and the map units on the viral genome are indicated. (Data from Hermiston *et al.*, 1987; Stenberg and Stinski, 1985; Stenberg *et al.*, 1985, 1989.)

repeat sequences and those sequences adjacent to the repeat sequences (Wathen and Stinski, 1982). Following viral DNA replication, abundant transcription from the long repeat and adjacent sequences continues throughout infection, and the viral RNAs are found on polyribosomes as poly(A) RNA. In contrast, transcripts from most of the long and short unique regions are found in relatively low concentrations on the polyribosomes at early times after infection (Wathen and Stinski, 1982). Even though CMVs have a very rapid IE phase of gene expression, the early phase extends for 24 hr or more (DeMarchi *et al.*, 1980; Stinski *et al.*, 1980; Wathen *et al.*, 1981).

2.4.3. Late (γ)

The overall level of transcription is directly proportional to the amount of viral DNA synthesis. However, most but not all of the late genes of CMV investigated to date do not require viral DNA synthesis for transcription. With human CMV, there is little distinction between the transcriptional activation of early and late genes. However, late RNAs may differ from early RNAs in their different 5′-end initiation

sites (Chang *et al.*, 1989a; Leach and Mocarski, 1989; Stenberg *et al.*, 1985). There appear to be several posttranscriptional mechanisms responsible for the delay in the appearance of late gene products. For example, a late gene of human CMV between 0.408 and 0.423 map units that encodes a structural tegument protein (pp64, described below) is significantly transcribed in the presence of inhibitors of viral DNA synthesis (Goins and Stinski, 1986). However, the viral RNA from this transcription unit remains preferentially associated with the nucleus until after viral DNA replication. The viral RNA that accumulates is precursor RNA. A 5′-end intervening sequence referred to as an intron is removed prior to viral DNA synthesis. However, a removal of a 3′ intron does not occur, and final cleavage and polyadenylation of the viral RNA do not occur until after viral DNA synthesis (Goins and Stinski, 1986).

These observations suggest that complete processing of these human CMV viral RNAs requires either a viral gene product that is not available until after viral DNA synthesis or a cellular gene or product that is induced only after viral DNA synthesis. Therefore, the precursor viral RNA is retained in the nucleus at early times. There are presumably other mechanisms of posttranscriptional regulation that control either the stability of viral RNAs, the transport of human CMV mRNAs to the cytoplasm, or the translation of these RNAs in the cytoplasm. There are some RNA transcripts at early times after infection that are not transported to the cytoplasm (DeMarchi, 1983a; Geballe *et al.*, 1986a). Other RNAs, such as the early RNAs from the repeat and adjacent sequences, are characteristically unspliced, and they are efficiently transported to the cytoplasm. Human CMVs may have cis-acting signals located either 5′ or 3′ on the RNA molecule that influence either stability, processing, or transport. Therefore, the betaherpesviruses may be very different from the alphaherpesviruses with regard to posttranscriptional regulation.

2.4.4. Organization and Expression of the IE1 and IE2 Genes

The two transcription units between 0.732 and 0.751 map units are designated IE1 and IE2 (Hermiston *et al.*, 1990; Stinski *et al.*, 1983, 1984; Wilkinson *et al.*, 1984). Transcription of IE1 and IE2 is influenced directly by the major IE promoter–regulatory region. The mRNA structures, sizes, predicted open reading frames (ORFs), and observed proteins associated with the IE1 and IE2 genes are summarized in Fig. 2.3. The IE1 gene, immediately downstream of the major IE promoter between 0.739 and 0.751 map units, codes for 1.95-kb poly(A) mRNA. The viral mRNA consists of a 5′ leader exon of 121 nucleotides (n) followed by three exons of 88, 185, and 1341 n (Akrigg *et al.*, 1985; Stenberg *et al.*, 1984). These exons have been designated exons 1, 2, 3, and 4, respectively (Fig. 2.3). The major ORF begins in exons 2, extends for 491 amino acids, and terminates near the 3′ end of exon 4. This viral protein is highly phosphorylated with a molecular mass ranging from 68 to 72 kDa (Blanton and Tevethia, 1981; Gibson, 1981a; Michelson *et al.*,

1979a; Stinski, 1978, 1983, 1984; Stinski *et al.*, 1982, 1983, 1984. There is a region rich in glutamic and aspartic acid residues positioned near the carboxy-terminal end.

The greatest degree of homology between human CMV and simian CMV or murine CMV is found in the upstream promoter–regulatory regions. Nevertheless, the IE1 mRNAs do exhibit a similar splicing pattern in which a major 3′ exon is linked to three smaller 5′ exons. Likewise, the first codon for initiation of protein synthesis is the second exon. The overall amino acid homologies between human and murine CMV are very weak. By an alternate splicing mechanism, exons 1, 2, and 3 of human CMV can be ligated to exon 5 in the region designated IE2 (Fig. 2.3). The IE2 has an in-frame intron. When the intron is present, the mRNA is 2.25 kb and can code for a large protein with an apparent molecular mass of 82 kDa (Hermiston *et al.*, 1987). If the intron is absent, a smaller 1.70-kb mRNA is predicted to code for a smaller IE2 protein with an apparent molecular mass of 54 kDa (Fig. 2.3). Smaller IE2 mRNAs can also result from the selection of a downstream acceptor site in exon 5 (Fig. 2.3) (Stenberg *et al.*, 1989; C. L. Malone and M. F. Stinski, unpublished data). At late times after infection, a 1.5-kb mRNA originates from IE2 that codes for a 40-kDa late viral protein (Stenberg *et al.*, 1989).

The IE1 and IE2 proteins have 85 amino acids in common, coded by exons 2 and 3 of the IE1 transcription unit. The major body of the IE2 protein is coded by exon 5. The coding sequences for the smaller IE2 protein of 28 kDa requires further investigation. However, deletion analysis suggests that the 28-kDa IE2 viral protein originates primarily from the carboxy-terminal half of the IE2 transcription unit (C. L. Malone and M. F. Stinski, unpublished data).

The IE1 and IE2 proteins can be detected in the nucleus of infected cells (Otto *et al.*, 1988). However, the IE1 protein accumulates first on intracytoplasmic membranes prior to transport to the nucleus. Consensuslike nuclear localization signals are not apparent in the amino acid sequence of the IE1 protein. However, the IE2 protein has two potential nuclear localization signals (Stenberg *et al.*, 1985). The relationship between IE2 proteins and the eventual transport of IE1 to the nucleus requires further investigation.

2.5. FUNCTIONS OF THE IE1 and IE2 GENE PRODUCTS

2.5.1. The IE1 Protein

At early times after infection, the IE1 viral protein is present in high abundance relative to the IE2 viral proteins. The relative abundance of these viral proteins is regulated at the transcriptional and RNA-processing level. The IE1 mRNA accumulates to approximately a fivefold higher level than the various IE2 mRNAs in

infected permissive human fibroblast cells and in Jurkat cells transfected with the IE1 and IE2 genes (Hunninghake *et al.*, 1989; Stinski *et al.*, 1983). In addition, the IE1 antigen is approximately five times more abundant than the IE2 antigens as demonstrated by immunogold electron microscopy of infected human fibroblast cells (Otto *et al.*, 1988).

One function associated with the IE1 protein is enhancement of downstream expression from some enhancer-containing promoter–regulatory regions. For example, the human CMV IE1 protein enhances expression trans from the cellular interleukin-1 receptor gene (G. Iwamoto, M. Monick, B. C. Clark, P. E. Auron, M. F. Stinski, and C. H. Hunninghake, unpublished data) as well as positively regulating its own expression (Cherrington and Mocarski, 1989; Malone *et al.*, 1989). In all cases, the level of enhancement of downstream gene expression is only five- to tenfold.

There is no conclusive evidence to suggest that the IE1 protein interacts directly with a cis site in the enhancer-containing promoter–regulatory region. Although Cherrington and Mocarski (1989) proposed that the IE1 protein acts via the 18-base-pair repeat element, we find that the 18-bp repeat placed as a single repeat element or as a triplicate upstream of the wild-type IE1 promoter containing a CAAT and TATA box does not respond to IE1 transactivation (M. F. Stinski and B. Liu, unpublished data). The IE1 protein may interact with eukaryotic transcription factors associated with the transcription complex. Therefore, the IE1 protein may act as a "bridging" molecule between eukaryotic transcription factors and RNA polymerase II.

Since the IE1 and IE2 genes are driven by the same major IE promoter–regulatory region, the IE1 protein also affects the level of IE2 expression by increasing the level of steady-state IE2 mRNA (Malone *et al.*, 1989). Mechanisms for this increase could include enhancement of transcription from the major IE promoter–regulatory region or stabilization of the IE2 mRNAs by the IE1 protein.

When wild-type and mutant IE1 proteins were tested for an ability to positively regulate the major IE promoter and thereby enhance IE2 expression, two essential domains of the IE1 protein were found to be required. Most of the amino acids encoded by exon 4 are required, and 52 amino acids encoded by exon 3 are required (Malone *et al.*, 1989). These broad regions might be related to the secondary (Stenberg and Stinski, 1985) and possibly tertiary structure of the IE1 protein. Because this viral protein appears to be highly folded, mutations in one location may significantly affect the structure of the viral protein in another location.

Although the positive regulatory effects of the IE1 protein have been detected in a variety of cells including human fibroblasts, Jurkat T lymphocytes, and monkey cells (Cherrington and Mocarski, 1989; Hermiston *et al.*, 1987; Hunninghake *et al.*, 1989; Tevethia *et al.*, 1987), the IE1 protein has a negative effect in monkey cells expressing the simian virus 40 large T antigen (Stenberg and Stinski, 1985). Therefore, the regulatory effect of the IE1 protein may require other viral and possibly other cellular transcription factors.

2.5.2. Transactivation of Early Viral Promoters by IE2 Proteins

As described above, several viral proteins are coded for by the IE2 transcription unit as a result of alternate splicing events. The IE2 proteins can independently enhance expression in trans from inducible heterologous (M. Davis *et al.*, 1987; Hermiston *et al.*, 1987; Pizzorno *et al.*, 1988; Tevethia *et al.*, 1987) or homologous (Chang *et al.*, 1989a; Malone *et al.*, 1989) promoters. The function of the human CMV IE2 proteins was first investigated using early adenovirus promoters, since CMV promoters were not yet characterized. The human CMV IE2 proteins alone could enhance expression from the adenovirus E2 or E3 promoters (Hermiston *et al.*, 1987; Pizzorno *et al.*, 1988). Maximum enhancement of the E2 promoter occurred when both the IE1 and IE2 proteins were expressed. However, the IE1 protein alone had no significant activity (Hermiston *et al.*, 1987). The effect of IE1 was referred to as augmentation of transactivation.

This phenomenon presumably extends to the other early adenovirus promoters because IE1 plus IE2 gene products could rescue adenovirus mutants d1312 or d11500 more efficiently than IE2 alone (Tevethia *et al.*, 1987). These adenovirus mutants are defective in the E1a gene, which is required for transactivation of the early adenovirus promoters. The adenovirus E2 promoter can be activated by a variety of viral transactivators including the simian virus 40 small t antigen (Loeken *et al.*, 1988). Recombinant plasmids expressing only exon 5 of IE2 could also enhance expression from the adenovirus E2 promoter (Hermiston *et al.*, 1987). However, recombinant plasmids expressing only exon 5 of IE2 were not sufficient for activation of a homologous human CMV early promoter designated E1.7 (Malone *et al.*, 1989). This promoter requires the large IE2 protein of approximately 82 kDa either alone or in combination with the smaller IE2 proteins for transactivation; i.e., the recombinant plasmids expressing exons 2, 3, and 5 are required (see Fig. 2.3). If the in-frame intron in exon 5 is spliced out to render a smaller IE2 protein of approximately 54 kDa, transactivation does not occur (Malone *et al.*, 1989).

A series of in-frame deletions or carboxy-terminal truncations were made to investigate the IE2 protein domains required for transactivation. These data indicated that two regions in the coding sequence of the IE2 proteins are essential for transactivation of the E1.7 promoter. One region is in the amino-proximal 52 amino acids coded by exon 3, and the other in the carboxyl-terminal 85 amino acids (Malone *et al.*, 1989). Splicing, which removes an in-frame intron, resulting in the intermediate-size IE2 protein of 54 kDa, would remove a putative zinc finger domain. Since the IE2 54-kDa protein also cannot enhance expression from the E1.7 promoter, it is proposed that both the carboxy-terminal end and the putative zinc finger are required IE2 protein domains. It is assumed that the IE2 proteins act synergistically with eukaryotic transcription factors (TFIIA, TFIIB, TFIID, TFIIE) and RNA polymerase II for expression of the downstream viral gene. The mechanism for this IE2 activation of transcription is not clear. Whether or not IE2 proteins bind to DNA is presently not known.

Although the IE1 protein alone has no significant effect on expression from the

E1.7 promoter, the presence of the IE1 and IE2 proteins allows for maximum expression from this viral promoter (Chang *et al.*, 1989a; Malone *et al.*, 1989). The IE1 protein positively regulates expression from the major IE promoter (Cherrington and Mocarski, 1989; Malone *et al.*, 1989) and thereby increases the steady-state level of IE2 mRNA about four- to fivefold (Malone *et al.*, 1989).

Other human CMV promoters that are relatively weak may require both IE1 and IE2 or other factors. The promoters upstream of two tegument genes that code for phosphorylated proteins of 65 and 71 kDa have been investigated. These genes are transcribed weakly at early times after infection (R. Stenberg, personal communication). Significant expression from the pp65 promoter was detected only when the IE1 and IE2 proteins were expressed (Depto and Stenberg, 1989). Interestingly, the pp65 promoter does not have a TATA box (Ruger *et al.*, 1987). An upstream octomer sequence, 5′ATTTCGGG3′, was proposed to be necessary for transactivation by IE1 and IE2 gene products (Depto and Stenberg, 1989). Whether this sequence has any relevance to other HCMV promoters remains to be determined.

The promoter upstream of an early gene that codes for a family of nuclear phosphorylated proteins of unknown function can also be transactivated by IE1 plus IE2 proteins (Stapans *et al.*, 1988). The effect of IE1 or IE2 proteins alone on this promoter has not been tested.

2.5.3. Negative Regulation of the Major IE Promoter by IE2 Proteins

When presented with the proper inducible promoter and other eukaryotic transcription factors, the IE2 proteins dramatically enhance downstream gene expression. However, when given the major IE enhancer-containing promoter–regulatory region, these IE2 proteins act antagonistically to repress downstream gene expression. This is perhaps one of the checks and balances in human CMV gene regulation that influence the dynamic balance between productive and persistent infection. Deletion analysis has demonstrated that the ORF for the large IE2 protein of 82 kDa is required for this antagonistic behavior, whereas the smaller IE2 protein of 54 kDa alone has no effect (Hermiston *et al.*, 1990). Deletions in the IE2 ORF or truncations towards the carboxy-terminal end demonstrated that 85 amino acids that include the putative zinc finger motif and the carboxy-terminal amino acids are required for negative regulatory activity (Hermiston *et al.*, 1990).

2.5.4. Sites in the Major IE Promoter–Regulatory Region Required for Negative Regulation by IE2

Two approaches were used to investigate negative regulation of the major IE promoter–regulatory region. (1) Internal as well as 5′-end deletions were made

upstream of the CAAT and TATA boxes as described previously (Stinski and Roehr, 1985). (2) A series of constructs were made starting with the minimal promoter (−68 to +7). One type of repeat element, either the 16-, 18-, 19-, or 21-bp repeat, or the combination of NF1/CBP and SRE cis sites was inserted 59 bp upstream of −68 as described previously (Hunninghake *et al.*, 1989). All constructs were functional for expression of a downstream gene product.

All deletions in the promoter–regulatory region were repressed by IE2. However, neither the minimal promoter (−68 or +7) nor the minimal promoter with three 16-bp repeats upstream was repressed by the IE2 gene products. In contrast, the presence of either the 18-, 19-, or 21-bp repeat or the NF1/CBP and SRE cis sites allowed for repression of downstream expression (Hermiston *et al.*, 1990). The 18-, 19-, or 21-bp repeat elements contain a consensus binding site for either NF-κB, ATF, or SP1, respectively (See Fig. 2.2B). These observations suggested that the mechanism of negative regulation by the IE2 proteins involves an antagonistic interaction with eukaryotic transcription factors that interact with upstream binding sites. The IE2 proteins might repress expression from the major IE promoter by inhibiting a linear diffusion or sliding of the cellular transcription factors from their initial entry site to the promoter. Since all of these test plasmids have the minimal promoter (−68 to +7), the role of these sequences in repression by IE2 was also investigated. Experiments indicated that a region around the IE TATA box is also required for negative regulation of IE2. Therefore, both an upstream cis-acting site and a downstream promoter-associated site are required for negative regulation by the IE2 proteins (Hermiston *et al.*, 1990). Because of the complexity of the major CMV IE promoter–regulatory region as well as the potential regulatory elements both upstream and downstream of the initiation site (+1) (P. Ghazal, personal communication), negative regulation by IE2 may function at early times after infection but not necessarily at late times after infection. Since CMVs characteristically replicate slowly and cause persistent infection in their host, there are presumably several sophisticated regulatory phenomena that influence the biology of this unique member of the herpesvirus classification group.

2.6. EARLY (β) GENES

Many of the human CMV early or late genes have been predicted by DNA sequence analysis (Chee *et al.*, 1989c). Two hundred eight individual ORFs have been recognized and are numbered according to location in the TR, IR, U_L, or U_S regions of the viral genome. The following discusses a few of the human CMV early genes, their transcription, or their role in DNA replication. The most abundant steady-state poly(A) RNA made during the human CMV infection originates from a diploid gene located in the large repeat sequences between 0.011 and 0.32 (0.805 and 0.816) map units (Hutchinson *et al.*, 1986; McDonough *et al.*, 1985) (Fig. 2.4A). Abundant transcription of this gene coincides with a general stimulation of host cell transcription (Stinski *et al.*, 1984). Under immediate early conditions, i.e,

in the presence of an inhibitor of protein synthesis, this gene is transcribed poorly. However, in the absence of inhibitors of protein synthesis, a 2.7-kb RNA is transcribed within approximately 2 hr after infection and continues to be expressed throughout infection. Even though there are two reports implying a role for the 5′ end of the 2.7-kb RNA in posttranscriptional regulation (Geballe *et al.*, 1986b; Geballe and Mocarski, 1988), no viral gene product has been related to this viral RNA, and it is not even certain whether it represents a mRNA (Hutchinson *et al.*, 1986b; McDonough *et al.*, 1985). Transcribed at lower abundance but in the same direction is a 1.2-kb early mRNA originating between 0.035 and 0.040 (0.792 and 0.797) map units (Fig. 2.4A). This viral mRNA has a single ORF of 254 amino acids in the Eisenhardt strain, but the viral protein has not been identified in virus-infected cells (Hutchinson and Tocci, 1986). In the AD169 strain, the 254-amino-acid reading frame is disrupted by three stop condons and two frameshifts relative to the Eisenhardt strain (Chee *et al.*, 1989c). Therefore, an ORF for this RNA cannot be predicted. It is possible that the early 2.7- and 1.2-kb RNAs are not mRNAs but represent structural RNAs. The role of these early viral RNAs in the infected cell requires further investigation. The strategic location of these genes and their duplicate nature and abundant transcriptional activity imply that they may have an important role in virus replication. However, mutations have not yet established a role for these viral genes.

Another early gene with three initiation sites for RNA synthesis maps between 0.225 and 0.243 map units (U_L44) (Fig. 2.4A). This gene encodes a single DNA-binding protein (DBP) of 52 kDa and is referred to as DBP52 or infected cell protein (ICP)36. Even though the gene is transcribed at early times, it most abundant transcription occurs late after infection (Mocarski *et al.*, 1985a).

Another early gene maps between 0.682 and 0.709 map units (U_L112, 113) (Fig. 2.4A). This gene codes for multiple mRNAs of approximately 2.2 kb that vary because of alternate splicing but have common 5′ and internal exons and alternative 3′ exons with coterminal 3′ ends. The gene codes for four phosphorylated proteins of 84, 50, 43, and 34 kDa (E pp84, 50, 43, and 34). All four proteins are immunoprecipitated by a polyclonal antiserum prepared against a synthetic peptide representing a stretch of amino acids near the amino-terminal end (Wright and Spector, 1989; Wright *et al.*, 1988). The function of these viral gene products is not known, but they accumulate in the nucleus. Since they are early gene products, they may have an important regulatory function. In addition, this gene has some cell-related sequences, and, therefore, it may have been acquired recently from the host cell. Alternatively, the protein may mimic a normal cellular function.

The early gene mapping between 0.370 and 0.385 map units (U_L57) is colinear and has homology with the HSV ICP8 gene (Fig. 2.4A). This early gene encodes a 140-kDa DNA-binding protein and is referred to as the major DNA-binding protein (MDBP140) or the ICP8 homologue (Anders and Gibson, 1988; Kemble *et al.*, 1987). A similar gene is present in the Epstein–Barr virus genome and in the simian CMV genome (Anders and Gibson, 1988; Kemble, *et al.*, 1987). In HSV, ICP8 is required for DNA replication (Chiou *et al.*, 1985; Conley *et al.*, 1981; O'Donnell *et*

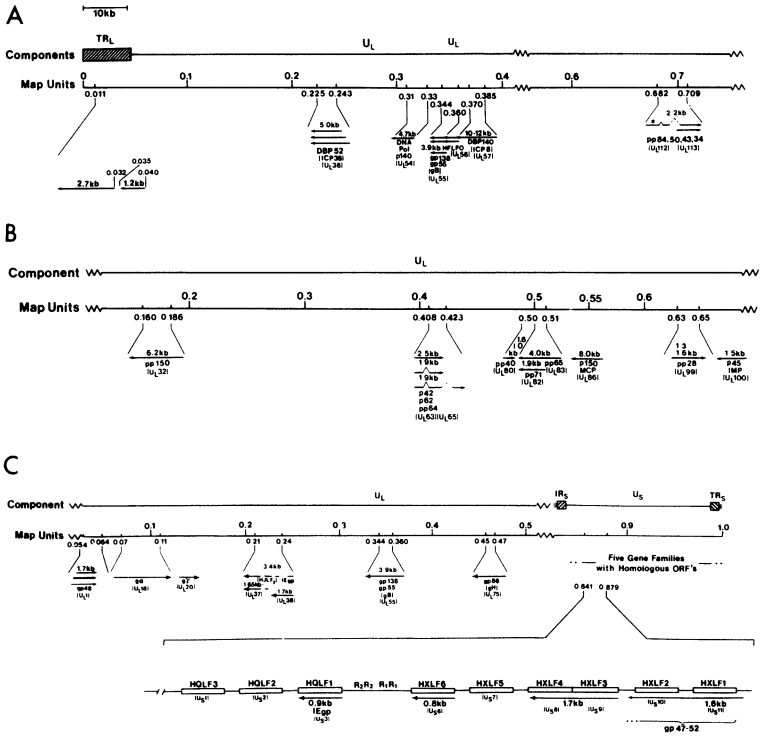

Figure 2.4. Early and late genes in the long and short components of the human CMV genome. Map units, mRNA sizes, and molecular masses of observed proteins when known are designated. When present, an asterisk indicates the location of sequences used for preparation of synthetic peptides to generate polyclonal antibodies. The relative amount of steady-state transcript observed is indicated by the thickness of the arrow. The ORF designation and location in the viral genome determined by DNA sequence analysis is designated as previously described (Chee *et al.*, 1989c). (A) Early genes of the human CMV. A cluster of human CMV genes in the U_L component with significant homology to other herpesvirus genes is designated between 0.310 and 0.385 map units. DBP, DNA-binding protein; HFLFO, the AD169 HindIII F left ORF; gB, the glycoprotein referred to as the HSV gB homologue; ICP, infected cell polypeptide; ICP8, the HSV ICP8 homologue. These gene products and the viral DNA polymerase protein of 140 kDa (DNA Pol p140) are described in the text (data from Anders and Gibson, 1988; Chee *et al.*, 1989c; Heilbronn *et al.*, 1987; Hutchinson and Tocci, 1986; Hutchinson *et al.*, 1986; Kemble *et al.*, 1987; Kouzarides *et al.*, 1987; McDonough *et al.*, 1985; Mocarski *et al.*, 1985b; Stapans and Spector, 1986; Wathen and Stinski, 1982; Wright and Spector, 1989; Wright *et al.*, 1988). (B) Human CMV structural genes in the U_L component. Genes are designated according to map units, mRNA sizes, and molecular masses (× 10) of the observed protein products. Bold and thin lines represent mRNA exons and their introns, respectively. A dashed line indicates that linkage of the exons has not been established by the criteria described in Fig. 2.3. The viral gene products are described in the text (data from Chee *et al.*, 1989b; Davis and Huang, 1985; Goins and Stinski, 1986; Jahn *et al.*, 1987a; Lehner *et al.*, 1989; Martinez *et al.*, 1989; Meyer *et al.*, 1988; Nowak *et al.*, 1984a; Pande *et al.*, 1984, 1988, 1989; Robson and Gibson, 1988; Ruger *et al.*, 1987). (C) Human CMV glycoprotein genes in the U_L and U_S components. The size of the mRNAs that code for g, gH homologue (gp86), HQLF3, and HQLF2 are not known. g, a potential glycoprotein with homology to the MHC class I chain; gB and gH, HSV homologues because these glycoproteins have some homology to HSV gB and gH; gp55 is derived from a precursor glycoprotein (gp138); IE gp, potential IE glycoproteins. The dashed line indicates that linkage of the exons was not established by the criteria described in Fig. 2.3. A multigene family in the U_S component that encodes the human CMV glycoprotein complex gcII (gp47–52) is designated.

al., 1987a; Quinlan *et al.*, 1984; Ruyechan and Weir, 1984), and it may be involved in regulating viral gene expression (Conley *et al.*, 1981; Godowski and Knipe, 1986). The human CMV DNA-binding protein is synthesized at early times after infection in relatively low abundance. It is localized in the nucleus, where it presumably plays a role in DNA replication (Knipe *et al.*, 1982; Lee and Knipe, 1985).

Another human CMV gene that has extensive homology with the other herpesviruses in the DNA polymerase gene between approximately 0.31 and 0.33 map units (U_L54) (Fig. 2.4A). This gene is transcribed as an unspliced 4.7-kb mRNA and codes for a protein of approximately 140 kDa (DNA Pol p140)(Kouzarides *et al.*, 1987). Approximately 24% of the predicted amino acid sequence of human CMV DNA Pol p140 is conserved relative to HSV or the putative EBV polymerase (Kouzarides *et al.*, 1987). Several other potential early genes have been identified by DNA sequence analysis. These include a potential ribonucleotide reductase (U_L95) (the α subunit), helicase–primase (U_L70), deoxy-UTPase (U_L72), DNase (U_L98), helicase (U_L105), and a uracil–DNA glycosylase (U_L114) (Chee *et al.*, 1989a). Most of the early genes described above are involved in viral DNA replication, but others may be involved in regulation of the viral genome or the host cell. Further research on human CMV early genes is required.

2.7. LATE (γ) STRUCTURAL GENES

The following discusses some of the human CMV structural genes. Electron micrographs of virions demonstrate a fibrous, granular array of proteins that are interfaced between an icosahedral capsid and the envelope (Stinski, 1983). This region is referred to as the tegument. Viral proteins that anchor the envelope to the nucleocapsid are referred to as matrix proteins. The capsid is approximately 100 nm in diameter and is composed of 162 capsomeres. The virions are pleomorphic and measure between 150 to 300 nm in diameter, in part because of variability in the thickness of the tegument. Human and guinea pig CMVs also form an aberrant particle that does not contain viral DNA or a nucleocapsid but does contain some of these viral proteins. These particles are referred to as dense bodies. Another aberrant form of the virus is a noninfectious enveloped particle that contains the central

---

Homologous gene family 2 or the HXLF gene family is one of five homologous gene families in the S component. The ORFs are designated by an open box. The HXLF1 and -2 and the HXLF3 and -4 genes code for bicistronic mRNAs of 1.6 and 1.7 kb, respectively; gp47–52 designates the molecular mass (× 10) of the glycoproteins as described in the text. The role of the other HXLF ORFs and the mRNAs coded by these genes is presently not known. Downstream of the HXLF6 gene are repeat sequences of enhancer elements designated R2 that are similar to the 18-bp repeat sequences upstream of the major IE gene. Another repeat sequence of unknown function is designated R1. HQLF1 and HQLF2 may also code for potential glycoproteins. Messenger RNA splicing occurs in HQLF1 (197), but only the unspliced mRNA is designated (data from Akrigg *et al.*, 1985; Chang *et al.*, 1989a,b; Cranage *et al.*, 1986, 1988; Gretch *et al.*, 1988b; Gretch and Stinski, 1990; Kouzarides *et al.*, 1988; Mach *et al.*, 1986; Spaete *et al.*, 1988; Weston and Barrell, 1986).

core, capsid, tegument, and envelope but lacks viral DNA (Irmiere and Gibson, 1983).

The human CMV genes that code for matrix–tegument viral proteins are discussed below. A protein found in both virions and dense bodies and referred to as the lower matrix protein because of its migration in denaturing polyacrylamide gels is a phosphorylated matrix–tegument protein of 65 kDa (pp65 or ICP 27) (Clark *et al.*, 1984). Another phosphorylated matrix–tegument protein (pp71), referred to as the upper matrix protein, migrates, more slowly (Roby and Gibson, 1986). The two genes are located in tandem between approximately 0.50 and 0.51 map units (U_L83, U_L82) (Fig. 2.4B). The lower matrix protein is coded for by a bicistronic unspliced 4.0-kb mRNA that could also potentially code for pp71, whose coding sequence is located toward the 3′ half of the mRNA molecule (Nowak *et al.*, 1984a; Pande *et al.*, 1984). A second monocistronic mRNA of 1.9 kb has been detected, and it may code for pp71. The two mRNAs have the same 3′ termini designated by a single polyadenylation signal. DNA sequence analysis predicts a 548-amino-acid protein equivalent to 61.5 kDa for pp65 and a 561-amino-acid protein equivalent to 62.9 kDa for pp71. In the infected cell pp71 is present in low abundance relative to that of pp65. Both proteins are believed to be present in virions, but dense bodies have little to no pp71 and an abundance of pp65 (Roby and Gibson, 1986; Ruger *et al.*, 1987). The abundant expression of pp65 may represent a property of laboratory strains of human CMV, since low levels of expression were found with wild-type strains (Jahn *et al.*, 1987b).

A matrix-tegument protein of 64 kDa that migrates in gels at approximately the same position as pp65 originates from a gene between 0.408 and 0.423 map units (U_L63, U_L65) (Davis and Huang, 1985). This gene codes for spliced mRNAs of 2.5 and 1.9 kb that code for proteins of 42, 62, and 64 kDa (Fig. 2.4B). The 64-kDa protein is phosphorylated (pp64) (Davis *et al.*, 1984; Goins, 1986). Posttranscriptional regulatory events that influence complete splicing and transport of this viral mRNA to the cytoplasm have been reported (Goins and Stinski, 1986) and are described in Section 2.4. Monoclonal antibodies have been use to immunoprecipitate pp65 and pp64. Both of these matrix-tegument proteins are associated with protein kinase activities (Britt and Auger, 1986b; Michelson *et al.*, 1984). However, it is not certain whether the viral proteins are protein kinases or, alternatively, the viral proteins are associated with protein kinases. The properties of these two viral-associated enzymes appear to be similar. They both require divalent cations and have optimal activity at pH 7 to 8. They both are cyclic nucleotide independent and phosphorylate threonine and serine but not tyrosine (Michelson *et al.*, 1984; Roby and Gibson, 1986). These viral protein kinases may play an important role in regulating transcription, viral DNA synthesis, or viral assembly as well as modifying host cell proteins.

Another potential viral protein kinase has been identified by DNA sequence analysis (U_L97) (Chee *et al.*, 1989c). In human CMV, there are two large late proteins of similar molecular mass of approximately 150 kDa. One is the major capsid protein, and the other is a basic phosphorylated matrix–tegument protein

(pp150) (Jahn *et al.*, 1987a,b). The major capsid gene maps at approximately 0.55 map units (U_L86) (Fig. 2.4B). The viral protein is 1370 amino acids, and it has approximately 25% homology to other human herpesvirus major capsid proteins (Chee *et al.*, 1989b). Another human CMV gene also has homology to the human herpesvirus major capsid protein and is located around 0.27 map units (U_L47) (Chee *et al.*, 1989c). The matrix–tegument gene for pp150 maps between 0.160 and 0.186 map units (U_L32) (Fig. 2.4B) (Jahn *et al;.*, 1987a; Pande *et al.*, 1989). This gene codes for an unspliced mRNA of 6.2 kb. DNA sequencing predicts a protein of 1048 amino acids, which would be equivalent to a molecular mass of 113 kDa. The protein is predicted to be a basic phosphoprotein with multiple β pleated sheets in hydrophilic amino acid clusters (Jahn *et al.*, 1987b). As a tegument protein, it has the unusual feature of an O-linked N-acetylglucosamine (O-GlcNAc) (Benko *et al.*, 1988). Although the function of the O-GlcNAc is not known, this viral protein could serve as a targeting signal for morphogenesis of the virion. It is interesting that the viral protein is absent from dense bodies, which lack a capsid, but present in virions or enveloped particles, which contain a capsid but no viral DNA. This suggests that pp150 is more associated with the capsid structure than with the envelope. Lastly, pp150 has highly antigenic properties (Hopp and Woods, 1981; Jahn *et al.*, 1987b).

Another matrix–tegument gene between approximately 0.63 and 0.65 (U_L99) map units is transcribed as an unspliced 1.3- to 1.6-kb mRNA that codes for a phosphorylated 28-kDa protein (pp28) (Fig. 2.4B). DNA sequencing predicted an ORF for a protein of 20.9 kDa. This viral protein is also extremely hydrophilic and highly immunogenic (Martinez *et al.*, 1989; Meyer *et al.*, 1988; Pande *et al.*, 1988; Re *et al.*, 1985).

Antibodies to the matrix–tegument proteins do not neutralize viral infectivity. However, these viral gene products may have an important role in the cell-mediated immune response.

The principal intranuclear capsid has been referred to as the B capsid because of its similarity to a previously described particle in HSV. A distinguishing characteristic of B capsids is a phosphorylated protein of 35–40 kDa (pp40) that has a role in capsid assembly and DNA packaging. Cytomegaloviruses have a similar protein, and the viral gene maps around 0.50 map units (U_L80) (Fig. 2.4B) (Robson and Gibson, 1988). The assembly protein is the herpesvirus counterpart of the bacteriophage scaffolding protein. Like the scaffolding protein, the herpesvirus assembly protein is an abundant constituent of capsids lacking viral DNA, but it is not present in the mature DNA-containing virions (Lee *et al.*, 1988).

New DNA sequence analysis indicates that human CMV has 54 ORFs with characteristics of glycoproteins for N-linked glycosylation. However, 22 of these potential glycoproteins lack a sequence for signal or membrane anchor domains. The following discusses some of the human CMV glycoproteins as well as membrane-associated or hydrophobic viral proteins. The viral envelope is composed of a lipid bilayer membrane containing proteins and glycoproteins. One of the human CMV envelope-associated proteins, referred to as the integrated membrane protein

(IMP), has approximately 20% amino acid sequence homology to other herpesviruses according to ORF analysis (Lehner *et al.*, 1989). The viral protein has a molecular mass of 45 kDa and is specified by a 1.5-kb mRNA (Lehner *et al.*, 1989). The viral gene maps adjacent to pp28 at approximately 0.65 map units (U_L100). The integrated membrane protein may be involved in membrane stability and structure. The viral antigen may have a role in the humoral and cellular immune response.

One of the human CMV genes that codes for a major envelope glycoprotein is located in the cluster of genes with homology to other herpesviruses. This gene is referred to as the gB homologue and maps between 0.344 and 0.360 (U_L55) (Fig. 2.4C). The gene has homology to the gB gene of HSV as well as to other herpesviruses such as EBV and VZV and other animal CMVs. This is an ancestral glycoprotein gene that is generally conserved in herpesviruses of all subgroups. The viral gene product is encoded by an unspliced mRNA of approximately 3.9 kb that codes for a protein of 906 amino acids, which is equivalent to approximately 95 kDa. One finds a cleaved glycoprotein of approximately 55 kDa (gp55) (Gretch *et al.*, 1988a) originating from the carboxy-terminal half of the gB gene (Britt and Vugler, 1989; Cranage *et al.*, 1986; Mach *et al.*, 1986; Spaete *et al.*, 1988). The gB homologue (gp55) is disulfide-linked to a viral envelope glycoprotein that migrates very diffusely in denaturing gels (Farrar and Greenway, 1986). Molecular mass estimates of this viral glycoprotein generally range between 93 and 130 kDa (gp93–130). Peptide mapping (Britt and Auger, 1986a) and immunologic data (Kari *et al.*, 1986) suggest that gp93 and gp130 are also derived from the gB gene. It has been proposed that the HSV gB functions to facilitate membrane fusion during viral infection. Since the gB genes are among the most highly conserved of herpesvirus genes, it is possible to speculate that the CMV gB homologue might also participate in membrane fusion events during infection.

Another human CMV glycoprotein gene, which maps between approximately 0.45 and 0.47 map units (U_L75), was identified by its slight homology to the HSV gH gene (Fig. 2.4C) (Cranage *et al.*, 1988). This human CMV gene has been referred to as the gH homologue. DNA sequence analysis predicted a protein of 743 amino acids with the typical hydrophobic amino-terminal signal sequence, potential N-linked glycosylation sites, and a hydrophobic membrane anchor region near the carboxy terminus. The homology with HSV gH is rather weak and is confined mainly to the C-terminal half of the protein (Cranage *et al.*, 1988). Monoclonal antibody to the gH gene homologue (gp86) will neutralize viral infectivity in the absence of complement (Rasmussen *et al.*, 1984), and therefore, the gH gene product may be necessary for infection and may also be an important immunologic determinant on the human CMV envelope.

A gene for an early glycoprotein maps between 0.054 and 0.064 map units (U_L1) (Fig. 2.4C). The ORF predicts a polypeptide of 17 kDa that contains signal and anchor domains as well as potential N-glycosylation sites (Chang *et al.*, 1989b). Antisera prepared against synthetic peptides derived from amino acid sequences within the ORF detect a viral glycoprotein of 48 kDa. The viral mRNA of 1.7 kb

and the viral glycoprotein are detectable in the presence of inhibitors of viral DNA synthesis. Since gp48 is also found in the virion, it is considered an early structural glycoprotein. The role of gp48 in infection or in the humoral or cell-mediated immune response is not known.

A potential human CMV glycoprotein (U_L18) has been identified by DNA sequencing between approximately 0.07 and 0.11 map units (Chee *et al.*, 1989c) (Fig. 2.4C). A potentially unspliced RNA derived from this region may code for a 350-amino-acid residue polypeptide that contains 13 potential N-glycosylation sites. In addition, the ORF has approximately a 20% homology with the MHC class Iα chain (Beck and Barrell, 1988). Therefore, the putative glycoprotein is referred to as the α chain homologue (gα). β2-Microglobulin (β2-m) binding may occur with this potential chainlike viral glycoprotein. Whether 2-β2-microglobulin or MHC class I antigen has a role in binding and entry of human CMV into the infected cell requires further investigation. It is also possible that the human CMV HLA-like molecule might be recognized by T-lymphocyte receptors.

DNA sequencing has identified another potential human CMV glycoprotein (U_L20) with some homology to the T-cell receptor γ chain. Potentially, the virus could interact via this glycoprotein with CD3 to infect T lymphocytes. Another human CMV gene identified by DNA sequencing (U_L73) predicts homology to the Epstein–Barr virus gp350/220 (Chee *et al.*, 1989c; Chee *et al.*, 1989b).

Three envelop glycoprotein complexes (gc) designated gcI, gcII, and gcIII are found in the viral envelope and have been fractionated by rate zonal contrifugation in sucrose gradients and characterized by electrophoresis in nondenaturing and denaturing polyarcylamide gels (Gretch *et al.*, 1988b) Both gcI and gcIII have also been detected on the membranes of human CMV-infected cells (Van der Voort *et al.*, 1989). The gcI is a large multimeric complex that can be separated from gcII and gcIII. It consists of the gB homologue (gp55, gp93, and gp130) as discussed above. The constituents of the gcII are described below. The gcIII complex migrates homogeneously in sucrose gradients as a complex of approximately 240 kDa. It consists of the HSV gH homologue (gp86) described above and a glycoprotein of 145 kDa (gp145). The location of the gp145 gene is presently not known, but it might represent the α-chain homologue or the T-cell receptor chain homologue. The gcIII forms very late in infection during morphogenesis of the virion. Other glycoproteins appear to be infected-cell-specific, and they may or may not be part of the viral envelope (Pereira *et al.*, 1982, 1984; Radsak *et al.*, 1985; Stinski, 1976, 1977, 1983; Stinski *et al.*, 1979b).

In the human CMV genome there are ten sets of homologous genes according to DNA sequence analysis. There are three pairs in the U_L and U_S components of the viral genome (U_L25 and U_L35; U_L82 and U_L83; U_S2 and U_S3) (Chee *et al.*, 1989c). There are also families of genes in the U_S, one partitioned between U_S and U_L, and one family distributed in U_L, U_S, and R_S (Chee *et al.*, 1989c). The U_S region of the human CMV genome is larger and more complex than analogous regions of other types of herpesviruses. DNA sequencing of the U_S component revealed at least 38 open reading frames (ORFs) (Weston and Barrell, 1986), which

can be grouped into at least five gene families (Fig. 2.4C). The origin and nature of these multigene families in the U_S component represent gene duplication and genetic drift.

One family, designated homologous gene family 2 or HXLF (AD169, HindIII X left reading frame), consists of five ORFs (U_S7–U_S11) that lie in tandem with varying degrees of homology (Fig. 2.4C). DNA sequencing predicted the gene products to have potential glycosylation sites, signal sequences for membrane insertion, and C-terminal hydrophobic domains for anchoring in cell membranes (Weston and Barrell, 1986). This multigene family codes for a set of envelope glycoproteins that range in molecular mass. The HXLF1 (U_S11) and HXLF2 (U_S10) genes code for proteins of 21 and 25 kDa and 20 kDa, respectively, that are glycosylated to glycoproteins of 47 to 52 kDa (gp47–52) (Gretch *et al.*, 1988c). These viral glycoproteins are disulfide-linked, forming different molecular weight glycoprotein complexes referred to as gcII (Gretch and Stinski, 1990). However, the contribution of gene products from each member of the gene family in forming the gcII is presently not known. Genes HXLF1 and −2 as well as HXLF3 and −4 are expressed from bicistronic mRNAs of 1.6 and 1.7 kb, respectively (Gretch *et al.*, 1988b). The role of bicistronic mRNAs in forming glycoprotein complexes requires further investigation. The contribution of each HXLF gene in forming the viral glycoprotein complexes may vary. These viral glycoproteins may also play an important role in the biology and immunology of human CMV.

There are enhancers and other repeat (R) sequences just downstream of the HXLF5 (U_S6) gene that may influence transcription. These cis-acting elements are located between the HQLF1 (U_S3) gene and the HXLF6 (U_S6) gene (Fig. 2.4C). The enhancer, regulatory element R2, has homology with the 18-bp repeats in the promoter–regulatory region of the major IE gene (Weston, 1988). Although not as strong as the major IE enhancer, the U_S enhancer influences transcription of the adjacent HQLF1 gene at immediate early times after infection (Weston, 1988) but not the adjacent HXLF1–6 genes (Weston, 1988). The HXLF genes are not transcribed until early and late after infection (Gretch *et al.*, 1990). The HQLF1 gene codes for one unspliced and three or four differentially spliced IE RNAs with common 5′ and 3′ ends ranging in size from 0.5 to 0.9 kb (Weston, 1988). Only the unspliced 0.9-kb RNA is designated. This mRNA is expressed at both IE and late times after infection. DNA sequencing suggests that the HXLF6, HQLF1, and HQLF2 genes may also code for viral glycoproteins, and HQLF3 may code for a nonglycoprotein of approximately 23 kDa (Weston, 1988). How the variety of glycoproteins from the HXLF gene family and the putative glycoproteins from HQLF genes influence human CMV infection requires further investigation.

Another potential glycoprotein gene has been identified by DNA sequencing between approximately 0.21 and 0.24 map units (U_L37 and U_L38) (Fig. 2.4C) (Kouzarides *et al.*, 1987). A 3.4-kb RNA that consists of four exons is transcribed only under IE conditions of infection. Although this is not a late gene, it is discussed here to develop continuity as well as comparisons among the various viral glycoprotein genes. The ORF predicts N- and C-terminal hydrophobic regions for a signal

sequence and for a membrane-anchoring segment, respectively. The ORF in the third exon (HJLF2) has an overall neutral charater and contains 17 potential N-linked glycosylation sites. This mRNA is predicted to code for a protein of 487 amino acid residues, equivalent to a molecular mass of 56 kDa (Kouzarides *et al.*, 1987). Upstream of the gene is a sequence element similar to the simian virus 40 enhancer that may influence the transcription of this IE glycoprotein gene. Since this RNA is detected only as an IE RNA, it is unlikely that this potential viral glycoprotein is incorporated into the envelopes of virions. Two additional viral RNAs of 1.65 and 1.70 kb also originate from the same region, but the potential N-glycosylation sites (HJLF2) would be eliminated because of the alternate use of 5′ or 3′ termini (Fig. 2.4C). The 1.65- and 1.7-kb RNAs could code for proteins of 55 and 37 kDa, respectively (Kouzarides *et al.*, 1987).

There are several families of membrane-associated human CMV proteins that are classified multiply hydrophobic (Chee *et al.*, 1989c). According to DNA sequence analysis, a family of multiply hydrophobic ORFs located in the U_S are predicted to be integral membrane proteins capable of spanning the membrane several times. The family of genes that is distributed in the U_L, U_S, and R_S have homology to U_S22 (U_L43, U_L22, and TR_S1) (Chee *et al.*, 1989c). Monoclonal antibody to U_S22, also referred to as ICP22, detects an early viral antigen in the nucleus (Mocarski *et al.*, 1988). Three ORFs (U_S27, U_S28, U_L33) have homology to be the opsin family of cell surface receptors (Chee *et al.*, 1989c). Again, the function of these potential membrane-associated proteins or glycoproteins in the human CMV-infected cell is presently not known.

At the predicted protein sequence level human CMV appears to be more related to Epstein–Barr virus, a gammaherpesvirus, than to the alphaherpesvirus. The conserved human CMV genes with homology to other herpesvirus genes are located in the U_L region of the viral genome. In contrast, the majority of the glycoprotein genes, multiply hydrophobic genes and gene families, are located in the U_S region of the viral genome (Chee *et al.*, 1989c).

2.8. VIRAL DNA SYNTHESIS

2.8.1. The Viral DNA Polymerase

The human CMV DNA polymerase is physiologically different from cellular polymerases. It can be distinguished from host cell enzymes by chromatographic behavior, template primer specificity, sedimentation behavior, and most notably by the requirement for high salt (Huang, 1975a; Nishiyama *et al.*, 1983). The viral DNA polymerase is a 140-kDa polypeptide that is associated with a 51- to 58-kDa polypeptide that is referred to as the polymerase-associated DNA-binding protein (Mar *et al.*, 1985a,b). The viral polymerase gene was described in Section 2.6. Like other herpesvirus polymerases (Knopf, 1979), human CMV DNA polymerase has

associated with it a 3′-specific exonuclease activity that prefers single-stranded DNA as a substrate (Knopf, 1979; Nishiyama *et al.*, 1983). The enzyme may play a role in editing the newly synthesized DNA.

2.8.2. The Rate of Human CMV DNA Synthesis

The time after infection required for the maximum rate of human CMV DNA synthesis is extremely long compared to HSV. DNA replication is initiated at approximately 12 hr after high-multiplicity infection, but maximum viral DNA synthesis does not occur until 72 to 96 hr. The reasons for this relatively slow accumulation of viral DNA are presently not known. Low levels of the viral DNA polymerase and other essential virus-specified proteins necessary for viral DNA synthesis provide one possible explanation. Transcriptional or posttranscriptional regulatory events may control the level of expression of these essential viral proteins. In addition, human CMV stimulates host cell macromolecular synthesis. It is possible that the human CMV DNA replication complexes do not compete well for limiting factors with the analogous host cell DNA complexes. Therefore, the low concentration of viral DNA relative to cellular DNA and the competition between the two synthetic events may cause a slow accumulation of viral DNA. Human CMV DNA synthesis follows a cyclic pattern with two peaks of DNA synthesis occurring at approximately 1 and 3 days after infection (Stinski, 1978, 1983). It has been proposed that viral DNA synthesis proceeds independently of host cell DNA synthesis because late viral synthesis was less likely to occur in the cells of which cellular DNA was synthesized (DeMarchi and Kaplan, 1976). Although cellular DNA synthesis is activated after infection, this activation is apparently not essential for viral DNA or viral antigen synthesis. Stimulation of cellular DNA synthesis could actually be a process that inhibits viral DNA synthesis (DeMarchi and Kaplan, 1976).

2.8.3. The Model for DNA Replication

Human CMV and HSV have a similar genome arrangement, i.e., long and short components with an *a* sequence at the ends of each component as described in Section 2.2. A rolling-circle model for the replication of the HSV genome has been proposed (Roby and Gibson, 1986; Roizman, 1979a), and it is likely that the same model applies to human CMV DNA replication. After treatment of the human CMV genome with exonuclease III, double-stranded circle with a circumference equal to the length of the viral genome were observed (Geelen and Westrate, 1981). This experiment demonstrated the existence of directly reiterated sequences at the termini, which permitted hybridization of the opposite strands to form a circular molecule.

Both the isomerizing and nonisomerizing CMV genomes have reiterated se-

quences at their termini that have been designated with *a* sequence. For the isomerizing genome, that *a* sequence is present at the termini of both the L and S components as well as at the L–S junction (Fig. 2.1) (Mocarski *et al.*, 1987). Experiments with HSV type 1 suggested that the *a* sequence is necessary for inversion of the L and S components (Spaete and Frenkel, 1985, 1982), and, therefore, the *a* sequence might act as cis site for inversion and recombination (Mocarski *et al.*, 1980, 1987; Mocarski and Roizman, 1981, 1982). However, others (Weber *et al.*, 1988) have proposed that the inversion of the L and S components of the HSV-1 genome do not require a site specific process and that isomerization is mediated by the viral gene products that replicate the viral DNA. Replacement of the HSV *a* sequence by the human CMV *a* sequence on a chimeric amplicon was found to be possible (Spaete and Mocarski, 1985).

All human herpesvirus sequences known to date have a short region of homology within the *a* sequence that is positioned near the site for cleavage of concatemers (Davison, 1984; Davison and Wilkie, 1981; Van den Berg *et al.*, 1984). The cleavage event occurs with 30 to 35 base pairs from this homologous sequence. These sequences for HSV and human CMV are illustrated in Fig. 2.5 and have been designated the "herpes pac homology" because they are presumably important in the cleavage/packaging process (Mocarski *et al.*, 1987). The sequences upstream of the cleavage site may contain more than one cis-acting signal that are presumably involved in the cleavage event (Marks and Spector, 1988). Whether a viral or cellular enzyme recognizes these sequences is currently not known. The packaging of only unit-size genomes suggest that the *a* sequence contains the necessary cleavage and packaging signals for encapsidation of the viral DNA (Spaete and Mocarski, 1985). The genomes of all herpesviruses carry similar cis signals that are required for cleavage of the concatemeric DNA and for packaging the progeny viral DNA into capsid (Frenkel *et al.*, 1984; Mocarski *et al.*, 1985a, 1980; Spaete and Frankel, 1985; Spaete and Mocarski, 1985; Stowe *et al.*, 1983; Varmuza and Smiley, 1985; Vlazny *et al.*, 1982; Wadsworth *et al.*, 1976).

The major difference between the isomerizing and nonisomerizing CMV genomes is that the *a* sequence is present internally at the termini of the L and S components of the isomerizing genomes but not the nonisomerizing genomes.

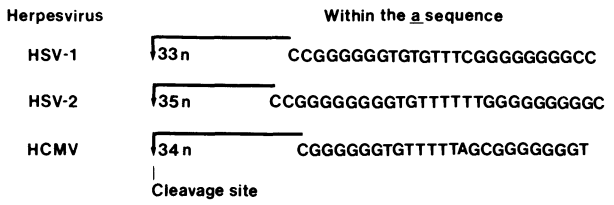

Figure 2.5. Sequence homology between HSV and human CMV in the *a* sequence. Cleavage of concatemeric DNA, designated by an arrow, occurs 34 to 35 n from the edge of the homologous region (data from Mocarski *et al.*, 1987).

Whether this sequence homology is present in all the nonisomerizing animal CMVs is presently not known, but it appears to be present in the murine CMV genome (Marks and Spector, 1988). Therefore, it is assumed that the animal CMVs, like human CMV, have *a* sequences that participate as cis replication signals for circularization and cleavage/packaging of the progeny viral DNA. Exonuclease activity presumably creates sticky ends at the complementary *a* sequences that are redundant in one or more copies in the terminal repeats. These viral genomes are circularized early after infection by hybridization of the sticky ends. Replication can be initiated in the L or S components and proceed in both directions to form a rolling circle, yielding a head-to-tail concatemer (O'Donnell *et al.*, 1987a). The missing *a* sequence, because of the initial circularization event, could be recovered by using the internal inverted repeat sequence as template for synthesis of the missing *a* sequence (Roizman, 1979a,b). Cleavage at the internal *a* sequence could result in inversion of the components following recombination at the homologous *a* sequence. As for HSV, four isomeric forms of the human CMV genome can form. Viral and/or cellular proteins presumably act in trans to mediate cleavage, inversion, recombination, and eventually genome length packaging. Human CMV has an *a* sequence position approximately the same distance from the cleavage site as does HSV (see Fig. 2.5). Since there is evidence for concatemers, replicative loops and forks, and four isomeric arrangements for the human CMV DNA (Geelen and Westrate, 1982; Jean *et al.*, 1978; Stinski, 1983), it is likely that human CMV replicates according to the model proposed for HSV.

2.9. EXPRESSION IN NONPERMISSIVE (NONPRODUCTIVE) CELLS

Cytomegaloviruses have the property of replicating preferentially in cells of the species from which they were originally isolated. However, even in these cells, different levels of viral gene expression may occur. For example, human CMV genome expression can be totally blocked or restricted to the IE and early genes in certain cell types. Present evidence indicates that viral DNA replication does not occur in the nonpermissive cells. The degree of permissiveness also differs with different cell types. For example, in human CMV replicates more efficiently *in vitro* in human fibroblast cells than in human epithelioid, transformed, or cancerous cells. Therefore, the type and physiology of the cell that the virus encounters can determine whether or not infectious virus is produced and the extent of infectious virus production.

In undifferentiated human teratocarcinoma stem cells, transcription of the human CMV IE genes is blocked (Gonczol *et al.*, 1984; also see Section 5.5.6). However, if the cells are induced to differentiate with retinoic acid, IE transcription and productive infection occur (Gonczol *et al.*, 1985; LeFemina and Hayward, 1986). This block to IE transcription occurs with human CMV but not with simian CMV (LeFemina and Hayward, 1986), even though the two viruses have many

similar repeat motifs in their promoter–regulatory regions. An analysis of the human CMV IE promoter–regulatory region in undifferentiated and differentiated human teratocarcinoma cells has demonstrated the induction of DNase-I-hypersensitive sites in the differentiated cell, primarily in regulatory region I (see Fig. 2.2) (Nelson and Groudine, 1986; Nelson *et al.*, 1987). This suggests a rearrangement in the chromatin structure caused by differential binding of proteins in the regulatory region where the NF1/CBP sites are located. These enhanced DNase I cleavage sites may be related to either the binding of transcription factors at specific locations in the regulatory region II or the removal of inhibitory factors.

The response of human peripheral blood cells to human CMV infection depends on the cell type. Monocytes are semipermissive, and, consequently, IE, early and late gene expression, and the production of infectious virus are at a low level compared to permissive cells (Turtinen *et al.*, 1987). A monocytic cell line is permissive for human CMV after stimulation and differentiation of the cell line to characteristics of mature macrophages (Weinshenker *et al.*, 1988). In contrast, evidence suggest that the T and B lymphocytes are nonpermissive (Rice *et al.*, 1984; Schrier *et al.*, 1985). During this latentlike state, transcription and translation of the IE1 gene occurs in the T cells (Rice *et al.*, 1984). The extent of IE transcription and translation or the extent of early gene expression is presently not known, but there is no evidence for viral DNA replication. A host cell protein(s) may be required for complete or efficient early CMV gene expression of a crucial protein(s) required for the initiation of viral DNA replication. Limited production of this crucial protein(s) in cells of different origin may influence either nonproductive, semiproductive, or persistent infection or latency.

Since the CMV genome can persist in cells for extended period of time without viral DNA replication or late gene expression, it is proposed that latency is associated with a nonproductive cell that permits only limited viral gene expression. In this regard, the T and/or B lymphocytes appear to be the best candidates. There is no evidence for integration of CMV DNA in these cells or other cells.

The restricted expression of one or more of the three virus gene classes may explain, in part, CMVs' unusual species specificity and extremely narrow host range. This may be important to the biology of the virus because restricted virus gene expression may be important in the establishment and maintenance of persistent or latent infection. Animal CMVs do not have the same level of restricted replication. Although human CMVs can replicate only in human cells, simian CMVs can replicate in both simian and human cells. However, simian CMVs cannot replicate in mouse cells. The block of human CMV replication in nonpermissive animal cells is not from a failure to stimulate host cell macromolecular synthesis (DeMarchi, 1983b,c). The regulatory mechanisms that influence nonpermissive infection are currently not understood, but they are presumably determined by viral as well as host cell factors (see also Sections 5.5 and 9.7.2).

3

Gene Products of Cytomegalovirus and Their Immunologic Significance

Lucy E. Rasmussen

3.1. INTRODUCTION

The protein composition of CMV is quite complex. The 230-kilobase genome has the coding capacity for over 200 proteins, each of which may be subject to post-translational modification by cleavage, phosphorylation, glycosylation, or sulfation. Our understanding of the immunologic structure of CMV has improved greatly in the last few years as a result of the development of monoclonal antibodies and recombinant gene expression technologies. The CMV gene products have been grouped by size in a recent review by Landini and Michelson (1988). The immune responses that occur following CMV infection and the importance of specific viral proteins in inducing these responses have also been reviewed recently (Rasmussen, 1989). In this chapter I first review what is known about the protein and glycoproteins of CMV. The gene products that are associated with immunologic activities are then described. The focus is on human immune responses; however, studies in animal models or with nonhuman CMV strains are included when they illustrate principles that may be important for subsequent studies in humans (see also Chapters 6 and 7).

Lucy E. Rasmussen • Division of Infectious Disease, Stanford Medical School, Stanford, California 94305.

3.2. INTRACELLULAR VIRAL PROTEINS

The proteins described in this section are those that occur in infected cells but are not detected on the virion or viral envelope. The sequential order of gene expression that is common to herpesviruses also occurs during CMV replication (Wathen and Stinski, 1982). The α and β viral proteins that are synthesized during the immediate early (0–2 hr) and early (2–24 hr) times after infection are important for regulation of virus replication. The immediate early and early proteins are synthesized before the onset of viral DNA synthesis. The late (after 24 hr) γ proteins are structural elements, although there may be exceptions. Most of the envelope glycoproteins are synthesized during the early and late periods after virus infection. These are discussed separately in Section 4 (see also Chapter 2).

3.2.1. Immediate Early (α) Proteins

The molecular biology of the IE genetic region coding for the α proteins has recently been summarized by Stinski (1984; see Chapter 2). The gene products may be important in regulation of latent CMV infection. For CMV (Towne strain), there are three IE gene-coding regions between 0.709 and 0.751 map units within the Hind III region of the unique long sequence. Region 1 (0.739–0.751) has been designated the major immediate early gene for IE1. The major gene product is a 491-amino-acid protein that is posttranslationally modified by phosphorylation, resulting in a polypeptide of 75 kDa. The phosphorylation accounts for the size variation among different strains of CMV such as Towne, AD 169, and Davis (Gibson, 1981a). The second region (IE2) from 0.732 to 0.739 codes for proteins that are found in low concentration. The third region between 0.709 and 0.728 codes for a 1.95-k mRNA that translates into a 68-kDa protein. A region of the genome at the junction between HindIII fragments Z and J of strain AD169 is also transcriptionally active at immediate early times (Wilkinson *et al.*, 1984). The sequence of this region is known, and three mRNAs have been identified by transcription analysis (Kouzarides *et al.*, 1988). It is of interest that the predicted translation product of one of the mRNAs has the characteristics of a membrane-bound glycoprotein. This would be the first example of a potential glycoprotein that is transcribed at immediate early times after infection. It is possible that such a membrane glycoprotein could be an important target for HLA-restricted cytotoxic T lymphocytes, as discussed in detail in Section 3.8.2.

3.2.2. Early (β) Proteins

3.2.2.1. DNA-Binding Protein

The major early CMV single-stranded DNA-binding protein in Towne strain of human CMV is 140 kDa (Gibson *et al.*, 1981; Anders *et al.*, 1986). The protein

appears to be the counterpart of the HSV DNA-binding protein ICP8 (Anders *et al.*, 1987; Kemble *et al.*, 1987). The open reading frame within the region between the CMV (AD169) EcoR1 (P/V) and HindIII (F/D) restriction fragments has predicted amino acid homology with BALF4 of EBV and ICP8 of HSV. For Towne strain the gene has been mapped to the EcoRI V and Q fragments (Anders and Gibson, 1988). The DNA-binding protein can be localized within the intranuclear inclusions characteristic of replicating CMV (Anders *et al.*, 1987).

3.2.2.2. The 72-kDa Protein

A 72-kDa protein has been isolated by immunoaffinity from virus-infected cells using a monoclonal antibody that detects early but not immediate early antigens (Rodgers *et al.*, 1987). The function of this protein or relationship to gene products of similar size is unknown at this time.

3.2.2.3. Viral DNA Polymerase

A DNA polymerase is induced after CMV infection that can be distinguished from host cell enzymes (Mar *et al.*, 1981, 1985a,b). Three groups have now reported the sequence and transcriptional analysis of the DNA polymerase gene (Kouzarides *et al.*, 1987; Heilbronn *et al.*, 1987; Aquila *et al.*, 1989). There is a close similarity of the CMV enzyme to the herpes group of virus-induced enzymes, since the genes for CMV, EBV, and HSV DNA polymerase show considerable amino acid homology and a number of highly conserved regions. The CMV DNA polymerase gene is an 850-base-pair region within the Eco R1 M fragment of strain AD169 in the U_L region with one continuous open reading frame. There is a highly conserved domain of 133 amino acids shared with HSV and the putative EBV polymerase sequences. One 5.4-kb early transcript appears to code for the 140-kDa polymerase protein.

3.2.3. Late γ Proteins: The 51–kDa DNA-Binding Protein

The 51-kDa DNA-binding protein (ICP36) is the major component of a family of gene products that are transcribed from a 2800-base-pair EcoR1 fragment (0.228–0.240) on both the CMV Towne and AD169 strain genome (Mocarski *et al.*, 1985b). It is phosphorylated but not a structural component of virus particles (Gibson, 1983).

3.3 STRUCTURAL PROTEINS

3.3.1. Development of Virions and Enveloped Infectious Virus

The development of infectious CMV begins in the nucleus of the host cells, where the nucleocapsids are formed. During the maturation process, the nucleocap-

sid acquires an envelope that is derived from both the internal nuclear membrane (Smith and DeHarven, 1973) and the endoplasmic reticulum (Severi *et al.*, 1979). There are more than 30 proteins that can be detected on the various forms of the extracellular virus (Kim *et al.*, 1976b; Gupta *et al.*, 1977; Stinski 1977; Gibson, 1983). The nucleocapsid proteins are the most abundant, but the membranes contain a discrete population of proteins and glycoproteins (Farrar and Oram, 1984; Stinski, 1977, Stinski *et al.*, 1979b).

The events in nucleocapsid assembly have been well studied by Gibson and colleagues using the simian CMV strain Colburn. Four types of virus particles can be identified within the CMV-infected cell. For purposes of comparison with the particles of HSV, they were given alphabetical designations. A capsids have the simplest structure with at least three protein species (145–145,34,28 kDa) and are present in the nuclear fraction of infected cells. The 145 to 155-kDa protein is 90% of the A-capsid mass, which is consistent with it being the major structural component of the icosahedral capsid (see Section 3.3.2.2). The 28-kDa protein appears to be important for maintaining structural integrity of the developing nucleocapsid. The B capsid, also present exclusively in the nuclear fraction of infected cells, has, in addition to the proteins on the A capsid, a 36-kDa protein that may be involved in DNA packaging and/or nucleocapsid envelopment. It is closely related to a 45-kDa protein present in much lower amounts (Irmiere and Gibson, 1985). The maturation of B capsids to nucleocapsids involves the modification or elimination of the 36-kDa assembly protein from the particle. Viral DNA packaging occurs subsequent to B-capsid assembly. Therefore, B capsids without DNA can be detected, and B capsids may accumulate when viral DNA synthesis is inhibited (Lee *et al.*, 1988). The gene for the primate cytomegalovirus assembly protein has been isolated and translated *in vitro* from Colburn-strain-infected human fibroblast RNA. Similar genetic sequences can also be detected in CMV strains Towne and AD169 (Roby and Gibson, 1986). The assembly protein is derived by cleavage from a precursor and is phosphorylated to give the 35- to 40-kDa protein. The noninfectious enveloped virus particles that are observed frequently in extracellular virus preparations appear to be enveloped B capsids.

The C capsid is yet more complex than either the A or the B capsid and is found exclusively in the cytoplasm of virus-infected cells. It has additional 205- and 66-kDa bands. The 66-kDa protein is the lower matrix phosphoprotein (see Section 3.3.2.1). There are at least an additional seven proteins found on the nucleocapsid that are not present in A, B, or C capsids. The 20 or more protein species on the nucleocapsids range in size from 20 to 200 kDa, with the lower matrix protein (Section 3.3.2.1) representing 70% of the capsid protein mass. It apparently serves as an interface between the nucleocapsid and the outer envelope proteins.

The involvement of these intracellular particles in the assembly pathway of the virus is not yet clear. It is possible that the empty A capsids may be precursors to the DNA-containing B and C capsids and nucleocapsids. We are just beginning to understand the protein composition of the extracellular virus forms. In the following section I discuss what is known about the proteins that are associated with the viral nucleocapsid. The glycoproteins of the viral envelope are described in Section 3.4.

3.3.2. Proteins Associated with Extracellular Virion Forms

3.3.2.1. Viral Matrix Proteins

The viral matrix is the area located in the virion between the nucleocapsid and the envelope (Gibson, 1981b). It is present in large amounts in virions, dense bodies (see Section 4), C capsids, and in the nucleoplasm of infected cells; however, it is not part of the intranuclear inclusion bodies that are so characteristic of CMV infection (Weiner *et al.*, 1986). There are three components of the viral matrix protein. The first is the lower matrix protein of 64–69 kDa; the second is an upper matrix protein of 69–71 kDa; and the third is a 150-kDa basic phosphoprotein. The "upper" and "lower" matrix proteins are designated according to their relative migration in sodium dodecylsulfate polyacrylamide gels.

The lower matrix protein, present on viral capsids, is detectable in both the nucleus and cytoplasm of infected cells since it is one of the most abundant proteins synthesized during the infectious cycle, representing about 15% of the total virus protein (Roby and Gibson, 1986). The gene is located within the Eco R1 A fragment of CMV Towne strain (Nowak *et al.*, 1984b; Pande *et al.*, 1984), and the sequence has been published (Ruger *et al.*, 1987). The gene for the lower matrix protein is transcribed late in the infectious cycle but in the absence of viral DNA replication and may be controlled by posttranscriptional events (Geballe *et al.*, 1986a). The lower matrix protein has been referred to as pp65 (Nowak *et al.*, 1984a,b), 69-kDa matrixlike protein (Irmiere and Gibson, 1983), HCMV gp 64 (Clark *et al.*, 1984), or ICP27 (Geballe *et al.*, 1986a). All of these are probably the same protein. The sequence of the lower matrix protein is not the same as the protein of a similar molecular weight described by Davis and Huang (1985) but is identical to that reported by Pande *et al.* (1984).

How the lower matrix protein is distributed on extracellular virus particles is not clear. Some have reported that the lower matrix protein is not detectable on nucleocapsids but is assembled only into cytoplasmic dense bodies (Gibson and Irmiere, 1984; Kim *et al.*, 1983; Landini *et al.*, 1987b). However, others have found that the lower matrix protein is an internal nonglycosylated virion phosphoprotein that is inaccessible to antibodies in virions or on the surface of infected cells (Britt and Vugler, 1987). It is of interest that in human sera the humoral immune response to the lower matrix protein, as detected by immunoblot analysis, is relatively weak (Jahn *et al.*, 1987a). Low-passage clinical isolates of CMV show considerable variability in the expression of the lower matrix protein in cell cultures (Klages *et al.*, 1989). The variability in expression may be dependent on the multiplicity of infection, with increasing multiplicities leading to overproduction of the protein and subsequent increased formation of dense-body particles. This may explain, in part, the low immune response in some patients.

The upper matrix protein is 71 kDa and is sometimes referred to as the 74-kDa protein (Roby and Gibson, 1986). It is transcribed from the same region of the genome as the lower matrix protein, but it is not as abundant in infected cells as the lower matrix protein (Gibson, 1983; Irmiere and Gibson, 1985). The lower matrix

phosphoprotein is coded by the 5′-terminal part of an abundant 4-kilobase mRNA, whereas the upper matrix phosphoprotein corresponds to the single translational reading frame from a rare nonspliced 1.9-kilobase mRNA that is coterminal with the 4-kilobase transcript. The upper and lower matrix proteins are immunologically distinct (Gibson, 1983).

A 150-kDa basic phosphoprotein is probably one of the matrix components of the virion (Gibson, 1983; Roby and Gibson, 1986). The gene is on HindIII fragments J and N (Jahn *et al.*, 1987a). Phosphoproteins of approximately the same size occur in the matrix of other herpesviruses (Lemaster and Roizman, 1980), but no sequence homology has been demonstrated. It is of interest that this protein appears to be particularly reactive with human sera when tested in immunoblots (Landini *et al.*, 1986, Jahn *et al.*, 1987a).

3.3.2.2. Major Nucleocapsid Protein

A protein of approximately 150 kDa is the main structural element of the viral nucleocapsid and constitutes approximately 90% of its total protein (Gibson, 1983). The major nucleocapsid protein comigrates on polyacrylamide gels with the basic phosphoprotein (see Section 3.3.2.1), also 150 kDa in size. However, the proteins can be well resolved by charge–size separation of the use of high-percentage polyacrylamide gels. The gene for the major nucleocapsid protein has been identified by its analogy to coding sequences for the major nucleocapsid genes of HSV, EBV, and varicella–zoster virus (Chee *et al.*, 1989c) and is located within the HindIII U fragment of strain AD169.

Despite its abundance on virions, the major nucleocapsid protein is weakly immunogenic in its natural host, since it is detected inconsistently by either acute or convalescent human sera (Jahn *et al.*, 1987b). In contrast, antibody to the major nucleocapsid protein of both HSV-1 and -2 can be detected in immunoblots of all individuals seropositive for the respective virus. Why this highly expressed protein fails to elicit an immunologically significant response is unclear. It is possible that antibodies to the major nucleocapsid protein could be either short-lived, or their persistence is highly dependent on frequent virus reactivation.

3.3.2.3. The 28- to 32-kDa Structural Protein

A 28- to 32-kDa structural protein is present in the cytoplasm of infected cells during the late phase of the viral replication cycle as well as in the extracellular virus particles (Re *et al.*, 1985). Monoclonal antibody detects antigen by immunofloures-cence 48 hr after infection, and antigen expression requires viral DNA synthesis. The protein is phosphorylated but not disulfide linked. It is not a target for virus-neutralizing antibody. Within infected cells, two protein bands of 27–28 and 32KDa can be detected with monoclonal antibody. However, only a single 32-kDa band is detected in virions (Pande *et al.*, 1988). The gene maps to the HindIII R fragment of strain AD169 and is transcribed into a late 1.3-kb RNA (Meyer *et al.*, 1988; Pande *et al.*, 1988; Martinez *et al.*, 1989). Parts of the 28-kDa polypeptide that were

expressed in *E. coli* as hybrid proteins are recognized by human sera (Meyer *et al.*, 1988). By immunoelectron microscopy the 28-kDa protein is present only in the outline of the cytoplasmic capsids (Landini *et al.*, 1987b), suggesting that it is a minor capsid protein. It is probably a structural component of the virions that is acquired in the cytoplasm and localized on the surface of the viral capsid. This 28- to 32-kDa protein is probably different from the 28-kDa protein described by Gibson (1981b) that appears only on intracellular capsid forms (see Section 3.3.1).

3.3.2.4. The 46-kDa DNAse

The 46-kDa polypeptide, present in extensively purified CMV particles, has been shown to have DNAse activity (Ripalti *et al.*, 1988). The monospecific anti-p46 serum produced in mice recognizes an enzymatically inactive 76-kDa polypeptide in both noninfected and CMV-infected cells showing that the enzyme is cellular.

3.3.2.5. Protein Kinase

Most herpesviruses have virion-associated protein kinases (Stevely *et al.*, 1985). All three extracellular CMV particles with a viral envelope, specifically noninfectious enveloped particles, dense bodies, and virions, have protein kinase activity (Roby and Gibson, 1986). A 67-kDa phosphoprotein with protein kinase activity has been immunoprecipitated from both the nuclei of infected cells and virion preparations (Davis *et al.*, 1984; Davis and Huang, 1985). Protein kinase activity has also been detected in virions and infected cells that have been immunoprecipitated with a monoclonal antibody to an abundant 68-kDa virion structural protein, probably the lower matrix protein (Britt and Auger, 1986a). A candidate gene for a protein kinase has been localized to a region between 0.37 and 0.39 map units (Davis *et al.*, 1984; Davis and Huang, 1985).

The function of virion-associated protein kinase activity is unknown. It may have an important role in virus replication, for example, uncoating or encapsidation of the viral nucleic acid. An immune response to such a potentially critical determinant of virus replication could clearly affect subsequent viral pathogenesis.

3.4. ENVELOPE GLYCOPROTEINS

The biogenesis of the viral envelope has been described in Section 3.3.1. There are at least three distinct species of extracellular enveloped particles: (1) complete infectious particles that contain DNA, capsid, matrix protein, and envelope, (2) particles that are structurally and compositionally similar to virions but contain no DNA and are referred to as noninfectious enveloped particles, and (3) large enveloped spherical aggregates, known as dense bodies, that are composed primarily of protein and contain neither DNA nor a capsid.

The envelope glycoproteins are of interest because of their importance as

antigens for both humoral and cellular immune responses. By analogy with other herpesviruses that have been well studied, they may be targets for virus-neutralizing antibody, mediate viral entry, and may play a role in the release of the virus from the host cell.

The outer envelope is osmotically fragile, and its proteins can be separated from those of the extracellular nucleocapsids. It has been estimated that there are three to eight glycoproteins present on the envelope of CMV (Fiala *et al.*, 1976; Kim *et al.*, 1976b; Stinski, 1976; Gibson, 1983; Nowak *et al.*, 1984b; Farrar and Greenaway, 1986). Farrar and Oram (1984) purified the envelope from virions and identified five glycosylated components of 52, 67, 95, 130, and 250 kDa after oxidation of purified virus with sodium metaperiodate and subsequent labeling with tritiated sodium borohydride. The most consistently identified polypeptides were the 52-, 95-, and 130-kDa species.

The development of monoclonal antibodies to human CMV has been of significant value for identifying viral proteins (Pereira *et al.*, 1982, 1984) and in particular viral glycoproteins that are targets for virus-neutralizing antibody. The use of monoclonal antibodies with biological activity, for example, virus-neutralizing capability, makes the identification of a target protein straightforward. With this approach it has been possible to identify some of the CMV envelope glycoproteins.

3.4.1. The gB Homologue

One of the major envelope glycoproteins, originally identified with virus-neutralizing monoclonal antibodies, is a complex of three immunologically related glycoproteins referred to as p130/55 (Rasmussen *et al.*, 1985, gA (Pereira *et al.*, 1982, 1984), glycoprotein complex I (gCI) (Gretch *et al.*, 1988c), and gB homologue (Cranage *et al.*, 1986). By informal agreement among laboratory groups, the name gB is used for the present because of the genetic homology to the gB glycoprotein gene of herpes simplex (Cranage *et al.*, 1986). The gB complex is detected by murine monoclonal antibodies that neutralize CMV infectivity in the presence of added complement (Pereira, 1982; Britt, 1984a,b; Rasmussen *et al.*, 1985).

The gB complex is derived from the intracellular processing and cleavage of a high-molecular-weight precursor. The primary form of the precursor protein is approximately 92 kDa and is rapidly glycosylated to give an intermediate form of approximately 160 kDa that is rapidly trimmed to form the stable precursor protein of approximately 130 kDa (Gretch *et al.*, 1988a). The high-molecular-weight precursor is then cleaved into products of approximately 55 kDa and 92 kDa that appear on the extracellular virion envelope in the absence of the precursor forms (Nowak *et al.*, 1984b; Law *et al.*, 1985; Rasmussen *et al.*, 1985; Cranage *et al.*, 1986; Benko and Gibson 1986; Mach *et al.*, 1986). The 55-kDa viral glycoprotein represents the C-terminal region of the gB 130 and contains epitopes for the virus-neutralizing antibody (Spaete *et al.*, 1988). The 92-kDa species is derived from the N terminus

of the precursor molecule (R. Spaete, personal communication), and it is unknown whether it has epitopes that are targets for virus-neutralizing antibodies.

The enzyme that cleaves the precursor molecule is probably cellular in origin, since processing of the product of an expression vector that contains only gB coding sequences takes place in CV-1, CHO, and COS cells that are normally nonpermissive for CMV replication (Spaete *et al.*, 1988). There also may be alternate cleavage patterns of the precursor protein. In one study the molecular weights of the deglycosylated forms, when added together, exceeded that of the polypeptide precursor (Gretch *et al.*, 1988a). Since the open reading frame for the gB gene predicts as many as seven dibasic protease recognition sites (Cranage *et al.*, 1986), alternative cleavage could explain the diversity of related products within the complex. It has been reported in one study that monensin, an ionophore that inhibits Golgi-dependent glycosylation of proteins, prevents the final processing steps of the gB precursor, including cleavage (Britt and Vugler, 1989).

The sugars on the gB homologue complex are predominantly high mannose and are added rapidly to the primary forms during translation on the endoplasmic reticulum (Rasmussen *et al.*, 1988). The 55-kDa product may also contain O-linked oligosaccharides (Benko and Gibson, 1986).

The gene coding for the gB complex maps to the right terminal sequence of the HindIII F fragment between map coordinates 0.344 and 0.380 of CMV (AD169) DNA (Mach *et al.*, 1986). The sequence of the gene has been determined by Cranage and co-workers (1986), who identified an open reading frame for a CMV gene that had glycoprotein characteristics as determined by nucleotide sequencing. This gB gene had homology with the gB gene of HSV, EBV, the gpII of varicella–zoster virus, and the pseudorabies virus gII as subsequently confirmed in other studies (Robbins *et al.*, 1987; Emini *et al.*, 1987). The sequence of the Towne strain of CMV gB gene has a 94% nucleotide similarity and a 95% amino acid similarity to the CMV AD169 gene (Spaete *et al.*, 1988), revealing that it is highly conserved among CMV laboratory strains.

The gB complex can first be detected on the plasma membrane of unfixed infected cells at 72 hr after virus infection when cells are stained with an anti-gB guinea pig antiserum (Landini *et al.*, 1987a). By immunoelectron microscopy the plasma membrane was positive only where the virus and dense bodies had budded through the membrane to form their own envelope. Extracellular virus particles, both viruses and dense bodies, were very strongly labeled on the external surface. The distribution of the gB complex on extracellular CMV particles has also been studied by high-resolution electron microscopy (Stannard *et al.*, 1989). Monoclonal antibody to the gB complex binds to the distal end of 12-nm-long slender spikes projecting from virion and dense body envelopes.

The gB complex will induce complement-dependent neutralizing antibody when injected into guinea pigs (Rasmussen *et al.*, 1985; Gonczol *et al.*, 1986). However, it has recently been reported that complement-independent antibodies are produced in low levels if the immunogen is recombinant gB from prokaryotic expression vectors (Britt *et al.*, 1988), suggesting that the glycosylation of the

protein may influence the type of neutralizing antibody that is produced. One conformational epitope that is a target for virus-neutralizing antibody has been localized to a 186-amino-acid fragment of the 55-kDa glycoprotein between amino acids 460 and 680 (Spaete *et al.*, 1988). In addition, there are two other conformation-dependent neutralizing epitopes that map in two separate domains of gb within a segment of 219 amino acids between residues 461 and 680 from the amino terminus of the molecule (Banks *et al.*, 1989). A third linear epitope for neutralizing monoclonal antibodies exists within the region encompassed by amino acids 608 and 625.

It is of interest that the epitope for nonneutralizing monoclonal antibody is in proximity to the neutralizing epitopes (Utz *et al.*, 1989). The nonneutralizing antibodies are able to inhibit the activity of the neutralizing antibody, pointing to the possible biological significance of antibodies directed to nonneutralizing sites on immunologically active gene products. This may argue for the use of gene products of limited size for immune prophylaxis of CMV infection. It has also been reported that synergistic effects on virus neutralization can occur between antibodies in different domains in virus neutralization assays. Moreover, nonneutralizing antibodies gave enhanced neutralizing responses in the presence of virus-neutralizing antibodies and two nonneutralizing antibodies in combination gave virus neutralization (Lussenhop *et al.*, 1988).

The function of the gB homologue during the viral replication cycle is unknown. It is of interest that the gB glycoprotein of HSV is involved in viral entry and cell fusion. It is required for viral infectivity at a stage after viral attachment but before the expression of the virus-specific proteins (Cai *et al.*, 1988).

3.4.2. The gH Homologue

The 86-kDa glycoprotein, originally described by Rasmussen and co-workers (1984) as a target for complement-independent neutralizing antibody, is currently called gH because of its genetic homology with the gH gene of HSV (Cranage *et al.*, 1988). It has recently been shown to be identical to the glycoprotein described as gc III by Gretch and co-workers (1988c). Biosynthetically, the gH glycoprotein is detected in the cytoplasm of infected cells at late times (24 to 48 hr) after infection. The glycoprotein contains only high-mannose linked oligosaccharides, which are added rapidly to a primary gene product of approximately 80 kDa (Rasmussen *et al.*, 1988). The oligosaccharides do not appear to be essential for induction of neutralizing antibody in guinea pigs but may play a protective or stabilizing role for the glycoprotein. The gH homologue is a single-gene product, and no precursors can be demonstrated. However a 145-kDa species can also be immunoprecipitated by monoclonal antibody from purified viral envelope preparations (Gretch *et al.*, 1988c). When purified by immunoaffinity, the gH will induce complement-independent virus-neutralizing antibody in guinea pigs (Rasmussen *et al.*, 1985).

The gene for the gH homologue is in the HindIII L region of the U_L segment of

the viral genome (Cranage *et al.*, 1988). The gene was identified by nucleotide sequencing of CMV genomic DNA and the subsequent identification of an open reading frame with the characteristics of a glycoprotein-coding sequence. The open reading frame produced an 86-kDa protein when expressed in a vaccinia virus vector. The predicted amino acid sequence of the gH homologue was homologous not only to the gH of HSV but also to the BXLF2 gene product of Epstein–Barr virus (Heineman *et al.*, 1988) and varicella–zoster gpIII (Keller *et al.*, 1987). The gH glycoprotein gene in Towne strain within the HindIII H fragment of the U_L is also virtually identical to the AD169 gene (Pachl *et al.*, 1989).

The gH glycoprotein may be involved in either intracellular spread or initiation of virus infectivity. The incorporation of monoclonal antibody into semisolid overlays of CMV-infected monolayers gives a reduction in the size of the microfoci initiated by the virus (L. E. Rasmussen, unpublished results). The effect on plaque size is similar to that reported for both monoclonal antibody to the HSV gH gene product (Gompels and Minson, 1986) and the varicella–zoster gH homologue (Keller *et al.*, 1987). For EBV, monoclonal antibodies to an 85-kDa gH homologue will inhibit the cell fusion reaction that is important for cell-to-cell spread of EBV but has no effect on virus attachment (Miller and Hutt-Fletcher, 1988). Antiidiotype antibodies that bear the internal image of a neutralizing epitope on the gH glycoprotein can inhibit the spread of CMV throughout the monolayer, suggesting that this viral glycoprotein may be interacting with cellular receptors (Keay *et al.*, 1988).

3.4.3. The gC II Glycoprotein

A third glycoprotein complex (gc II) detected on the viral envelope has been described by Kari and co-workers (1986). There are two components of 93 kDa and 450 kDa in the unreduced state. After reduction the two most abundant species are 50–52 kDa and greater than 200 kDa. The 50- to 52-kDa protein has precursor proteins of 25–32 kDa. Minor species of 90, 116, and 130 kDa are also detected. Neutralizing monoclonal antibodies that react with the complex are complement independent but also cross-react with herpes simplex and adenovirus in immunofluorescent assays. Peptide maps of the 50- to 52-, 93-, 200-kDa species that had been isolated by high-performance liquid chromatography show identical patterns, indicating that the three components are structurally similar (Kari and Gehrz, 1988). The glycoproteins in the gC II complex are heavily sialated, and the 52-kDa component appears to have a unique phenotype characterized by a large amount of O-linked oligosaccharides.

The gene for the gC II complex is located within the HXLF family of five genes that are arranged in tandem within the U_S region of the CMV genome (Weston and Barrell, 1986; Gretch *et al.*, 1988b). Monoclonal antibody to the gC II complex will immunoprecipitate the *in vitro* translation products of an abundant 1.62 bicistronic mRNA from this region. The amino acid composition of the 50- to 52-kDa glycoprotein purified from virion envelopes has highest similarity to the

predicted amino acid composition of HXLF1 plus HXLF2 open reading frames but is more similar to HXLF2 than to HXLF1. The glycoprotein can also be synthesized by monocistronic 0.8-kb mRNA encoded by the HXLF2 gene.

3.4.4. Integral Membrane Glycoprotein

Rabbit antiserum produced against purified CMV envelope preparations has been used to identify a 45-kDa structural component of the virus that has multiple membrane-spanning domains (Lehner *et al.*, 1989). The gene for this envelope glycoprotein is located in the HindIII R fragment of AD169 DNA. There is predicted homology, based on amino acid sequence of the hypothetical proteins, from reading frames BBRF3 of Epstein–Barr virus, UL10 of herpes simplex virus type I, and ORF50 of varicella–zoster virus. The IMP gene is transcribed into a 1.5-kb RNA that is detectable only late in the course of infection. The presence of several membrane-spanning domains makes the IMP a unique type of protein among the known constituents of the of the CMV envelope.

3.4.5. The gp48 Glycoprotein

An envelope glycoprotein that is synthesized in the absence of viral DNA replication but accumulates to high levels at late times after infection has recently been described (Chang *et al.*, 1989b). It is the product of an early gene that maps between 0.054 and 0.64 map units and has three temporally regulated promoters (Chang *et al.*, 1989a). The primary gene product is 17 kDa and is modified by N- and possibly O-linked glycosylation to give the final 48-kDa form. There was no detectable genetic homology between the gp48 gene and other known herpesvirus glycoproteins as determined by computer-aided analysis.

3.5. PROTEINS IDENTIFIED BY SEQUENCE ANALYSIS ONLY

3.5.1. The MHC Class I Antigen Homologue

The ongoing sequence analysis of the CMV genome by Barrell and co-workers at Cambridge University indicates that there are many open reading frames for potential proteins and glycoproteins that have yet to be detected immunologically. There is a CMV gene that can code for a glycoprotein that appears to be homologous to the MHC class I antigens (Beck and Barrell, 1988). Infectious CMV can bind β_2-microglobulin, a protein that is normally found in association with the class I major histocompatibility complex. It thought that one mechanism for CMV entry may be via binding or displacement of β_2-microglobulin (Griffiths and Grundy, 1988) on target cells. Two CMV envelope proteins of 36 and 65 kDa have been

identified that bind to β_2-microglobulin. It is of interest that the gene for the class I HLA-like molecule can also code for a 65-kDa protein, assuming that seven or eight of the 13 potential glycosylation sites are actually glycosylated *in vivo*.

3.5.2. Phosphotransferase

Chee *et al.*, (1989a) have identified a gene sequence in the HindIII S fragment (positions 3776 and 5896) that could specify a product related to cellular protein kinases. These sequences are distinct from that reported by Davis and Huang (1985) for a gene specifying a protein kinase. The predicted size of the gene product is approximately 78 kDa. Whether this putative gene product is related to any other protein kinase associated with virions (see Section 3.2.5) is not clear.

3.6. PLASMA MEMBRANE PROTEINS

Cytomegalovirus infection results in profound alterations of the host cell. Morphological, biological, and antigenic changes occur following virus infection. It is important to understand the nature of the CMV-specified constituents on the infected cell surface, as they are of potential immunologic importance. Some of these virus-specified neoantigens on the surface of infected cells may be primary targets for a host's immune defense. Human sera have antibodies, predominantly IgM, that react with the membranes of CMV infected cells (The and Langenhuysen, 1972; The *et al.*, 1974) (see also Section 6.10.4). Anti-CMV membrane antigen antibodies are rarely detected in latently infected normal donors but are induced during the acute phase of symptomatic CMV infection (Middledorp *et al.*, 1984).

The CMV-specified components that appear on the cell surface during productive infection are currently being studied. It is possible to detect virus-induced proteins on the surface of virus-infected cells that are distinct from those on virions (Sullivan-Tailyour and Garnett, 1986). At least eight virus-induced proteins were shown to be unique to the membrane of CMV-infected cells. All of these unique proteins were reactive in immunoblot assays with pooled human sera, pointing to a possible contribution to the host immune response. The envelope glycoproteins gB and gH can also be detected on the surface of virus-infected cells and can be recognized by CMV antibody-positive human sera (Van der Voort *et al.*, 1989).

3.7. VIRUS-INDUCED HOST CELL PROTEINS

3.7.1. Class I HLA Molecules

Cytomegalovirus gives a significant increase in the level of cytoplasmic and cell surface class I HLA antigen expression on human fibroblast monolayer cultures

(Grundy *et al.*, 1988a,b). Although the major part of the observed enhancement is caused by the action of interferon released by CMV-infected cells, some increase in class I HLA was still observed in the presence of anti-β-interferon. The increase in class I HLA was accompanied by increased binding of CMV to cells, suggesting that CMV is using the class I HLA molecules as a cellular receptor (see Section 3.5.1) (Grundy *et al.*, 1987a). The Class I HLA-associated virus binding may also be a potential mechanism for concentrating CMV on the surface of infectable cells for subsequent interaction with the cells of the immune system.

3.7.2. Fc Receptors

Cytomegalovirus, as well as other herpesviruses, induces receptors for the Fc portion of IgG in the cytoplasm and cell membrane (Furukawa *et al.*, 1975b; Keller *et al.*, 1976; Rahman *et al.*, 1976; Westmoreland *et al.*, 1976). An Fc-binding virus-encoded glycoprotein called gE can be detected in herpes-simplex-infected cells and virions (Para *et al.*, 1982). It is not known whether the CMV-induced Fc receptor is virus or cell specified. It is unlikely that the Fc receptor induction is a secondary effect resulting from the interferon that is induced during virus infection, since Fc receptors are increased in Vero cells that cannot produce interferon (Keller *et al.*, 1976). The Fc receptors are specific for IgG. A 42-kDa protein from CMV-infected cells has been shown to have Fc-receptor-binding activity (Sakuma *et al.*, 1977). More recently proteins of 130, 65, 50, 38 kDa have been identified in CMV-infected cells as Fc receptors (Xu-Bin *et al.*, 1989). Interestingly, all four Fc receptor proteins reacted with a murine monoclonal antibody to HSV gE, suggesting immunologic as well as possible genetic conservation of this biological activity among some herpesviruses.

Although the biological significance of the development of Fc receptors is unknown, many possibilities have been discussed, in particular with respect to herpes simplex virus (Kerbel, 1976). Westmoreland and Watkins (1974) and Costa and Rabson (1975) suggest that coating of infected cells with IgG molecules or immune complexes by the Fc region of IgG might protect cells from cytotoxic antibodies and lymphocytes. Lehner *et al.*, (1975) state that double binding of antiviral antibodies on cell surfaces by both Fab and Fc fragments would render the Fc end unavailable for complement binding or for Fc receptors on killer lymphocytes (as in antibody-dependent cell-mediated cytotoxicity).

Fc receptors may not always work in favor of virus infection. Rager-Zisman *et al.*, (1976) found that HSV-infected cells could be nonspecifically lysed by Fc-receptor-positive killer cells. This phenomenon might be mediated by cross-linking of Fc receptors on effector and target by nonspecific aggregated immunoglobulins or soluble immune complexes. Adler *et al.*, (1978), however, showed that aggregated IgG bound to Fc receptors protected HSV-infected immune lysis. Most of these phenomena remain to be tested or worked out in the HCMV field. There is no reason to believe that the results will be less plentiful or complex. In the case of

CMV, it has been shown that the Fc receptors facilitate bacterial adherence to human embryonic lung cell monolayers infected with CMV (Mackowiak *et al.*, 1984). It is possible that these finding may provide one explanation for the high incidence of secondary bacterial and fungal infections among patients undergoing primary CMV infection (Rand *et al.*, 1978). It has also been suggested that the binding of nonimmune IgG to the CMV virion or to infected cells through Fc receptors might lead to protection of the virus from immune mechanisms (Xu-Bin *et al.*, 1989).

3.8. IMMUNE RESPONSES TO CMV GENE PRODUCTS IN HUMANS

3.8.1. Viral Gene Products That Stimulate Humoral Immunity

Serum antibody is likely to play an important role in the control of CMV infection. For example, a fetus is protected from symptomatic infection if maternal antibody is present (Stagno *et al.*, 1982a) (see Section 11.3.3). Passively transferred CMV hyperimmune globulin may also provide protection against CMV-associated interstitial pneumonitis in bone marrow transplant recipients (Winston *et al.*, 1987; Huart *et al.*, 1987) (see Section 14.1). In humans exposed to CMV, serum antibody to at least 15 CMV-associated proteins can be detected by either immunoblot analysis (Gold *et al.*, 1988; Mirolo *et al.*, 1987; Landini *et al.*, 1988, 1989) or immunoprecipitation of radiolabeled CMV-infected cells (Pereira *et al.*, 1982, 1984; Zaia *et al.*, 1986; Hayes *et al.*, 1987; Alford *et al.*, 1988). However, the complex nature of the polypeptide profile of CMV makes it difficult to interpret the importance of reactivity to a specific protein to the protective immune response. The use of genetically pure or immunoaffinity-purified viral proteins is helping to solve the problem of identifying the immunologically significant proteins.

Cytomegalovirus polypeptides captured by monoclonal antibodies in the solid phase have been used to study the humoral antibody response (Cremer *et al.*, 1985). Antibodies with specificities for the different polypeptides were detected after seroconversion. This could mean that detection of some antibodies may be useful for evaluating the course of infection, as has been shown with antigens of Epstein-Barr virus (Henle *et al.*, 1974) and hepatitis B (Dienstag, 1982). However, we now know that even with immunoaffinity chromatography there may be copurification of immunologically unrelated antigens, so caution must be taken in interpreting assays without visualization of the protein of interest.

The basic phosphoprotein (see Section 3.3.2.1) that is a component of the viral matrix has been shown to be a useful marker for assessing CMV immune status (Landini *et al.*, 1986). With recombinant-derived p150, it was shown that antibody to the p150 is widespread in the CMV-seropositive population who are remote from recent infection. However, antibody to the basic phosphoprotein is undetectable

during acute infection (Ripalti *et al.*, 1989). Antibody to the basic phosphoprotein may be a useful marker in situations where it is important to distinguish between primary and recurrent infections, for example, during pregnancy.

The 28-kDa protein (see section 3.3.2.3) is immunogenic in humans since it is reactive with human sera having anti-CMV antibodies (Re *et al.*, 1985).

The human antibody response to six isolated DNA-binding proteins found in CMV-infected cells, probably similar to the intracellular DNA-binding protein family described in Section 3.2.3, was studied by immunoblotting (Gergely *et al.*, 1988). In sera from patients with acute infection, reactions with all six proteins were detected. The strongest reactivity was the 52- and 35-kDa proteins. The sera from some healthy CMV-seropositive donors reacted only with the 52-kDa DNA-binding protein. In another study, high titers of antibody to the p52 protein, isolated as a recombinant *E. coli*-derived fusion protein, were associated exclusively with acute infection (Ripalti *et al.*, 1989).

The lower matrix protein (see Section 3.3.2.1) has been isolated by high-performance liquid chromatography and can induce antibody in both humans and rabbits (Forman *et al.*, 1985). However, the antibody does not strongly neutralize virus infectivity.

Both the envelope glycoprotein complex gCII and gB homologue have been isolated from gradient-purified Towne strain of CMV by anion-exchange high-pressure liquid chromatography. Sera from a small group of seropositive individuals were studied and found to have antibody that immunoprecipitated both of the glycoprotein complexes, but the cellular response as measured by lymphocyte proliferation was inconsistent (Liu *et al.*, 1988). Recent studies using immunoaffinity-purified gB and gH homologues as antigen in immunoblot assays to test sera from patients at various stages of CMV infection have shown that neither of these glycoproteins is highly immunogenic after initial exposure to the virus. Repeated reinfections, such as occur in allograft recipients, may be necessary to evoke a detectable immune response (Rasmussen and Merigan, unpublished work). Some individuals immunized with Towne vaccine strain will weakly immunoprecipitate a 58-kDa protein from CMV envelope preparations that is probably the gB glycoprotein (Gonczol *et al.*, 1989).

Some of the characteristics of the envelope glycoproteins that are targets for virus-neutralizing monoclonal antibody *in vitro* are summarized in Table 3.1. Virus-neutralizing antibodies are produced during CMV infection (Stalder and Ehrensberger, 1980), but how they are involved in protection is unclear. In view of the ability of some antibodies to the viral glycoproteins not only to neutralize virus infectivity but also to inhibit cell-to-cell spread, it is possible that virus-neutralizing activity may not be the primary function of the specific antibody *in vivo*. We do not yet know which of the CMV glycoproteins are the most important antigens in humans for virus-neutralizing antibody production. The gB homologue is probably immunodominant as compared to gH, since it can be more readily detected in human sera. Also human neutralizing monoclonal antibodies to the gB have been isolated, indicating that gB is antigenic for this response in humans (Matsumoto *et al.*, 1986).

TABLE 3.1
Characteristics of CMV Glycoproteins That Are Targets for Virus-Neutralizing Antibody

Glycoprotein	Mol. mass (kDa)[a]	Precursor (kDa)	C′ for neutralization[b]	Function of glycoprotein during virus replication	Gene locus	Gene expression in:
gB	130/92/55	160–130	+/−	?	Hind III F(AD169) Hind III D(Towne)	*E. coli* Vaccinia, COS cells CHO cells
gH	86/145	None	−	Cell–cell spread? Binding to cell receptor?	Hind III L(AD169) Hind III H(Towne)	*E. coli* Vaccinia COS cells
gcII	50–52/200	25–32	+/−	?	Hind III X(AD169) (HXLF gene family)	Rabbit reticulocytes

[a] Under reducing SDS-PAGE conditions.
[b] +, complement dependent; −, complement independent; +/−, both complement dependent and independent.

3.8.2. Viral Gene Products That Stimulate Cellular Immunity

The importance of cellular immunity in CMV has been made clear from numerous clinical observations. Patients undergoing immunosuppressive therapy or with the acquired immunodeficiency syndrome (AIDS) have profound defects in cell-mediated immunity but normal or even elevated levels of circulating antibody (see Chapter 13). Symptomatic CMV infections such as interstitial pneumonitis, retinitis, colitis, hepatitis, and encephalitis, are common in these groups. The delayed hypersensitivity response and functioning T lymphocytes are probably critical for keeping latent infection in check (see Chapter 7).

T helper cells are important both for antigen recognition as measured by lymphocyte proliferation and as a primary source of lymphokines such as interferons and interleukins (see Section 7.2). The lower matrix protein (Section 3.3.2.1) of CMV has been shown to induce a proliferative response in seropositive humans that is associated with both IL-2 secretion and IL-2 receptor expression (Forman *et al.*, 1985). A 72-kDa protein (Section 3.2.2.2) can also stimulate T helper lymphocytes (Rodgers *et al.*, 1987) as measured in assays for both humoral and cellular immunity. It can also stimulate cytotoxic class I restricted $CD8^+$ T lymphocytes. The envelope glycoprotein, gB homologue, can stimulate T-lymphocyte blastogenesis in hyperimmunized guinea pigs (Gonczol *et al.*, 1986) and in normal seropositive humans (Gonczol *et al.*, 1987), indicating that it interacts with T helper cells both *in vivo* and *in vitro*.

Cytotoxic T lymphocytes (CTL) probably play a pivotal role in CMV infections, since the ability to develop a specific cytotoxic response is correlated with successful resolution of disease in patients (Rook *et al.*, 1984a; Meyers, 1984; Quinnan *et al.*, 1982) (see Section 7.3.2.3). It was previously thought that viral glycoproteins were involved exclusively in the induction of protective host responses. However, we now know that in addition to the cell membrane glycoproteins, there are proteins located in the nucleus of the infected cell that specify immunodominant determinants recognized by CTL in conjunction with class II and class II major histocompatibility glycoproteins. This area has recently been reviewed (Rouse *et al.*, 1988). Both class I (Borysiewicz *et al.*, 1983; 1988a,b) and class II restricted (Lindsley *et al.*, 1986) cytotoxic T lymphocytes can be demonstrated in humans infected with CMV.

The role of CTLs in CMV infection has been studied in detail in the murine CMV model of infection and is described in detail in Chapter 16. It is not clear whether the IE gene products will have the same immunologic significance for this response in humans as has been shown for murine CMV. Immediate early, early, and late CMV antigens can all serve as targets for class I restricted cytotoxic T lymphocytes (Borysciewicz *et al.*, 1983; Charpentier *et al.*, 1986). However, as in the mouse model, the CTLs recognizing CMV-infected cells prior to viral DNA replication are the predominant cell type (Borysiewicz *et al.*, 1988b). The T lymphocytes specific for the gB homologue are only a minor fraction of the cytotoxic population. However, other viral glycoproteins have yet to be evaluated as targets for CTL. In

TABLE 3.2
Summary of Immune Responses to Identifiable CMV Gene Products

Gene product	Size or alternative names	Humoral		Cellular			Acute (A) or convalescent (C) response
		Neutralizing antibody	Binding antibody[a]	Cytotoxic T cells	Helper T cells	Lymphokines	
Intracellular							
1. IE Antigens	72-kDa	?[b]	+[c]	+	?	?	A
2. Late: DNA-BP	51-kDa, ICP36	?	?	?	?	?	A
Structural proteins							
1. Viral matrix	Lower, 69- to 71-kDa, ICP27	−	+	?	+	IL-2	?
	BPP, 150-kDa	?	+/−[d]	?	?	?	C
2. Major nucleocapsid	150-kDa	?	+	?	?	?	?
3. 28–32 kDa	−	?	+	?	?	?	?
Envelope glycoproteins							
1. gB	p130/55, gcl gp 55–116	+	+	+	+	?	C
2. gH	86, gc III	+	+	?	?	?	C
3. gcII	−	+	+	?	?	?	?

[a] Binding antibody detected by either immunoblot or immunoprecipitation.
[b] ?, unknown.
[c] +, response detectable.
[d] +/−, response variable.

herpes simplex virus type I-infected mice, a significant proportion of the class I HLA restricted cytotoxic T cells from the draining lymph nodes of acutely infected mice recognized IE gene products in target cells but are not immunodominant (Martin *et al.*, 1988). They account for less than 30% of the total anti-HSV cytotoxic response. Thus, it appears that there will be differences in both the types of antigens needed for stimulation of protective cytotoxic T-lymphocyte responses as well as the phenotypes of the primary effector cell among the herpesviruses.

3.9. SUMMARY

Significant progress has been made in the last few years in the understanding of the antigenic structure of human CMV. We are beginning to identify the proteins that are associated both with the nucleocapsid during the process of virus assembly and with the enveloped virions. As our ability to isolate the viral proteins improves, and to express the genes in appropriate systems, we will be able to associate immune responses with specific viral components. For example, we know that the IE antigens are likely to be important targets for CTLs and that viral envelope glycoproteins are associated with the induction of virus-neutralizing antibody. Our current state of knowledge regarding immune responses associated with specific viral proteins is summarized in Table 3.2. Only the viral antigens that have been shown to stimulate some type of immune responses are shown. Our knowledge of the association between viral antigens and immune responses is only fragmentary at this time. We do not have a clear understanding of the immune function of many of the viral proteins that have been identified. Moreover, we do not know which antigens are associated with acute- and convalescent-phase responses, either humoral or cellular. The immune responses that are critical for protection from primary infection or from virus reactivation are not yet well understood. With the technologies now available for obtaining sufficient quantities of purified viral proteins, as well as the improved methods for diagnosis of CMV infection, we should make rapid progress in solving some of these problems.

4

Characteristics of Cytomegalovirus

4.1. HERPESVIRIDAE

The main criterion for including a virus in this enlarging group of viruses is viral morphology. The unique morphology of herpesviruses was unknown until Wildy *et al.* (1960) introduced the negative staining technique to electron microscopy. They found that herpes simplex virus did not have the spherical shape thought, up to 1960, to be common to many viruses. In sections prepared by negative staining, the naked virus particle or capsid was shown to be an icosahedron (20-sided body) in shape, containing 162 subunits or capsomeres, with five capsomeres along each edge of the triangular facet of the icosahedron. The capsid of herpes simplex virus had an overall diameter of 105 nm, and the capsomeres were either pentagonal or hexagonal and 10 nm in diameter. Within the hollow capsid was the core, which contained the viral genome. The structure of the core was less well defined by electron microscopy. Surrounding the capsid, some particles, presumably the complete infectious unit, possessed an outer membrane or envelope. This envelope increased the diameter of the complete virion to about 180 nm. It is now known that all viruses belonging to the herpes group share this morphology. The morphology of human CMV (CMV), described by Wright *et al.* (1964), is illustrated in Fig. 4.1.

In 1970, the International Committee on Nomenclature of Viruses agreed that the herpes group should be given the taxonomic rank of genus and named *Herpesvirus* (cryptogram = [D/2:54-92/7:S/S:V/O]) (Wildy, 1971). Virus families are designated by terms ending in -viridae, and the herpesvirus family is called Herpesviridae. The group definitive description was as follows:

> Contain double-stranded DNA, molecular weight 54 to 92 × 10^6. G + C content 57 to 74%. Virus particle about 100 to 150 nm diameter. Capsid icosahedral with 162 hollow capsomeres, 100 nm diameter, with a lipid-containing membrane and therefore sensitive to lipid solvents. The DNA is about 7% of the particle weight. Buoyant density (Cs_2Cl):

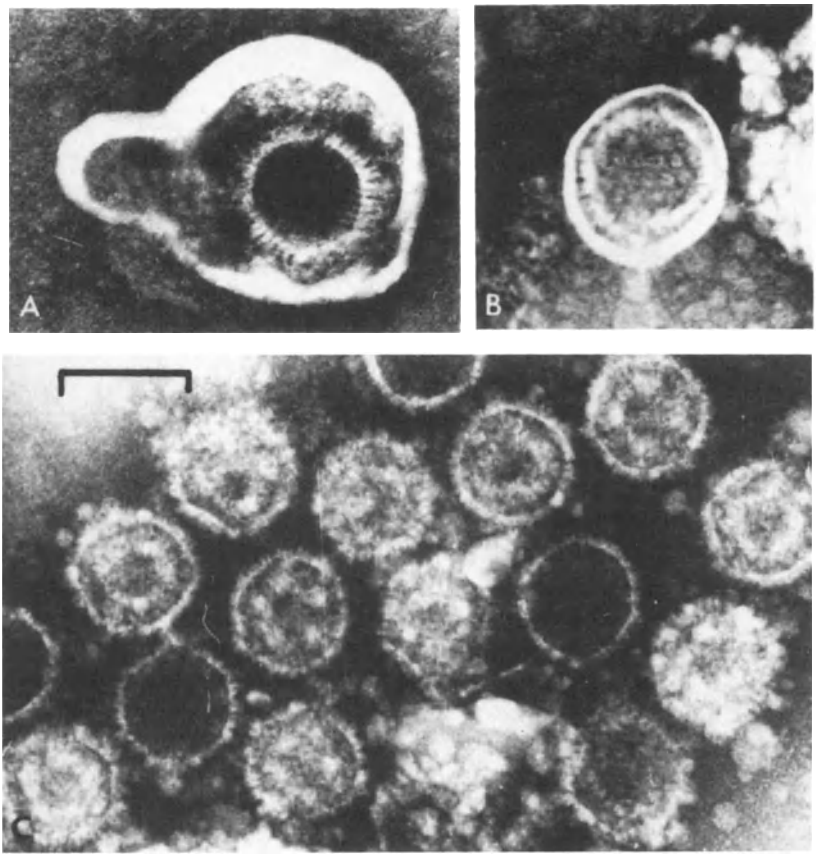

Figure 4.1. Electron micrograph of human cytomegalovirus. (A) An enveloped particle, although it is "empty." (B) A "full" enveloped particle. (C) Two "empty," many "full" but unenveloped particles. The bar is 100 nm. (Wright *et al.*, 1964. By permission of H. T. Wright, Jr., C. L. Goodheart, and editors of *Virology*.)

1.27 to 1.29 g/cm^3. Development begins in the nucleus and is completed by the addition of protein membranes as the virus passes into the cytoplasm. Intranuclear inclusion bodies formed. Separate subgroups have been proposed for viruses which are not readily separable from the cell and for the cytomegaloviruses. There are, however, such gradations in the properties that neither proposal is practical. The type species given was herpes simplex virus. [S/S means particle and nucleocapsids are "essentially spherical," V/O means vertebrates are infected and no vector is involved.]

Attempts to subclassify herpesviruses have been more controversial. Melnick *et al.* (1964) proposed two patterns based on infection in cell cultures. One pattern is produced by herpes simplex virus, which is easily recovered from tissue culture fluid. The group producing this pattern was called A. Group B are those viruses that are not recovered readily from infected tissue culture fluid. They tend to have a

restricted host range. Plummer (1967) pointed out that this distinction is not clear-cut, since viruses form a spectrum of ease of release from cells. The distinction may also vary with the host cell and experimental conditions.

Weller (1970) lists three criteria for identifying cytomegaloviruses: (1) a tendency to cause infection in the salivary gland; (2) a tendency to grow slowly only in cell cultures derived from their natural species; (3) a tendency to form cytoplasmic inclusions in addition to intranuclear inclusions. None of these criteria is hard and fast.

Wildy (1973) suggested that further classification should be based on biochemical properties of the structural components of the virus such as (1) properties of virus proteins including their antigenic properties, serology, polypeptide analysis, amino acid sequences, and genetic complementation tests; and (2) properties of viral DNA. These include base composition, nearest-neighbor analysis, nucleic acid hybridization tests, and genetic recombination.

Now murine and human CMV DNA can indeed be differentiated from herpes simplex virus on the basis of molecular weight (see below), and studies of other properties are being pursued. Some physiochemical properties have not been very helpful. For example Plummer *et al.* (1969) studied the DNA of 14 different members of the herpesvirus group by CsCl equilibrium gradient centrifugation and derived the G + C ratios of the viral DNAs. The lengths of eclipse following infections in cell cultures were also compared. There was no clear division of these viruses based on these criteria. Some well-known human herpesviruses such as herpes simplex type 1 and 2 have unusually high molar proportions of guanosine and cytosine (68% G + C). These two closely related types are slightly different in their G + C molar ratios, and it is gratifying that their separation into different types is thus substantiated. However, in terms of G + C molar ratios, cytomegaloviruses of various species do not form a distinct group. For example, human and murine CMV have lower (58%) ratios than herpes simplex virus, but the G + C molar ratio of vervet monkey CMV seems closer to varicella–zoster virus than to other CMVs and is even lower. Herpesviruses have a greater range of G + C molar ratios in the base the compositions of their DNA than the entire vertebrate group of animals (Plummer *et al.*, 1969).

Since there was no precise virological definition of "cytomegalovirus," the report of the Herpesvirus Study Group of International Committee for the Nomenclature of Viruses (ICNV) in 1973 decided not to use the term at all (Roizman, 1973). They recommended that each herpesvirus, including all the cytomegaloviruses, be named after the taxonomic unit—the family—to which its primary natural host belongs and given in Arabic number. This method of classification has been applied to the naming of a new human lymphotropic virus, human herpesvirus 6 (HHV-6) (Salahuddin *et al.*, 1986). The classification of human herpesviruses is as follows

Human herpesvirus 1 = Herpes simplex virus type 1
Human herpesvirus 2 = Herpes simplex virus type 2
Human herpesvirus 3 = Varicella–zoster virus

Human herpesvirus 4 = Epstein–Barr virus
Human herpesvirus 5 = Human cytomegalovirus
Human herpesvirus 6

In 1979, the ICNV swung back toward a rehabilitation of the cytomegalovirus group. The family of Herpesviridae was divided into three subfamilies representing the herpes simplex viruses (Alphaherpesvirinae), cytomegaloviruses (Betaherpesvirinae), and the lymphoproliferative virus groups (Gammaherpesvirinae). The main characteristics of the CMV group were (Mathews, 1979) the following:

1. Properties of the virus particle. Its DNA has a molecular weight of 130–150 $\times$ 10^6 with 56% G + C. Sequences from either or both termini may be present in an inverted form internally. (see Section 2.2.1).
2. Replication. The CMV group have a relatively slow reproductive cycle (>24 hr), forming slowly progressing foci in cell culture. Enlargement of the infected cell occurs *in vivo* and often *in vitro* (cytomegalia). Inclusion bodies containing DNA may be present in the nuclei and cytoplasm late in infection. Latent virus infection is frequently present in the salivary gland and/or other tissues.
3. Biological aspects. The host range is narrow; in cell culture, CMV usually grows best in fibroblasts, but exceptions exist.

The genus *Human cytomegalovirus group,* and type species *Human (beta) herpesvirus 5* (human cytomegalovirus) have the following main characteristics: "DNA molecular weight = 150 $\times$ 10^6. Virus recovered only from human infections. Experimental host range narrow; grows best in human fibroblasts and less well in certain human lymphoblastoid cells" (Mathews, 1979).

We expect that further studies of both the DNA and proteins will provide better criteria for future classification of herpesviruses and cytomegalovirus subgroups. Data from powerful new techniques such as DNA fragment mapping and restriction endonuclease digestion have yet to be applied.

4.2. CYTOMEGALOVIRUS AND OTHER HUMAN HERPESVIRUSES

The six human herpesviruses present a wide spectrum of properties and biological activities, but they share basic characteristics that make them unique.

On the surface, what could be more different than the prototype diseases caused by CMV, the two herpes simplex viruses, varicella–zoster (VZ) and Epstein–Barr (EB) viruses, i.e., congenital cytomegalic inclusion disease, herpes labialis and genitalis, chicken pox, infectious mononucleosis, and Burkitt's lymphoma? These agents also behave differently in the laboratory in terms of ease of growth and recognition. Herpes simplex virus was one of the first viruses cultured, and it grows in practically any culture of human or nonhuman cells of either

epithelial or fibroblast origin. The culture may be a primary explant or from a continuous line. It infects the gamut of experimental animals and shows little of the species specificity characteristic of the other three agents.

Both CMV and VZ virus are much more restrictive than herpes simplex virus. Both grow best on human cells or fibroblast origin. Varicella–zoster virus has also been grown in cultures of simian tissue (Weller *et al.*, 1958) and guinea pig embryonic tissue (Söltz-Szöts, 1964). Cytomegalovirus has been grown in cell cultures in animals of other species, frequently abortively or in low titer (see Section 5.2.1). Cytomegalovirus also takes longer to replicate, but it is usually less cell associated than VZ virus.

Cytomegalovirus is slower to develop cytopathology and, in addition to the nuclear inclusions characteristic of this group, produces paranuclear cytoplasmic inclusions. It should be noted, however, that VZ may also produce cytoplasmic inclusions (Weller *et al.*, 1958).

The most restrictive of these five agents is EB virus, which grows only in human lymphoid cells and in such a way that no diagnostic cytopathology can readily be observed (Henle *et al.*, 1979).

Perhaps the firmest biological criterion that distinguishes herpesviruses is their antigenic distinctiveness. Despite diversity, the six agents have important biological as well as structural similarities. They are all to varying degrees cell associated. That is, they are best propagated in cell culture in the form of infected cells rather than as cell-free virus. This is least apparent with herpes simplex virus and most apparent with VZ virus. These viruses may spread from cell to contiguous cell, presumably via intercellular bridges, even in the presence of antibody in the extracellular phase ("type 2 spread"; Notkins, 1974). This characteristic may be related to a common characteristic of these agents, that is, their ability to produce latent infection that reactivates under appropriate stimuli.

Perhaps the best-characterized form of reactivation is herpes zoster, which results from reactivation of VZ virus latent in the dorsal ganglia following a primary infection of chicken pox (Head and Campbell, 1900). In normal subjects, according to Hope-Simpson (1965), herpes zoster increases with age, such that the rate of activation at age 80 is about five times higher than that at age 40. Herpes zoster also increases in frequency when cell-mediated immunity is suppressed, as in Hodgkin's disease, or following the administration of cytotoxic drugs. The conclusion seems inescapable that immunologic surveillance, perhaps the type of cell-mediated immunity that holds herpes zoster in check, declines with aging.

The recurrences of herpes labialis and genitalis also represent activation of latent virus infections, presumably, as in the case of herpes zoster, in neurons in the dorsal ganglia (Cook *et al.*, 1974). It is, however, less clear what the exciting trigger event of such activation is. Suppression of immunity is not always involved, although such suppression, as after renal transplantation, does indeed reactivate herpes simplex infection when measured by laboratory methods or when clinical observations are made (Ho, 1977).

The situation with EB virus as regards latency is even more intriguing. Most

primary EB virus infections, as is the case with the other three agents except VZ virus, are inapparent. The prototype primary disease when it does occur is EB virus mononucleosis. When we follow laboratory parameters of EB infection in immunosuppressed patients, there is evidence of activation, both primary and reactivation infection. These may be silent, but after transplantation, a spectrum of lymphoproliferative diseases ranging from severe mononucleosis to frank lymphomas associated with EBV have been described (Ho *et al.*, 1985, 1988).

Latency and reactivation of CMV in cell cultures are discussed in Chapter 5. Reactivation of latent CMV infection in the host occurs when immunity is suppressed and has not been shown to occur sporadically, as in the case of herpes labialis or genitalis. For example, latent CMV infection is often activated following renal and other major solid organ or marrow transplantation and in patients with AIDS. This is discussed in Chapter 13.

To summarize: these herpesviruses manifest a spectrum of diseases in their hosts. They differ in cell permissiveness, in the incubation time required for replication, and in the degree of cell association. They may produce latent or persistent infections in man. But latency occurs in different target cells, and the stimulus for reactivation of each virus infection is different. The effect of immunosuppression on each virus infection also varies in terms of the frequency of activation of infection and the severity of clinical manifestations.

4.3. MORPHOLOGICAL EVENTS OF REPLICATION

The morphological development of CMV resembles that of other herpesviruses such as HSV with some outstanding differences (Table 4.1).

The adsorption and penetration of CMV take about the same time as those of herpes simplex virus, but subsequent replicative events are greatly prolonged (Smith and De Harven, 1973). Herpes simplex virus replicates and releases progeny virus in 8 hr, but CMV requires 4 days. Why CMV is so "slow" is not known.

In an electron micrographic study, Stern and Friedman (1960) showed that CMV, like HSV (Morgan *et al.*, 1959), is formed in the nucleus and that it acquires its envelope from the reduplicated nuclear membrane. Virus particles enter the cytoplasm from the nucleus by rupture of virus-containing intranuclear vacuoles, by formed invaginated membranes, or, more probably, by extension on the whole vacuole (see Section 4.3.2).

Herpes simplex virus causes condensation and margination of chromatin and has a greater tendency to produce membrane alterations. Cytomegalovirus, on the other hand, characteristically produces "dense bodies."

By *in situ* cytohybridization, in which tritiated RNA complementary to viral DNA was used to locate copies of viral DNA in cells by autoradiography (Fig. 4.2), Huang *et al.* (1976) found 24 hr after infection two acrocentric areas of DNA synthesis in the nucleus. This is significantly earlier than viral DNA patches first observed by acridine orange staining 48 hr after infection (McAllister *et al.*, 1963;

TABLE 4.1
The Timing of Major Morphologic Events in the Replication of CMV and HSV

Event	Time first detected after infection (hr)	
	CMV	HSV
Early cytopathology (rounding)	6–12	6
Alteration of Golgi apparatus	24	8
Viral eclipse	48	2
Nuclear immunofluorescence	24–48	5
Viral DNA patches in nucleus	48	4–7
Cytoplasmic Fc receptors and immunofluorescence	48	3–8
Condensation of chromatin	None	3
Capsids in nucleus	72	4
Viral DNA in cytoplasm	72	6
Naked capsids in cytoplasm	96	6
Dense bodies in cytoplasm	96	None
First released virus	120	8
Late cytopathology	120–168	24

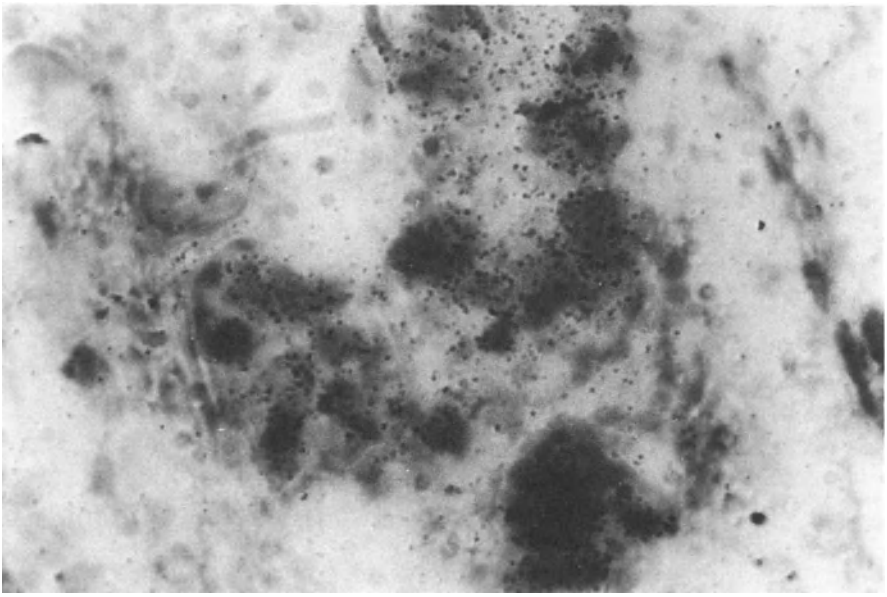

Figure 4.2. Complementary RNA–DNA cytohybridization *in situ* with CMV [^{3}H]cRNA applied to infected kidney cells. After fixation of cells, DNA was denatured by 0.1 N NaOH. Labeled cRNA was made from a purified CMV–DNA template. After hybridization in a moist chamber at 70°C for 20 hr, the tritium label was exposed for autoradiography. Grains signifying presence of CMV DNA may be seen concentrated in cell nuclei. (Huang *et al.*, 1976. Courtesy of Dr. Huang and by permission from the editors of *Yale Journal of Biology and Medicine*.)

Goodheart *et al.*, 1964). Seventy-two hours after infection, viral capsids can be demonstrated in the nucleus by immunoflourescence, and viral DNA synthesis can be demonstrated by autoradiography. This is followed at 96 hr by the presence of capsids and dense bodies in the cytoplasm. Free extracellular virus is first detected 24 hr later, or about 5 days after initial infection.

Severi *et al.* (1988) observed a great deal of interaction between viral progeny and the Golgi apparatus. On the basis of prior hypotheses (see above) and a consideration of cellular response to injury, they postulate three cytoplasmic routes taken by viral nucleocapsids. During passage from the nucleus, capsids acquire and lose a temporary envelope. In the cytoplasm, the naked viral particle may go through the Golgi region, egress directly through the cytoplasmic membrane with acquisition of an envelope, or end up as part of a dense body (see Section 3.3).

In the Golgi region, the capsid is sequestered in a vacuole that can (1) fuse with the plasma membrane and be released like a secretory product, (2) fuse with a lysosome—the resulting phagolysosome seldom seems to discharge its contents extracellularly—or (3) fuse with a dense particle, which is equivalent to a "black hole."

These observations concerning the Golgi apparatus and phagolysosomes are consistent with cell behavior in sublethal conditions (Trump and Arstila, 1975). They also apply to other herpesviruses except for the formation of the dense bodies, which is unique for human CMV.

4.3.1. Early Antigens and Cytopathology

Before viral DNA is synthesized, CMV produces in both permissive and nonpermissive cells a number of early antigens (see Sections 2.4 and 2.5), and an early cytopathic effect (CPE) is seen before any synthesis of viral DNA or structural protein. It is now clear that antigen may appear significantly earlier than the CPE. Michelson-Fiske *et al.* (1977) reported that when human diploid lung fibroblasts were infected at a multiplicity of infection (m.o.i.) of 1 PFU per cell and examined by indirect immunofluorescence, an antigen appeared 20 min after infection and disappeared by 45 hr. It was homogeneously distributed in the nucleus and was called an "immediate early antigen." Reynolds (1978) used human and specific animal sera against MCMV to detect an "immediate" early antigen in nuclei of infected cells 1 hr after infection by indirect and anticomplement immunofluorescence. The development of this antigen was not affected by actinomycin D but was inhibited by cycloheximide, an inhibitor of protein synthesis. He suggested that perhaps a virus-specific nonvirion antigen specified by the input virus was being produced. Detection of immediate early antigen is the basis of the current rapid shell-vial assay for CMV (see Section 5.1.1).

An "early" cytopathology observed by most authors is cellular rounding. For example, Furukawa *et al.* (1973) infected WI-38 cells at a m.o.i. of 50 and detected this type of CPE 6–12 hr later. This early CPE was prevented by neutralization of

the virus with antiserum but not by UV irradiation of the virus. Inhibitors of DNA synthesis, such as FUdR, BUdR, and ara-C, when applied to infected cells, did not prevent early CPE, but actinomycin D did. When convalescent human serum was used in a fluorescence test, a granular cytoplasmic fluorescence was seen as early as 3 hr after infection. Nuclear and perinuclear fluorescence appeared 6 hr after infection. These data suggest that one or more new proteins are synthesized before DNA, but transcriptive activity of input viral DNA is required (see Section 2.4).

Albrecht *et al.* (1980) studied the cytopathology of five laboratory strains of HCMV (AD169, C-87, Davis, Esp., and Kerr) in human embryonic cells derived from thyroid, skin and muscle, and lungs. Early CPE, consisting of cell rounding and early cytoplasmic inclusions, was observed 5 hr after infection. Whether these inclusions correspond to the development of early antigen described above is not clear. Only later, 72 hr after infection, do cytoplasmic inclusions develop into dense eosinophilic beads, most likely corresponding to the dense bodies and viral subunits that evolve from the nucleus, as described by most authors (McGavran and Smith, 1965; Kanich and Craighead, 1972).

Nuclear inclusions also undergo a sequence of development. Inclusion subunits were first observed with the C-87 strain 24–48 hr after infection and 48–72 hr after infection with the four other strains. Homogeneous dense nuclear inclusions were observed much later.

AD169-, C-87-, and Esp.-infected cells largely recovered from early CPE (cell rounding) 48 hr after infection, whereas Davis- and Kerr-infected cells did not recover until 72–96 hr after infection. Cells infected with the latter two strains exhibited reniform nuclei in contrast to the fibroblastoid morphology and oval nuclei of cells infected with the first three strains (see Section 5.1).

The location of immediate early (IE)1 proteins of CMV was investigated by fractionation and immunocolloidal gold electron microscopy (Otto *et al.*, 1988). As expected, both IE1 and IE2 were detected in the nucleus. The IE1 protein was associated with intracytoplasmic membranes and membranes of the endoplasmic reticulum 6 hr after infection. However, no IE antigen was found to be associated with plasma membrane or nuclear membrane. In view of the postulated importance of IE antigen in the recognition of specific cytotoxic response (Sections 3.8.2 and 7.2.3), this is relevant information. But by itself, it does not reject the hypothesis.

4.3.2. Nuclear Changes and Nucleocapsid Formation

Following infection, the nucleocapsid, the complete virus without its surrounding membrane, is synthesized in the nucleus along with viral DNA. Within 5 min of infection, Smith and De Harven (1974) detected the arrival of input CMV capsids by electron microscopy. In 20 min to 1 hr, immediate nuclear early antigens could be detected by immunofluorescence (see Section 4.3.1). At least some of the early nuclear antigens could be shown to be newly synthesized (Geder, 1976; Reynolds, 1978). Albrecht *et al.* (1980) detected by light microscopy early nuclear changes

with development of inclusion subunits 24 hr after infection. The full development of such inclusions does not take place until much later, usually about 96 hr after infection.

The biochemical assembly of nucleocapsids has been well studied and is described in Section 3.3.1. There is more uncertainty about how nucleocapsids become enveloped. Usually, no fully enveloped particles are observed in the nucleus, although both enveloped and naked nucleocapsids may be observed in the cytoplasm. The prevailing theory is that the envelope is acquired by the nucleocapsids from the nuclear membrane during egress from the nucleus (Stern and Friedman, 1960; Smith and De Harven, 1974). Severi *et al.* (1979, 1988), however, believe on the basis of electron microscopy that nucleocapsids bud into the enlarged cisternae, acquiring a temporary envelope, but that as they leave the cisternae and enter the cytoplasm, they lose the envelope. The capsids acquire a final envelope in the cytoplasm by budding into vacuoles or by becoming wrapped in lamellar folds (see Section 4.3).

4.3.3. Cytoplasmic Inclusions and Dense Bodies

Cytoplasmic inclusions were early noted to be hallmark of CMV infection (see Section 1.1). Cytomegalovirus produces perinuclear cytoplasmic inclusions, a unique feature among herpesviruses. Albrecht *et al.* (1980) saw evidence 5 hr after infection of early cytoplasmic inclusions that gradually evolved into the classical structural changes. McAllister *et al.* (1963) and Goodheart *et al.* (1964) noted that 24 hr after infection of human fibroblasts, unstained areas surrounded by an RNA-rich "halo" were noted in the juxtanuclear cytoplasm. By 72 hr after infection, DNA as well as free virus could be detected in the cytoplasm. They showed by autoradiography that this viral DNA was not synthesized in the cytoplasm. Viral DNA was only made in the nucleus. Presumably viral DNA in the cytoplasmic inclusions migrated from the nucleus.

Smith and De Harven (1979) reported that phenanthrenequinone (PQ) staining of WI-38 cells infected with hCMV revealed an intense fluorescence corresponding to the location of cytoplasmic dense bodies. Phenanthrenequinone is a nonfluorescent compound that reacts specifically with monosubstituted guanidines such as arginine to form a fluorescent condensation product. Since dense-body proteins contain viral structural proteins that fail to assemble into normal viral particles, one or more arginine-rich dense-body proteins may actually be structural proteins. This is consistent with findings with HSV, which do not form dense bodies but contain one or more arginine-rich structural proteins (Olshevsky and Becker, 1970).

After making electron microscopic observations, McGavran and Smith (1965) thought that the cytoplasmic inclusion was an aggregate of lysosomes and that the "dense bodies" observed in these inclusions were lysosomes. One piece of evidence offered was the pattern of distribution of lysosomal enzymes. Specific staining for

acid phosphatase in infected cells was three times that found in uninfected cells. They thought induction of lysosomal enzymes to be a unique feature of CMV infection.

Ruebner *et al.* (1964, 1965) observed dense bodies in both mCMV- and hCMV-infected cells and also thought they were lysosomes. They made the interesting observation that in the salivary gland of mice infected with mCMV, dense bodies commonly found in liver or spleen were not seen. They postulated that persistence of infection in the salivary gland may be related to the low lysosomal activity of this organ.

That dense bodies contained viral subunits and not just cellular substructures was first realized by Craighead *et al.* (1972). Dense bodies were described as homogeneous electron-dense materials, two to three times the diameter of enveloped viruses, which accumulate in cytoplasmic inclusions in association with virions. Cytoplasmic inclusions were homogeneous masses of dense bodies, viral particles, and microtubules. Dense bodies formed in proximity to microtubular membranes and the Golgi apparatus. Like nucleocapsids, they budded in tubules and acquired a limiting membrane demonstrable with periodic acid–methanamine stain. Dense bodies were considered to be proteinaceous substances that contained no acid phosphatase, lipids, or polysaccharides and to be unrelated to lysosomes. A significant finding was that by immune electron microscopy, specific antibody clumped both virions and dense bodies. Hence, the membranes of dense bodies possessed viral antigens. This view has been substantiated by more recent biochemical findings that dense bodies and viral capsids are composed of essentially the same structural proteins (Sarov and Abody, 1975; Fiala *et al.*, 1976; Kim *et al.*, 1976a,b). Labeling of the DNA of infected cells showed that dense bodies contain no DNA (Sarov and Abody, 1975).

The relationship of dense bodies and virions to lysosomes has now been further clarified in as more recent electron microscopic study by Smith and De Harven (1978). They agree with Craighead *et al.* (1972) that dense bodies are not lysosomes. But they found that enveloped capsids, naked capsids, as well as dense bodies acquired an envelop by budding directly into lysosomes, which contain acid phosphatase and arylsulfatase. It is unclear why Craighead *et al.* (1972) only observed dense bodies budding into nonlysosomal vacuoles. The problem raised by budding into lysosomes is why virions are not inactivated by intimate exposure to lysosomal enzymes. The same type of lysosomal budding may occur during replication of HSV (Smith and De Harven, 1978) and VZ viruses. In the case of VZ virus, perhaps as a result of exposure to lysomal enzymes, extensive degradation of virions does occur. This reaction has been thought to account at least in part for the difficulty of obtaining infective extracellular, non-cell-associated virions (Gershon *et al.*, 1973).

As pointed out in Sections 2.7 and 3.7.2, dense bodies are particles that do not contain viral DNA or nucleocapsid but consist of matrix and tegument structural proteins that have now been largely identified.

4.4. BIOCHEMICAL EVENTS

The rapid advances in our knowledge of the structure and expression of the CMV genome and its proteins have already been described (see Chapter 2 and 3). Here we describe some of the biochemical characteristics of the infected cell (Table 4.2).

4.4.1. Protein and DNA Synthesis

In general, subconfluent, actively growing cells yield more infectious virus than confluent, contact-inhibited cells. Cytomegalovirus is different from herpes simplex virus, whose replication is not cell-cycle dependent (DeMarchi and Kaplan, 1977). Although CMV takes 72 hr or longer to produce progeny virus, interesting biochemical events occur much earlier (Table 4.2). As mentioned, within minutes of hours after virus adsorption, viral antigen is synthesized, and early cytopathology develops in both permissive and nonpermissive cells, requiring RNA transcription and new protein synthesis.

Unlike certain cytopathic viruses, including herpes simplex virus, hCMV does not completely shut off host protein synthesis. This renders difficult the task of sorting out specific viral synthesis. According to Stinski (1978), protein synthesis following CMV infection may be divided into two stages. There is an early stage with a peak of synthesis that is predominantly (70–90%) host specific, although some specific viral proteins are also made. Specific viral DNA synthesis initiates a second stage of protein synthesis, which is predominantly viral, but 40–50% of the protein synthesized is still of host origin.

Much interest was evoked by the finding that CMV stimulates cellular DNA synthesis (St. Jeor *et al.*, 1974). This is because CMV produces latent infection and can transform cells to the malignant state (Albrecht and Rapp, 1973) and because stimulation of host cell DNA seems to be a common property of a number of oncogenic viruses, such as polyoma, SV-40, EB virus, but not herpes simplex virus. St. Jeor *et al.* (1974) observed that hCMV stimulated cell DNA synthesis in human

TABLE 4.2
Biochemical Events of CMV Replication

Event	Hours after infection
Immediate early protein synthesis	0–4
Early protein synthesis	2–20
Induction of DNA polymerase	20
Stimulation of cell DNA	20
Viral DNA synthesis and late protein synthesis	15–72
Synthesis of IgG receptors	36

embryonic lung cells or human embryonic kidney cells made permissive by pretreatment with IUdR. Nonpermissive Vero cells similarly treated could also be stimulated.

Furukawa *et al.* (1976) showed that in the presence of depleted medium, hCMV infection of WI-38 cells resulted in an increase in cell DNA synthesis 16 hr after infection. The increase was in synthesis of supercoiled mitochondrial rather than nuclear DNA. The significance of this observation is unknown.

Furukawa *et al.* (1975a) also showed that nonpermissive guinea pig skin muscle cells infected at a m.o.i. of 10 with the Towne strain of CMV had increased cellular DNA and RNA synthesis. The stimulatory effect was not eliminated when the infecting virus was treated with heat or UV irradiation.

The significance of the stimulation of cell DNA is still disputed. De Marchi and Kaplan (1976) do not think that it is essential for subsequent viral replication. By autoradiography, using [^{3}H]thymidine and specific immunofluorescence, they distinguished individual cells that were making cell or viral DNA. Relatively few cells (3–6%) were synthesizing both cell and viral DNA. Cells in which cell DNA synthesis was enhanced did not go on to make viral antigen. Virus stocks that were enriched by undiluted passages for noninfectious defective particles were better able to stimulate cell DNA. Hence, the observation that CMV stimulates cell DNA may be a "population" phenomenon more representative of abortive infections. This may be another problem produced by heterogeneity of CMV preparations. We may be ascribing to CMV properties that arise from the major noninfectious component of such preparations.

4.4.2. Induction of Enzymes

A number of herpesviruses induce new DNA polymerases in infected cells. This has been described with herpes simplex virus (Keir and Gold, 1963), Marek's disease virus (Boezi *et al.*, 1974), and pseudorabies virus (Halliburton and Andrew, 1976). With the exception of the polymerase induced by Marek's disease virus, the activity of such herpesvirus-stimulated polymerases can be enhanced by high salt concentration, whereas DNA polymerases native to the host cell are not affected.

Huang (1975a) reported that in WI-38 cells infected with hCMV, three host cell cytoplasmic and nuclear polymerases were stimulated, and in addition, a new polymerase was found. It was separated and purified from host enzymes by DEAE-cellulose and phosphocellulose column chromatography. It was enhanced by 0.03–0.06 M ammonium sulfate or 0.06–0.12 M sodium chloride.

Hirai *et al.* (1976) confirmed the induction of a new polymerase in the nuclei but not cytoplasm of infected WI-38 cells. The polymerase was induced 20 hr after infection, before stimulation of host cell DNA and viral DNA synthesis. It was not inhibited by DNA inhibitors but was blocked by cycloheximide, which suggests that it is an early protein coded for by input hCMV genome.

In an *in vitro* essay system, Huang (1975b) showed that his new polymerase

was specifically inhibited from incorporation of [^{3}H]thymidine by phosphonoacetic acid. Host cell DNA polymerases were only slightly affected. This is thought to be the basis of action of this antiviral drug, which inhibited hCMV DNA synthesis as measured by virus-specified nucleic acid hybridization. It did not affect the development of early CPE, which requires the synthesis of early proteins. The synthesis of virus-specific polymerase was not determined. Presumably it was unaffected. As described in Section 2.8.1, CMV viral DNA polymerase is a 140-kDa polypeptide associated with a 51- to 58-kDa polymerase-associated DNA-binding protein. It is an early β gene that lies between 0.31 and 0.33 map units (Fig. 2.4A).

Herpes simplex virus induces a number of other nonstructural enzymes besides DNA polymerase: thymidine kinase, deoxycytosine kinase, ribonucleotide reductase, and a DNase. Thymidine kinase (Estes and Huang, 1977; Zavada *et al.*, 1976) and ornithine decarboxylase (Isom, 1979) levels are also increased in CMV-infected cells. The production of the latter appears to be an early event detectable 12 hr after infection and does not require viral DNA replication. So far, only DNA polymerase has been found to be specified by hCMV. Thymidine kinases in infected cells were studied with respect to their phosphate donor specificities, pH optima, thermostability, and salt inhibition. No difference was found between kinases produced in infected and uninfected growing cells. Presumably hCMV, unlike HSV, does not induce a new thymidine kinase (Estes and Huang, 1977). This is relevant to the action of antiviral agents that specifically inhibit viral-induced thymidine kinase, such as acyclovir (Elion *et al.*, 1977) (see Section 14.1).

In addition to the above viral and cellular enzymes, a plasminogen activator is also increased in permissive human embryo fibroblasts or nonpermissive hamster embryo fibroblasts infected with hCMV (Yamanishi and Rapp, 1979). Plasminogen activator converts a serum plasminogen to plasmin, which in turn hydrolyzes fibrin. This enzyme is associated with transformation of cells by oncogenic RNA and DNA viruses and may be a marker for such transformation (Unkeless *et al.*, 1973; Ossowski *et al.*, 1974). Human CMV that was strongly UV-irradiated could still stimulate production of plasminogen activator. Such irradiated virus was previously shown to transform hamster embryo fibroblasts (Albrecht and Rapp, 1973).

4.5. STABILITY OF CYTOMEGALOVIRUS

Human CMV, like other cytomegaloviruses and all herpesviruses, is an enveloped virus that is sensitive to fat solvents and extreme physical conditions. It is sensitive to diethyl ether (Rowe *et al.*, 1956) and chloroform (Hamparian *et al.*, 1963) and is inactivated at pH 3 (Hamparian *et al.*, 1963).

Deibel *et al.* (1965) observed a 2-log-unit drop in titer after lyophilizing hCMV. However, when the hCMV was stored at 4°C, no further drop was observed for 3 months, Jung and Krech (1970) found no titer drop if the virus was lyophilized at 0°C in the presence of human serum albumin and calcium lactobionate. The titer of the virus was maintained if it was stored at −20°C but not at 4°C.

Choi *et al.* (1978) found a number of cytomegaloviruses to be sensitive to heparin. In the presence of 100 units of heparin in the culture, the titers of hCMV and mCMV were both reduced 1.5 logs. The titers of green monkey and guinea pig CMV were reduced 3.5 logs. As little as 0.1 units in culture medium reduced the titer of guinea pig CMV by 1.0 log. When heparin was added to culture medium after adsorption had taken place, reduction was negligible. Heparin seemed to act directly on the virus. Human CMV was one of the more resistant cytomegaloviruses, which is consistent with the fact that heparin has been used extensively as an anticoagulant in buffy coat samples to be tested for CMV (Lang *et al.*, 1968).

4.5.1. Stability of Cytomegalovirus at Different Temperatures

In addition to the usual lability of viruses at high temperatures, the stability of hCMV preparations varies strikingly at a given temperature depending on conditions of storage and the source of the virus.

Hildebrandt *et al.* (1968) preserved CMV in liquid nitrogen for 3 years. Storage at −60° to −80°C was also satisfactory, particularly if 25–35% sorbitol was added (Weller and Hanshaw, 1962). Feldman (1968) observed that storage at −20°C resulted in complete loss of infectivity within a few days. With addition of sorbitol, such loss could be delayed for a few months. At 4°C CMV was stable for several days.

A number of investigators found that hCMV was more labile at 4°C than at higher temperatures. Krugman and Goodheart (1964) observed that the virus was rapidly inactivated at 37°C but that the inactivation curve was erratic at 23°C. Intracellular virus in infected human lung fibroblasts released by sonic disintegration was less stable at 4° and 10° than at 22°C, according to Plummer and Lewis (1965). Extracellular virus was not tested. There was a plateau of the rate of inactivation at 36° and 22°C lasting about 2–6 hr during which no loss of viability was detected.

Vonka and Benyesh-Melnick (1966) also reported CMV was labile at 4°C. The following strains diluted in Tris buffer were inactivated at 4°C with increasing order of lability: AD169, Davis, Kerr, and C-87 strains. The AD169 strain was more or less stable for 4 hr, after which time more than 90% of Kerr and C-87 strains were inactivated. Distilled water stabilized the virus at 4°C. Calf serum stabilized the virus at 37° but not at 4°C. Virus was unstable at 4° and 37°C in either Tris buffer or Eagle's medium with bicarbonate. Virus harvested early (day 5) was ore labile at 37° and at 4°C than virus harvested late (day 11).

Somewhat different results were obtained by Wentworth and Gloyd (1968). The Davis, UW1, and UW2 strains grown in human embryonic tonsil fibroblasts were diluted either in distilled water or in transport medium (TM) containing trypticase soy broth and bovine albumin. Rates of inactivation at 37°, 25°, or 3°C were about the same. The virus was somewhat more stable at these three temperatures when suspended in water. But whereas Vonka and Benyesh-Melnick (1966) noted

90% or more inactivation of CMV at 4°C in 1–4 hr, this degree of inactivation took about 4–8 days with virus in TM. Virus in water maintained its titer for 7–10 days without reduction. At 37°C, there was reduction of about 1 log per day. Storage at 23° for 4 days essentially produced no inactivation.

There is no ready explanation for all these discrepancies. The salt concentrations and pH of the suspending medium were clearly important. But differences were also noted when the virus was suspended in water. Since purified virus was not used in any of these studies, varying amount of cellular contaminants may have been present in the virus preparations. Cytomegalovirus is closely associated with lysosomes (see above, Section 4.3). Such enzymes may be present in virus aggregates or in virus preparations. They may also vary in concentration depending on the stage of virus replication in cells, which might explain why Vonka and Benyesh-Melnick (1966) found that virus harvested early was more labile than that from late harvests.

4.5.2. Stability of Cytomegalovirus in Urine or Blood

In contrast to the unpredictable stability of crude virus suspension from cell cultures, hCMV is quite stable in urine. Feldman (1968) found that the titers of hCMV in samples of urine to which only 0.2 ml of an antibiotic "cocktail" had been added remained essentially stable for 10 days at 4°C. In the only case where there was precise documentation, a 0.5-TCD_{50} reduction in urine titer took place in 9 days, and 1.0 log in 16 days.

Suspension of urine in 25% sorbitol did not improve stability at 4°C but did prolong infectivity at −20°C. Sorbitol also failed to help at −60°C, where there was about a 1.5-log decline in 87 days. In a study on rapid diagnosis of hCMV in the urine of infants, Lee *et al.* (1978) stored or shipped specimens for several days at 4°C without loss of activity.

The stability of this virus at 4°C in urine contrast markedly with the lability of cell-culture-passed material at this temperature. Cytomegalovirus is cell-free in urine, which again suggests that cellular material is the basis of rapid inactivation in crude suspensions. Urine is normally free of protein and presumably also of hydrolytic enzymes.

Armstrong *et al.* (1971a) demonstrated that AD169 tissue-culture-passed virus persisted in cell culture medium containing 5×10^6 human red cells per ml for 21 days. At both 4° and 25°C, there was no decay in titer over a 5-day period. Foster and Jack (1968) isolated hCMV by inoculating white cells of a blood sample on fibroblast cultures. The sample came from a blood donor whose blood was given to a patient who developed CMV mononucleosis. After standing for 6 days, the sample again yielded virus, but the amount was less. No virus could be isolated 24 days from the time the sample was taken. Despite a great deal of interest in transmission of CMV by fresh and stored blood, the stability of cell-associated and free virus in blood has not been adequately studied (see Section 9.7). The virus in

viremic blood is probably cell associated and may be more stable than free virus. Earlier studies on the risk of transfused blood emphasized that fresh blood was a greater risk, but this is now not thought to be a significant factor (see Section 6.6.1).

McKeating *et al.* (1987) reported that β2 microglobulin (β2-m) may be bound to CMV virus particles in urine. Coated CMV could not be neutralized by hyperimmune globulin or monoclonal murine antibodies that could neutralize CMV grown in cell culture. β2-microglobulin is a non-polymorphous component of HLA class I antigen commonly found in body fluids. The virus may evade the immune system by binding to it (Grundy *et al.*, 1989a, 1988a,b).

5

Virological Diagnosis and Infections in Cells and Tissues

5.1. VIROLOGICAL DIAGNOSIS OF CYTOMEGALOVIRUS INFECTION

In this section we discuss practical approaches to the virological diagnosis of CMV, that is, the demonstration of CMV in clinical samples by isolation of the virus or demonstration of its nucleic acid or antigens in tissues and cells. Practical knowledge is emphasized. The scientific background of this material is found in other sections, especially from Chapters 2 to 4. Technical details are not presented fully but may be found in other texts (Reynolds *et al.*, 1979b).

Cytomegalovirus infection is diagnosed by isolation of the virus or by demonstrating its presence by serologic or molecular techniques. Serologic diagnosis (see Chapter 6) is accurate and specific but may be of limited usefulness in acute medicine because the required changes in antibody state or titers may take too long to demonstrate.

5.1.1. Routine Virus Assay

The virus may be recovered from body fluids or from tissues obtained by biopsy or at autopsy. Human CMV has been isolated from urine, blood, throat washings, saliva, tears, milk, semen, stool (Cox and Hughes, 1974), and vaginal or cervical secretions. In the clinic or hospital, where symptomatic CMV infections occur in the neonate or in the normal or immunosuppressed adult, urine, blood, or throat specimens will yield the virus, although a positive culture may not be sufficient for diagnosis.

Almost all clinically significant CMV infections are associated with viruria

despite the fact that symptomatic urinary tract infection or renal failure is unusual. Viruria at birth is the essential and sufficient finding for the diagnosis of congenital CMV infection. Urine in 0.2-ml amounts can be directly inoculated in human fibroblast cultures. Occasional urine samples may be toxic for cells. To obviate this, the culture medium can be changed 24 hr after inoculation.

Viruria may be an incidental finding in older children and occasionally in adults. However, except in immunosuppressed individuals, and occasionally in sexually active females and male homosexuals, it is not common in adults.

Viremia is often associated with CMV mononucleosis and other forms of CMV infection in the adult, particularly in the immunosuppressed patient after transplantation or with AIDS. To collect "buffy coat" specimens, 10 ml of blood is collected in a tube containing 0.1 ml heparin (100 U/ml). It is then centrifuged at 400 × *g* for 15 min, and about 1×10^6 cells of the leukocyte-rich "buffy coat" are inoculated into tube or well cultures.

For throat cultures, a dry cotton swab is rubbed over the inner side of both cheeks opposite the upper molars and over the posterior pharynx and floor of the mouth. The swab is then promptly rinsed in 2 ml of culture medium and inoculated.

All samples submitted to the laboratory for virus isolation should be processed within a few hours after collection. As indicated above (Section 4.5), the virus is surprisingly stable at 4°C or at room temperature in urine and probably in other tissue fluids. For storage, −70°C is preferable to −20°C, at which temperature many enveloped viruses are inactivated.

Human CMV does not grow adequately in any laboratory animal. Nigida *et al.* (1975) found that 10^7 PFU of CMV stimulated antibodies in white-lipped marmosets, but no infectious virus was produced. Virus could be recovered by cocultivation of the kidney tissue. This animal, even if generally available, cannot be used for diagnostic purposes. All specimens submitted for virus growth or identification must be inoculated in cell cultures.

Practically speaking, the only cells that should be used in the diagnostic laboratory to grow CMV are human fibroblasts. Fibroblast cultures may be obtained as primary cultures prepared from foreskin or embryonic skin and muscle, lung, testes, or myometrium. Serially propagated diploid fibroblast cell strains, either developed in one's own laboratory or obtained commercially, such as MRC-5, WI-38, or MA-184 human embryonic fibroblasts derived from lung or foreskin, are satisfactory. Low-passage (10–24) cultures are more susceptible.

Fibroblast cultures inoculated with virus-containing material may show CPE in 3–4 days but usually in 1–2 weeks or longer, depending on the concentration of virus in the specimen. There is evidence that MRC-5, a fibroblastic cell line that has a normal diploid karyotype and was derived from male embryonic lung, is more satisfactory than WI-38 because cytopathic effects were observed earlier. Gregory and Menegus (1983) tested clinical specimens of urine, cervical vagina specimens, and salivary specimens. Of all positive samples, 98% were positive in MRC-5 cultures, and only 85% were detected in WI-38 cells. In addition, all MRC-5 cultures became positive after 2 weeks of inoculation, but by that time only 88% of the eventually positive WI-38 cultures were positive.

Smith (1986) showed that replication of CMV was not restricted to cell cultures of fibroblast lineage. A well-differentiated diploid epithelial cell line, HCMC, derived from normal human colonic mucosa, replicated CMV to titers equal to that obtained in MRC-5 fibroblast cells in about the same time with production of cytopathology. It was concluded that the status of differentiation and chromosomal ploidy were more important factors. Thus, WI-38 VA13, a hyperploid (chromosome number 73 to 78) cell line derived from SV-40 transformation of the CMV-permissive WI-38 euploid fibroblast cell line, was no longer permissive.

Initially, foci of enlarged, rounded refractile cells will appear in the center rather than the periphery of the cell monolayer (Fig. 5.1). Affected cells characteristically follow the linear pattern in which fibroblasts align themselves. These foci will enlarge, coalesce, and may eventually destroy the monolayer, a process that may take 2–3 weeks. At times, foci of infection will enlarge but never involve the entire monolayer unless the infected cells are passed. Occasionally, CPE will not be produced in the originally inoculated cultures. When such cultures are trypsinized and planted on a fresh monolayer, CPE may occur. The pattern and progression of cytopathology are different from those of herpes simplex and VZ viruses. The CPE of herpes simplex appears sooner and progresses more rapidly. It may be visible in 24–48 hr, and cell destruction may be complete in 3–4 days. Varicella–zoster virus, on the other hand, produces CPE at an intermediate rate. Varicella–zoster virus may also grow in human epithelial cell cultures in addition to fibroblasts, and in the laboratory, the virus cannot be maintained in the cell-free state.

As discussed in Section 4.3, two prototype laboratory strains of CMV, Davis and AD169, produce somewhat different cytopathic effects. The Davis, Esp., and Kerr strains as well as most clinical isolates cause early cell rounding. The nuclei are pushed to the periphery of the cell and are often distorted. A single prominent eosinophilic paranuclear inclusion develops (Fig. 5.2). In contrast, AD169-infected cells retain their fibroblastic shape. Nuclear morphology is maintained except for margination. Cytoplasmic inclusions are less distinct and are often bipolar (Fig. 5.3).

The cytopathology of CMV is usually sufficiently characteristic for identification in the laboratory without further serologic confirmation. When there is doubt, antigen may be prepared from the infected culture for identification by the complement-fixation (CF) test using known antiserum, or the neutralizing capacity of a known antiserum on the unknown virus may be tested. Alternatively, the isolate may be cultured in a shell vial and identified by demonstration of CMV early antigen using commercially available monoclonal antiserum against this antigen (see below).

5.1.2. Virological Diagnosis in the Presence of Antibody

One of the hallmarks of CMV infection is persistence of the virus in various body fluids despite the development of serum neutralizing antibodies (see Sections 11.2 and 12.3). One possible reason is the protective role β_2-microglobulin might

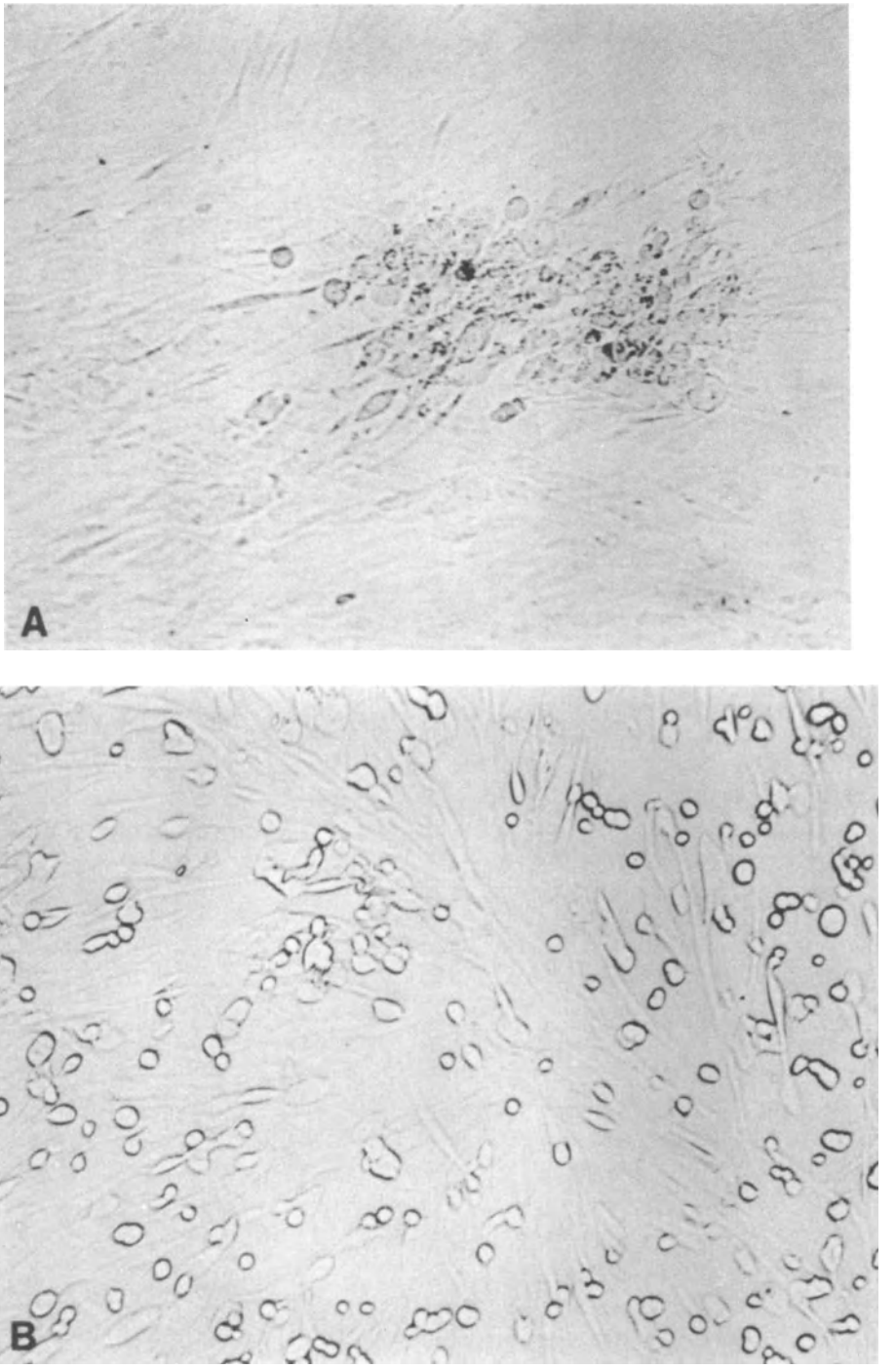

Figure 5.1. Cytopathic effect of clinical isolates of hCMV in human foreskin fibroblasts (unstained preparations). (A) Advanced focus 14 days after inoculation. (B) Specimen with high concentration of virus, 5 days after inoculation. (Reynolds *et al.*, 1979a, in Lennette and Schmidt, 1979. By permission of the authors and the American Public Health Association.)

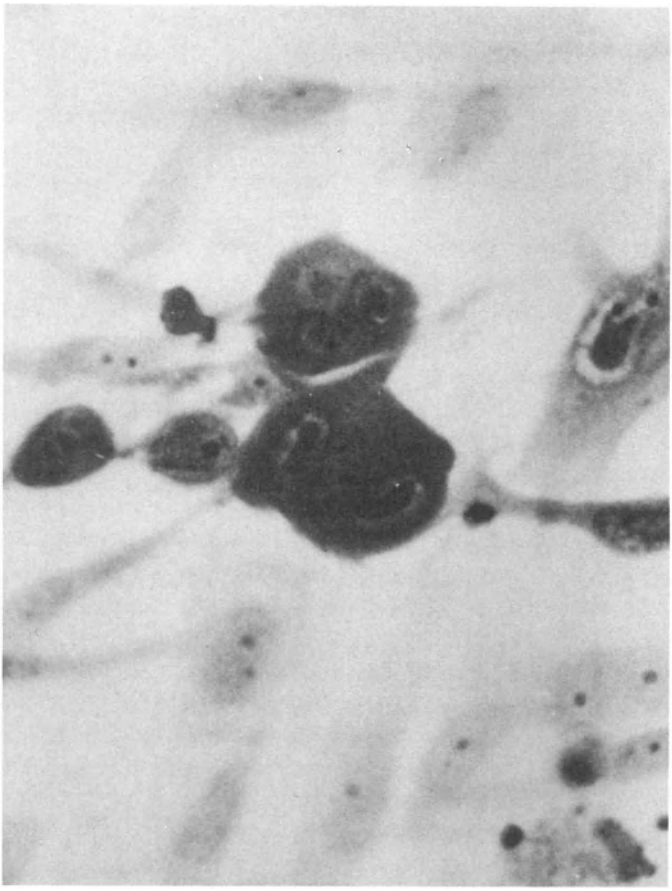

Figure 5.2. Cytopathic effect of the Davis strain of hCMV in human foreskin cultures. Hemotoxylin–eosin stain, ×500. (Reynolds *et al.* 1979a, in Lennette and Schmidt, 1979. By permission of the authors and the American Public Health Association.)

play against serum neutralization (McKeating *et al.*, 1987) (Section 4.5.2). In CMV mononucleosis, whether arising spontaneously or after transfusion, and particularly in CMV infection in the immunosuppressed patient, viremia may persist for months despite the presence of serum antibodies (Stulberg *et al.*, 1966; Lang and Hanshaw, 1969). There have been studies on the coappearance of CMV and antibodies in other body fluids. Cytomegalovirus viremia is common in patients with AIDS, who are almost invariably seropositive for CMV.

Tamura *et al.* (1980a) studies saliva for the presence of neutralizing antibodies against hCMV. A microculture plaque assay without complement was performed. Out of 54 infants from 3 months to 3 years of age who were viruric or seropositive, 41 (75%) had neutralizing antibodies in their saliva despite presence of CMV in the

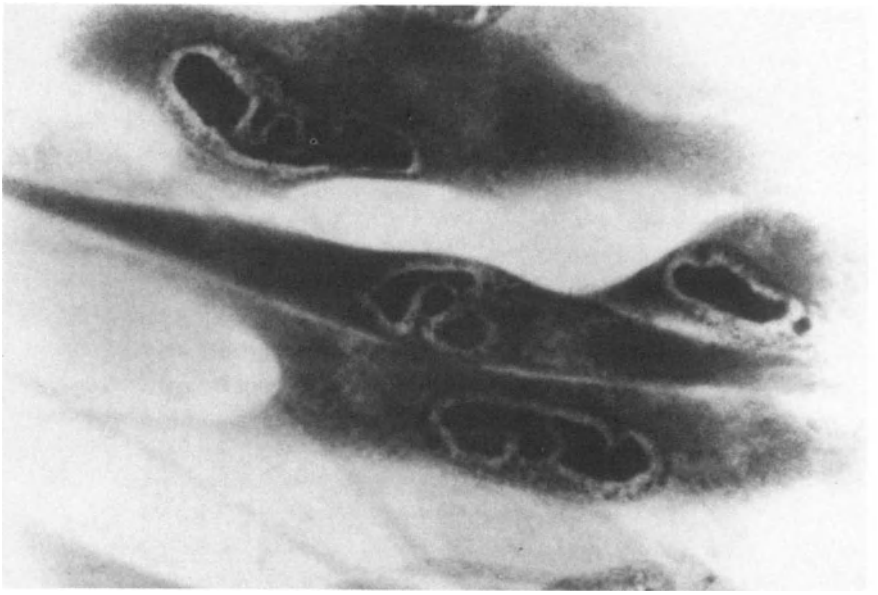

Figure 5.3. Cytopathic effect of the AD169 strain of HCMV in human foreskin fibroblasts. Hematoxylin–eosin stain, ×500. (Reynolds *et al.*, 1979a, in Lennette and Schmidt, 1979. By permission of the authors and the American Public Health Association.)

saliva. No antibodies to virus were found in the saliva of 11 seronegative controls. The neutralizing titer of saliva was 1 : 4 to 1 : 32, whereas corresponding serum neutralizing titers were 1 : 32 to 1 : 512. The amount of antibody in saliva was apparently inadequate to neutralize the virus.

Waner *et al.* (1977) studied 102 specimens of cervical secretions for IgG and IgA antibody to CMV detected by immunofluorescence. Specimens from six of ten patients who were excreting CMV contained IgG antibody. Four of the six samples also contained IgA antibodies against CMV. Only three samples positive for IgG antibodies were from patients who were not virus excreters, although it is possible that viral excretion might have been detected if more samples were cultured from these patients. As in saliva, the presence of antibody in cervical secretion may be associated with the presence of virus.

5.1.3. Rapid Virus Assay

The availability of effective antibodies against CMV antigens has stimulated many methods to circumvent the observation of cytopathology in inoculated cultures. Using a pool of seven CMV-specific monoclonal antibodies determined by

immunofluorescence, Griffiths *et al.* (1984) detected CMV in human embryonic lung fibroblasts grown in slide chambers 4 hr after inoculation of specimen from immunosuppressed patients. This method resulted in identification of CMV in 12 of 15 culture-positive specimens and a sensitivity of 80%. There were no false positives among 115 specimens that were negative by conventional assay.

The currently popular rapid assay method for CMV depends on early recognition of infected cells based on the detection of early antigen and centrifugal absorption of the virus to increase sensitivity. Susceptible cells such as MRC-5 are seeded and cultured in 1-dram shell vials containing a 12-mm round coverslip until use. Before inoculation the medium is removed from the shell vial and an aliquot of a specimen is added on the monolayer and cytospun at 500–700 $\times$ *g* for 45 min to 1 hr. After as early as 16 hr of incubation at 36°, a monoclonal antibody to early antigen, such as pH 2.4, is added to the coverslip preparations, and antigen-containing cells are detected by indirect immunofluorescence or enzyme-linked detection (Shuster *et al.*, 1985).

The appropriate centrifuging step is essential for increased adsorption of the virus (Osborne and Walker, 1968; Hudson *et al.*, 1976) (see Section 16.2). Comparative experiments showed that uncentrifuged specimens showed fewer immunofluorescing foci, and specimens spun at 1500 $\times$ *g* gave confusing results because of cell damage.

In an early evaluation of this method Gleaves *et al.* (1985) tested 770 specimens of urine, blood, lung tissue, and sputum at the Mayo Clinic. Of 124 isolates detected by this rapid method, only 88 or 71% were recovered by the conventional cell culture method in which the inoculum was not cytospun and virus was detected by cytopathology. In addition to higher sensitivity, this method yielded results more rapidly. Immediate early antigen in infected cells can be detected as early as 3 hr after adsorption of laboratory-adapted strains such as AD169 strain, and from 6 to 12 hr after inoculation with clinical specimens containing the virus. Routinely most specimens are incubated 16 hr or overnight after inoculation.

More recently, Janssen *et al.* (1988) compared the conventional virus isolation method with the rapid assay of low-speed centrifugal inoculation followed by early antigen detection by IFA. The monoclonal antibody against immediate early antigen (72 kDa) (Shuster *et al.*, 1985) was used. The monoclonal antibody was applied after 1 (EA-1) and 6 (EA-6) days of cultivation. Of 68 positive samples, 49 (72%) were detected with EA-1, 58 (85%) were detected by EA-6, and only 43 (63%) were detected by conventional virus isolation. It is interesting to note that no method covered all the positive specimens. However, the combination EA-1 and EA-6 showed positive results with 66 samples (97%), which was significantly better than conventional virus assays. There were only two samples that were positive by conventional assay and negative by early antigen detection. It is possible that a small number of isolates do not express the same early antigen as that detected by the monoclonal used. Still, it is clear that the early antigen method is a significant advance in that it has produced a rapid test without losing much in sensitivity.

5.2. DIRECT DEMONSTRATION OF CMV BY MONOCLONAL ANTIBODIES

With the demonstration that potent antisera against CMV EA could detect both lytically and latently infected cells, monoclonal antibodies have also been used for direct tissue diagnosis.

Cytomegalovirus is a common cause of pneumonia, particularly in bone marrow transplant patients, and this is an example of a clinical condition that requires rapid diagnosis (see Section 13.3.1). The diagnosis is usually made by culturing CMV from tissue specimens or by demonstrating characteristic inclusions in histological sections of the lung. Recently the use of bronchoalveolar lavage (BAL) has become a common method in the diagnosis of nonbacterial causes of pneumonia when sputum and expectorate specimens or biopsy specimens are not available. Emanuel *et al.* (1987) showed a correlation between the presence of CMV-specific early antigen (EA) in alveolar macrophages obtained from BAL specimens obtained from patients with interstitial pneumonia and the histological or cultural diagnosis. Martin and Smith (1986) diagnosed CMV infection in 19 subjects using a monoclonal antibody to detect immediate early antigen of CMV in BAL specimens. Direct cytospun preparations from BAL yielded positive results in six (31.6%). When specimens were inoculated by centrifugation on MRC-5 cell cultures and cultured, 94% (18/19) were positive when tested with the monoclonal antibody. In contrast, conventional tube cell culture methods were positive in only 11 of the 19 subjects (58%), and these results were obtained after an average of 9.3 days. These results show that although the rapid culture assay was more sensitive than routine cultures, diagnosis using direct cytospun specimens from BAL without incubation was less sensitive.

In a prospective study Paradis *et al.* (1988) studied the comparative sensitivity and specificity of three methods of diagnosing CMV pneumonia by using BAL specimens. These were routine culture, direct immunoperoxidase detection of early antigen, and histological evidence of inclusions. Virus was isolated in BAL specimens from all patients with CMV pneumonia (sensitivity: 100%), but some specimens from patients who did not have CMV pneumonia were also positive (specificity: 70%). Cytomegalovirus inclusions were found in three of 15 specimens from patients with CMV pneumonia (sensitivity: 21%) and in one patient who did not have pneumonia (specificity: 98%). Thus, the positive culture and positive cytology virtually confirmed CMV pneumonia, and the negative culture excluded it. Direct immunoperoxidase detection proved to be particularly useful when the culture was positive and the cytology negative. This situation-specific labeling of CMV early antigen from monoclonal antibody was found in nine of 11 patients with confirmed CMV pneumonia (sensitivity: 82%).

In conclusion, it appears that the rapid assay using cytospin inoculation and detection of the 72-kDa early antigen with monoclonal antibody is a rapid, sensitive, and specific method for detection of CMV. It is possible that a small number

of CMV-positive specimens would go undetected if routine cytopathology were discarded. However, the numbers are small, particularly if cultures are evaluated after 1 and 6 days of incubation.

5.3. DEMONSTRATION OF CMV BY NUCLEIC ACID HYBRIDIZATION

Nucleic acid hybridization can detect viral genomes (DNA) or messenger RNA in cells and tissues. In addition to detecting the presence of complete lytic virus, it may be capable of detecting viral genomes in latently or abortively infected cells. *In situ* hybridization detects either the genomic or DNA or RNA transcripts in individual cells of tissues. The usefulness of this method has been suggested by the finding that cells and circulating leukocytes may contain either CMV DNA or mRNA (see Section 5.5.3). The question whether nucleic acid hybridization offers advantages over other methods of virus assay must be precisely posed. When only complete lytic virus is probed, the assay is unlikely to be more sensitive than conventional techniques. Chou and Merigan (1983) compared CMV DNA hybridization with routine virus assay in urine specimens. They ultracentrifuged the urine, immobilized the sample on nitrocellulose filters, and hybridized it with ^{32}P-labeled cloned probes consisting of *Eco* RI restriction fragments of CMV strain AD169. Although the test could be performed rapidly in 24 hr, it was less sensitive than virus culture. Only 34 of the 48 culture-positive specimens were positive by DNA hybridization, and samples with virus titers less than 500 infective units were negative. The specificity was excellent. So Spector *et al.* (1984a) probed clinical urine specimens with ^{32}P-labeled cloned fragments of CMV strain AD169. They reported a better sensitivity (92%, 22 of 24 culture-positive specimens) and a specificity of 88% (23/26). In a prospective study of 67 blood buffy coat specimens from bone marrow recipients, 13 (93%) of 14 culture-positive samples were detected. Interestingly, out of 53 culture-negative specimens, 21 were positive by hybridization. It was suggested that 20 of these 21 specimens represented infections that were not detected by culture but were detected by hybridization. This may be related to circulating latently infected white blood cells detected by probing for immediate early antigen (see Sections 5.5.3 and 9.7.3).

Myerson *et al.* (1984) evaluated *in situ* hybridization for the diagnosis of CMV pneumonia. Biotin-labeled CMV DNA was hybridized to formalin-fixed tissues and detected by an immunoperoxidase procedure. The sensitivity of this technique was similar to that of viral culture and of viral antigen detected by monoclonal antibody. Results could be obtained quickly, and fixed tissues with no culturable virus particles could be used. Another bone marrow transplant group (Churchill *et al.*, 1987) used homogenates of lung tissue immobilized on nitrocellulose filters that were hybridized with ^{32}P-labeled CMV DNA probes. Compared with virus cultures, the hybridization assay had a sensitivity of 91% and detected 520 infectious unit equivalents. However, the specificity of the assay was only 78%.

In summary, nucleic acid hybridization methods are less sensitive than cultural methods for detecting complete lytic virus. They are specific and may be more rapid. They may also detect the presence of latent virus, but accuracy and usefulness remain to be shown.

5.3.1. Polymerase Chain Reaction

The polymerase chain reaction (PCR) amplifies minuscule amounts of DNA in a clinical specimen and makes them detectable. It takes advantage of a heat-stable DNA polymerase, which assembles a complementary strand from a defined segment in a strand of DNA, a primer. In order to do this, PCR requires first that a double-stranded DNA be denatured. Then the primer segment is added, annealed to a denatured DNA strand, and extended in the presence of DNA polymerase. Saiki *et al.* (1988) described a system for amplifying and concentrating specific sequences as much as 10^6-fold. It has become a powerful tool and has facilitated the detection, characterization, and sequencing of increasing numbers of viruses, including human papilloma virus, HIV, HTLV-1, HTLV-II, and lentiviruses (Schochetman *et al.*, 1988). Shibata *et al.* (1988) detected CMV DNA in the blood of 14 of 27 patients with HIV infection or AIDS. It was more sensitive than standard culture assays and used only 20 μl of blood. Interestingly, no CMV DNA was detected in the blood of healthy seropositive individuals. This is surprising in view of the reports that mononuclear cells of normal seropositive subjects contain CMV transcripts (Schrier *et al.*, 1985; see Section 5.5.3). Demmler *et al.* (1988) reported results of testing urine samples from 44 congenitally infected infants. Synthetic oligonucleotide primer pairs were used to amplify DNA from the immediate early and late antigen regions of the CMV genome. Amplified products were detected by dot–blot hybridization. When compared with tissue culture, sensitivity and specificity were 100%. Detection by gel electrophoresis provided a sensitivity of 93%. Both IEA and LA primers were needed to detect virus in all 46 samples.

Hsia *et al.* (1989) selected for amplification fragments of 130 and 152 base pairs (bp) at two opposite ends of *Eco* RI fragment D of the AD169 strain of CMV. This fragment is transcribed early and also late in abundance. After amplification of the 152bp DNA, only one CMV-infected fibroblast cell or 0.01 pg of fragment D DNA was detected. All 35 culture-positive urine samples were detected, and two culture-negative samples were positive only by PCR. Urine samples from these two patients also became positive later, suggesting that PCR was more sensitive than routine cultural methods.

The full potential of PCR has not yet been realized. One of the major problems in the CMV field is the difficulty of predicting transmissibility of CMV from the blood or tissues of seropositive subjects. Blood from seropositive donors does not always transmit CMV (see Sections 9.7.1 and 13.1.1). A similar problem exists in the case of transmission of CMV by donor organs or tissues in transplantation (Section 13.1.1). It is possible that an appropriate type of quantitative PCR or

reverse transcriptase PCR in which RNA transcripts rather than DNA are amplified may be helpful. It is also possible that in detecting blood, blood products, or other tissues that might harbor transmissible CMV, seropositivity of the donor may also miss certain cases of occult infection. Stanier *et al.* (1989) reported that five blood samples from 25 PCR-positive specimens came from seronegative individuals.

5.3.2. Immediate Early Antigen Transcripts

Stocke *et al.* (1988) evaluated *in situ* hybridization with a probe specific for immediate early antigen (IEA) (EcoRI J fragment of AD169) for detection of CMV RNA transcripts in peripheral blood mononuclear cells (PBM).

When 84 out of 212 samples of PBMs were positive for CMV by conventional cultural methods, only 57 were positive by hybridization (sensitivity of 68%). On the other hand, 34 of the 128 culture-negative samples were hybridization-positive. Some of these may have represented latent infection in normal subjects (Schrier *et al.*, 1985; see Section 5.5.3), but others may have represented significant infection before detection by cultural methods was possible. The authors suggest that quantitative evaluation of hybridization results may be useful and that "five positive cells in a given sample always correlated with productive infection." Unfortunately, no supporting data are provided.

5.4. DEMONSTRATION OF CMV BY OTHER METHODS

For rapid diagnosis, a drop of urine or urinary sediment may be smeared on a slide and fixed for 20 min with equal parts of ether and alcohol. After being stained with hematoxylin and eosin, Giemsa, or Papanicolaou stains, the slide is examined for inclusion-containing cells (Fetterman, 1952) (Fig. 5.4). This simple early procedure may be used in suspected cases of congenital infection or cytomegalic inclusion disease, but it is not now in general use. It lacks both sensitivity and specificity.

Electron microscopy has been used as a rapid and reliable method of diagnosis for certain types of virus infections, notably, smallpox, rotaviruses, Norwalk agents of diarrhea, and hepatitis A and B viruses (Schmidt and Emmons, 1989). The feasibility of diagnosing CMV infection in infants by electron microscopy was studied by Lee *et al.* (1978). Examination for CMV in urine after preparation of the specimen by the "pseudoreplica" technique was completed in 15 to 30 min. This technique permits tenfold concentration of the specimen. When the virus concentration in the urine was greater than 10^4 PFU/ml, this method was consistently reliable. However, if the virus concentration was lower, or if a distinction from other herpesviruses (such as herpes simplex virus) was required, then it was less satisfactory. The specificity of this method may be improved by immune electron microscopy. These methods are not in general use.

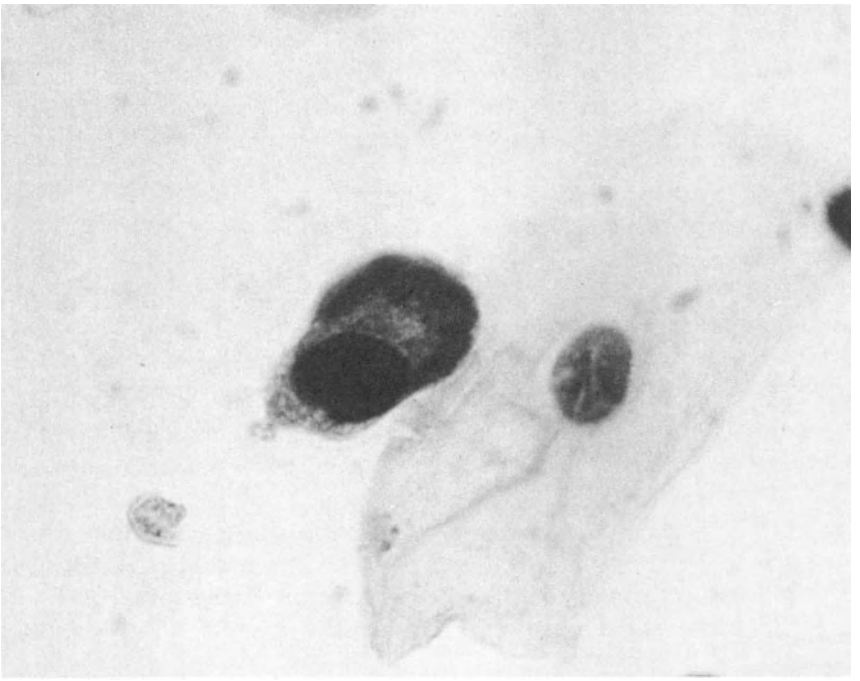

Figure 5.4. Urine sediment of a 3-week-old male infant with C.I.D. Left: Typical cytomegalic cell with intranuclear inclusion occupying entire nucleus with no halo around inclusion but with perinuclear chromatic margination. Note diffuse, amorphous cytoplasmic inclusion. Cell on right is a squamous epithelial cell.

Antigenemia has been tested for its ability to be a surrogate for positive blood cultures. Van der Bij *et al.* (1988) used a mixture of different monoclonal antibodies to detect antigenemia in patients with viremia. Inclusion of antibodies against IEA was found to be essential for sensitivity. They assessed 139 blood samples from 15 transplant patients. Cytomegalovirus viremia was documented in 23 samples, and antigenemia was found in 44 samples. All positive samples were from nine patients diagnosed as having active CMV infection. Twenty-one of 23 viremic samples were also antigenemic. Of the remaining 23 antigenemic samples, 20 came from the previremic phase of acute disease, and three came from two patients with serologic evidence of infection. These results suggest that direct detection of CMV antigens in a cytospin preparation of peripheral leukocytes is specific and possibly more sensitive than routine blood cultures, particularly before the onset of viremia. The procedure is also rapid and potentially more cost effective. Positive cells were mostly polymorphonuclear cells with nuclear or perinuclear staining (see also Section 5.5.3). Support for these conclusions have been provided by Wunderli *et al.* (1989) and D'Antonia *et al.* (1988). In both cases cells containing IEA were de-

tected in viremic patients, frequently before the onset of viremia. This method should be applied prospectively to test its clinical utility.

5.5. ABORTIVE, PERSISTENT, AND LATENT INFECTIONS IN CELL CULTURES

The study of CMV infection in cell cultures is important for the detection of virus in clinical samples and for the quantitative assay of the virus. Infections in cell cultures may also be a model for the study of infection in the host. This is particularly applicable to the problem of persistence and latency of CMV. These terms are defined operationally in this book as follows.

Lytic infection indicates infections in which complete virions are produced during a lytic replicative cycle and cells are destroyed while exhibiting cytopathology. Persistent infection indicates the occurrence of lytic infection and virus production in a cell culture, but where cytopathology is incomplete and there is simultaneously enough cell regeneration for the culture to survive indefinitely.

Abortive infections indicate infections in which no virions are produced and the virus replicative cycle is not completed. Abortive infection may be a feature of latent infection. Some viral genes, for example, early antigens, may be expressed.

Latent infection indicates that cells are infected without production of virus. There may or may not be transcription or translation of some of the viral genes. An essential component of latently infected cells is that if they divide, the virus genome is carried from cell generation to cell generation.

In this section we also discuss the permissiveness of cells to CMV depending on the species origin of cells, their phenotypic function, and the role of cell differentiation and agents that enhance differentiation.

5.5.1. Infections in Nonhuman Cells

Infection by human CMV is not strictly restricted to cells of human origin. Although viral replication has not been reported in nonhuman cells, CMV will produce abortive infections in many such cells. Fioretti *et al.* (1973) reported that CMV produced a cytopathic effect in guinea pig cells. A similar phenomenon in bovine and Vero (simian) cells was found by Waner and Weller (1974). The cytopathic effect appeared early, 5–34 hr after infection. Refractile cells, cytoplasmic inclusions, but no nuclear inclusions were noted. Both nuclear and cytoplasmic viral antigens were found by immunofluorescence. Infectious virus was not produced unless, as in the case of infected Vero cells, they were cocultivated with human fibroblasts.

Albrecht *et al.* (1976) reported that 24 hr after infection of hamster embryo fibroblasts with CMV at a multiplicity of three, only 1% of the cells showed

cytopathology. When the infected cells were "arrested" by prior treatment with IUdR, they became detached, and cytoplasmic inclusions developed. When tested with convalescent human serum, 90% of the cells showed both nuclear and cytoplasmic fluorescence. In nonarrested cells, only cytoplasmic antigen was found. Significant increases were observed in the uptake of [^{3}H]methylthymidine in cellular DNA in arrested but not in nonarrested cells 0–24 hr after infection. Infective as well as UV-irradiated virus had similar effects. Irradiated virus was more stimulatory 24–48 hr after infection. Mitotic activity was correspondingly increased. To eliminate DNA stimulation by UV irradiation of the virus, the dose required was fivefold greater than the dose needed to eliminate infectivity.

Abortive infection of hCMV in rabbit lung fibroblasts was described by Farber *et al.* (1979). During a 4-week period after infection, typical nuclear and perinuclear inclusions developed which contained specific hCMV antigens as shown by indirect immunofluorescence. Cytopathic effects were also seen. These changes occurred despite inhibition of DNA synthesis by cytosine arabinoside, which suggests that they were associated with the development of early antigen (see Section 2.4.2). Infective virus was only obtained when infected rabbit cells were cocultivated with human fibroblasts.

The effect of hCMV in mouse cells and in mouse–human heterokaryons was studied by Boldogh *et al.* (1977). Abortive viral replication and viral persistence took place in these nonpermissive cells. Early antigen could be demonstrated in mouse cells infected with hCMV strain AD169 for 2 weeks. Twenty to 50 days after infection, infected cells were fused with human fibroblasts. Virus-specific antigens became visible in the cytoplasm and cell membranes of heterokaryons 12 hr to 40 days after fusion. This indicates that the viral genome had persisted in mouse cells. Cycloheximide (10 μg/ml) added 5 hr after fusion inhibited the appearance of immediate early antigen, but ara-C did not, suggesting that early antigen required protein but not DNA synthesis. It may be coded for by parental viral DNA.

Infectious virus was produced in heterokaryons if the heterokaryons or the mouse cells used for fusion were treated with IUdR. In that case, both viral specific nuclear and cytoplasmic antigens were diffusely distributed in the cytoplasm and in the nucleus 3–4 days after fusion. Perhaps a late viral function was actively suppressed in the isolated mouse cell. Similar activation of virus infection after fusion with permissive cells has been shown in other systems. Rous sarcoma virus antigens were expressed, and the virus was "rescued" in heterokaryons of Rous sarcoma virus-transformed mammalian cells and chick fibroblasts (Donner *et al.* 1974). Epstein–Barr virus was recovered from somatic hybrids of EB-virus-negative Raji cells and sternal marrow cells (Glaser and Rapp, 1972).

5.5.2. Infections in Human Epithelial Cells

The nonpermissiveness of epithelial cells such as HeLa and KB cell lines for CMV was already noted by Rowe *et al.* (1956). More recent evidence suggests that

restriction is not absolute and that the status of differentiation and chromosome ploidy may be more important factors. This hypothesis is more consistent with the extensive invasion of epithelial tissues by CMV in the body (see Chapter 10).

Human CMV can replicate in human epithelial cells, although not as efficiently as in fibroblasts. Michelson-Fiske *et al.* (1975) reported that CMV replicated in lung epithelial cells. Knowles (1976) found that CMV could grow and produce late cytopathology 3 weeks or more after infection in human thyroid cell monolayers. Attempts to use these cells to isolate CMV from urine were unsuccessful, presumably because the amount of virus in such samples was inadequate to infect thyroid cells. Different lots of thyroid cells also varied in their sensitivity.

Human diploid BAMB cells derived from amniotic fluid cells infected with CMV (Vonka *et al.*, 1976) formed large multinucleated giant cells with more than 100 nuclei. Cytoplasmic dense bodies were produced. Cytomegalovirus replicated, and the majority of particles acquired their envelopes by budding into cytoplasmic vacuoles as opposed to replication in permissive cells in which virus buds from nuclear membranes. This is an interesting finding because, as stated in Section 4.3.2, Severi *et al.* (1979) believe that this is the normal method of envelope formation. Concentration of viral macromolecules in dense bodies rather than the nucleus in BAMB cells may account for the lower efficiency of viral replication in these cells. Similar observations were made by Figueroa *et al.* (1978). After high-multiplicity infection of primary human amnion cells, large multinucleated syncytial giant cells developed. Inclusion bodies and specific antigens developed in the nucleus and cytoplasm. Viral DNA, identified by its density in isopycnic centrifugation, and infectious virus were both produced. Virus titers were about 1 log lower than in infected fibroblasts, and replication was slower.

As pointed out above (Section 5.1.1), Smith (1986) described the rapid and efficient replication of CMV in HCMC, a diploid epithelial cell line from human colonic mucosa, comparable to MCR-5, a popular fibroblast cell line for the demonstration of CMV in the diagnostic laboratory.

5.5.3. Infections of Human Leukocytes

A great deal of attention has been focused on infection of lymphocytes or other white cells by CMV. Work with murine CMV suggests that lymphocytes may be at least one of the carriers of latent infection (see Section 16.5). Viremia in man has been shown to be carried by white cells rather than plasma. Nonlymphoid white cells have been implicated (see Section 9.7.1). There is overwhelming evidence that blood cells carry CMV, and they may transmit the virus during transfusion (see Section 9.7). However, attempts to demonstrate CMV by coculture or even by nucleic acid hybridization were usually negative until 1984.

Attempts to infect or immortalize or transform human lymphocytes with CMV have also met with indifferent success. Huang and Pagano (1974) tried to infect four B and two T lymphoblastoid cell lines after failing to infect fresh normal lympho-

cytes. Cytomegalovirus DNA synthesis, detected 68–72 hr after infection, reached its peak by 5 days. Viral DNA was no longer detectable by 22 days. Furukawa *et al.* (1979) successfully infected a B-cell line (SB), which continually produced 2×10^4 to 3×10^5 PFU/ml. This line also carried EB virus nuclear antigen (EBNA). It is not known if the presence of EB virus genome made lymphocytes permissive for the replication of CMV.

Tocci and St. Jeor (1979) infected with CMV two B-cell lines, the Simpson plasma cell line free of EBV and the Raji cell line containing EBNA, and one T-cell line (Molt-4) and followed the infection by DNA–DNA reassociation kinetics. About 1% of cells from infected Simpson or Raji cells produced infectious virus. These two cell lines also showed increased CMV-induced cellular DNA synthesis. Over 15 days, Simpson cells maintained a fairly constant number (about 56–59) of CMV DNA copies per cell. However, 168 and 360 hr after infection of Raji and Molt-4 cells, the number of CMV genome equivalents gradually declined.

Saltzman *et al.* (1988) used the Xbal fragment "C" of the Towne strain of CMV for detection of CMV DNA in blood leukocytes of viremic patients. After separation by centrifugation in Ficoll–Hypaque, 17 out of 17 samples enriched for polymorphonuclear leukocytes (PMNs) grew out CMV, and 16 (94%) had CMV DNA by dot–blot hybridization. Viral DNA was also present in 94% of mononuclear cells, even though only 47% yielded virus. Virus could be recovered from PMNs but usually not from mononuclear cells, suggesting that CMV exists within granulocytes in a mature infectious form. Whether they are synthesized in these cells or scavenged from other sites is unknown. This is confirmation of earlier data that PNMs are the primary source virus in viremic patients (Rinaldo *et al.*, 1977).

By 1984, several new developments facilitated the demonstration of abortively or latently infected blood leukocytes. Einhorn and Ost (1984) showed that fresh clinical isolates of CMV, but not laboratory strains such as AD169, could regularly infect leukocytes and produce early antigen (EA) as detected by a polyclonal antiserum against CMV DNA polymerase. No late antigen or intact virions were demonstrable. Most infected cells were monocytes. Both peripheral blood leukocytes and bone marrow cells became more permissive to abortive infections after 6 days of *in vitro* growth.

Rice *et al.* (1984) confirmed these findings using monoclonal antibodies against IEA. They showed more clearly that leukocytes with specific markers of monocytes (Mo2), NK cells (HNK-1), B lymphocytes (Anti-SIgG), and OKT4 and OKT8 lymphocytes could all express the major 72-kDa IE antigen with varying degrees of efficiency. Monocytes were most effective (20%). Only 1–6% of the other cell types were infected. They also showed that despite the low number of demonstrably infected leukocytes, specific lymphocyte or NK function was compromised (see Chapter 7).

Schrier *et al.* (1985) then used the Eco RI J DNA fragment of the CMV genome, which contains the genes for IE antigen, to prove by *in situ* hybridization infection of peripheral leukocytes of eight healthy seropositive individuals. The signal appeared to be RNA transcripts of IEA. Strong hybridization in 2% of the

cells was found in two out of eight individuals, and weaker hybridization in 0.3–1% of the cells was found in the other subjects. A higher percentage of OKT4 than OKT8 cells were infected. In addition, monocytes were also thought to be infected. An earlier report had also identified T cells as being infected (Garnett, 1982).

This paper suggests that latently and abortively infected leukocytes are carried by normal seropositive individuals. How frequently this occurs and the extent of gene expression remain to be confirmed. The question also arises as to when and how such cells may be activated to produce lytic virions.

5.5.4. Persistent Infections in Other Human Cells

Under certain conditions, permissive human fibroblasts may also become refractory to the cell-destructive effect of CMV and carry the virus for long periods of time.

Mocarski and Stinski (1979) observed infected fibroblasts for over 20 weeks after infection with hCMV and found viral persistence. Two types of cells developed, both nonfibroblastic in appearance. About 70% were nonproductively infected and were cuboidal in appearance. These contained no viral structures in the cells, but virions and dense bodies were shown to be adsorbed on their cell surfaces. About 30% were of the second type and were productively infected. They were rounded cells that contained cytoplasmic inclusions, virions, and dense bodies.

The nonproductively infected cells could be separated from the others by Ficoll–Hypaque gradient centrifugation. Although they contained no morphologically evident virus structures, they contained about 120 genome equivalents of viral DNA per cell as determined by DNA reassociation kinetics. The DNA was uniformly distributed in cell nuclei as determined by *in situ* hybridization (see Fig. 4.2). These cells did not synthesize the full complement of viral proteins but, like nonpermissive cells of other species, made early virus-induced proteins and antigens as measured by polyacrylamide gel electrophoresis and by immunofluorescence. The generation time of these cells was four to six times longer than normal cells. A small proportion of them apparently slowly and spontaneously reverted to productive viral replication.

The production of infectious virus in persistent cultures could be slowly eliminated by repeated passage and cultivation in medium containing antiserum. Fibroblast morphology was also gradually restored. Although early antigen was no longer detected, the cells continued to contain about 45 genome equivalents per nucleus. After withdrawal of antiserum, productively infected cells appeared within 2 days.

This cell system suggests that viral persistence may take place in permissive cells when, for unknown reasons, they are unable to show expressive productive infection. Perhaps a limiting host protein is not made.

The persistence of virus in permissive cells, the replication of CMV in nonpermissive cells if they are treated with IUdR, and the accumulation of dense bodies rather than nuclear virions in some inefficiently infected cells may be examples of

how a partially completed or prolonged viral replication cycle can account for viral persistence in the human host.

Because CMV produces a slow infection, many attempts have been made to produce persistence or transformation in cell culture. Lang *et al.* (1974) grew human fibroblasts infected with the AD169 strain in agarose medium for several generations before they lysed and died. There was evidence of contact inhibition but no permanent transformation. Infection with UV-irradiated CMV was also effective, but uninfected fibroblasts did not grow in agarose.

A number of persistent infections have been described in association with studies on the oncogenicity of CMV (see Section 5.6). Long-term persistence was established in human embryonic lung cultures infected with the Mj strain of CMV (an isolate from the prostate), with transformation of cells occurring on two occasions (Geder *et al.*, 1976). Human embryonic lung cells inoculated with K9kV, a CMV strain isolated from Kaposi's sarcoma (Giraldo *et al.*, 1972a,b), were carried without loss of cell viability up to 22 passages (Glaser *et al.*, 1977). Virus titers as high as 10^6 PFU/ml were obtained. Study of persistently infected cultures may provide clues not only to viral latency but also to viral transformation and oncogenicity.

5.5.5. "Latent" Infection in Human Cells

Cockley and Rapp (1986) established a "latent" infection in human embryo lung fibroblasts by pretreatment with 200 units of α-interferon and 300 μM acyclovir before infection. After such treatment for 23 days, virus "latency" (prevention of virus production) was maintained by increasing temperature of incubation from 37° to 40.5° for over 100 days. The CMV could be reactivated by returning incubation temperature to 37°.

Tanaka *et al.* (1987) described a similar "latent" infection in a line of human thyroid papillary carcinoma cells (TPC-1). These cells were fully permissive and virus producing if incubated at 37°. However, if they were preheated at 40.5° for 48 hr prior to infection and maintained at that temperature for as long as 65 days, no virus was produced, but more than 95% of the cells expressed immediate early nuclear antigen. Virus-specific DNA polymerase was not produced. After return to incubation at 37°, a small proportion (0.0002% to 0.2%) of the cells were "activable" and produced virus, and about 38% showed evidence of producing viral membrane antigen and were lysed by immune serum against CMV and complement. They further found that indomethacin and tetracaine, inhibitors of prostaglandin synthesis, could inhibit the growth of CMV or the reactivation of CMV when incubation temperature was returned to 37°. These agents did not affect the expression of IE antigen (Tanaka *et al.*, 1988).

This model is interesting because it demonstrates mechanisms by which abortive infection can be maintained and how a replicative cycle may be brought to completion. There is no clear indication in any of the cell culture models how reliably "latently" infected cells could divide and multiply.

5.5.6. Differentiation of Cells and Permissiveness to CMV

Earlier, Dutko and Oldstone (1981) showed that murine CMV was produced in murine teratocarcinoma cell lines only after differentiation was chemically induced by dimethyldiacetamide. Human teratocarcinoma stem cells from embryonal carcinoma (ED) are also thought to resemble pluripotent cells of the early embryos. Such a cell line may undergo extensive and irreversible differentiation when exposed to retinoic acid (Strickland and Mahdavi, 1978). Gonczol *et al.* (1984) found that one such embryonal cell line (NT2/B9) that expressed embryonal antigen SSEA-3 was not permissive for CMV even though virus could adsorb and penetrate these cells such that infectious centers could be formed on susceptible cells. Only rare cells (<2%) became antigen positive after infection as measured by a polyclonal antiserum, and there was little evidence of viral replication. After undergoing differentiation on addition of retinoic acid with loss of SSEA-3, 50% of these cells replicated viral antigen, and lytic virus was found in culture medium 7 days after infection.

LaFemina and Hayward (1986) studied the molecular nature of the block in CMV replication in these teratocarcinoma cells. After infection of undifferentiated cells with CMV, the immediate early (IE) 68-kDa protein was not expressed, and input viral genomes were not replicated. The block was at the transcriptional level, since no IE messenger RNA could be detected. After exposure to retinoic acid and differentiation, this block was released, IE antigen was expressed, and viral replication proceeded. Interestingly, infection of undifferentiated cells with simian CMV (sCMV) resulted in productive viral replication and expression of sCMV IE antigen. Comparison of the structure of the IE genes of human CMV and sCMV reveals that sCMV has 23 tandemly repeated regulatory sequences that bind nuclear factor I proteins, whereas hCMV has only four. These are the binding sites for cellular nuclear factor 1. The binding of this cellular promoter–enhancing factor in the case of sCMV allows expression of IE antigens and may be the key to the permissiveness of the cells to this virus.

Monocyte differentiation or activation has been clearly related to permissiveness to CMV. Brautigam *et al.* (1979) observed that mouse macrophages activated with thioglycolate were more permissive to murine CMV (see Section 16.6.2.2).

Weinshenker *et al.* (1988) studied four different human leukocyte cell lines. Only one line (HUT 102) was susceptible, and only IE gene products were produced. However, a monocyte line (THP-1) was rendered fully permissive to CMV after being treated with 12-O-tetradecanoyl phorbol-13-acetate (TPA). The TPA induced differentiation so that 90% of cells became adherent, and cells proliferated, enlarged, and developed vacuoles. Complete virions were demonstrated by electron micrographs, and late antigens were demonstrated.

12-O-tetradecanoyl phorbol-13-acetate is a pleitropic substance that can influence signal transduction (Hamilton and Adams, 1987). It is known to activate EBV replication in lymphoblastoid cell lines (zur Hausen *et al.*, 1979). NF-kB, an intracellular protein, has been identified as mediating the up-regulation of Ig light chain

k synthesis by TPA (Nabel and Baltimore, 1987). Thus, viral transcription induced by TPA or cell differentiation may also be dependent on the presence of an enhancer sequence for the transcription-promoting protein such as NF-kB.

5.6. HUMAN CYTOMEGALOVIRUS AND CELL TRANSFORMATION

Under certain laboratory conditions, hCMV can transform or immortalize non-human and human cells, a capacity it shares with two other human herpesviruses, herpes simplex and EB viruses. Both of these have been implicated to a greater or lesser degree in human malignancies (A. S. Evans, 1976).

Albrecht and Rapp (1973) first produced transformation by hCMV in hamster embryo fibroblasts. A cell line, designated CX-90-3B, was produced after hamster cells were infected with UV-irradiated hCMV. It produced a poorly differentiated fibrosarcoma in weanling Syrian hamsters. About 0.5% of CX-90-3B cells contained CMV-specific antigen detected by indirect immunofluorescence diffusely distributed in the cytoplasm. A bright membrane fluorescence seen with human convalescent serum was also found in some cells. Serum from tumor-bearing animals did not contain neutralizing antibodies against CMV but did react with infected hamster embryonic fibroblasts by indirect immunofluorescence. These observations are similar to those made on herpes simplex virus and hamster embryo fibroblasts (Duff and Rapp, 1971).

Human CMV can also transform human cells, although we have already referred to the failure of laboratory strains to transform human fibroblasts (Lang *et al.*, 1974). A strain of CMV (Mj) isolated from the prostate of a 3-year-old boy was inoculated in human embryo lung cells (Geder *et al.*, 1976). It replicated slowly, and fewer than 50% of the cells became infected. After about 26 passages, the inoculated cultures underwent a crisis and transformed to a mixed epitheloid and fibroblastoid culture designated CMV-Mj-HEL-1. By immunofluorescence, CMV-specific membrane antigen as well as perinuclear and occasionally intranuclear staining were detected. Spleen cells from hamsters immunized with CMV were cytotoxic for the transformed cells. Spleen cells from tumor-bearing hamsters were cytotoxic for the transformed cells, the tumor cells, and normal human cells. Hence, passage in the animal did not lead to the loss of CMV-specific antigens. The transformed cells produced tumors in 62% of nude mice inoculated after a latent period of 19 days. They consisted of poorly differentiated epithelial cells that contained CMV-specific intracellular and membrane antigens (Geder *et al.*, 1977).

In addition to transforming embryonic hamster and human lung fibroblasts, hCMV plays an as yet undetermined role in transformed lymphocytes. Joncas *et al.* (1975) established two lines of transformed lymphocytes from the blood of a 9-month-old infant with cytomegalic inclusion disease. Both contained the nuclear antigen of EB virus (EBNA), which is found in lines of lymphocytes transformed by this virus. In addition, one of the lines contained eight to nine CMV genome

equivalents demonstrated by complementary RNA–DNA membrane hybridization. In 1977, the same group (Joncas *et al.*, 1977) described another patient with the stigmata of C.I.D. who showed serologic evidence of dual EB virus and CMV infection. Three lines of transformed lymphocytes were derived from this boy, of which two again contained CMV genomes. Another such infant was described in 1981 (Joncas *et al.*, 1981), who died on the third day of life with pathological stigmata of congenital CMV infection. Lymphocyte lines established on the first day of life contained EB virus cRNA. The liver contained CMV DNA by DNA–DNA molecular hybridization. Although no virus was isolated, this was considered more conclusive evidence for EBV and CMV congenital infection (see also Section 11.2).

Nelson *et al.* (1982) identified a fragment of DNA from strain AD169 CMV that transformed NIH 3T3 and rat embryo cells so that they developed "anchorage" independence. The transformants were able to form colonies under 1.2% methylcellulose and tumors in athymic Balb/c nude mice. Additional experiments with deletion mutants of this fragment permitted identification of the minimum size fragment required to initiate transformation (Nelson *et al.*, 1984). To delineate the boundaries of the transforming region pcm 4000, deletion fragments were constructed by digestion with exonuclease III and S1 nuclease, recombined in plasmids, and used to transfect primary rat cells. The results indicated that the left-hand boundary of the smallest transforming fragment must be between 490 bases (pcm 4115) and 318 bases from the Hind III site of pcm 4000.

Clanton *et al.* (1983) identified in the Towne strain of CMV a Towne *Xba*I fragment E that was capable of transforming Syrian hamster embryo cells and causing tumor formation of NIH 3T3 cells. This fragment lacked homology with the transforming fragment described above but was homologous to *Bg/II*-c transforming fragment of type 2 herpes simplex DNA (Jariwalla *et al.*, 1980). When fragment E was further digested with *Bam* HI and assayed by focus formation on NIH 3T3 and Rat-2 cells, transforming activity was localized within the terminal fragments EJ and EM (El-Beik *et al.*, 1986). Virus-specific EM sequences were detected at less than one copy per cell in fragment E transformed cells. In contrast, EJ sequences and AD169 transforming sequences are not retained in cells transformed by these fragments (Galloway *et al.*, 1984).

Jariwalla *et al.* (1989) studied the interaction between the EJ and EM fragments. Large transformed foci in Rat-2 cells were induced at a tenfold higher frequency by EJ plus EM than by either DNA fragment alone. Focus-derived cell lines also produced tumors in rats at a faster rate than lines transformed by EJ or EM alone. EJ is not detected in the transformants, but it encodes a major immediate early 72-kDa (IE1) protein implicated in transactivation and autoregulation. This protein or any other protein coded by this fragment is not expressed in the transformants.

In summary, three transforming domains have been mapped in human CMV DNA, one a 558-base-pair fragment of strain AD169 (pcm 4127) and two located in *Xba*I fragment E (EM and EJ) of strain Towne. As yet there is inadequate evidence

that CMV or any one of these fragments plays a role in human diseases by transforming cells *in vivo* or playing a role in cancer.

5.7. RELATIONSHIP OF HUMAN CMV TO CANCER

The evidence that CMV is associated with cancer is circumstantial and of two types. First, as noted in Section 5.6, CMV can transform hamster and human cells, which then can produce tumors in experimental animals. By itself, this is inadequate evidence that CMV is oncogenic in man. Certain human adenoviruses have similar biological properties, but none has been found to cause cancer in man (Green, 1970). The second type of evidence is that the virus or its genome has been found in some cancer tissues or that the presence of the cancer is associated with CMV infection. The strength of these observations may be questioned. In addition, in terms of the etiology of cancer, whether this is the "smoking gun" or a coincidence remains to be seen.

Sanford *et al.* (1977) established 34 lines of human prostatic adenocarcinoma in culture. Two developed CMV-specific membrane antigens. These authors also reported that 95% of 35 patients with prostate cancer had antibodies that reacted with CMV-infected cells. In contrast, only 78% of patients with bladder cancer and 70% of patients with benign prostatic hypertrophy had such antibodies. Laychock *et al.* (1978) compared prevalence of immunofluorescent antibodies against CMV-specific antigens in CMV-transformed human embryonic lung cells in patients with prostate cancer and age-matched controls. More patients with prostatic cancer were positive than patients with benign prostatic hypertrophy or patients without cancer. But patients with transitional cell carcinoma of the bladder also had elevated antibody titers. These data together with isolation of the Mj strain, which transformed human embryonic lung cells (Geder *et al.*, 1976), are at best presumptive evidence that hCMV causes prostatic cancer.

Huang and Roche (1978) looked for CMV DNA in adenocarcinomas of the colon and in inflammatory bowel disease using membrane hybridization with complementary RNA–DNA sensitive enough to detect 0.8 genome equivalents per cell. Twenty-four specimens from 14 patients were analyzed. Four out of seven with carcinoma of the colon were positive. Normal colon, inflamed colon from three patients with Crohn's disease, and rectal carcinoma from two patients were negative. One out of two patients with familial polyposis and two out of three patients with ulcerative colitis were also positive. Both are conditions that predispose to carcinoma of the colon. Only one patient with polyposis was seronegative for prior CMV infection; all others were seropositive. Hashiro *et al.* (1979) isolated CMV from cell cultures derived from three out of 16 surgical specimens of adenocarcinomas of the colon. Possibly, CMV produced a latent infection in the colon that was cocarcinogenic in the development of adenocarcinoma of the colon. The possibility also exists that CMV infection was coincidental or that it followed the development of the cancer.

There is evidence that CMV may also be found in other types of cancer.

Melnick *et al.* (1978) isolated hCMV from two of ten biopsies of cervical cancer. Five serial passages of an established cell line were made before cytopathology of CMV infection appeared. These observations remain to be confirmed and extended.

Kaposi's sarcoma is an angioproliferative neoplasm localized mainly in the skin but may invade the viscera. It is a curious tumor that is difficult to classify. It consists of vascular, lymphoid, and fibroblastic elements and has been classified as a fibrosarcoma, endothelial sarcoma, or lymphoma (Penn, 1979).

Giraldo *et al.* (1972b) first observed herpes-type particles in five out of eight tissue culture lines from cases of Kaposi's sarcoma from two equatorial African regions, Congo and Uganda. An isolate from a line designated K9V was indistinguishable from CMV except that, like the Mj isolate, it produced a slow spread of cytopathology. The part of Africa where Kaposi's sarcoma is found is also endemic for Burkitt's lymphoma. All sera from African patients with Kaposi's sarcoma contained antibodies against CMV (Giraldo *et al.*, 1975). A sample of Europeans and Americans with Kaposi's sarcoma had a 75–100% rate of seropositivity, which was significantly higher than that of the normal population (Giraldo *et al.*, 1978). The mean antibody titers against CMV in these patients were high, similar to those in African patients. African as well as control subjects had high background titers, so that they were not significantly different. Giraldo *et al.* (1980) detected CMV early antigen in seven out of 31 biopsies of the tumor and in early passages of some cell lines derived from them. The presence of CMV DNA was also detected in three out of eight biopsies tested. The line from which the strain K9V of CMV was isolated looked transformed and produced a fatal disease involving the lymphoid tissue in Old World monkeys.

The first indication that Kaposi's sarcoma is related to immunodeficiency is its increased frequency after organ transplantation. Penn (1979) found that in renal transplant recipients, who already have an increased incidence of malignancies, 3% of the malignancies are Kaposi's sarcoma, which ordinarily causes only 0.6% of all cancers. These tumors occur on the average 37 months after transplantation. A significant number (45%) have involvement of internal viscera. It has also been reported after cardiac transplantation (Lanza *et al.*, 1983). It has been repeatedly noted that withdrawal of immunosuppression led to the regression of lesions. Stribling *et al.* (1978) noted that four of five patients in whom a diagnosis was made preterminally responded favorably to withdrawal of immunosuppressive agents. Zisbord *et al.* (1980) reported complete remission of cutaneous and visceral involvement in a renal transplant recipient. Little *et al.* (1983) controlled Kaposi's lesions without rejection in a renal transplant recipient after reducing cyclosporine to 100 mg per day. Kaposi's sarcoma has been noted under other conditions of immunosuppression, for example, as a complication of corticosteroid therapy in temporal arteries (Leung *et al.*, 1981).

It is clear that Kaposi's sarcoma under as yet undefined conditions may be brought on by immunosuppression and that it may be controlled by reduction or elimination of such suppression. Recent studies indicate a close relationship between the development of Kaposi's sarcoma and the presence of unique growth factors. One view is that it is not a true malignancy but a multicentric proliferation

of normal endothelial and lymphatic elements stimulated by known and as yet unknown cytokines. Virus such as HIV or CMV may facilitate production of these growth factors, and under immunodeficiency growth of tumors is tolerated (Salahuddin *et al.*, 1988).

Kaposi's sarcoma of AIDS or epidemic Kaposi's sarcoma was one manifestation of the AIDS epidemic recognized in 1981 (Centers for Disease Control, 1981). It is different clinically from endemic sarcoma in that it affects a younger age group, and it is not restricted to the skin but may affect the viscera and lymph nodes. Besides the observations suggesting that there may be a relationship between CMV infection and endemic African Kaposi's sarcoma cited above, the possibility that CMV infection may somehow be etiologically responsible for epidemic Kaposi's sarcoma was widely considered. It was already evident that most of the gay males who bore the brunt of the epidemic were infected with CMV, and there was a circumstantial relationship among CMV, AIDS, and Kaposi's sarcoma (Hymes *et al.*, 1981; see also Section 13.2.1). Drew *et al.* (1982) noted that viral cultures of tumors were negative, but CMV RNA was demonstrated by *in situ* hybridization, and six out of nine tumors had detectable CMV-related antigens by ACIF.

Because cell-related DNA sequences have been found in the CMV genome (Peden *et al.*, 1982; D. H. Spector and Vacquier, 1983), the hybridization results, particularly when the number of gene copies was low, were regarded with increasing skepticism.

D. H. Spector *et al.* (1984) detected CMV-specific sequences in tissues of Kaposi's sarcoma at a level that varied from one gene copy per two to 50 cells in five out of five autopsy specimens and one out of seven biopsy specimens. The specificity of their results was thought to be enhanced by southern blot hybridization. Similar results were obtained by Drew *et al.* (1984). Others, however, used identical methods including probes that contained DNA sequence coding for immediate early antigen as well as putative transforming sequences (see above, Section 5.6), but no evidence of CMV DNA was found in tumor tissue (Ruger *et al.*, 1984b; Ambinder *et al.*, 1987). There was a similar lack of agreement in the results *in situ* hybridization.

These conflicting results may be explained by the frequent presence of CMV DNA in the tissues of immunosuppressed patients, particularly in AIDS patients in whom CMV genomes may be widely distributed without causing any clinical disease (see Section 13.2).

The conclusion is that there is as yet inadequate evidence to incriminate CMV infection as the cause of either endemic or epidemic Kaposi's sarcoma or as an essential factor in its development.

5.8. HUMAN CMV AND ATHEROGENESIS

Fabricant *et al.* (1978) showed that an avian herpesvirus, Marek's disease virus, could initiate atherosclerosis in chickens. Melnick *et al.* (1983) demonstrated

CMV antigens by indirect immunofluorescence with polyclonal mouse or guinea pig serum in the cytoplasm of smooth muscle cells cultured from atheromatous and uninvolved arterial tissue surgically removed from patients with severe arterial disease. No nuclear fluorescence and no evidence of viral replication by electron microscopy or by cocultivation with permissive cells was demonstrated. The same group also detected in such cultured cells CMV-specific nucleic acid sequences by *in situ* hybridization using the *Bam* HI A and B fragments of the Towne strain as probes. Nucleic acid sequences were detected only in the cytoplasm, as was the case of CMV antigen. No specimen directly obtained by frozen section was positive for either CMV nucleic acid or antigen. The hybridization test was more sensitive than the antigen test using immunoperoxidase, as some antigen-negative cell cultures were positive by hybridization. It was postulated that the entire CMV genome was present in the arterial cells, but it was not always detectable because of lack of sensitivity of the hybridization method. Messenger RNA and antigen formation in the cytoplasm occurred only during active cell growth.

Yamashiroya *et al.* (1988) demonstrated the presence of CMV and herpes simplex virus (HSV) nucleic acid and/or antigen in the coronary arteries and thoracic aortas of young trauma victims. *In situ* DNA hybridization and immunoperoxidase methods were used. Out of 20 subjects, the coronary arteries of six were positive for HSV; two were positive for CMV. The aortas of five were positive for HSV, and two were positive for CMV. Virus was, in occasional cells, associated with early or advanced artheromatous changes in coronary arteries.

Tumilowiscz *et al.* (1985) prepared smooth muscle cell cultures from arteries of the umbilical cord. Cytomegalovirus strain AD169 replicated in these cells, as demonstrated by electron microscopy and by cocultivation. Virus persisted in cell cultures over a 3- or 4-month period, with growth and cytopathic effect occurring concurrently. Smiley *et al.* (1988) also showed that endothelial cells were permissive to viral replication, but it took more time. Cytomegalovirus could also transform endothelial cells to anchorage-independent growth, but an immortalized cell line was not obtained.

The method by which CMV or other herpesviruses might contribute to atherogenesis is still speculative, but future research will consider the following possibilities (Petrie *et al.*, 1987). (1) The cytopathology produced by the virus on the endothelial layer could expose smooth muscles to platelets and serum factors implicated in atherogenesis (Ross, 1986). (2) Periodic reactivation of a latent infection could lead to chronic immune injury. (3) Transforming genes could potentiate abnormal proliferation of smooth muscle cells important in plaque formation (Ross, 1986; Tumilowicz *et al.*, 1985; Thomas and Kim, 1983). (4) Virus infection could alter lipid and cholesterol metabolism. Clearly, additional experimental data and confirmation are needed before any of these hypotheses can be proven. These observations take on added importance when we consider that there is circumstantial evidence that CMV may contribute to the development of coronary atherosclerosis in late rejection after cardiac transplantation (Section 13.1.4).

6

Serologic Tests for Cytomegalovirus Infections

6.1. INTRODUCTION

In this chapter we consider the various serologic tests that have been described for the diagnosis of CMV infection. The principles and background of each test are presented along with its utility. The technical details of each procedure are not described, as they have been admirably updated elsewhere (Lennette and Schmidt, 1979; Schmidt and Emmons, 1989). Tests for cellular immunity against CMV and their changes during and after various infections are described in Chapter 7.

Antibody responses to CMV infection are considered here and under the description of separate infections and diseases (Chapters 9, 11–13). It might be noted at the onset that production of antibodies following viral infections is almost a universal response. In the usual immunosuppressed patient, the general antibody response to CMV remains intact unless there is serious or fatal disease (see Sections 13.1 and 13.3.2). Even severe congenital infection invariably stimulates some antibodies in the neonate. The integrity of responses to specific viral proteins (Chapter 3) remains to be elucidated. In contrast, cellular immunity is easily and frequently suppressed (see Section 7.3.2).

6.2. COMPLEMENT-FIXATION TEST

In this test, complement is bound by a complex formed by antigen and antibody after they have reacted. A prepared antigen is incubated with serum or other samples containing unknown amounts of antibody. The reaction produces antigen–antibody complexes. A known amount of complement is then added to the mixture, part of which adsorbs to the antigen–antibody complexes. The more antigen–

antibody complexes present in the mixture, the smaller is the amount of residual complement that will be left. This is measured by a given quantity of sensitized sheep cells, which lyse in the present of complement. The degree of red cell lysis, which is readily visible, measures indirectly the amount of antibody in the serum sample tested.

The complement-fixation (CF) test was historically the most important test in detecting the antibody response to CMV infection. For many years, it was the standard test to which others were compared. There is no comparable body of work about any other test; hence, the complete review is retained in this edition. Perhaps the greatest utility of the CF test has been in seroepidemiologic surveys (see Chapter 9). From its inception, the test has been criticized for lack of sensitivity, and in laboratory practice it has now been superseded by more convenient and sensitive tests.

6.2.1. The Nature of Complement-Fixation Antigen

Rowe *et al.* (1956) first used sonicated, infected cell culture fluids as CF antigen, Benyesh-Melnick *et al.* (1966b) showed that CF antigens were produced only late in the replicative cycle of the virus. Krech *et al.* (1971b) showed that glycine extraction of infected culture fluid antigen improved its reactivity. Some of the early reports of the insensitivity of the CF test, for example, in the diagnosis of C.I.D. in neonates, were partly a result of the inadequacy of the antigen used (see below).

Cremer *et al.* (1975) centrifuged freeze-thawed (FT) and glycine-extracted (GE) antigens from AD169 virus-infected culture fluids in sucrose density gradients. Most of the CF activity was found in the lighter fraction containing naked nucleocapsids and not in one containing complete enveloped virions.

In similar experiments, Waner (1975) did not find CF activity in the fractions containing particles, but in the supernatant. The responsible antigen was characterized by rate zonal centrifugation and Sephadex G-200 filtration to be 67,000 to 85,000 daltons. Two polypeptides, one of 140,000 daltons (? major capsid proteins) and another a glycopeptide of 66,000 daltons (? lower matrix proteins), both with CF activity, were identified by Kim *et al.* (1977) (see also Chapter 3). The CF antigen was stable at 4°C and withstood repeated cycles of freezing and thawing, but potency was lost at 37°C and after boiling. The precise biochemical nature of the CF antigen(s) remains to be confirmed.

Antisera raised against purified CF-reactive supernatant antigen did not neutralize CMV by the fluorescing cell assay method (Waner, 1975). This result suggested that the CF antigen is not the same as neutralizing antigen or envelope antigens. When used in the indirect immunofluorescence test against CMV-infected cells, the antiserum produced specific fluorescence confined to intranuclear inclusions. No cytoplasmic fluorescence was seen. Antisera raised by supernatant antigens of AD169 or Davis strains were equally active against cells infected against

Davis, AD169, Kerr, or C-87 strains. Hence, supernatant or CF antigen may represent a common virus-determined antigen synthesized and shared by all strains. These results may explain why strain differences are not ordinarily detected by the CF or IFA tests.

Many experiments show that the CF antigen is specific. Stern and Elek (1965), before undertaking an epidemiologic study in London, determined the CF reactivity of seven strains of CMV, including the three prototypes of Weller *et al.* (1960), against known human sera. They found that the seven strains cross-reacted and were essentially indistinguishable.

Generally, the most popular strain for preparation of CF antigens has been AD169, although other strains have been used and appear just as effective. Stern and Elek (1965) used the Kerr strain for their studies. Carlstrom (1965) used the AD169 strain for a seroepidemiologic study of 242 children and adults in Stockholm.

Starr *et al.* (1967) tested the AD169 CF antigen against sera that showed diagnostic rises to *Mycoplasma,* measles, adenovirus, varicella, mumps, herpes simplex, and respiratory syncytial viruses. They also tested paired sera that showed rises from $<1:4$ to $>1:256$ against AD169 antigen against the CF antigens of *Mycoplasma pneumoniae,* measles, parainfluenza types 1, 2, and 3, influenza types A, B, and C, adenovirus, varicella virus, respiratory syncytial virus, and herpes simplex virus. No cross reactivity was observed in any one of these two groups of tests. Rifkind (1965) tested sera from three patients with a rising anti-CMV titer against herpes simplex and varicella–zoster antigens. No cross reaction was found. Andersen *et al.* (1971) tested paired sera from 36 patients against varicella–zoster, herpes simplex viruses, and CMV. Sixteen patients showed a rise to varicella or herpes zoster, two had high titers against varicella–zoster, four patients had clinical cold sores, and 12 patients had CF rises against CMV. In three cases of varicells or zoster and one case of cold sores, the well-known cross reaction between herpes simplex and varicella–zoster virus was observed (Weller, 1979). Except for three cases of apparent double infection, there was no evidence of cross reactions in the CF test between CMV and the other herpesviruses, in contrast to the IgM immunofluorescent test (see Section 6.9.1).

6.2.2. The Nature of Complement-Fixation Antibody

The type of antibody detected by most CF reactions belongs to the IgG class. This dogma has been extended to the CMV field, and a number of reports suggest that IgM antibody cannot fix complement with CMV antigens (Hanshaw, 1969; Hanshaw *et al.*, 1968; Lang and Hanshaw, 1969). However, herpesviruses may actually be exceptional. Tokumaru (1966) found CF antibodies in IgM as well as IgG fractions of sera of patients with herpes simplex infection. Schmitz and Haas (1972) recognized CF activity in the IgM fraction of patients with CMV infection.

In a comparison of the indirect hemagglutination assay (IHA) and the CF test,

Cremer *et al.* (1978) tested the IgM and IgG fractions of 20 serum pairs. Specific IgM antibodies were detected by the CF tests in late (convalescent) sera and in some early ones, which suggests that under certain conditions IgM binds complement. Some of the binding may have been by IgG–rheumatoid factor complexes, as prior adsorption by aggregated IgG reduced the CF titers of the IgM fractions.

That specific IgM may be detected in the test was also shown by Booth *et al.* (1980). In their study, the CF activity in the IgM fractions from five patients could not be adsorbed by aggregated IgG, and the activity was destroyed by 2-mercaptoethanol. Complement-fixing IgM antibodies were found to react against both sonicated and freeze-thawed crude antigen or against purified enveloped virion antigen, but only IgG antibodies reacted against soluble antigen obtained after sedimentation of virus particles.

It appears that although the CF test may detect a certain amount of IgM antibody, it is not a sensitive test for it. The IHA test and specific IgM tests are more sensitive (see Sections 6.5 and 6.10.1). This explains the relative lack of sensitivity of the CF test in early infection (see Chapters 11–13).

6.2.3. Constancy of the Complement-Fixation Titer

Since the CF test has been used in so many epidemiologic studies, it is important to know if the titer is constant. If it wanes rapidly, a significant number of false negatives would result from the assumption that no titer indicates no infection. As early as 1956, Rowe *et al.* (1956) obtained constant titers on sera of three adults taken over a 4- to 7-year period. This may, however, represent the constancy of antibody levels at a time far removed from the initial episode of infection.

Stagno *et al.* (1975a) showed that the CF antibody level declined steadily in the neonate infected with CMV, such that in 11 out of 55 children, it disappeared after 2 years. This was more common in neonates with lower antigenic loads, i.e., the perinatally as opposed to congenitally infected (see Section 11.2). There was some fluctuation in titers in this group between 1 : 8 and 1 : 32 (Reynolds *et al.*, 1979a). It should be pointed out that the tests done before 1975 were often performed with sonicated, freeze-thawed, and not glycine-extracted antigens, which form a significantly better reagent (see Section 6.2.4).

The CF test has also been criticized because of fluctuations. Medearis (1964a) followed sera from three mothers who had each given birth to a child with C.I.D. The titers of one varied from 1 : 64 to 1 : 640. He attributed these to repeated episodes of infection followed by waning of antibody titers. More disturbing were the results of Waner *et al.* (1973), who followed the CF titers of 50 donors undergoing plasmapheresis over an 18-month period. Multiple fluctuations of the CF titer were seen in some subjects, even wild swings between high titers and complete seronegativity. This has not been reported in other subjects and may represent a peculiar reaction of this population.

Starr *et al.* (1967) used sonicated AD169 antigen to follow 21 seropositive

first-grade school children over 3 years; most of their titers remained positive. Only two out of 78 determinations from this group were $<1:4$, showing that precipitous disappearance of antibody did not occur. Nevertheless, these children were not followed as intensely as the subjects of Waner *et al.* (1973), and they were followed with only one antigen.

Fluctuations in CF titers have not been reported in transplant patients, who are usually followed for at least 6 months to a year (see Section 13.1). Betts *et al.* (1976) reported no fluctuation in titer when glycine-extracted antigen was used.

At the moment, my conclusion is that CF titers of the normal individual are relatively constant if the proper antigen is used. The titers reach a peak following an acute infection, after which they reach a stable, lower level. In a number of such subjects, they may disappear altogether. The precise magnitude of this group, particularly following inapparent infection, is unknown but is probably not high. Hence, in a seroepidemiologic survey, there may be a small number of false negatives.

6.2.4. The Complement-Fixation Test in Cytomegalovirus Infections

Weller *et al.* (1960) were unable to find CF antibodies in sera of infants with C.I.D., although they detected neutralizing antibodies. In other cases diagnosed by virus isolation, Weller and Hanshaw (1962) also found no CF antibodies. These findings cast an early doubt on the ability of the CF test to diagnose neonatal infection.

Medearis (1964a), however, used sorbitol to stabilize antigen extracted by homogenization of infected cells and obtained significant titers in the CF test. He followed the CF antibody titer of neonates with C.I.D. at 3 and 7 months of age and showed a significant rise in titer. Sera from two babies with C.I.D. were tested against 14 isolates including the four prototype strains of Weller *et al.* (1960), the AD169, Davis, Kerr, and Esp. strains. The early 3-month sera reacted in high titers against homologous strains. They reacted in lower titers to other strains, consistent with a heterologous response. These results suggested that there were antigenic differences between the strains. But when sera from the same babies were tested at 7 months of age, all strains reacted equally well, with titers exceeding 1 : 1000. There was thus a broadening of seroactivity after prolonged antigenic stimulation. This was the first demonstration that the CF antibodies developed in C.I.D., although it was apparent that it could not be used as a rapid test.

The height of the CF titer also varied with whether or not active infection or disease was present. Infants with C.I.D. from whom virus was frequently isolated from the throat or urine had titers of 1 : 199, and their mothers had titers of 1 : 132. Infants without known CMV infection and their mothers had mean titers of 1 : 20 and 1 : 64, respectively. These findings have been extended by Stagno *et al.* (1975a).

Krech *et al.* (1971b) compared glycine-extracted antigen to "native" antigen prepared by homogenization of infected cells. Out of 17 sera from 12 children under 1 year who were excreting virus, eight were negative when native antigens of all three prototypes were used, but they were all positive when tested against glycine-extracted antigens. Titers from tests in which glycine-extracted antigens were used were two to 16 times higher. Thirty-eight sera out of 39 from virus excreters under 4 years of age, or 97%, were CF positive when tested against extracted antigen (Davis); only 50% were positive against unextracted antigen; 92% were positive by immunofluorescence, and 79% by neutralization tests.

Later studies using glycine-extracted antigen show that the CF response of the infant is unsatisfactory in the diagnosis of both perinatal and congenital CMV infection (Reynolds *et al.*, 1979a). It is frequently undersensitive, there being a 12–15% false-negative rate (Stagno *et al.*, 1975a; also see Section 11.2).

Cremer *et al.* (1975) titrated, with both antigens, 442 sera from children and adults; 39–44% showed a fourfold or higher titer against GE antigen. Many titers were ten to 100 times higher than titers using freeze-thawed antigen. Glycine-extracted antigen detected a fourfold or greater antibody rise in 16 serum pairs, whereas FT antigen detected only 11.

Betts *et al.* (1976) compared the indirect immunofluorescent and CF tests using freeze-thawed (CF-FT) and glycine-extracted (CF-GE) AD169 antigen. There was no problem with specificity if only nuclear fluorescence alone was considered positive. Sera from 71 dialysis patients before renal transplant and from 33 donors were tested. The IFA and CF-GE tests detected about the same number of seropositive subjects in both groups (48% and 49%). The CF-FT test was less sensitive. It only detected 31% seropositive subjects in the pretransplant and donor groups. Serial serum samples from 12 individuals who developed primary infection after transplantation were also tested. The FA test became positive 20–60 days after transplantation. Seroconversion by the FA test was 2–14 days earlier than by the CF-GE test. The CF-GE test was the last to become positive, sometimes by as long as 24 days after the other tests had become positive. Thus, FT antigen was clearly inferior. Although the results of the FA and CF-GE tests were similar, the FA test is superior because some sera are anticomplementary (12%) and could not be tested by the CF test. The FA test is also faster to perform, titers are higher, and it becomes positive faster in certain patients. The more recent indirect hemagglutination (IHA) test and the ELISA tests compare favorably with the CF tests (see below).

No fluctuations in titer occurred with serial tests with CF-GE, as reported by Waner *et al.* (1973). Fluctuations were reported to occur with the CF-FT test, but no data were presented.

To summarize, the following comments may be made about the CF test: (1) There is little evidence of important strain variation of CMV by the CF test (also see Section 5.3). (2) The CF test is specific but not as sensitive as the indirect immunofluorescent, anticomplement immunofluorescent tests, ELISA, and other newer tests (see below). But if the glycine-extracted antigen is used, it compares favorably with other tests. (3) The CF test is not very useful for the diagnosis of congenital

infection. Compared to other tests, such as IFA, IHA, and specific IgM ELISA tests, it is unsatisfactory to detect IgM antibodies. The CF titers rise reliably but significantly later. (4) The CF test is now superseded by more convenient tests, but historically is was the basis of much of the serologic work on CMV before the 1980s.

6.3. NEUTRALIZATION TESTS

It is assumed that glycoproteins of the herpesvirus envelope are responsible for stimulating neutralizing antibodies. In the case of herpes simplex virus, at least three have been identified (Spear, 1985; Gromples and Munson, 1986). Similarly, three viral glycoproteins of CMV stimulate neutralizing antibodies (Section 3.4). These are to gB homologue (or gcI, p130/55), gc II (93 kDa and 450 kDa), and gH homologue (gcIII, 86 kDa). Neutralizing antibody produced against gB homologue in guinea pigs after immunization is complement dependent, whereas prevalent antibody to the gH homologue is independent. Which set of antibodies is most important or prevailing in response to infection is still not clear.

The neutralization test is useful in virology because practically all viruses can be neutralized by specific antibody, and the reaction is quite specific. Circulating neutralizing antibodies are assumed to be functional in host defense. In the case of CMV, however, the neutralization test has certain limitations. It is technically demanding and takes at least 10–12 days. The titer is also almost always low, even with the addition of complement. Hence, the neutralization test has not been popular in diagnostics.

The reason neutralizing antibody titers after infection are low is not that such antibodies are absent but perhaps that they are not efficient. One cause of inefficient neutralization *in vivo* may be the masking of viral determinants by finding of the host protein β_2-microglobulin (McKeating *et al.*, 1987, see also Section 4.5.2). Rundell and Betts (1981) found that antigen–antibody complexes consisting of CMV and IgG anti-CMV could still be infective. Such complexes may help account for persistence of virus infection in the presence of circulating antibody. They may also produce immunopathology (see Section 10.5). The infectivity of such complexes may be significantly reduced in the laboratory by the addition of antihuman IgG antibody. A similar phenomenon has been shown with other poorly neutralizable viruses that produce chronic infections in mice, such as lymphocytic choriomeningitis and lactic dehydrogenase viruses (Fenner *et al.*, 1974).

Weller *et al.* (1960) used the classical tube dilution method for neutralization titrations. Dilutions of serum to be tested were incubated with about 100 PFU of virus and then inoculated in susceptible human fibroblast tube cultures. They were observed for 28 days for the development of cytopathology. This CPE reduction test was generally used until 1964, when it was largely replaced by the plaque reduction test.

Plummer and Benyesh-Melnick (1964) introduced plaque titration of CMV and the plaque neutralization test. This method increased sensitivity and efficiency.

Plaques developed under methylcellulose overlay and were observable after staining with methylene blue 14–18 days after inoculation. Neutralization titers of antiserum by plaque reduction were three to 30 times higher than titers obtained by CPE reduction in tube titration (Weller *et al.*, 1960).

The plaque reduction neutralization test was modified by Schmidt *et al.* (1976). They noticed that only 8 days after infection, foci of infected cells stained with neutral red more intensely than uninfected cells. Virus or mixtures of virus and antiserum were adsorbed on cell monolayers, which were then overlaid with medium containing 0.5% agarose. After 7 days of incubation, another overlay containing neutral red was added, and the foci or plaques were counted 24 hr later. Reduction in focus formation by the dilution of antisera was computed. The advantage of this test is that it reduced the time of observation by about one half.

Waner and Budnick (1973) devised a "fluorescing cell assay" (FCA) for counting infected foci and for neutralization. After infection for 3 days with about 200 PFU, foci could be demonstrated by indirect immunofluorescence. Inoculation of virus neutralized by antiserum resulted in absence of fluorescence. This test reduced the time required to get a neutralization titer to only 3 days. Its main disadvantage is that many variables affect fluorescence. Both the neutral red and FCA tests have sensitivities identical to that of the plaque reduction test. The addition of complement to reactions with human sera increases titers about two- to fourfold.

Stalder and Ehrensberger (1980) described a microneutralization test. Preincubated dilutions of serum and virus were placed in flat-bottomed wells of 96-well plates. A suspension of human fibroblasts and guinea pig serum followed. Plates were read in 10 days. Sequential sera from 11 patients with primary CMV infection showed that no neutralizing antibodies were detected before 7 weeks after onset of illness, but all sera were positive by 10 weeks. This is significantly later than the development of IgM-immunofluorescent, IgG-immunofluorescent, and CF antibodies (see Section 12.3).

The introduction of the shell vial assay for CMV by detection of infectivity by presence of immediate early antigen (Shuster *et al.*, 1985) should further reduce the time needed for neutralization tests to 24–48 hr.

6.4. FLUORESCENT ANTIBODY TECHNIQUES

Schmitz and Haas (1970) first reported the application of the indirect fluorescent assay (IFA) for antibody against the Davis strain of CMV in human sera. In general, infected fibroblast cultures placed or grown on slides are used as the "antigen." Various dilutions of the serum to be tested are added to the antigen, and finally a "conjugate" consisting of an antiserum against human globulin that is tagged with a fluorescent material, usually fluorescein isocyanate, is added to detect antigen–antibody complexes. The IFA titers of 100 sera from normal subjects were higher than CF titers. Titers over 1 : 160 were unusual in undiseased subjects. Out of 62 CF-negative subjects, only 43 were IFA negative, which suggests that IFA is

more sensitive. Six symptomatic clinical cases who were urine excreters of CMV and whose CF titers were 1 : 8 to 1 : 64 had IFA titers of 1 : 1280 and 1 : 2560. Hence, higher titers suggested the presence of disease. Nuclear fluorescence was considered diagnostic. Cytoplasmic fluorescence detected in sera diluted to 1 : 40 or less was considered nonspecific. In retrospect, it occurred because of the development of Fc receptors in infected cells and was correctly discounted (see Section 3.7.1).

This German paper (Schmitz and Haas, 1970), not much quoted in the English literature, recognized the main advangates of the IFA test. Its utility was confirmed by studies of Chiang *et al.* (1970), Nagington (1971), and Stagno *et al.* (1975a).

A significant advance of the IFA is the anticomplement immunofluorescence (ACIF) test. The antigen and the serum to be tested are treated in the same way. The third reagent added is complement, usually in the form of fresh guinea pig or human serum. It adsorbs to the antigen–antibody complexes formed in the cells. The final reagent is fluorescein-tagged anticomplement. The main advantage of this test is that nonspecific Fc receptors, even in the presence of IgG, do not fix complement (Keller *et al.*, 1976) (Fig. 6.1).

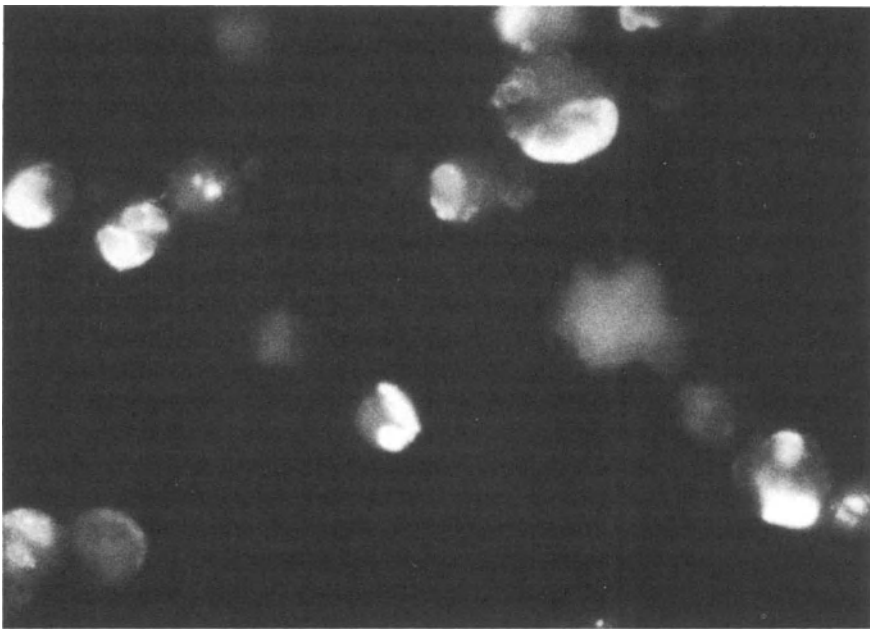

Figure 6.1. Anticomplement immunofluorescence (ACIF) antibody test against hCMV. Human foreskin fibroblasts infected with the Davis strain were used as an "antigen." These were incubated with a dilution of heat-inactivated human serum followed by human complement and fluorescein-labeled goat antihuman complement. Note nuclear fluorescence and absence of cytoplasmic fluorescence at Fc receptor sites. (Courtesy of Dr. W. W. Atchison.)

Betts *et al.* (1976) compared the IFA and glycine-extracted CF tests for measuring CMV antibodies in sera from renal transplant recipients and donors. The IFA method gave higher titers then the CF tests, but both tests detected the same number of seropositive sera (see Section 6.2.4). Rao *et al.* (1977) made the same observation in a similar patient group, comparing results from the CF and immunofluorescence tests. In primary CMV infection, the IFA and ACIF antibodies appeared earlier and had significantly higher titers than corresponding CF titers. Griffiths *et al.* (1978a) directly compared 406 sera from women who presented for prenatal care by the CF (glycine-extracted antigen), IFA, and ACIF tests. The titers obtained by the two fluorescence tests were about eight times higher, but each test was of equal specificity and detected over 98% of the positive ones. It should be noted that Rao *et al.* (1977) and Griffiths *et al.* (1978a) found that the titers of the CF and immunofluorescence tests were not well correlated, suggesting that different antigens were being detected. The ACIF and IFA tests were of similar sensitivity and specificity, but the ACIF test was easier to read.

6.5. INDIRECT HEMAGGLUTINATION TEST

The principle of this test is to adsorb viral antigen to tanned sheep red cells, which then agglutinate in the presence of antibodies against the antigen. It is called the indirect hemagglutination test (IHA), since cytomegalovirus or its antigens do not directly agglutinate red cells.

Bernstein and Stewart (1971) prepared glycine–NaOH (pH 9.0) buffer-extracted antigen from fibroblasts infected with AD169 for both CF and IHA tests. The IHA titers of normal subjects were five to ten times higher than CF titers. Sera negative by one test were also negative by the other. All positive sera were detected by both tests.

The IHA test, in contrast to the CF test, is sensitive for the detection of specific IgM. Hence, in acute infections, a higher and earlier titer may be obtained by the IHA test (Cremer *et al.*, 1978). At times, Bernstein and Stewart (1971) showed some sera to have disproportionately low IHA titers compared with the CF results. Although the crude antigen they used was the same in both tests, nothing is known about the components of this preparation, which may react differently in the two tests. To explain these results, the authors suggest that antigens that sensitize red cells may be "soluble" and that both soluble antigen and virions participate in the CF test (Benyesh-Melnick *et al.*, 1966b).

Fuccillo *et al.* (1971) used freeze-thawed antigens in a similar comparison and obtained slightly different results. Nineteen grade-school children excreting CMV were tested. Eight were positive by the CF test (42%), 78% were positive by the neutralization test, and 17 (89%) were positive by the IHA test. This suggests that IHA was the most sensitive test. The CF test, however, may have lacked sensitivity because glycine-extracted antigen was not used, and the antigens used for the CF and IHA tests were not the same lot. They do show, however, that neonates with C.I.D. may have negative CF tests but positive IHA, presumably because of the

presence of IgM antibodies. The IHA test has been used successfully in many laboratories.

One of the limitations of the IHA test is that CMV-sensitized red blood cells cannot be stored and must be freshly prepared from time to time. This disadvantage can now be overcome. Yeager (1979) used human group O red blood cells fixed in glutaraldehyde and stored frozen at −70°C. Cabau *et al.* (1981) first fixed group O cells with either glutareldehyde or formalin in whole blood, "tanned" them with tannic acid, and then "sensitized" them by incubation with CMV antigen. Such cells could be stored indefinitely after being lyophilized without loss of activity.

Although the IHA test has been consistent in its reliability and accuracy, it, like the IFA tests and CF test, is slowly giving way to the enzyme-linked immunosorbent assay (ELISA) tests (see below).

6.6. ENZYME-LINKED IMMUNOSORBENT ASSAYS

The ELISA tests constitute a significant advance in all areas in serology. Castellano *et al.* (1977) compared antibody titers of 30 serum samples assayed by the ELISA and the indirect hemagglutination assay. Antigen was extracted by freeze-thawing of AD169-infected WI-38 cells. It was adsorbed and coated to plastic wells. The serum or a dilution thereof is incubated in these wells. A conjugate is then applied, such as rabbit antiserum to human IgG labeled with alkaline phosphatase, and then the substrate *p*-nitrophenyl phosphate. In all 17 positive samples, the titers by ELISA were two- to tenfold higher. Only two sera positive by IHA were negative by ELISA at the beginning dilution of 1 : 50. Otherwise, the two tests were in close agreement.

Schmitz *et al.* (1977) described an ELISA for the detection of specific IgM antibody against CMV. In this type of test, a conjugate that consists of a specific antiserum against human IgM is applied (see Section 6.10 below). They found good correlation between the immunofluorescence assay and ELISA if, in the latter, a nuclear antigen was used. Cappel *et al.* (1978) used commercial AD169 antigen to develop ELISA against both specific IgG and IgM antibodies (see IgM tests, Section 6.10.1). The ELISA for specific IgG was more sensitive than the CF test. Interestingly, they also found that four out of 12 seropositive renal transplant patients who showed no elevation of their IgG titers developed viruria and specific IgM by ELISA. This suggests that the ELISA for IgM detected reactivation as well as primary infections and confirms the appearance of IgM in reactivation infections (Nagington, 1971).

Forghani and Schmidt (1980) described an enzyme immunoassay utilizing purified CMV virion and dense-body antigens, which consist primarily of matrix and tegument structural proteins (see Sections 2.7 and 3.3). The latter did not improve sensitivity or specificity. Neutralization tests were more strain specific. Yolken and Stopa (1980) compared seven different enzyme immunoassay systems using different enzyme-labeled immunoreactants and antigen detection systems. The sensitivity, ease of operation, and popularity of ELISA tests suggest that they

will be used widely. Its comparison with other tests is described below (Section 6.11).

The above are so-called "indirect" ELISA tests. As in the case of the IFA test, the "conjugate" consists of a labeled antiserum against immunoglobulin. Another approach is the "antibody capture" ELISA in which the conjugate is an enzyme-labeled CMV nuclear antigen. Individual wells of ELISA plates are coated with antihuman IgM, IgE, IgA, or IgG. Single dilutions of serum to be tested are incubated in the wells to capture the specific immunoglobulin. Then an enzyme-linked CMV antigen is added as a conjugate. In CMV mononucleosis and primary infection after bone marrow or renal transplantation, CMV antibodies of all four antibody classes developed. They persisted for a year or more except for IgA. The main advantage of this type of ELISA was lack of false IgM reactions caused by rheumatoid factor. The main disadvantage of this test is that it is a one-dilution test that may lack sensitivity, especially if a one-class test is used alone. Also, non-specific antibodies against cellular antigens could be detected.

6.7. RADIOIMMUNOASSAY

Knez *et al.* (1976) described an indirect solid-phase microradioimmunoassay (RIA) to measure anti-CMV IgM and IgG antibodies. Glycine-extracted CMV antigen was desiccated onto wells of microtiter plates. Serial dilutions of human sera to be assayed were added. After incubation, residual unadsorbed antibody was measured by binding to ^{125}I-labeled goat antihuman IgG or IgM. For accurate measurement of specific IgM, rheumatoid factor, if present, had to be adsorbed out with glutaraldehyde-insolubilized IgG. Otherwise, as in the case of IgM immunofluorescence tests, it produced a false-positive result (see Section 6.10.1). Kimmel *et al.* (1980) compared the RIA and CF tests on 100 sera. ^{125}I-Labeled goat antihuman IgG was used. The RIA was more sensitive than the CF test. In 66 healthy adults, only three were positive by RIA but negative by CF, and three were positive by CF but negative by RIA. Four out of seven patients with diagnostic serologic rises of CF antibody had diagnostic rises ($>0.5 \log_{10}$) in the RIA. The test was also shown not to cross with herpes simplex and varicella–zoster antigens. Hence, it was considered to be both sensitive and specific. The advantages of the RIA test are its convenience, its rapidity, and its objective end point. Its disadvantages are the expense of reagents and equipment, the difficulty of preparing radioactive probes, and the extremely short half-life of ^{125}I.

6.8. LATEX AGGLUTINATION TEST

The ability of a variety of antigens and antibodies to bind to latex particles has resulted in many simple tests to detect either antibody or antigens. Latex particles

coated with CMV antigens have become a popular serologic tool to detect antibodies by agglutination (Adler *et al.*, 1985). This test has been particularly unduly advocated in screening of blood donors (Adler *et al.*, 1985; LaRocco *et al.*, 1986; McHugh *et al.*, 1985; Traswell *et al.*, 1986; Gray *et al.*, 1987). Some authors report that the test compares favorably in sensitivity and specificity to standard tests such as the ELISA and IHA (Adler *et al.*, 1985; Sererat *et al.*, 1986; LaRocco *et al.*, 1986). However, Chou and Scott (1988) report it may lack sensitivity. Seropositive kidney donors who transmitted CMV to recipients were missed by the assay. It is unclear how much variation is related to different manufacturers and how much to the assay itself.

6.9. OTHER METHODS FOR DETECTING ANTIBODIES

Other methods for assaying antibodies against CMV that are less commonly used are described below.

6.9.1. Platelet Agglutination Test for Cytomegalovirus Antibody

Platelet agglutination (PA) induced by antigen–antibody complexes is the basis of a test for rubella infection (Myllylo *et al.*, 1969). Washed platelets are added to a mixture of antigen and dilutions of serum to be tested, and their pattern of sedimentation is observed. Penttinen *et al.* (1970) used the freeze-thawed AD169-infected fibroblasts in the PA and CF tests and found the PA titers were about ten times higher than CF titers. Twelve out of 13 CF-negative sera were also negative according to the PA test. In testing batteries of sera from 17 patients who had developed CMV mononucleosis after open heart surgery, similar results were obtained. However, three out of eight patients who were CF negative prior to surgery were positive by the PA test. These patients may have activated a latent infection following surgery. The main disadvantage of the platelet agglutination test is that one must have a constant, reliable supply of platelets. This test has no special advantage.

6.9.2. Immune Adherence Hemagglutination Assay

In immune adherence, indicator human red cells are agglutinated in the presence of complement. Surface receptors of red cells adhere to the third component of complement (C3), which also binds to antigen–antibody complexes and complement components C1, C4, and C2. Dithiothreitol is added to protect C3 from C3 inactivator. Nelson (1963) coined the term "immune adherence" to describe a phenomenon recognized much earlier. The immune adherence hemagglutination assay (IAHA) has been used since 1971 in Japan to detect hepatitis B surface antigen

and antibody and later elsewhere to assay for antigen and antibodies associated with hepatitis A virus (Miller *et al.*, 1975). It is as specific as the CF test but four to 20 times as sensitive (Lennette and Lennette, 1978; Schmidt and Emmons, 1989).

Dienstag *et al.* (1976) used CMV antigen from disrupted cells infected with AD169 to perform the CF and IAHA tests on serum samples from 36 multiply transfused patients who had undergone open heart surgery. Titers produced by the IAHA test were two to eight times higher than CF titers. Some sera that were negative by the CF test were positive by IAHA. No difference was noted between the kinetics of CF and IAHA antibody development as was noted in the case of CF and IFA tests. The IAHA seems to be an efficient, sensitive alternative for the detection of antibodies against CMV. Its main advantage is ease of performance given properly controlled reagents.

6.9.3. Other Enzyme-Linked Assays

Other methods using enzyme-linked reagents have been described. These may be particularly useful for the histological detection of antigens in cells and tissues. Gerna *et al.* (1976a,b) describe the immunoperoxidase test for human and murine CMV for detection of either virus or antibody. Immunoglobulins are coupled with a conjugate that consists of the enzyme horseradish peroxidase. In the direct test, it is coupled to serum containing antibodies against CMV. In the indirect tests, as in the case of IFA, the conjugate (goat antihuman IgG) is reacted with the antigen–antibody complex. In both types of tests, the coupled enzyme localized on infected cells is detected by exposure to a substrate containing 0.01% hydrogen peroxidase and a reagent.

Sixteen virus isolates were examined. The direct immunoperoxidase technique was found unsatisfactory because of excess background staining, and only the indirect method was subsequently used. Confirmation of 13 isolates identified by the indirect ELISA was achieved by IFA and electron microscopic examination. Both nuclear and cytoplasmic localization were seen. The technique is more sensitive than visual observation of cytopathic effect and is specific if one uses a known serum, although 8 days were required for the first positive result.

Gerna *et al.* (1976a) also used the indirect immunoperoxidase (IPA) technique to detect antibodies against CMV. Cytomegalovirus-infected WI-38 cells were incubated for 4 days in wells of chambered slide cultures, fixed in acetone, and incubated with dilutions of test serum. Sera from 114 blood donors and four babies with C.I.D. and their mothers were tested. The results using the IPA test for IgG antibodies were comparable to the IFA test for total antibodies against CMV. Titers by IPA and IFA were generally higher than CF titers. Some sera negative by CF and IPA tests were positive at low dilution by IFA antibody test, particularly during the first few weeks after infection, when IgM is detectable. The IPA test, like the IFA test, can be rapidly performed in 90 min. The test, however, does not need to be read immediately, and it can be read with a regular light microscope.

6.9.4. Detection of Antibodies by Counterimmunoelectrophoresis

Fortunato *et al.* (1977) placed commercial AD169 CF antigen in the cathodal well; serum samples known to contain high-titer IgM and total antibodies against CMV were placed in the anodal well, and counterimmunoelectrophoresis (CIEP) was carried out. Specific precipitin lines developed. The lowest specific IgM titer in this group that was positive by CIEP was 1 : 40, and the lowest CF titer was 1 : 128. The main defect of this method was that sera whose CF titers were lower than 1 : 128 were not reactive. Sera to be tested could not be diluted beyond 1 : 4. It is at best a qualitative test that is significant when positive and not contributory when it is negative. False-positive reactions occur particularly when the patient's serum contains rheumatoid factor.

Niebojewski *et al.* (1977) similarly used dilutions of serum to react by CIEP with a live hCMV antigen. An 89% correlation with the CF test was obtained. The main advantage of this test is its rapidity.

6.9.5. Detection of Antibodies by Gel Precipitins

Trlifajova *et al.* (1972) demonstrated specific precipitins in sera of CMV-infected individuals. They tested 554 Czech subjects for precipitin antibodies and found 90% positive among those 60 years or older, which was higher than expected. Comparison with the CF titers showed that 83 out of 94 gave the same results. The significance of multiple precipitin lines was not elucidated. Fioretti and Pollini (1978) prepared antigen by sonication of WI-38 cells infected with the Towne strain of CMV. Antigen and dilutions of serum were placed in wells made in 0.4% agarose and incubated at 4°C for 1 week. In comparison with the CF test, the precipitin titers were four- to eightfold lower, and blood donors with a CF titer less than 1 : 16 were not detected, although most serum pairs of patients with diagnostic serologic rises were correctly diagnosed. This method is simple but probably inadequate.

6.10. TESTS FOR ANTIBODIES TO SPECIFIC CMV ANTIGEN OR ANTIBODY TYPES

With better knowledge of specific active antigens and responding antibody classes (see Section 3.8.1) it will be possible to develop specific ways to test for these. The purpose will usually be to enhance diagnostic capability concerning specific clinical conditions (see Section 6.11). Most of these tests have been developed using the immunofluorescent techniques or the rapid and frequently more sensitive ELISA techniques. Some of the currently available tests are described below.

6.10.1. Immunoglobulin M Antibody Test

One of the main disadvantages of serologic tests in the diagnosis of acute infections is that a single titer is frequently not diagnostic. This is a serious problem in the case of infectious agents such as CMV that produce inapparent infections in large segments of the population. The problem may be illustrated further by the diagnostic tests for neonatal infection by CMV. Virus isolation, introduced as a diagnostic method *intra vitam* by Weller *et al.* (1957), remains the most sensitive and reliable method, but it is not always available, and it is time consuming. The main limitation of most serologic tests is that IgG antibodies largely responsible for a positive test may be passively transferred from the mother.

Hanshaw *et al.* (1968) introduced the fluorescent test for IgM antibody with the hope that this antibody, which is not transferred across the placenta, may provide a specific diagnosis in one determination. Dilutions of test serum were incubated with slide cultures of WI-38 cells infected with the AD169 strain of virus, as in the IFA test. Adsorbed IgM was detected with goat antihuman IgM antiserum conjugated with fluorescein isothiocyanate. All 50 persons up to 20 years old excreting virus were positive for specific IgM. Two symptomatic neonates were positive, but three of six asymptomatic ones with viruria were negative for IgM antibodies. The test was only positive in the more symptomatic patients. This early report already suggested that the IgM test is not as sensitive as viruria in detecting neonatal infection.

Another important defect of the specific IgM test is that it is nonspecifically positive in EB virus infections. Out of 43 heterophil-positive sera, 42 were positive for anti-CMV macroglobulin (Hanshaw *et al.*, 1972). Absorption of the heterophil agglutinins did not alter the result of the macroglobulin test. Only seven of the 43 had CF antibodies against CMV, suggesting that the positive macroglobulin test under these conditions did not denote CMV infection. Horwitz *et al.* (1977) also found that 21 out of 55 heterophil-positive sera had a $\geq 1:16$ IgM titer against CMV.

Cytomegalovirus-reactive IgM was not detected in 14 sera from patients with herpes simplex infection. However, six out of ten sera from patients with moderate to severe varicella–zoster infection contained CMV-reactive macroglobulin. These results suggest that anti-CMV IgM cross-reacts with antigens developed during other herpesvirus infections such as EB and VZ virus infections.

An IgM test may also be falsely positive in the presence of rheumatoid factor, which is an IgM antibody against IgG present in the blood of patients with certain collagen–vascular diseases (Reimer *et al.*, 1975) and also frequently seen in pregnancy. Rheumatoid factor may complex with specific IgG and adsorb to specific antigen. It may then react with anti-IgM to produce a false-positive test. This reaction probably creates problems for the detection of specific IgM in all tests, whether by IFA, CF (Cremer *et al.*, 1978), radioimmunoassay (see Section 6.7), or ELISA. One may attempt to adsorb out the rheumatoid factor, which may reduce sensitivity. Another approach to detect IgM is to test the serum specimen on two antigens. Riggs

and Cremer (1980) found that IgM does not react with early antigen. Early antigen may be produced in bromodeoxyuridine-treated infected culture. This procedure would require the preparation of two different antigens to determine IgM.

Langenhuysen *et al.* (1970) adapted the method described by Hanshaw *et al.* (1968) to the diagnosis of CMV infection in adult patients. Generally, they obtained somewhat higher titers and greater sensitivity by using a three-layer technique. AD169-infected fibroblasts were first layered with dilutions of test serum, then with rabbit antihuman IgM, and, finally, conjugated goat antirabbit serum was added. Nine patients suspected of posttransfusion mononucleosis were studied. Immunoglobulin M antibodies were detected in all nine patients when the CF test was still negative. Diagnostic rises were recorded in seven. The IgM antibodies were detected 1–7 days after onset of illness, and titers reached a height of 1 : 64 or greater in 11 to 15 days, after which they gradually declined. In 31 control sera from subjects not suffering from CMV infection, IgM antibody titers did not exceed 1 : 8. It was suggested that a single IgM titer of 1 : 16 was significant for recent infection.

Schmitz and Haas (1972) confirmed and extended some of these findings. In one patient with proven CMV infection, IgM titers rose from 0 to 256 up to 10 weeks after infection and declined to undetectable levels by 34 weeks. Hence, if IgM antibodies are detectable in a single serum sample, infection presumably had taken place in the preceding 4–8 months. The authors believe that whereas in neonates the IgM test may be falsely negative, it is reliable in infection in adults. Their test stresses incubation of cell antigen with 0.4% KCl to facilitate penetration of IgM and a double diffusion technique using anti-μ rabbit serum and swine antirabbit IgG conjugate (Schmitz *et al.*, 1977).

In summary, the IgM test is useful but neither completely specific nor sensitive. Unfortunately, it is not as sensitive or specific for the diagnosis of neonatal infection as had been hoped. Its use in epidemiologic surveys to detect primary infection in pregnant mothers and congenital infections is described in Section 11.3.1. It plays an auxiliary role in the diagnosis of adult infection in view of the length of persistence of IgM and in view of the fact that it may occur during reactivation (Nagington, 1971).

More recent studies have concentrated on the evaluation of the IgM antibody response against CMV using the enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay. To eliminate nonspecific IgM activities, Joassin and Reginster (1986) evaluated four different methods of eliminating anti-IgG IgM from serum. These methods were binding of IgG by protein A, serum fractionation, and absorption of rheumatoid factor with IgG-coated latex beads or with heat-aggregated human IgG. For these comparisons, the ELISA test was used in which CMV antigen (the CF type of reagent) was adsorbed onto plastic plates. Dilutions of the patient's sera were incubated on the plates, followed by samples of alkaline phosphatase-coupled antihuman IgG or IgM. After this, fresh solutions of inactive dye such as 4-nitrol phenol phosphate was added, and the plates were kept for 1 hr at 37°. Finally, the enzyme reaction was stopped by sodium hydroxide. Adsorption of sera with

latex or aggregated IgG was inadequate in eliminating nonspecific IgM samples. *Staphylococcus aureus* increased the nonspecific activity of some sera and in other cases removed or impaired specific activities. The most successful method was one in which hyperimmune antihuman IgG serum from sheep was used in a concentration that provided a binding capacity of 50 mg of IgG per milliliter. Treatment of samples with this serum eliminated nonspecific activity and preserved the specific effects. However, other methods have been satisfactorily applied in other studies. For example, Stagno *et al.* (1985) used an IgM ELISA test in which sera from pregnant women suspected of primary infection with CMV were adsorbed with staphylococcal protein A. This test was compared to an IgM radioimmunoassay in which the serum was adsorbed initially with latex beads containing human IgG to eliminate nonspecific activity. Both tests were satisfactory. It was found that 78% in the general and 86% in the female population tested within 16 weeks of seroconversion were positive by these two methods.

Chou *et al.* (1987a) evaluated two commercially available ELISA tests with an in-house test for IgM antibodies for CMV. One commercial test used pretreatment with *Staphylococcus aureus* to adsorb IgG, and the other pretreatment with a sheep hyperimmune antihuman IgG serum. The in-house ELISA test developed by the authors used CMV antigen extracted with glycine buffer from human foreskin fibroblasts infected with the AD169 strain of CMV. The in-house test also used preadsorption with sheep antihuman IgG serum. The two commercial assays both did well in comparison to the in-house assay in sensitivity in identifying IgM antibodies in patients with recent primary infection and seroconversion, but the commercial test was less specific. This could be traced to the use of different antigens.

The studies of Stagno *et al.* (1985) maintained that the RIA test for specific IgM was superior for diagnosis of congenital infection. In infections that were diagnosed by culturing urine at birth, the RIA test had a sensitivity of 89% and a specificity of 100%. In contrast, the specific IgM ELISA test had a sensitivity of 69% (42/61) and a false-positive rate of 5.7% (4/70) as determined in uninfected control newborn infants. The ELISA IgM test was only 43% successful detecting congenitally infected infants born of mothers with recurrent infection but more successful in detecting congenitally infected infants born to mothers with primary infection (77% sensitivity). The specificity of the ELISA IgM test for detecting congenitally infected babies was 94%. Both the ELISA IgM and RIA tests were superior to the indirect immunofluorescence test for IgM antibodies for detecting congenitally infected babies. The latter test identified only 45–80% of infected babies and showed a false-positive reaction of 20–33% as ascertained by tests on sera from uninfected controls (Griffiths *et al.*, 1982, 1984).

The higher positivity rate in congenitally infected babies from primary infections in mothers was thought to result from the larger amount of infection and antigen in such babies. Although these studies showed that the ELISA IgM test was slightly less sensitive than the RIA test, it has several technical advantages. The test is commercially available, has a long shelf life, and does not require radioactive probes, which in the case of iodination must be used within 72 hr of preparation.

The IgM tests may be used in combination with CMV-specific IgG tests for monitoring CMV infection in pregnant women. Serum samples could be followed for a positive IgM test or seroconversion. An initial positive IgM test without additional data or serologic follow-up would be difficult to interpret.

Ahlfors *et al.* (1987) reported less satisfactory results with the RIA tests. By testing 68 cord sera from babies proven to be congenitally infected by cultures, only 50% were shown to be positive.

In summary, the ELISA and RIA systems offer improved tests for detection of specific IgM antibodies in pregnant mothers, neonates, and other patients. With appropriate elimination of nonspecific factors accounting for false-positive tests, specificity appears to be satisfactory for the diagnosis of active infection in neonates. One must note, however, that in adults, the duration of the response may be prolonged. The test still suffers from lack of sensitivity. In adult immunosuppressed patients, IgM cannot be used to distinguish primary from secondary infection.

6.10.2. Immunoglobulin A in Cytomegalovirus Infection

There has been some interest in determining whether IgA is useful in diagnosing CMV infection. McCracken (1965) studied eight infants 2–11 days of age with clinical signs of CMV infection and found nonspecific IgA in the cord serum of all. Serum from uninfected control infants had none.

Mason *et al.* (1976) studied 953 infants born of primiparous mothers all of whom were screened within 48 hr of birth for viruria. Four hundred fifty cord sera were analyzed for IgM and IgA using low-level radial immunodiffusion plates. Of nine infected infants defined by viruria, three (33.3%) had elevated IgM levels (> 21 mg/100 ml), and all had IgA. However, 53 (12%) of 441 noninfected infants had elevated IgM, and 33 (7.5%) had IgA. The authors suggest that the association with IgA may be a more sensitive screening test for congenital infections that IgM. The test is a qualitative rather than a quantitative test and is simple to perform. However, it cannot be used to diagnose CMV because it is not specific.

Schmitz and Haas (1972) reported that specific IgA antibodies against CMV rose in parallel with IgM in an adult with proven CMV infection, although their decay was slower. Either IgA or IgM antibodies may be present in the absence of a CF titer because these two types of globulins do not fix complement well.

Levy and Sarov (1980) showed that CMV IgA antibody was produced in 90% of primary infections but not detected in any of 50 control sera from healthy seropositive individuals. However, in the case of renal transplant recipients, IgA antibodies were found to persist as long as 66 weeks after transplantation.

I. Sarov *et al.* (1981) devised a CMV IgA ELISA test using frozen-thawed antigen from AD169 CMV-infected fibroblasts. The IgA was determined with peroxidase-linked antihuman IgA specific for α chain. Ten patients after renal transplantation with demonstrated recurrent CMV infection proven by elevation of CF antibody titers were tested for IgA antibodies. Nine patients produced CMV IgA in high titers, and only one did not produce any IgA antibodies. In three of the nine

patients from whom closely spaced samples were available, specific CMV IgA antibodies were detected before the rise in CF titers.

In another study, these workers (I. Sarov *et al.*, 1982) compared solid-phase radioimmunoassay, ELISA, and slide immunoperoxidase techniques for the detection of IgA antibodies in renal transplant recipients. Of eight patients in whom a significant rise in IgG was demonstrated, seven showed a rise in IgA antibody by RIA and ELISA, and six by immunoperoxidase tests. The problem with both of these papers is that only patients with active infection and no negative controls were tested; thus, the frequency of false positives could not be determined.

Strand and Hoddevik (1984) studied further the diagnostic significance of IgA antibodies. Their prevalence was determined in 228 healthy blood donors and 112 patients suspected of CMV infection. Specific IgA antibody was found in 50 out of 62 (81%) patients who had specific IgM antibodies. Out of 112 patients who were suspected of having CMV infection, six of 30 who had IgM antibodies also had IgA antibodies. Only 1.8% of healthy blood donors had CMV-specific IgA antibodies. This suggests that their prevalence in a normal population is low. None of the sera negative for IgM antibodies were positive for IgA antibodies. Unfortunately, it was not possible from this paper to determine which of the 112 patients clinically suspected to have CMV infection actually had the infection. Hence, the sensitivity rate and specificity rate for this test could not be determined. It might be correlated with IgM test, but the IgM test itself is not entirely satisfactory. Morris *et al.* (1985) confirmed the finding that IgA antibodies for CMV were found in the sera of 68 gynecological patients only when IgG antibodies were present. They were found in 40% of the CMV IgG-positive sera. In conclusion, the significance and utility of assaying for IgA antibodies against CMV in either primary or recurrent infection have not been demonstrated.

6.10.3. Immunoglobulin E Assay

Nielsen *et al.* (1987) used an antibody capture ELISA method to detect CMV-specific IgE antibodies. Individual wells were coated with rabbit anti-E (E chain). One dilution (1 : 10) of a test serum was added, followed by a CMV conjugate labeled with horseradish peroxidase. Serum samples from viruric infants including umbilical serum were tested for the IgM and IgE antibody capture test using the saline CMV conjugate. The sensitivity for the IgE test was 82% (72/92), and 66% for the IgM test. There were no false positives. The IgE tests gave higher absorbances even though IgM concentrations in serum are significantly higher than IgE. The usefulness of this test remains to be confirmed by others.

6.10.4. Membrane Antigens

Ordinarily, membrane antigens may be demonstrated by indirect immunofluorescence in infected coverslip cultures that are not fixed. The and Lan-

genhuysen (1972) demonstrated antibodies to membrane antigens in CMV-infected fibroblasts. By using a serum with known antibodies, membrane antigen was shown to appear 48 to 72 hr after infection of cells, earlier than virion antigen in the nucleus. Antibodies to this antigen were detected in 11 out of 12 patients with proven CMV infection. They appeared 1 to 13 days after illness and persisted about 1 year after onset of illness. Antibodies to this antigen were mainly of the IgM class.

Membrane antigens may differ in related strains of different herpesviruses. Membrane antigens of herpes simplex types 1 and 2 are different (Geder and Skinner, 1971). Earlier, Andersen (1972) found no difference between AD169 and Davis strains with the same sera by neutralization kinetics, but the membrane antigens of the three strains were different. Hyperimmune rabbit antisera against AD169, C-87, and Davis strains were used in box titrations of antibody titers for membrane antigen against cells infected with these strains (Gonczol and Andersen, 1974). Each serum titered highest against the homologous strain. Davis and C-87 seemed most disparate. Serum against AD169 titered 1 : 640 against the homologous strain but only 1 : 40 against Davis and C-87.

The usefulness of this test in the diagnosis of CMV infection has not been confirmed. A major problem is the development of Fc receptors on the membranes of CMV-infected cells, which gives a type of immunofluorescence similar to membrane fluorescence (Whitley *et al.*, 1976).

6.10.5. Early Antigens

As mentioned before, despite the relatively slow replication of CMV, early antigens (EA) can be demonstrated in infected cells within hours of infection (see Section 4.3.1). The importance of detecting EA in accelerating virus diagnosis has already been described (Section 5.1.3). Most tests describe antibodies against as yet molecularly undefined early antigens developed in CMV-infected cells with no requirement for DNA synthesis. Cells are infected at a multiplicity of 1 and incubated for 72 hr in medium containing 20 μg/ml cytosine arabinoside to prevent synthesis of late antigens (Reynolds *et al.*, 1979a), which, by definition, requires the synthesis of viral DNA (see Section 2.5.1 and 2.7). The prepared antigen preparation is then reacted with antiserum and is stained with conjugate as in the IFA test. If a defined early antigen should prove to be meaningful, an ELISA-type test will undoubtedly be developed.

The *et al.* (1974) first studied antibodies to early antigens in cases of primary infection following blood transfusions. The presence of this type of antibody at a titer of 1 : 80 was thought to indicate current primary infection.

Stagno *et al.* (1975a) followed the antibody titers of infected neonates measured by the IFA, IFA-EA, neutralization, indirect hemagglutination, and CF tests. The titers in cord serum reflected those of the mother. When elevated, they decreased after birth. It was noted that the perinatally infected child will significantly elevate his EA antibodies after the initial decrease. Stable elevated IFA-EA antibody levels were a helpful indication of congenital CMV infection (see Section 11.2).

Gerna *et al.* (1976a,b) developed an immunoperoxidase test to detect IgG antibody against EA. Neonates with congenital infections, their mothers, leukemic children, and pregnant women were studied. A close correlation was found between virus isolation and antibodies against EA in all of these patient groups whether they had congenital, perinatal, primary, or recurrent infection. In one case, EA antibodies disappeared when virus excretion cleared. Hence, it was thought that EA antibody is an indication of current infection rather than primary infections.

The utility of antibodies to CMV EA may be questioned because it may remain elevated as long as 250 days after a proven CMV mononucleosis in a normal patient (Ten Napel and The, 1980). Out of 15 renal transplant patients, eight converted 47 to 137 days after transplant, and five were already positive at time of transplant. In some patients, CMV EA titers remained high 4–8 years after transplantation (The *et al.*, 1977). Unfortunately, no virological data were presented. Ten Napel and The (1980) believe that antibodies against CMV EA are produced in primary infection (The *et al.*, 1974), congenital infection (Gerna *et al.*, 1978), and following reactivation of a latent CMV infection (The *et al.*, 1977; Gerna *et al.*, 1978). They believe the common denominator to be that EA denotes active infection, whereas in its absence, antibodies to late antigens denote inactive or latent infection. This working hypothesis remains to be proven. There is no consistent relationship between cervical CMV excretion and antibodies against EA (Reynolds *et al.*, 1973; Griffiths *et al.*, 1978b; also see Section 6.3).

A promising modification of the test uses early antigen developed in human fibroblasts by vervet monkey CMV in the presence of cytosine arabinoside (Swack *et al.*, 1977). This antigen is superior technically because cytoplasmic fluorescence is reduced. More important, sera from patients with active infection reacted with this antigen, but most of the sera from normal donors that were positive by the CF or regular CMV EA test did not. Apparently, the monkey antigen discriminated better between active and past infection (Waner and Biano, 1979).

As mentioned above (Section 4.3.1), a "preearly" or immediate early antigen may appear in the nucleus of infected cells even before "early antigen." The diagnostic significance of antibodies to this antigen is still unclear. Michelson-Fiske *et al.* (1977) and Reynolds (1978) report the development of a nuclear antigen 20 min to 1 hr after infection. Michelson *et al.* (1979b) report an indirect immunofluorescent study of "immediate early," "early," and "late" antigens in different groups of subjects. Antibodies against immediate early and early antigens were more often associated with specific IgM-positive, and presumably acutely infected, individuals. Further differentiation of the responses to "immediate early" and "early" antigens was not made. These results do not seem to conform to those of Chiba *et al.* (1980; see below). Perhaps different antigens were being detected.

Chiba *et al.* (1980) prepared "preearly" antigen by infecting cells at a multiplicity of 0.5–1 and fixing them after 3 hr of incubation. Antibodies were detected by the anticomplement immunofluorescence test. Sera from ten congenitally or perinatally infected infants were tested. In contrast to the more rapid development of antibodies to early antigen (EA), membrane antigen (MA), and late antigens (LA),

antibodies to preearly nuclear antigen (PENA) did not develop before 1 month after birth. The IgM antibodies to MA declined first, followed by a decrease in anti-EA, anti-PENA, and then anti-LA titers. Whereas antibodies against EB virus nuclear antigen (EBNA) develop late and then persist (Henle *et al.*, 1979), anti-PENA declined and disappeared in 18 months.

More data are clearly needed before the full significance of antibody response to early antigens is clear. It will be necessary to define which early antigen or gene product is involved before the situation is clarified. These are now well defined (see Sections 2.5 and 3.2.1).

6.10.6. Cytolytic Antibody

Betts and Schmidt (1981) described a complement-dependent antibody that lysed CMV-infected cells. Cytolytic antibody was present in 35 out of 36 patients with primary CMV infection and in only five of 96 controls. Its presence was correlated with symptomatic infection. When it was present in patients with primary infections or in four with reactivation infections, they were symptomatic. The antibody appeared within 1 week of onset of illness and persisted for only 2–4 months.

The antibody appeared to be IgM. The complement had to be of human or rabbit origin but not from guinea pigs. Presumably, one may diagnose acute disease with a single sample. However, these findings have not been confirmed, and more data on this test would be desirable.

6.11. COMPARISONS AND PATTERNS OF COMMON TESTS FOR ANTIBODIES

In view of the large number of tests and commercially available kits that use different detection methods and poorly described antigens, it is difficult to conclude which is the best test. Reliable data on sensitivity, specificity, and technical feasibility are not always available. Because of ease of operation, long shelf life, and convenience and rapidity, the ELISA tests and other forms of rapid tests are supplanting the more complex complement fixation tests, the immunofluorescence tests, and the indirect hemagglutination assays for detection of antibody to CMV. LaRocco *et al.* (1986) compared three different types of ELISA tests, with the latex agglutination test, automated immunofluorescence test (FIAX), the complement fixation, and the immunofluorescence tests. In addition to sensitivity and specificity, the positive and negative predictive values were obtained. The latex agglutination test had a lower negative predictive value (81%) than the ELISA, IFA, or FIAX tests. That is, it had more false-negative results. Still, it was pointed out that the latex agglutination test is so easy to perform that it may serve as a useful screening test.

Phipps *et al.* (1983) screened a group of 120 sera by the complement fixation test (CF), immunofluorescence assay (IFA), solid-phase fluorescence immunoassay (FIAX), enzyme-linked immunosorbent assay (ELISA), and indirect hemagglutination test (IHA). In terms of accuracy, sensitivity, specificity, false-positive rate, and false-positive/false-negative rates, the IHA test scored best. This was followed by the ELISA, FIAX, CF, and finally IFA, in that order. The CF test was particularly low in sensitivity (75%), and the IFA test was the lowest in specificity (69%). This is reflected in the high false-negative rate of the CF test (25%) and the high false-positive rate of the IFA test (32%). The results of these authors in the IFA test probably reflect their subjective difficulty in interpreting this test. False-positive interpretations may result from cytoplasmic straining because of Fc receptors (see Sections 6.4 and 3.7.1). It is to be noted that although the IHA test was the best in the series of tests, the ELISA tests were not far behind. Brandt *et al.* (1984) performed a similar comparison among the CF test, a commercially available ELISA assay, an IFA test, and a modified IHA test. Forty-three serum samples were tested by each of the above procedures. The ELISA, IFA, and indirect hemagglutination tests were in close agreement. The CF test was the least sensitive.

Both groups felt that the CF test not only was less sensitive than the other tests but also has other problems such as samples that are anticomplementary and long turnover time required by overnight fixation at 4°. Although the performance of the IHA test was among the best, it too has a number of difficulties. About 3–4% of the samples show nonspecific agglutination, and they had to be excluded. The source of red cells is always a problem, although this can be partially offset by the use of glutaraldehyde-fixed human O cells. The commonly used glycine-extracted antigen does not work with tanned cells, and tannic acid titration is exacting. Brandt *et al.* (1984) concluded that the most practical method and the one offering the highest degree of sensitivity was the ELISA test system.

Table 6.1 presents an overview of the patterns of responses as measured by the more useful and established tests. This table is much simplified for clarity.

Five major categories of infections are presented. "Past infection" is the most common condition and is indicated by the presence of IgG antibody. There is as yet no test to detect the presence of latent infection, which we can only assume. "Carrier infection" denotes past infection that is accompanied by persistence of demonstrable virus at certain sites such as in the urine, respiratory tract, semen, or cervix. These two conditions are important for the study of epidemiology and transmission of infection. No single serologic test can distinguish clearly between "past" and "carrier infection." Virus isolation remains the most useful way to diagnose them. Both IgM antibodies and antibodies against early antigen and perhaps against other antigens remain to be evaluated as useful indicators. The clinical significance of antibodies against defined viral proteins remains to be elucidated but will gradually become apparent.

Specific categories of clinically important infections may be diagnosed serologically with varying degrees of success. Details concerning the serologic diag-

TABLE 6.1
Patterns of Serologic Responses to Cytomegalovirus Infections[a–c]

Type of infection	Early antigen (EA)	Specific IgM (ELISA, RAI)	IFA and ELISA	IHA	CF	Neutralization (NT)	Comment
Past infection (latent infection)	–	–	P	P	P	P	Results from normal inapparent infection, usually latently infected
Carrier infection (urine, cervix, semen carrier)	±	±	P	P	P	P	No good serologic distinction from above
Congenital infection	P, stable for 6 mo	P, cord blood	P	P, R	P, R, 4–6 mo	P, R	IgM helpful, other tests may be persistently high or show rises
Perinatal	R, 2–3 mo	R, cord blood	R, 2–4 mo	R, 2–6 mo	R, 3–6 mo	R, 4–6 mo	Rise of EA helpful in distinguishing from congenital infections
Acquired infection (postnatal)[d]	R, <1 mo	R, 0.5–1 mo	R, 1–2 mo	R, 1–2 mo	R, 1–2 mo	R, 2–3 mo	Sequence of appearance of rises: IgM, EA, IFA, ELISA, IHA, CF, NT

[a] P, persistently positive; R, rise (conversion or elevation) in antibody; time following R is rise when first observed; –, negative; ±, may be positive or negative.
[b] IFA, indirect fluorescent antibody assay; ACIF, anticomplement immunofluorescent assay; IHA, indirect hemagglutination assay; CF, complement fixation.
[c] Antibodies against membrane antigen (MA) and complement-dependent cytolytic antigen, probably IgM antibodies. Response to MA parallels IgM IFA; development of cytolytic antibody is said to predict symptomatic infection (see text).
[d] Primary *de novo* infection is distinguished from reactivation infections by serologic conversions rather than mere elevation of titers.

nosis of congenital and perinatal infections are given in Chapter 11 (Section 11.2). More details on the serologic diagnosis of acquired primary or reactivated CMV infections in normal, transplant, and immunosuppressed patients are given in Chapters 12 and 13. The responses of cell-mediated immunity to infection are discussed in Chapter 7.

7

Cell-Mediated Immunity to Cytomegalovirus Infection

7.1. INTRODUCTION

We have already discussed the humoral immune response to CMV infection in Chapter 6. Immunopathology mediated by specific antibody and humoral substances is discussed in the chapter on pathology (Chapter 10). The immunosuppressive effects of CMV infection in the human and in mice are treated, respectively, in Sections 13.1.5 and 16.6.3. Here we concentrate on changes in specific and nonspecific cell-mediated immunity (CMI) following infections with CMV.

Cell-mediated immunity is of particular interest because it is presumed to be the main host defense against CMV infections. The evidence for this is largely indirect. Since antibodies are usually normally produced following apparent and inapparent infections, even in the severely immunosuppressed subject (see Section 13.1), and infection is often accompanied by clinical disease despite the presence of circulating antibodies, one assumes that they are inadequate to prevent latent infection and disease manifestations. The most impressive indirect evidence that specific CMI is indeed important in man is the frequency of CMV infection following its suppression after transplantation and in the HIV-infected subject (Sections 13.1 and 13.2).

As in the case of antibodies, where different antibodies have different functions, one should not speak of a test for CMI but of tests for different functions. All of them are significantly more complex than titrations for antibodies, and their meaning and significance are often disputed. Nevertheless, many important tests have been described. As we improve our understanding of the different components of cellular immunity, we can expect more and better tests.

7.2. TESTS FOR CELL-MEDIATED IMMUNITY

By specific cell-mediated immunity against CMV, one thinks of immunologic responses mediated by cells and not by antibodies. They are usually measured by complex tests that are difficult to control and are liable to error. Practically all tests of CMI require fresh lymphocytes, for which it is difficult to devise controls. Some tests also require fresh target cells, which compounds the extent of variability. The reacting cell is usually a non-B lymphocyte, which is presumed to react against one or more components of the virion or virus-specific antigen(s) exposed on the surface of an infected cell. It is frequently forgotten that in the usual test one does not or cannot isolate the putative reacting lymphocyte, but one uses a suspension of mixed lymphocytes obtained directly from the subject. The so-called "antigen" may consist of suspensions of infected cells or cell extracts containing many antigens or purified or cloned antigens. Assumptions concerning these tests should be constantly reviewed, as they may not be strictly valid. For example, early tests of lymphotoxicity were assumed to be mediated by specific T lymphocytes (Section 7.2.2). Very likely many were mediated by nonspecific NK cells or K lymphocytes. In examining a procedure, one should always know (1) which type of lymphocyte or molecular subset is reacting and (2) what antigen is being recognized.

Cell-mediated immune effects may be separated into those based on recognition and effector effects (Oldstone and Perrin, 1979). Recognition tests are based on the phenomenon that sensitized lymphocytes from infected subjects recognize the proliferate in the presence of the antigen to which they are sensitized. Recognition is usually indicated by proliferation, measured by the amount of radioactivity incorporated by the cells in the form of tritiated thymidine. In the lymphocyte blastogenic, proliferation, or transformation test, peripheral lymphocytes are reacted against a specific virus antigen or nonspecific mitogen, and DNA synthesis of the responding lymphocyte is measured by uptake of tritiated thymidine.

Other tests, which require the measure of an effector event as well as recognition, have an extra dimension of complexity. In such tests, recognition is not measured but assumed. If it occurs without the effector event, it would result in a negative test. The event may demonstrate a biologically important function such as killing of infected cells, inhibition of chemotaxis, or production of lymphokines or cytokines.

7.2.1. Blastogenic Response of Lymphocytes to Cytomegalovirus

The specific lymphocyte proliferative response is accepted as an *in vitro* correlate of cell-mediated immunity. Although both B and T lymphocytes participate in it, reactivity to viral antigens is initiated only after specific antigen recognition by sensitized T cells (Chess *et al.*, 1974). A positive test indicates the presence of circulating T memory cells specific for CMV.

In the usual test, the precise reacting antigen, as in the case of antibody test, is not specified. Pollard *et al.* (1978) studied the proliferative response against antigens of David, AD169, and C-87 strains of CMV prepared by three methods. Sonicated antigens consisted of infected cell suspensions that were sonicated and heated at 56°C for 45 min. To produce supernatant antigen, supernatants from infected cell cultures were collected, and the virus was pelleted and inactivated. For glycine extraction, a 1 : 20 dilution of 0.1 M glycine buffer (pH 9.5) was used to extract detached infected cells.

Lymphocytes from seropositive normal donors were significantly more reactive against sonicated antigen than against supernatant or glycine-extracted antigen. Sonicated antigens from the three strains were equally reactive. Commercial CF antigen from AD169 was somewhat less reactive than sonicated Davis antigen, which was used in the remainder of the tests.

Ninety percent of normal seropositive individuals had detectable lymphocyte transformation responses (mean transformation index, TI, 7.9 ± 1.2), whereas none of the normal seronegative patients had a TI > 3.0.

Significantly increased transformation responses to CMV (TI of 12 to 21) occurred 1–12 months after normal individuals developed CMV mononucleosis (see below).

7.2.2. Cytotoxicity Tests against Cytomegalovirus

The cytotoxic tests are examples of tests in which both the recognition and effector systems operate. The responder lymphocyte kills or lyses a target cell that is virus-infected. Viral antigens must be expressible on the plasma membrane, although internal viral proteins, frequently nonstructural, may also be recognized. Expression of some such antigens may take place 1–2 hr after infection of the target cell (Ada *et al.*, 1976). Targets may also be constructed by transfection with DNA coding for the epitope in questions using SV40, vaccinia, and other vectors. Very often in order to stimulate specific cytotoxic T cells, the stimulating antigen must be presented by cells. For example, infected cells must be used, and free virus may be inadequate (Borysiewicz *et al.*, 1988b).

The target cells are then labeled with ^{51}Cr, usually in the form of sodium chromate (Na_2CrO_4). This label has the property of being eluted when the labeled cells die or are lysed, providing a convenient measure of killing, although spontaneous elution must be taken into account.

The test is performed by mixing lymphocytes and targets at various effector-to-target ratios and calculating specific lysis, taking into account the proper controls.

The classical T-cell cytotoxic response is one in which a specific class of T lymphocytes, e.g., $CD8^+CD4^-$ or $Leu3^-Leu2^+$ subset, reacts. It requires responder and target cells that are compatible in one or more major histocompatibility complex (MHC) antigens (Blanden, 1977; Zinkernagel and Doherty, 1979). In man, one or more class I antigens, e.g., HLA-A or HLA-B antigens, must be shared.

More recently, restriction by class II (DR) antigens has also been described for CMV (Lindsley *et al.*, 1986). Cytotoxic T lymphocytes (CTLs) of this type are $CD4^+$. To prepare genetically compatible targets, one must either prepare and propagate cells, such as skin fibroblasts or monocytes from the patient himself or from HLA-compatible subjects, or one can use the patient's own lymphocytes as targets. This is possible even though lymphocytes are not readily infected by CMV. B lymphocytes transformed by EBV are transfected with desired regions of viral DNA (e.g., the IE region in CMV) using vaccinia virus as a vector (Borysiewicz *et al.*, 1988b). The cytotoxic T-cell response has been shown in the mouse to be a limited immunologic response lasting 1 to 2 weeks after infection (see Section 16.6.2.1). Unlike many other parameters of humoral and cellular immunity, it does not persist indefinitely. Thus, CTL against viruses including the herpesviruses such as CMV have been shown to be present in normal seropositive subjects only as precursors that must be specifically stimulated *in vitro* with the viral antigen for a number of days before activity can be demonstrated. Infection with HIV seems to be an exception. Circulating CTLs specific against a number of HIV proteins may be found at all times in infected subjects (Walker *et al.*, 1988).

Earlier reports did not clearly distinguish the specific T-lymphocyte cytotoxicity from the other types of cytotoxicity (Steele *et al.*, 1973). Lymphocyte-mediated cytotoxic tests were described for rubella (Steele *et al.*, 1973), mumps (Speel *et al.*, 1968), measles (Labowskie *et al.*, 1974), Sindbis (McFarland, 1974), and lymphocytic choriomeningitis virus (Marker and Volkert, 1973), most of which shared the confusion. Thong *et al.* (1976) first described a cytotoxicity test using cryopreserved virus-infected target cells (WI-38) that were infected for 24 hr. However, the importance of HLA restriction was not appreciated even though the work in mice was described in 1974 (Zinkernagel and Doherty, 1974). Later, McMichael *et al.* (1977) demonstrated more rigorously that HLA-restricted T lymphocytes were generated *in vitro* by stimulation of peripheral leukocytes with influenza virus. During active measles infection, Kreth *et al.* (1979) detected circulating HLA-restricted, Fc-receptor-negative virus-specific cytotoxic lymphocytes.

Quinnan *et al.* (1980, 1981, 1982) reported clearly for the first time a cytotoxic T-lymphocyte test using HLA-compatible syngeneic hCMV-infected targets. A number of renal transplant patients were tested. Untyped fibroblasts were used as targets for the NK response. The following hallmarks of cytotoxic T lymphocytes were noted: (1) The requirement of HLA-compatible targets distinguished specific cytotoxic T lymphocytes from nonspecific NK and K cells. (2) Unlike NK and K cells, which may be constantly present, cytotoxic T lymphocytes were only stimulated for 1–2 weeks following infection with CMV. The cytotoxic T lymphocyte response in transplant recipients is described below (see Section 7.3.2).

There are other cytotoxic tests that are not mediated by specific lymphocytes and do not require genetic compatibility between effector and targets.

One is the antibody-dependent cell-mediated cytotoxic (ADCC) test. The infected target cell, which need not be HLA restricted, is reacted with specific antibody. The main requirement for the ADCC effector cell is that it is a killer cell

(K cell) that possesses an Fc receptor so that antibodies may be attached. The ADCC test does not require complement. Other types of antibody-mediated cytotoxicity reactions may involve killing targets in the presence of complement. These tests then measure either a type of cytotoxic antibody or the status of the K cell in the individual. Since three of four variable elements may be involved (e.g., effector cell, target cell, antibody, complement), one must keep all variables constant except the one that is being measured. The ADCC response in which CMV-infected targets are treated with antibody against CMV has not been successfully demonstrated except possibly in one report. Kirmani *et al.* (1981) reported that non-T (non-sheep-red-cell-rosetting) Fc-receptor-bearing lymphocytes lysed CMV-infected fibroblasts and that such lysis was enhanced if the targets were pretreated with specific antibody. These studies need further confirmation.

Another type of cytotoxic response is mediated by the natural killer (NK) cell (Haller *et al.*, 1977; Moller, 1979; Herberman, 1980). This is a non-B, non-T lymphocyte that belongs to the large granular lymphocyte population representing about 5% of peripheral blood lymphoid cells and that carries Fc receptors for IgG (FcR) on its surface. They contain the antigen clusters CD16 and HNK1 (Leu7). Antibody is not required for this test, and one needs only the effector and the target cells. It is not clear what type of recognition is involved in this reaction, but it does not involve immune recognition as usually understood. The target cell may be a tumor cell or a virus-infected cell. The NK cell is present in normal blood but is stimulated by interferon. Probably as a result of interferon production, the NK response is elevated in the early stages of many virus infections (Welsh, 1978). The NK cell response has been studied in many different types of CMV infection (Section 7.3).

7.2.3. Targets Recognized by Human CTL

Borysiewicz *et al.* (1983, 1988a) showed that there are precursors of CMV-specific cytotoxic T cells (CTL) present in peripheral blood of seropositive individuals and that most of these are directed against nonstructural immediate early antigens.

To demonstrate that such precursors exist, Borgsiewicz *et al.* (1983) first stimulated production of CTLs by coculturing CMV infected fibroblasts with peripheral mononuclear leukocytes (PBL). The fibroblasts were obtained by skin biopsies of donors of PBL and hence were autologous and HLA compatible with the effector cells. Predominantly Leu $2a^+$ lines of CTL were generated, which lysed ^{51}Cr-labeled CMV-infected cells in a virus-specific and HLA-restricted manner.

They further showed the frequency and specificities of these precursor cells. By limiting dilution analysis, CMV-specific CTLs were present in peripheral blood lymphocytes of seropositive subjects at a frequency of one in 5000 to 20,000 E-rosette-positive lymphocytes. To differentiate CTLs recognizing different viral specificities, targets that expressed only immediate early (IE) or early (E) antigen

were produced by infecting fibroblasts in the presence of phosphonoformate to inhibit viral DNA synthesis and late antigen (LA) synthesis. Targets that expressed LA were produced in the absence of phosphonoformate. The majority (60%) of CTL clones developed from peripheral lymphocytes lysed cells expressing only IE and E antigen. Although a minority of clones selectively killed only cells expressing LA, there was considerable overlap between killing of autologous targets expressing IEA and EA alone and those also expressing LA. Results consistent with these were obtained by using another approach. Targets were first made consisting of B cells expressing either the 72-kDa IEA or the viral structure protein glycoprotein B (gpB) by infection with a recombinant vaccinia virus infection. The CTL precursor cells of two subjects were analyzed (Borysiewicz *et al.*, 1988a). It was found that 43% and 58% of the CMV CTL precursors lysed IEA-expressing targets, whereas less that 6% lysed targets expressing gpB. The high frequency of recognition of IEA by CTL in normal subjects suggests that IEA is a major recognition element and that such CTLs may play a role in preventing reactivation.

7.2.4. Interferon and Other Cytokine Responses

Lymphokines and cytokines are functional proteins produced by lymphocytes and other cells that contribute to the expression of both specific and nonspecific immunologic responses. Their production during an immunologic reaction may be considered as an expression of effector cells. As cytokines become better understood, their measurement may serve as tests of effector activity in tests of cell-mediated immunity.

Lymphocytes from sensitized individuals are exposed to specific antigens to which the individual has been previously exposed. Or lymphocytes are exposed to nonspecific antigens or mitogens such as phytohemagglutinin, concanavalin A, and pokeweed mitogen. Interferon and many other lymphokines such as IL-2, tumor necrosis factor, etc. are produced and may be measured. For example, it appears that the production of γ interferon by lymphocytes in the presence of specific and nonspecific mitogens is one valid measure of immune competence. Lymphocytes from patients with AIDS, who suffer greater destruction of their immune system than asymptomatically infected patients or patients with ARC (AIDS-related complex), are less able to produce γ and α interferon (Murray *et al.*, 1985; Rinaldo *et al.*, 1988).

However, by and large the significance and interpretation of production of cytokines as tests of cellular immunity are still open to discussion. In the case of interferon, there is at least one compounding factor that frequently is not taken into account. Interferon production is known to be modified by prior exposure to an interferon inducer. "Hyporeactivity" is the inability of an animal host to produce circulating interferon for about 1 week after the animal has been exposed to inducer (such as a virus or polynucleotide) or after it has been infected (e.g., murine cytomegalovirus in mice) (Ho *et al.*, 1965; Stringfellow and Glasgow, 1972). The

capacity to produce interferon is often decreased in transplant patients with HIV infection and in patients with AIDS. The compounding factor is that truly immunosuppressed individuals are also those who are most likely to be infected by an opportunistic virus.

7.3. PATTERNS OF CELL-MEDIATED RESPONSES IN PATIENT GROUPS

At this time, because of the lack of adequate test procedures or their difficulty, we do not have a complete picture of the known cell-mediated responses in the many categories of CMV infection. Some of the responses occur early during infection, before patients are available for study. This makes accumulation of complete data particularly difficult. Still, because of rapid technical advances, progress in this area of knowledge has been and will continue to be striking.

7.3.1. Response in Cytomegalovirus Mononucleosis

In the normal subject with CMV mononucleosis, two conflicting forces are at work. On the one hand, the patient is specifically stimulated by the invading virus, and on the other, the infection itself suppresses cellular immunity.

Data are available on the proliferative, interferon, lymphocyte subset, and cytotoxic responses. Levin *et al.* (1979) and Rinaldo *et al.* (1977) found that the mean stimulation indices (S.I.) of the proliferative response to glycine-extracted AD169 antigen measured 9–26 days after onset of illness was 2.9, which is about ten times lower than the S.I. for normal seropositive subjects (25, range 7–68). It was still less than 10 until 60 days after illness. By 85–274 days after onset of illness, it reached a mean value of 32, which is similar to the response of immune subjects. The S.I.s against glycine-extracted herpes simplex and varicella–zoster antigens were also subnormal during the first 2 months after CMV infection. Although the lack of response to CMV may be attributed to late stimulation of lymphocyte reactivity, the depression of response to other antigens suggests that immunosuppression played a role.

Hence, although CMV antibodies peaked by the second month, the proliferative response against heterologous antigens was inhibited during the first 2 months and did not attain its maximum until after 3 months. These results are similar to what is found after mCMV infection of mice, except that the time scale is expanded about threefold in man (Section 16.6).

Ten Napel and The (1980) also found that in primary acutely infected patients, the development of lymphocyte proliferative reactivity lagged behind the development of immunofluorescent antibodies. In 14 patients, the median was 54 days after onset of illness. Antibodies to CMV early antigen developed in less than 30 days. Once positive, lymphocyte reactivity remained positive. Subjects who were positive

for both late and early CMV antigen had high lymphocyte reactivity except those over 60 years old. This may be related to decreased immunologic competence with aging. The relationship of lymphoreactivity with pregnancy, congenital infection, and transplantation is discussed below (Sections 7.3.2 and 7.3.3).

Blood cells from acute CMV mononucleosis patients were hyporesponsive to certain nonspecific mitogens (Rinaldo *et al.*, 1977). The patients' cells responded poorly to optimal concentration of pokeweed mitogen and concanavalin A (Con A) compared to cells from convalescent patients or normal subjects. Reactivity to phytohemagglutinin (PHA) was normal.

There was an interesting difference in the blastogenic response to PHA between blood cells from patients with community-acquired or posttransfusion CMV mononucleosis (Rinaldo *et al.*, 1980). Cells from the latter patients were hyporeactive to all three mitogens during the acute phase of illness. This may be related to other possibly immunosuppressive events in these patients, e.g., surgery, blood transfusion, or to a more severe CMV infection.

Levin *et al.* (1979) reported that the interferon response of blood cells from CMV mononucleosis patients to CMV antigen was diminished. The mean titer was 55 units/ml compared to normal subjects whose cells produced 190 units/ml. Interferon production in response to CMV antigen increased twofold during convalescence, but it was still below that noted with seropositive normal donors. In contrast, HSV and VZV antigens induced equal amounts of interferon in blood cells from three donors. It is not clear which type of interferon was produced.

Rinaldo *et al.* (1980) also reported that the production of interferon by cells from patients with CMV mononucleosis in response to the three mitogens was diminished two- to fourfold compared to normal cells. During convalescence, production was normal. Interferon stimulated by these agents was usually γ-interferon.

Oill *et al.* (1977) reported a deficiency of T cells in a case of CMV mononucleosis. The patient had 7% T cells in the peripheral lymphocytes measured by sheep red cell rosettes 3 weeks after onset of illness compared to 65–70% in normal subjects. This situation is different from EB virus mononucleosis in which there is an absolute increase in both T and B cells during acute illness (Pattengale *et al.*, 1974). The patient also lost skin reactivity to mumps antigen during the acute illness.

Alterations in T-lymphocyte subsets without marked change in the total number of T cells have been observed during acute CMV mononucleosis as in the case of EBV mononucleosis (Carney *et al.*, 1981). There was a marked increase in CD8 suppressor cells and some decrease in CD4 helper cells, such that the ratio of CD4 to CD8 cells was less than 1 (0.1 to 0.5). The usual ratio was 1.5. Similar changes were found in helper (Lyt 1.2) and suppressor (Lyt 2.2) T lymphocytes in mice after murine CMV infection (Sell *et al.*, 1985) (see also Section 16.6.2). These modest changes are quite different from that seen after HIV infection and AIDS, where there is primarily an absolute decrease in CD4 cells. Interestingly, abnormalities in lymphocyte subsets persisted as long as 10 months, whereas they lasted only about 2 months in EBV mononucleosis (Reinherz *et al.*, 1980).

Functional changes accompanied these alterations (Carney *et al.*, 1983).

Though more numerous in circulation, the CD8 cells from patients with CMV mononucleosis were hyposensitive to the mitogenic stimulus of concanavalin A, and they were less able to develop specific cytotoxicity against Laz 156 cells, an EBV-infected B-cell line, after *in vitro* stimulation with these cells. Lymphocytes with CD8 and Ia ("activated") or Leu 2 markers were selectively depleted on *in vitro* culture (Carney *et al.*, 1983; Rinaldo *et al.*, 1983). Rinaldo *et al.* (1983) also found that a new T-cell subset, Leu 1^+ $2a^-$ $3a^-$, emerged after preculture.

As in the case of EBV mononucleosis, the atypical lymphocyte seen during CMV mononucleosis was shown to be largely CD8 in phenotype (about 70%). Possibly these represent immunologically committed cytotoxic or suppressor T cells (Felsenstein *et al.*, 1985c).

The base-line as well as interferon-stimulated NK cytotoxic response against nonspecific K562 was normal during acute CMV mononucleosis (Rinaldo *et al.*, 1983). This is different from the frequently low NK response during CMV infection after transplantation (see below). Lymphocytes of the patient with HIV infection or AIDS and CMV infection may also be markedly defective in lysing CMV-infected fibroblast targets or nonspecific NK cell targets (Rook *et al.*, 1983, 1984b, 1985).

Taken together, the evidence is that active CMV infection depresses cell-mediated immunity. Lymphocyte responses to antigens of CMV and to nonspecific mitogens is decreased. There is also a decrease in the capacity of peripheral leukocytes to induce interferon and a decrease in the concentration of inducer, helper T lymphocytes and an increase in the concentration of suppressor, cytotoxic T lymphocytes. The specific cytotoxic response has not yet been studied in immunologically normal subjects who have CMV mononucleosis.

7.3.2. Response in Transplant Patients

As is pointed out below (Section 13.1), recipients of organ or tissue transplantation are of special interest because of their susceptibility to infection by CMV. All aspects of their immunologic responses are of interest, but particularly responses of CMI, as these have more frequently been abnormal. It should be noted that almost all transplant recipients are on cytotoxic immunosuppressive therapy, which may affect such responses, and there are no good indicators for the efficacy of such drug therapy. It may be particularly difficult to determine whether an observed effect is caused by immunosuppression or viral activity.

7.3.2.1. Proliferative and Interferon Responses

Linnemann *et al.* (1978) described the results of a lymphocyte proliferation test in 15 renal transplant recipients using as targets mitomycin-treated, 4-day-infected WI-38 cells. Lymphocytes were incubated with these targets for 3 days, and [^{3}H]thymidine incorporation was measured. A stimulation index ≥ 3 represented a specific response.

Lymphocytes of 11 patients (one seronegative) responded before operation,

but all became negative during the first postoperative month. In the subsequent month, six regained their reactivity. Serologic rises (both IFA and CF) occurred in 12 patients. During the first month after transplantation, PHA stimulation index dropped from 175 to 18. Humoral and cellular immune responses were dissociated.

Haahr *et al.* (1979) studied lymphocyte responses to HSV and CMV antigens in 59 renal transplant patients 1 day to 7 years after transplantation. Lymphocyte transformation against CMV was depressed at all times tested. No difference in transformation indices against HSV was noted in patients seropositive for HSV type 1, 2–12 months after transplantation.

The depression of specific lymphocyte transformation was dramatically reduced up to 18 months after renal transplantation, according to Pass *et al.* (1981a). It was worse in patients who received antithymocyte globulin, who also had more viremia and CMV-related diseases (see Section 13.1).

Heat-stable interferon formation produced by lymphocytes stimulated by HSV was suppressed up to 12 months after transplantation. Haahr *et al.* (1979) used CMV to induce lymphocytes from seropositive recipients. No interferon was made during the first month after transplantation, and it was suppressed when tested at 2–12 months and more than 12 months after transplantation. For reasons not stated, cells from seronegative subjects did not produce interferon in any of the three periods. Presumably, immune or gamma interferon alone was involved, although it was not specifically tested for.

Pollard *et al.* (1978) followed 12 patients after cardiac transplantation of whom nine were seropositive for CMV. All became infected as proven by virus isolation by 6 months after transplantation, and many had serologic (CF) rises. Specific lymphocyte transformation, however, was inhibited to the level of normal seronegative controls. Only in the group that survived for more than 3 years were responses more like those of immune normals. Interferon produced by lymphocytes induced by CMV *in vitro* was also suppressed in the infected.

In contrast, the PHA response in cardiac transplant patients was only about 50% suppressed (transformation index was 23 versus 58 in normal subjects) up to 3 years after cardiac transplantation (Pollard *et al.*, 1978). Lopez *et al.* (1974b) found that washed leukocytes of renal transplant patients responded normally to PHA. These data suggest that as in the normal subject with CMV mononucleosis, the PHA response is less easily suppressed (see Section 7.3.1).

Levin *et al.* (1978) described the lymphocyte proliferation and interferon responses in patients after bone marrow transplantation. These patients are different from renal and cardiac transplant recipients because their lymphocytes are derived from a normal donor, and they are frequently undergoing a graft-versus-host response, which in itself may be immunosuppressive. Their lymphocytes proliferated normally and produced interferon in response to PHA and pokeweed antigen. Cells from nine patients who were seropositive or undergoing CMV infection reacted abnormally. Two did not proliferate in response to CMV antigen. In the late transplant period (>13 weeks), lymphocytes of three out of five patients did not produce interferon after stimulation with CMV. In normal controls, lymphocyte proliferation and interferon formation in response to CMV went together.

A total of 158 patients undergoing marrow transplantation were studied for their specific CMV proliferative response (Meyers *et al.*, 1980c). It was depressed in seropositive patients 20–40 days after the procedure. Isolation of CMV was associated with further depressed responses. Later, they increased, particularly if there was a serologic rise. Survivors had normal responses. Interestingly, seronegative recipients who received seropositive cells had negative responses unless they became infected.

Strong *et al.* (1978) found that plasma and spinal fluid from a marrow transplant recipient with evidence of CMV infection specifically blocked the production of migration-inhibition factor and the proliferative response by lymphocytes in response to CMV. Fractionation and immunologic inactivation studies suggested that the blocking factor contained IgG and C3 and that it may be an antigen–antibody complex. Lopez *et al.* (1974b) also found that serum of renal transplant patients inhibited the PHA response. The significance of these findings is still unclear.

Witherspoon *et al.* (1979) studied the responses of lymphocytes of 51 marrow graft recipients to PHA and to irradiated allogeneic lymphocytes in a mixed lymphocyte culture. Patients who developed interstitial pneumonia had lower responses in both tests. These tests were helpful in identifying patients at risk.

These results suggest that an accentuation of the abnormalities of the proliferative and interferon responses described in acute CMV infection occurs in the transplant recipient. Depending on the severity and duration of CMV infection, there may be absence or loss of specific lymphocyte proliferative response to CMV. There is an associated loss of interferon production stimulated by CMV. The proliferation and interferon responses to HSV are less disturbed, and the responses to nonspecific mitogens, particularly PHA, may be close to normal.

7.3.2.2. Changes in Lymphocyte Subsets during CMV Infection after Transplantation

The two major problems after transplantation are rejection and infection. It may be difficult to distinguish between rejection and infection with CMV, which is one of the most important categories of infection after transplantation. Cosimi *et al.* (1981) suggested that perhaps rejection episodes may be diagnosed by monitoring T-helper to T-suppressor cell ratios, which were frequently greater than 1 during acute rejection. Schooley *et al.* (1983) from the same institution extended their observations on the effect of CMV infection on lymphocyte subsets in normal subjects (see above) to renal transplant recipients on azathioprine and prednisone. They reported that during primary and reactivated CMV infection as well as other herpesvirus infections after transplantation, CD4 lymphocyte counts in such patients were decreased, such that inversions (values of less than 1) of the helper-to-suppressor cell ratios occurred. Fiche *et al.* (1984) reported from France that 39 renal transplant patients did indeed have the reported changes in subset ratios during acute CMV infection but not during acute rejection. Maher *et al.* (1985) reported that a significant number of the CD8 cells also have a Leu 7 phenotype, which is the

marker for natural killer cells. O'Toole *et al.* (1986) reported that in cardiac transplant recipients on cyclosporine and prednisone, inversion of T-cell subset ratios preceded the rise in titer of antibody to CMV. Inverted ratios persisted in chronically infected patients, as determined by persistent urinary excretion of CMV for as long as 3 years. Infected patients had a greater than tenfold increase in T-suppressor cells and a 2.5-fold decrease in T-helper cells compared to reinfected controls.

However at other institutions, and particularly in patients on cyclosporine, monitoring lymphocyte subset counts or ratios was not helpful in diagnosing CMV infection. Rinaldo *et al.* (1986) studied a group of 16 renal transplant recipients on cyclosporine and prednisone at the University of Pittsburgh. Only four with viremia had subset ratios less than 1. From the same institution, Dummer *et al.* (1984) studied 11 cardiac recipients on cyclosporine and prednisone of whom only three had subset ratios less than 1. These patients had CMV viremia and concomitant acute EBV infections.

In conclusion, acute CMV infection does produce lymphocyte subset changes. This becomes more apparent when patients are studied as a group. The changes are not sensitive or clear-cut enough to be of diagnostic assistance in individual patients. At the University of Pittsburgh, as probably at most other centers, subset monitoring is no longer *de rigueur* for the diagnosis of either herpesvirus infections or acute rejection.

7.3.2.3. Cytotoxic Responses in Transplant Patients

Transplant patient recipients are particularly suitable for observing specific and nonspecific cytotoxic responses because CMV infection may be followed during its entire course in this group. Up to now, CMV-specific cytotoxic T-cell (CTL) responses have only been described in transplant patients, and even so, by only one group of investigators. There are three reasons for the paucity of reports. First, this immune response usually occurs within a week or two after infection and is transient in nature. Therefore, it will usually be missed in a sporadic infection. Secondly, HLA-compatible infected target cells are essential for the assay of this response, and they must be prepared in advance. Thirdly, the CTL assay is technically difficult to carry out. Essential controls and the required number of cells may be difficult to come by. To prove conclusively that CTL are involved, one must also exclude activity of NK cells or non-HLA-restricted cytotoxic cells.

Quinnan *et al.* (1982) first studied the CTL responses in 58 bone marrow transplant recipients. Syngeneic fibroblast cell targets infected with CMV were prepared first. Nonrestricted cytotoxicity was measured using allogeneic CMV-infected targets. The NK-cell activity was measured against K-562 targets and ADCC by cytotoxic activity against antiserum-treated Chang liver cells. Cytomegalovirus infection developed in 43 patients. During infection, CMV-specific CTL responses developed in nine out of 18 patients who survived and in none of the ten who died of CMV interstitial pneumonia. The NK cell or ADCC responses were enhanced in the other nine survivors and in only two of those who died. Thus,

their conclusion was that both specific CTL and nonspecific cytotoxic responses were associated with recovery from CMV infection.

Rook *et al.* (1984a) studied the cytotoxic responses during CMV infection after renal transplantation. They found that 20 out of 30 recipients developed CMV infection diagnosed by virus isolation or serologic rise. Fourteen developed CMV-specific CTL, usually before virus isolation (7/9) and before antibody rises (10/14). Thus, the CTL response may be one of the earliest specific laboratory responses after CMV infection. Apparently it occurred in both primary and secondary infections, although this point was not specifically addressed. No CTL response occurred when there was no evidence of CMV infection.

As in the bone marrow transplant recipients, the CTL response in kidney recipients was associated with a more benign clinical course with no complications. Patients having a CTL response did not have as much fever, leukopenia, thrombocytopenia, and elevated liver function tests as those who did not. Cytomegalovirus infections complicated by pneumonia (one), death (one), or nonviral superinfection (three) were seen among the six patients who had no CTL responses. However, unlike bone marrow recipients, none died.

An interesting finding was that acute allograft rejection during infection was experienced by four of the six patients without CTL responses but only one (1/14) with responses. The meaning of this is unclear. The relationship of graft rejection to CMV infection is complex (see Section 13.1.4).

The NK response against K-562 cells and ADCC responses against Chang liver cells were significantly higher in patients with CTL responses than in patients without responses.

Bowden *et al.* (1987) confirmed the finding that NK responses are diminished in marrow transplant patients with CMV infection. Using CMV-infected fibroblasts as targets, he found that patients with severe or fatal CMV infections had lower NK-cell responses than asymptomatic virus shedders. Patients with GvH reactions also had lower responses. Uninfected subjects had the highest response of all.

The NK-cell response to nonspecific targets such as K-562 cells has been studied by many other groups in transplant recipients. Normal subjects usually have significant circulating NK-cell activity, which can be further enhanced by *in vitro* incubation with interferon. Gui *et al.* (1983) found that the mean NK activity of renal transplant patients on either azathioprine and prednisone or cyclosporine and prednisone studied at various times after transplantation was significantly decreased, as was the ability of interferon to enhance NK activity. In the cyclosporine group, base-line levels increased, and enhancement by interferon recovered, when cyclosporine doses were decreased later after transplantation or when there was no CMV viremia. As noted above, Quinnan *et al.* (1982) and Rook *et al.* (1984a) correlated low NK activity with poor response to CMV infection in marrow and renal transplant patients. Rook (1988) believes, however, that cyclosporine–prednisone drug regimens by themselves produce little or no suppression of NK activity or ADCC-type cytotoxicity, whereas patients on azathioprine–prednisone exhibit marked abnormalities in these modalities 1 week after transplantation. This may

come about because azathioprine is reported to decrease the number of circulating large granular lymphocytes (Ramsey *et al.*, 1984).

In summary, so far only one group has provided data on specific CTL response in transplant patients. Their results indicate that it is critical to effective recovery from CMV infection. However, the number of patients studied so far is small, and confirmation of their work is needed. On the other hand, there is some evidence that the NK response is diminished in transplant patients, and in marrow recipients, it seems to be inversely correlated with severity of infection.

7.3.3. Response in Infants with Congenital Cytomegalovirus Infections and Their Mothers

As is shown in Section 11.3, transplacental infection does not always follow maternal infections, and not all congenital infections are accompanied by symptomatic disease. We know little about the determinants of these outcomes.

Gehrz *et al.* (1977) studied four children, 5 months to 4 years old, with active CMV infection demonstrated by elevated antibody titers and viruria. Although they had normal numbers of T lymphocytes and normal responses to PHA, Con A, and pokeweed antigen, they responded poorly to CMV in the proliferation tests. Three of the four mothers studied shortly after delivery (5–11 months) also had low lymphocyte proliferation responses. One patient who had delivered more than 3 years before responded normally. These abnormal responses of the mother are consistent with the findings of Starr *et al.* (1979) (see below).

Rola-Pleszczynski *et al.* (1977) described a specific lymphocyte cytotoxicity test against CMV-infected cell targets to measure CMI. Since HLA compatibility between effector and target cells was not considered, as in the test of Thong *et al.* (1976) (see Section 7.2.2), it is not known whether specific cytotoxicity T cells or NK cells were measured. Using this test, the authors found that eight mothers of congenitally infected babies and two adult nulliparous women with persistent viruria had depressed responses. The test results of eight congenitally infected infants, 6 to 27 months old, were normal. These results are not in line with those using the usual proliferative tests.

More recently, Gehrz and Rutzick (1985) found circulating HLA-restricted cytotoxic T cells (CTL) against CMV-infected fibroblast targets in some congenitally infected babies as well as NK-cell-like activity against these targets. There were not enough data to determine whether there was any defect in the CTL response. They also found a CTL response in blood leukocytes of normal seropositive individuals after *in vitro* stimulation with CMV antigen. Charpentier *et al.* (1986) described a system of development autologous target cells by creating these with CMV antigen and using these to stimulate specific anti-CMV CTL. This system has not been further evaluated.

Reynolds *et al.* (1979a) reported that lymphocytes from five out of six symp-

tomatic, congenitally infected children did not respond in a specific lymphocyte transformation assay. Only six out of 24 other children who had silent congenital or perinatal infection did not respond, but those who did had lower responses that normal immune adults. Thus, viruria and presence of disease correlated strongly with absence of response. Four children seropositive for herpes simplex virus also showed low proliferative response to herpes simplex antigen. Lymphocytes of the mothers of congenitally infected babies also showed an impaired response to CMV but reacted normally to HSV-1 antigen. Mothers of children with symptomatic congenital infection were most impaired. The responses to mitogens of lymphocytes of the mother and children were normal. It is impossible to say whether this immunologic deficiency is the cause or the result of infection.

Starr *et al.* (1979) studied the lymphocyte proliferative and interferon response to CMV of eight symptomatic, congenitally infected children. Normal seropositive adults responded positively in both tests. Six viruric children responded poorly in both assays. Two older nonviruric children responded in the blastogenesis assay, and lymphocytes from one of them produced interferon. The blastogenic responses were significantly depressed in mothers whose congenitally infected infants were less than 9 months old. The interferon response was suppressed in seven of the eight mothers. Normal seropositive postpartum women responded as did other normal adults. Impairment in children did not appear to be permanent, as older children and most of the mothers responded. It did not correlate with the extent of neurological damage in this symptomatic group.

Gehrz *et al.* (1981) found that the CMV-specific lymphocyte proliferative response was significantly depressed in pregnant women during the second and third trimesters. T-lymphocyte counts and mitogen-induced proliferation and humoral responses were not affected. This selective depression of cell-mediated immunity appears to be physiological.

Tamura *et al.* (1980b) compared CMV-specific lymphocyte transformation in unselected subjects of various ages. All seropositive subjects responded, but the stimulation index (mean: 5.7) of lymphocyte from children less than 1 year of age or between 1 and 5 years of age was significantly lower. Similar results were reported by Pass *et al.* (1981b), who noted, however, that lymphocytes from children with evidence of CMV infection, but less that 1 year old, did not respond at all (none of eight times), whereas older children responded better. A stimulation index of over 3 was considered a positive response. Most patients were clinically asymptomatic. Two conclusions were drawn: (1) The immunosuppressive effect of CMV infection as measured by specific lymphocyte proliferation is more severe in infants. (2) This suppressive effect is virus-specific, since neonates with herpes simplex infection responded to herpes antigen.

In summary, as in the case of other CMV infections, there are gradations of immunosuppression in the congenitally infected depending on the severity and duration of infection. Mothers of congenitally infected babies also show deficiencies in their lymphocyte proliferation and interferon responses. How infection and pregnancy interact to produce these abnormalities is still unclear. At least some of

TABLE 7.1
Immunologic Stimulation and Suppression by HCMV Infection[a]

Condition of CMV infection	Stage	Antibody response	Proliferative responses to					Interferon response to		Reference
			Specific antigen	Other antigen	Con A	PWM	PHA	CMV	Others	
Inapparent infection		N	N	N	N	N	N	N	N	
Community mononucleosis	Acute	N	D	D	D	D	N	D		Rinaldo *et al.* (1979a)
	Convalescent	N	N	N	N	N	N	N	N	
Transfusion mononucleosis	Acute	N	D	D	D	D	D			Rinaldo *et al.* (1979a)
	Convalescent	N	N	N	N	N	N			
Mothers of congenitally infected			D	D	N		N	D		Gehrz *et al.* (1977) Starr *et al.* (1979)
Women with viruria			D							Gehrz *et al.* (1977) Starr *et al.* (1979)
Congenital infection	Symptomatic	N	N,D	N,D	N		N	D		Reynolds *et al.* (1979a)
	Asymptomatic	N	N							Starr *et al.* (1979) Gehrz *et al.* (1977)
Perinatal infection		N	N		N		N			Reynolds *et al.* (1979a) Starr *et al.* (1979) Gehrz *et al.* (1977)
Posttransplant	Acute	N,D	D,A	N	D		D		D	Linnemann *et al.* (1978)
	Convalescent	N	D,N	N					D	Pollard *et al.* (1978) Haahr *et al.* (1979)

[a]N, normal; blank space, no data; D, depressed; A, absent.

the observed effects in the mother and perhaps in the child are a normal response during pregnancy. Table 7.1 is a summary of immunologic stimulation and suppression by various types of CMV infection. So far there still are not enough data to determine whether the HLA-restricted cytotoxic T-cell response is affected in any of the patient groups listed except the transplant recipient (see Section 7.3.2.3).

8

Variants of Human Cytomegalovirus

8.1. VARIANTS ACCORDING TO NEUTRALIZATION TESTS

Antigenic variations of human cytomegaloviruses were first detected by cross-neutralization tests. Weller *et al.* (1960) reacted sera from two babies (named Davis and Esp.) with isolates from themselves, with isolates from each other, and with AD169, an isolate from adenoids (Rowe *et al.*, 1956). The homologous serum–virus pairs produced the highest titer. Serum from Davis did not neutralize the Esp. and AD169 isolates, whereas it neutralized the Davis isolate at a high dilution (Table 8.1). The Davis and AD169 strains were designated types 1 and 2, representing the most diverse strains, and Esp. and Kerr isolates were designated type 3. The Kerr isolate (not shown in the table) appeared to be closely related to the Esp. strains. These type designations have not been generally accepted.

The significance of the observed differences has been difficult to evaluate. The following factors have prevented a clear resolution of the problem of types on the basis of serologic tests: (1) low neutralization titers of most human sera; (2) difficulty in raising antiserum in animals; (3) possible heterogeneity of human serum antibodies because they may represent a response to multiple infections by more than one strain; (4) change of avidity of both human and animal serum with intensity of antigenic stimulation; and (5) possible change of antigens with prolonged *in vitro* passage of virus strains. Many of these difficulties have gradually been overcome in the last 20 years, but there is still no clear consensus regarding the problem of types. For clarity, I state my tentative conclusion at the outset and present the data subsequently. There are antigenic variations among CMV isolates, but these are not great enough or clear enough to warrant the designation of different types of human cytomegaloviruses. Other methods of analysis, such as restriction endonuclease digestion patterns and nucleic acid hybridization studies, confirm the variability of the virus genome but do not indicate that variations are great enough or uniform

TABLE 8.1
Antigenic Variation of hCMV According to Neutralization Tests

Antiserum from baby	Neutralization titer against three strains[a]		
	Davis	Esp.	AD169
Davis	512	<16	<16
Esp.	64	128	10

[a]Data from Weller *et al.* (1960).

enough to assign different prototypes (see Sections 2.2.2 and 8.7). So far, study of antigenic viral peptides have not produced support for types of CMV (see Chapter 3). For practical diagnostic purposes, there is no need to worry about type variations. The ELISA, CF, immunofluorescent, and other tests are sensitive and specific enough to diagnose most CMV infections. The serologic tools for the diagnosis of CMV infection are about as good as those for any other infection. However, the important question, how much cross immunity there is in terms of resistance to other strains following infection by one, remains unresolved.

Following the report of Weller *et al.* (1960), numerous attempts to improve serologic diagnosis of CMV infection by using many types of antigens did not improve sensitivity. In the field or in the clinic, the neutralization test has not been found to be more discriminatory than the CF test.

Medearis (1964a) followed the CF and neutralization titers of four babies with C.I.D. The neutralization titers were low and frequently absent, whereas CF titers were always positive and diagnostic.

Carlström (1965) found that one strain, AD169, detected neutralizing antibodies in all cases of C.I.D. he collected. He also showed that neutralizing and CF antibody positivity coincided in a study of seroprevalence in 242 children and adults in Stockholm, although in all age groups, there were slightly more subjects with neutralizing antibodies ($\geq 1:10$).

Andersen (1970) studied CMV infection after renal transplantation in five adults and tried to determine if different strains were involved. The CF titers against homologous isolates or against AD169 were identical in all five patients. The earliest CF titer rise was detected 29 days after transplant. The neutralization titer always lagged behind, especially in cases of primary infections, where the patient was seronegative before transplant. In four out of five patients, strain AD169 was as effective as the homologous strain in detecting neutralizing antibodies. In one, the neutralizing titer was fivefold higher when the homologous strain was used in the test. The neutralizing titers were more sustained, and it was suggested that their late rise may be helpful in cases where the CF titer is already high.

8.2. DEVELOPMENT OF SPECIFIC ANTISERUM

Monospecific animal serum is essential to a proper analysis of serotypes. Early failures (Medearis, 1964a) to immunize animals against mCMV resulted from lack

of antigenic mass and failure to use adjuvants. Failure to add complement in the neutralization reaction may also have obscured the presence of antibody. Mäntyjäryi (1968) used glycine buffer (pH 9.0) to extract AD169-infected human embryonic fibroblasts. When administered with Freund's adjuvant, this antigen raised antisera in rabbits with CF titers around 1 : 128–256 and neutralization titers around 1 : 18. Krech and Jung (1969) injected a similar antigen in guinea pigs. Their best serum reduced the infectivity titer of the Davis or AD169 strains ten- to 100-fold at dilutions of 1 : 5 and 1 : 10.

Graham *et al.* (1971) used complement to enhance the neutralization reaction, a phenomenon well known in virology and already described for the herpes simplex virus (Yoshino and Taniguchi, 1964). They noted that high-titer monkey antiserum, with titers around 1000 to 2000 against hCMV, could be raised if complement were added in the neutralization test. Without complement, no neutralizing antibodies were evident. The complement requirement extended to antibodies that were raised early or late during immunization, and to 7 S or 19 S γ-globulins. Two human strains, AD169 and C-87, were used to immunize baboons and monkeys in order to develop monospecific serum. Monkey antiserum was used in cross-neutralization tests, and these two strains were indistinguishable. Sera from baboons immunized with either strain also cross neutralized the heterologous strains, but the titer of the homologous serum–virus pair was three to four times higher. These two strains were earlier shown to be different by the CF test (see Section 8.3; Dreesman and Benyish-Melnick, 1967).

Andersen (1971) immunized rabbits with AD169 or T27, a new isolate. Titers as high as 1 : 800 against AD169 were obtained in the presence of complement. The antiserum against AD169 failed to distinguish the two strains, but the serum against T27 had a higher neutralization titer against the homologous virus. As in the case of the CF reaction, AD169 appeared to have a broader antigenic spectrum (see Section 8.3).

Later, Andersen (1972) prepared hyperimmune rabbit serum against AD169, Davis, Esp., and T27 and tested these by cross neutralization. He found that each strain was neutralized by all four types of sera. The T27 seemed most different, but there was no difference between the Davis and AD169 strains, the types 1 and 2 of Weller *et al.* (1960). Fifty CF-negative and 50 CF-positive human sera were also screened. Each positive strain was neutralized by AD169, Davis, or T27 strains. No CF-negative serum had neutralizing activity.

With the development of monoclonal antibodies, it has become relatively commonplace to develop murine antibodies against viral structural and nonstructural proteins (see Chapter 3).

Volpi and Britt (1985) used a panel of 20 monoclonal antibodies reactive against a variety of viral structural proteins, including envelope glycoproteins, tegument, and capsid proteins, to distinguish serologic differences between strains of CMV. Only one such antibody was found. The monoclonal antibody "11-1-1" reacted against a 90,000-dalton "late" virus-encoded protein. It reacted against all strains of CMV except for the Towne strain, which is a clinical isolate that has been subsequently used for vaccine development (see Section 14.2).

8.3. VARIANTS ACCORDING TO COMPLEMENT-FIXATION TESTS

Dreesman and Benyesh-Melnick (1967) approached the problem of antigenic heterogeneity by testing African green monkey antisera developed against two monkey CMVs (2598 and 2757) with human CMV strains in the CF test. Monkey CMVs have some relationship to CMV since they can be propagated in human embryonic lung fibroblasts. Ninety percent of 29 monkey sera positive for anti-2598 reacted with AD169, but none reacted with C-87, a human strain isolated by Benyesh-Melnick *et al.* (1964). Approximately 50% reacted with human strains BC-13 and L38E, and 25% reacted weakly with Davis. Hence, on the basis of reactivity with monkey antiserum, AD169, Davis, and C-87 seemed clearly differentiated. There was no crossing between monkey and human hCMV by the neutralization test, although complement was not used. The cross by the CF test was unidirectional. No human serum that contained antibodies against hCMV reacted with the two monkey antigens either by the CF or neutralization tests. The authors suggested that the variability of hCMV may be determined by a "mosaic" of antigens that cross-react to a varying degree. The AD169 seemed to share more antigens with monkey CMV than with other strains, which may be a reflection of its broader reactivity.

Huang *et al.* (1974) prepared antiserum in guinea pigs stimulated by purified extracellular virus free of cellular antigen (Huang *et al.*, 1973). High-titer antisera to C-87, AD169, and Simian GR-2757 strains were prepared. By the CF test, the sera against AD169 and C-87 crossed with each other. Titers were 1 : 640 against heterologous and 1 : 2560 against homologous strains. No significant difference was detected. Only one serum out of three against GR-2757 fixed complement at the low titer of 1 : 20 in the presence of human CMV strain C-87, and no antiserum made against AD169 crossed with the monkey virus. Immunofluorescent antibody titers were higher (1 : 25,600 to 1 : 10,240), and relatively little interspecies cross reactivity was evident.

8.4. VARIANTS ACCORDING TO KINETIC NEUTRALIZATION TESTS

The avidity of neutralizing antibody for a virus can be studied in the kinetic neutralization test, which has been used to distinguish closely related viruses such as vaccine and wild community strains of poliomyelitis (McBride, 1959). The neutralization constant is an expression of avidity.

Andersen (1972) first reported an analysis of strain differences in hCMV using neutralization kinetics. An antiserum was calculated to have a neutralization constant K, according to the formula

$$K = \log (V_0/V_t) (2.303D/t)$$

TABLE 8.2
Segregation of Strains Using Neutralization Kinetics

Rabbit antiserum	Percentage relatedness to homologous strains, NK[a]		
	75–100	50–75	25–50
Davis	Kerr	Esp.	AD169
	C-87		Towne
	Davis		
Esp.	Kerr	Davis	
	C-87	Towne	
	Esp.		
AD169	Kerr	Davis	Towne
	C-87		
	Esp.		
	AD169		

[a]NK, neutralization kinetics. Strains in *italics* are homologous. Data from Waner and Weller (1978).

where V_0 = initial virus concentration, V_t = virus concentration after t minutes of neutralization reaction, and D = dilution of serum. K is an expression of potency or avidity of the antiserum. K values were determined for human sera and the rabbit sera raised against AD169, Davis C87, and T27 strains. The K values were relatively low, i.e., 0.1 to 3.0. Andersen first found that all sera neutralized the four strains to some extent. Hyperimmune rabbit serum neutralized the homologous strain best, except that the serum against the Davis strain neutralized all strains almost uniformly. No difference was found between the AD169 and Davis strains, contrary to the earlier findings of Weller *et al.* (1960).

A more complete study of neutralization kinetics was reported by Waner and Weller (1978). More potent hyperimmune rabbit serum was obtained after injections of antigens extracted with glycine-buffered saline (pH 9.0) and Freund's adjuvant. Antisera against homologous virus with K values of 10 or greater were developed against the strains listed in Table 8.2.

Differences in the immunogenicity of various strains were shown. Esp. induced antisera of greater activity; Kerr was neutralized better by heterologous than homologous strains. Sera obtained 4 weeks after the immunization were more specific than sera taken after 12 weeks. Three of the four early sera against the Davis strain titered fourfold or higher against Davis than against AD169; among four later sera, only one was different to this degree. Hence, strain differences were most apparent in early sera. The IgG and IgM fractions of serum were studied separately for their neutralizing activity. The 7 S antibodies were enhanced by complement, although they did neutralize without complement. Preparations of 19 S antibodies did not neutralize virus without complement. These were monospecific and did not cross (Davis and AD169 tested), but their titers were too low for the fraction to be useful in further analysis. The highest titer was only 1 : 16.

To determine strain antigenic variations, early antisera made against Davis, Esp., and AD169 strains were used for cross-neutralizing tests (Table 8.2). Anti-

genic relatedness against six strains of virus was determined by comparing *K* values, using the *K* against the homologous strain as 100%. Based on this relationship in 25% increments, the Davis and AD169 antisera differentiated three groups, whereas Esp. antiserum differentiated two. The Esp. assumed a position intermediate between Davis and AD169. Thus, extremes of variability represented by the AD169 and Davis strains, as originally suggested by Weller *et al.* (1960), were confirmed. Whether a more extensive analysis of other isolates, such as T27 of Andersen (1971), would reveal a greater variation or spectrum remains to be determined.

Zablotney *et al.* (1978) produced hyperimmune guinea pig antiserum against 17 strains of CMV and conducted 10-min kinetic neutralization tests. Based on *K* values obtained in crossed tests, there was a suggestion that four antigenic groups could be created. The isolates in each group were unrelated epidemiologically in terms of time, place, or site of isolation. Interestingly, the most unrelated strain was AD169; this finding may reflect its being a laboratory strain passed more than 300 times.

These observations are best interpreted as demonstrating variation in a number of shared and unshared neutralizing virion antigens, and there is probably a continuous spectrum of a variable mosaic of antigens among different strains.

8.5. HETEROGENEITY OF INDIVIDUAL RESPONSES

Another interesting and unusual demonstration of the existence of different strains was provided by Waner *et al.* (1973). Fifty donors undergoing plasmapheresis were followed at monthly intervals for 18 months and tested for CF antibodies against the Davis, AD169, and Esp. strains of CMV. Of 510 sera examined, 321 (63%) reacted with one or more of the antigens; 96% with Esp., 94% with Davis, and 83% with AD169. Of the 321 sera, 81% reacted with all three antigens. No serum reacted only to AD169, which suggests that it was the least sensitive or least prevalent antigen.

Patterns of reactivity were determined with 20 of the 39 seropositive donors. A mean of 14 sera were examined per subject. Multiple fluctuations of titers were observed in 19, including 11 subjects who at one or more times were seronegative. All 20 subjects showed a fourfold or greater fluctuation in titer of their serum to one or more strains. Multiple episodes of fluctuation were seen in 19. Some of the changes occurred only against one or two of the three strains.

The CF titers against herpes simplex virus showed no concomitant fluctuations. The tests were also carefully controlled, and results of repeated assays of the same serum were quite consistent. These results suggest that the host–virus relationship permits periodic antigenic stimulation. Such stimulation seems to be strain specific. Strain variation existed, and AD169 was not necessarily the most broadly reacting. A single serum sample from subjects in this population would have yielded, at best, a most incomplete picture. It would be desirable to publish the

patterns on all 20 subjects studied rather than just three. Rises in titers may be attributed to superinfection, perhaps with a strain to which the subject was not immune. But precipitous falls in titers are more difficult to explain. In one of the three subjects, after significant titers were attained against all three antigens, the titers suddenly became negative to all three during multiple observations for 12 months before rising again.

At the moment, our conclusion is that the process of plasmapheresis may underlie some of the changes observed by Waner *et al.* (1973). A similar study should be undertaken in a normal population of seropositive subjects, preferably with a number of other serologic tests in addition to the CF test. It should be pointed out that Waner (1975) detected no strain difference using the indirect immunofluorescence test (see Section 6.2.1).

8.6. VARIANTS ACCORDING TO IMMUNODIFFUSION

In screening sera by immunodiffusion (Jung *et al.*, 1973; Fioretti and Pollini, 1978) or counterimmunoelectrophoresis (Fortunato *et al.*, 1977; Niebojewski *et al.*, 1977), no more that five precipitin lines were observed. Sweet *et al.* (1979) used the AD169 strain against a serum pool from a patient with toxic megacolon selected from 2000 blood donors. As many as 20 antigens were detected by electroimmunodiffusion (EID). It reportedly had superior resolving ability, especially when an antibody gradient was used. About 14 antigens could be reproducibly seen.

Electroimmunodiffusion runs using high-passage stock C-87, Davis, and low-passage local isolates VD 14, 1694, and 1723 against the same serum resolved 15, 15, 13, 11, and 11 antigens. At least 16 antigens were common to all strains. The AD169 did not lack any antigens seen in other preparations. There was no clear-cut reaction of nonidentity with any strain. A high degree of antigenic homology of the various strains was suggested.

8.7. VARIANTS ACCORDING TO DNA STRUCTURE

A powerful new tool in the study of genetic variants of herpesviruses is the pattern of DNA fragments following digestion by one or more restriction endonucleases (Huang *et al.*, 1980b) (Fig. 8.1). This is particularly true of herpesviruses that have genomes with molecular weights in excess of 100 million (see Section 2.2.1). Coelectrophoresis of Eco R.R-1 or Hind III digests shows considerable matching among human CMV isolates. The Colburn isolate, isolated from the brain of a 6-year-old boy, showed significant matching with GR-2757, a simian strain. By DNA–DNA reassociation kinetics, the latter two strains showed 90% homology. They also crossed strongly by the CF test. The Colburn strain is hence not considered a human strain but more akin to simian CMV (Huang *et al.*, 1976, 1978).

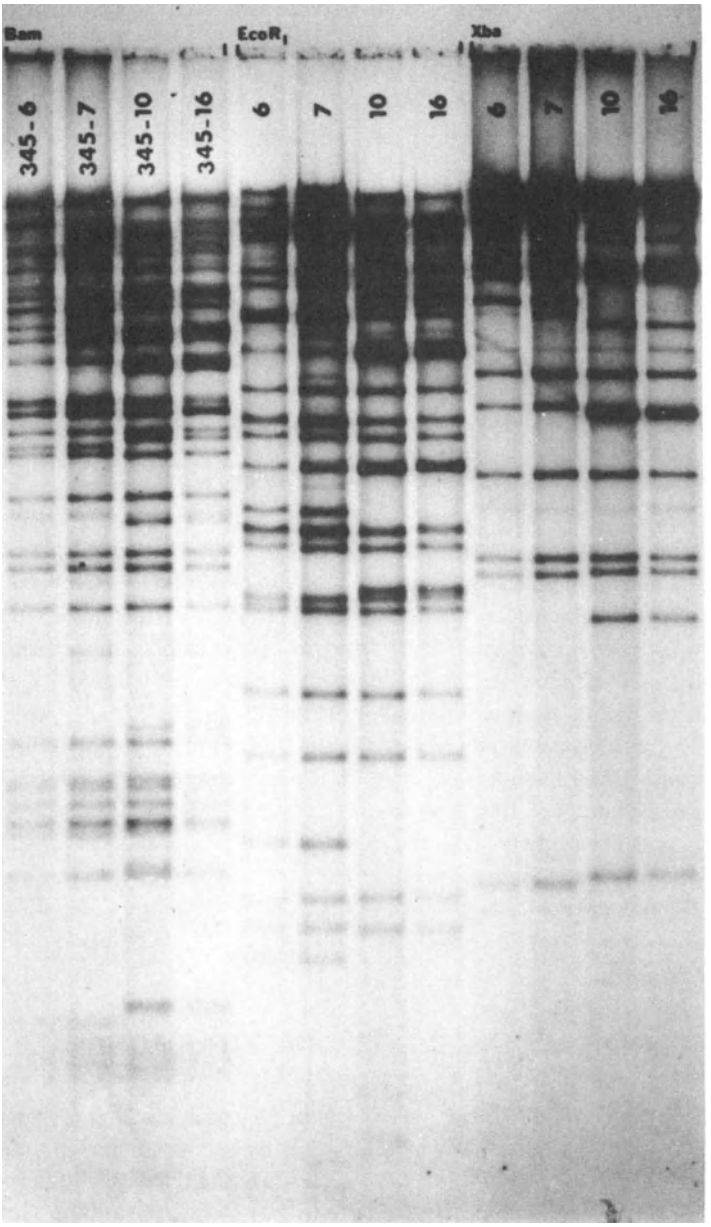

Figure 8.1. DNA restriction fragment length polymorphism of two pairs of CMV strains isolated from semen of two asymptomatic homosexual men (345–6 and 345–7; 345–10 and 345–16). Restriction enzymes Bam HI, EcoR1, and Xbal were used. Note similarity of each pair of isolates. By permission of E. S. Huang and Masson Publishing Co. (Huang, 1984).

Eleven different strains of human CMV were cleaved by HinD III and Eco R-1 nucleases. The fragments produced reproducible patterns by electrophoresis in 1% agarose slab gels. In Hind III digests, three fragments comigrated among all isolates and six among most. In Eco R-1 digests, nine fragments comigrated among all and five among most digests (Kilpatrick *et al.*, 1976).

Although no two human isolates had completely identical patterns (Fig. 8.1), study of interstrain nucleic acid homology revealed that the human CMV strains analyzed shared at least 80% DNA sequence homology with prototype AD169 (Huang *et al.*, 1976). The degree of homology existing among CMV strains is greater than that between HSV type 1 and type 2, which is about 47–50% (Kieff *et al.*, 1972). Cytomegalovirus strains isolated from the genital tract cannot be distinguished from strain isolates from other sites with regard to sequence homology.

What is the significance of the variability of the hCMV genome despite the fact that human CMV strains do share at least 80% of genetic information? The heterogeneity in restriction enzyme fragment patterns among various strains may be largely related to absence or gain of restriction endonuclease sites and not to major DNA rearrangements or major sequence duplications (see Section 2.2.2). So far genetic as well as serologic analysis has failed to reveal distinct hCMV types.

9

Epidemiology of Cytomegalovirus Infection in Man

9.1. THE DISTRIBUTION OF HUMAN CYTOMEGALOVIRUS INFECTIONS

In this chapter we consider the prevalence of CMV infection in human population groups and the major mechanisms of transmitting the virus. Transplacental transmission is separately treated in Chapter 11, along with clinical and laboratory aspects of congenital infection.

9.1.1. Prevalence of Antibodies in the General Population

The complement-fixation test was used to measure antibodies against CMV in the general population very soon after human cytomegalovirus was isolated in cell culture. Rowe *et al.* (1956) prepared CF antigen by sonic inactivation of cell culture fluids infected with their AD169 strain and used it to measure antibodies of inhabitants of the Washington, D.C., area. The results are shown in Table 9.1. It was soon evident that infection by this virus is common in the general population.

Important features of the epidemiology of CMV infection, subsequently amplified, were already apparent from their data. The frequency of antibodies in cord blood (71%), presumably a reflection of transplacental transmission from the mother, was higher than the frequency in early childhood. The frequency in 18- to 25-year-old women was higher than that in males. Rowe *et al.* (1956) also showed that all sera positive for CF antibodies also had neutralizing antibodies. Titration of

TABLE 9.1
Prevalence of Complement-Fixing Antibodies in Washington, D.C.[a]

Age of subject	No. positive/No. tested	Percentage positive
Cord blood	12/17	71
6 weeks	5/17	29
6–23 months	3/21	14
2–4 years	11/36	31
5–9 years	11/33	33
10–15 years	22/49	45
18–25 years	52/98	53
>35 years	42/52	81

[a]Data from Rowe *et al.* (1956).

serial sera in three adult males indicated that CF antibody levels remained stable for at least 4–7 years. Sera of older adults (>35 years) that were negative for CF antibodies often had neutralizing antibodies.

Stern and Elek (1965) used antigen from the Kerr strain to study the prevalence of CF antibodies in London (Table 9.2). Their data were different from the data from Washington, D.C., suggesting geographic variation of CMV infection. (1) The peak prevalence (54%) was significantly lower than in Washington (81%). (2) There was a relatively slow rise in early childhood, so that only 15% were infected at 10 years of age, whereas in Washington, D.C., 31% were infected by 4 years of age. The lower conversion rate in London in early childhood may be a reflection of a smaller population reservoir for transmission. Apparently, the greater the pool of positive adults, the more rapid is the acquisition in childhood.

Another interesting finding in this paper is the sociological basis of antibody prevalence. The prevalence of seropositivity was 18% and 29% in two day schools, but 80% or 40 out of 50 subjects tested were positive in a boarding school where increased contact among children was conducive to infection. This suggests that crowding, irrespective of socioeconomic status, is a factor. A study from Israel

TABLE 9.2
Prevalance of Complement-Fixing Antibodies and Rate of Increase with Age in London[a]

Age group (years)	No. positive/No. tested	Percentage positive	Rate of increase (%/year)
0–0.5	3/9	33	
0.5–5	4/93	4	
5–10	15/97	15	2.1
10–15	54/257	21	0.8
15–25	47/130	36	2.0
25–35	62/114	54	1.8
35–75	46/85	54	0.0

[a]Data from Stern and Elek (1965).

TABLE 9.3
Prevalence of Complement-Fixing Antibodies in Various Regions of the World[a]

City	Country	Percentage with CF antibody
Lyon	France	40
Freiburg	Germany	42
St. Gallen	Switzerland	45
Albany	USA	45
Melbourne	Australia	54
Houston	USA	79
Buenos Aires	Argentina	81
Hong Kong	China	94
Sendai	Japan	96
Manila	Philippines	100
Morocco	Morocco	98
Entebbe	Uganda	100

[a]Data based on summary of Krech (1973).

showing a higher seroprevalence in kibbutz children than in urban Jewish or Bedouin children supports this hypothesis (Sarov *et al.*, 1983). Infection with CMV is in striking contrast to infection with EB virus, which probably requires more intimate contact to be contracted and was essentially nonexistent in a study of British boarding school children (Evans and Niederman, 1976).

It is now clear that the prevalence of antibodies and hence the prevalence of prior infection by CMV vary with the socioeconomic basis of the population group. The relevant data in population groups over the age of 35 were summarized by Krech (Table 9.3; Krech, 1973). The prevalence is lower in Europe, Australia, and parts of North America, whereas it is significantly higher in "less developed" areas. Some regions with warmer climates such as Uganda, the Philippines, and Tanzania have a prevalence of 100%. It is, however, unlikely that warm weather *per se* is a decisive factor, as prevalence among Eskimos is also high (81%; Sinha and Pauls, 1971). Lang (1975) reported early universal acquisition of antibodies in Melanesian people living in eastern New Guinea and on islands of the New Hebrides and Solomons. He thought frequent and close interpersonal contact between the virus shedder and the susceptible subject was needed for transmission.

9.1.2. The Effect of Age

Carlström and Jalling (1970) studied the prevalence of CF antibodies in Stockholm (Table 9.4). The prevalence data are similar to those found in London. Peak prevalence in adults was 53–57% in both regions. The Swedish data provide more information on the rate of increase in early life. In terms of percentage serologic

TABLE 9.4
Cytomegalovirus Seroprevalence and Rate of Increase with Age in Sweden[a]

Age	No. positive/ No. tested	Percentage positive	Rate increase (%/year)
1–3 mo	6/32	19	
2–3 yrs	11/58	19	1.60[b]
4–5 yrs	10/44	23	
6–10 yrs	26/94	28	1.43
11–15 yrs	26/96	27	0
16–30 yrs	49/50	33	0.60
31–50 yrs	42/74	57	1.37
>50 yrs	43/81	53	0

[a]Data from Carlström and Jalling (1970).
[b]Calculated by dividing differences in percentage by difference in mean age.

conversion per year, it was highest between 6 months and 5 years of age and between 16 and 50 years of age. There is thus evidence for two peaks of rate increases, early childhood and early adulthood.

Wentworth and Alexander (1971) studied 4824 sera from a predominately white middle-class urban American population in the state of Washington. The ages of the population varied from 5 to 60. There was a constant rate of conversion of about 1.3% per year from age 5 to about 43. Beyond ages 43 to 57, there was a leveling off of the rate of conversion so that about 70% of the population was seropositive at the older extreme. In populations with higher infection rates, the age when infection levels off occurs earlier. They also showed conclusively that more females were seropositive than males, a point that could be gleaned from the earlier data of Rowe *et al.* (1956). This difference could not be accounted for by pregnancy.

The pattern of increasing positivity with age up to and beyond adulthood contrasts strikingly to varicella and other common childhood diseases, against which antibodies develop early in life, reach their peak around age 13, and remain level or decline slightly thereafter.

Age also affects virus shedding. Viruria and respiratory shedding are relatively frequent during the perinatal period (see Section 9.1.3). They may occasionally be found during childhood, but viruria is rare in healthy adults (see Section 9.7.3). Cervical shedding and viruria may occur in women with varying frequency, increasing with sexual activity and decreased socioeconomic status (see also Section 9.2.1). Knox *et al.* (1979) found in a predominantly indigent black population that genital shedding and urinary tract shedding were 8.9% and 3.8%, respectively. Interestingly, shedding decreased steadily with age, from peak rates of 15% and 8% at 11–14 years to undetectable levels at 31 years of age or older. This decrease is probably not related to sexual activity or the development of circulating antibodies, as most of this population is seropositive at an early age.

9.1.3. Frequency of Perinatal Infections

The acquisition of CMV infection during the first year of life is an important part of its epidemiology. Congenital infections are, by comparison, relatively uncommon and are treated separately in Chapter 11. The frequency of perinatal infections seems to vary according to geographic and cultural region rather than socioeconomic well-being. The practice of breast feeding may be an important factor.

Table 9.5 lists the frequencies of viruria at various times during the first 2 years of life as an index of perinatal infection. Viruria is a more reliable indicator of infection in this period of life than serum antibodies because transferred maternal antibodies remain in circulation in early life. By and large, the frequencies should be considered cumulative because viruria, once present, persists for long periods of time. A small proportion of babies had viruria at birth, signifying congenital infection. There are cumulative increases in the frequency of viruria beginning with the first months and peaking in most instances at about 6 months (Table 9.5). These increments represent perinatal infection. The frequencies at 6 months of age illustrate three recognizable patterns: (1) a high frequency of perinatal infection in developing countries, such as Thailand and Guatemala (42–55%); (2) a high frequency of infection in some developed countries such as Japan and Finland (35–56%); (3) a low frequency of infection in other developed countries (England, United States) whether among middle-class (Seattle, Washington) or economically deprived populations such as in Birmingham, Alabama (8–13%).

The common denominator accounting for frequent perinatal infection irrespective of socioeconomic status is unknown, but transmission by maternal milk is suspected (Hayes *et al.*, 1972; Stagno *et al.*, 1980). A high proportion of "immune" mothers might carry CMV in their milk and then transmit their infection to their offspring if breast feeding is common. On the other hand, in Birmingham, even though most mothers are latently infected, breast feeding is not as common as in other countries, and hence, the perinatal infection rate is lower (Stagno *et al.*, 1980). This role of breast milk is discussed in greater detail below (Section 9.2.2).

9.1.4. The Effect of HLA Type

Whether the HLA genotype is connected with susceptibility to CMV infection is still unproven. Although there are a few unconfirmed studies, Pereira *et al.* (1978) noted in screening donors in connection with a marrow transplant program in London that subjects with HLA-BW 15 had a higher proportion with CF and IFA antibodies against CMV (73%) than did controls (49%). Antibodies against other herpesviruses were not different. This HLA type has been reported to be associated with systemic lupus erythematosus (McDevitt and Bodmer, 1972). Renvoize *et al.* (1979) studied a group of patients with Alzheimer's presenile and senile dementia.

TABLE 9.5
Frequencies of CMV Viruria during the First 2 Years of Life[a]

Authors	Locality	Total no. of infants studied	Percentage excreting CMV according to age (months)							
			At birth	1	2	3	6	9	12	24
Numazaki *et al.* (1970)	Japan	257	—[b]	6	10	20	56	44	22	7
Olson *et al.* (1970)	Thailand	140	—	—	—	38	55	18	15	—
Cruz *et al.* (1977)	Guatemala	109	—	—	—	23	42	40	35	—
Leinikki *et al.* (1972)	Finland	105	2.3	—	12	23	35	25	33	41
Granström *et al.* (1977)	Finland	148	2	—	16	32	36	—	39	—
Ahlfors *et al.* (1978)	Sweden	326	1	12	—	—	—	—	23	—
Levinsohn *et al.* (1969)	U.S., Seattle	92	1	3	—	11	13	—	11	—
Stern (1968)	England	118	2.5	—	—	—	9	—	—	—
Collaborative study (1970)	England	1395	0.4	—	1.8	3.2	—	5.8	—	4
Reynolds *et al.* (1973)	U.S., Birmingham	154	1.3	2	4	7	8	8	8	9

[a] Adapted from Stagno *et al.* (1980).
[b] —, No data available.

These patients had a higher frequency of HLA-BW 15 and CF antibodies against CMV.

9.1.5. Antibody and the State of Infection

We assume that the presence of antibodies with CMV implies a past episode of infection with the virus and that it endows some immunity against subsequent infection. This immunity is by no means absolute, as reactivation of the original infecting strain as well as reinfection by a new strain are both possible. We do not have a good estimate of false negatives, i.e., patients who were infected but did not develop antibodies or patients whose antibody titers waned to the point where they were undetectable. Despite one unconfirmed report of the erratic appearance and disappearance of CMV antibodies (Waner *et al.*, 1973), aggregate evidence suggests that antibodies persist quite well.

Seropositivity does not distinguish a cured past infection from a latent, persistent, or active infection. Active and inactive infections will eventually be discovered and differentiated by more sophisticated serologic and virological methods, including DNA and RNA hybridization and polymerase chain reaction (Section 5.3). As yet there is no precise, practical method to do this or to diagnose latent infection.

Seropositive subjects almost always become actively infected with CMV after organ transplantation and immunosuppression or when they develop AIDS (see Sections 13.1 and 13.2). Most such individuals reactivate an original infection that had become latent, but some became reinfected.

De novo reinfections or secondary infections in immune individuals have been shown in long-term studies of repeated isolates from immune virus shedders. Huang *et al.* (1980a) studied the restriction endonuclease patterns of repeated isolates from the urine and genital tract of five immune women in Alabama. The isolates were obtained 1–6 years apart. In one case out of five a repeat isolate from the genital tract was not identical to the original. Reinfections have also been demonstrated by similar methods after kidney and bone marrow transplantation and in sexually active homosexual males (see Sections 13.1, 13.2, and 9.5).

Taken together, the present evidence suggests that an original CMV infection is rarely if ever cured. It remains latent and may reactivate during immunosuppression. Epidemiologically all seropositive subjects are potential sources of virus transmission under special circumstances, i.e., pregnancy, blood transfusions, organ transplantation, and possibly sexual intercourse (see respective sections in this chapter).

9.2. THE MECHANISMS OF PERINATAL TRANSMISSION

There are two basic modes of transmission of CMV from a carrier to a susceptible subject: vertical and horizontal. Vertical transmission of CMV from mothers

with primary or reactivation infection to the infant *in utero* is considered in Chapter 11. Perinatal transmission, i.e., horizontal transmission during and shortly after birth, is considered in this section. Transmission by exposure to the infected cervix, breast milk, and other routes are considered in Sections 9.2.1 to 9.2.4.

We know of no nonhuman sources of CMV infection. Since human CMV is a species-specific virus that does not readily replicate in nonhuman hosts, it is unlikely that animals and lower life forms can serve as sources of infection. Weller (1971) pointed out that in view of the relatively high concentration of the virus in urine, objects contaminated by infected urine could theoretically serve as a source of infection. This may be important in newborn nurseries and in day care centers (see Sections 9.2.4 and 9.3). Against this possibility is the limited amount of virus on such subjects and the lability of the virus that, like all enveloped herpesviruses, is easily inactivated by extremes of temperature and pH. Neither do we have any direct evidence of respiratory transmission, a commonly assumed route.

A study by Cabau *et al.* (1979) points out the close association between the serologic status of the mother and acquisition of infection by the child during the first year of life, which suggests that the most important source of infection may be the mother. In a study of 10-month infants in France, no seronegative mother had a seropositive child. The prevalence of seropositivity in samples of French mothers from low and high socioeconomic groups were, respectively, 67% and 48%, and their offspring at 10 months of age had respective ratios of 39% and 11%. Breast feeding enhanced chances for infection. Little increase in antibody prevalence was noted between 10 months and 2 years of age. Hence, acquisition of CMV infection seemed to center around the perinatal period. The role of breast feeding and the transmission of CMV in the early postnatal period are discussed separately below.

9.2.1. Perinatal Transmission: Infected Cervix

Reynolds *et al.* (1973) followed babies for CMV infection and correlated this with maternal cervical CMV secretion and showed that timing was important. Of babies born to mothers excreting CMV from the cervix during the first or second trimester, 12.5% became infected. Thirty-seven percent of babies born of third-trimester excreters became infected. In 14 of these, whose mothers also shed virus post-partum and who were presumably shedding at birth, the infection rate rose to 57% (Table 9.6). Interestingly, none of the babies of urine shedders who were not shedding from the cervix was infected. Almost all cervical shedders had antibody in their serum, which was transmitted transplacentally to the offspring. As in the case of most perinatal infections, such antibody did not prevent infection of the baby. Most of the infants showed serologic rises by indirect humagglutination test 30–60 days after birth. The only infant who had no antibody in the umbilical cord serum specimen had an earlier rise in antibody than the others. As in the case of reactivation of CMV infection in adult transplant recipients, the presence of maternal

TABLE 9.6
Association between Source of CMV in Mother and Subsequent Infection of Neonates[a]

Sole source of CMV	No. neonates infected/ No. exposed (%)	
1. Breast milk (CMV positive)		
Infant on breast milk	11/19	(58%)
Infant on bottle	9/9	(0%)
2. Cervix CMV positive at		
Third trimester and post-partum	8/14	(57%)
Third trimester	18/68	(26%)
First and second trimesters	1/8	(12%)
3. Urine CMV^+ (third trimester)	0/11	(0%)
4. Saliva CMV^+ (1 day post-partum)	0/15	(0%)
5. Mother CMV-negative for 1–4 months		
Infant on bottle	0/125	(0%)
Infant on breast milk	1/11	(9%)

[a] Adapted from Stagno *et al.* (1980).

antibody may have delayed the serologic response. No neonatal disease was detectable in any of the babies.

Numazaki *et al.* (1970) studied early childhood infection in Sendai, Japan, where 81% of babies were seropositive by 1 year of age. Cytomegalovirus was not isolated from mouth swabs, urine, or milk of mothers. Maternal serum was 85% positive, and cord serum was 94.5% positive for CF antibodies, a rate similar to that in the black Alabama population studied by Reynolds *et al.* (1973). Vaginal swabs were frequently positive during the second (9.6%, %62) and third (27.8%, 17/61) trimesters, and they showed that the cervical secretion rate rose during the third trimester. It is, however, unlikely that the 81% infection rate was all accounted for by virus transmitted from the uterine cervix. The possibility that infected milk may play a role should be reconsidered (see below).

9.2.2. Perinatal Transmission: Breast Milk

Hayes *et al.* (1972) considered maternal breast milk as a possible source and found CMV in some samples of milk. In view of the high rate of perinatal infection in their follow-up of Japanese neonates, Numazaki *et al.* (1970) also suspected breast milk but were unable to isolate the virus from milk.

Granstrom *et al.* (1977) followed 148 children from birth and found that 39% became infected during the first year of life. This rather high frequency of perinatal infection was thought to be related to breast feeding. Out of 148 babies, 100 were breast fed; not all but most (72) stopped breast feeding before 2 months of age. At 2 months of age, the perinatally infected were significantly more often breast fed than not (69% versus 41%). No attempt at virus isolations from milk were made.

Leinikki *et al.* (1978) studied the epidemiology of CMV infections during pregnancy and infancy in Finland. Immunofluorescent antibody determinations and virus isolations from urine were obtained from 200 typical Finnish mothers and their offspring at intervals up to 1 year post-partum. Three infants (2%) were congenitally infected, whereas 30% of the children became perinatally infected as indicated by onset of viruria 2 to 4 months after birth. Twenty-three mothers (10%) were seronegative, and none of their offspring became infected with CMV. The presence of maternal antibodies did not protect the children from acquiring perinatal infection. The authors also thought breast milk may have been the vehicle of virus transmission. Granstrom *et al.* (1977) also found that more than 36% of 148 Finnish infants were infected by 6 months of age. No difference was found in the socioeconomic status of the perinatally infected and uninfected babies.

Stagno *et al.* (1980) provided the best evidence so far that perinatal CMV infection is associated with breast feeding (see Table 9.6). Infants of 28 mothers who shed CMV in their milk were studied prospectively. Nine who were bottle fed did not become infected, whereas 11 of 19 (58%) who were breast fed became infected. The risk of breast milk was about the same as the risk of passage through a genital tract shown to be infected during the third trimester and post-partum (57%). It is interesting that in Japan, Thailand, Guatemala, and Finland, where the rate of perinatal infections is high, as shown by viruria during early life (39–56%), the practice of breast feeding is common, and the majority of childbearing women are seropositive (Table 9.5). The missing link in this hypothesis is a demonstration that most samples of milk of seropositive mothers are infected. Transmission of CMV by the oral route has not been shown in later life. It is possible that in early infancy, gastric acidity may be inadequate to inactivate CMV and that the infantile gastrointestinal tract permits transmucosal infection. I know of no data on this issue.

Table 9.6 summarized prospective studies conducted at the University of Alabama on the relative importance of CMV in breast milk, cervix, urine, and saliva in perinatal transmission from seropositive mothers to newborn infants (Stagno *et al.*, 1980). Uninfected newborns of women who were positive at the indicated sites were followed prospectively for viruria. More than half of the babies who were fed infected mother's milk or whose mother had evidence of CMV in the uterine cervix during the third trimester and after parturition became infected. Cytomegalovirus in the urine or saliva of mothers did not appear to be a risk factor. Conversely, only one baby out of 125 whose mothers were tested negative at all sites became infected. Possibly this one exception may have resulted from a false-negative milk sample. These studies argue strongly that breast milk and the infected uterine cervix are the main sources of perinatal CMV infection, at least in the Alabama black population. It should be noted, however, that those mothers whose milk (13%) or cervix (10%) tested CMV positive were still the minority of all seropositive mothers. And in certain populations (see Table 6.4) practically all babies of seropositive mothers became infected perinatally. Hence, other mechanisms of perinatal transmission are still possible and have not yet been ruled out (see below). In view of the asymptom-

atic nature of most perinatal infections, these modes of transmission produce natural immunity and need not be controlled or prevented.

9.2.3. Perinatal Transmission: Other Factors

Numazaki *et al.* (1970) found much oral secretion of CMV in infants between 1 and 12 months of age. Of 257 babies tested up to 2 years, none was positive at birth. Sixty percent were secretors at 5 months of age, all secretors being seropositive. Secretion remained high up to 9 months of age (44%) and had ceased by 1 year. In what way such secretion may have been the source of infection for siblings or other infants is unknown.

Levinsohn *et al.* (1969) collected oropharyngeal and urine specimens for CMV from 100 babies at term and up to 1 year of age in Seattle, Washington. One positive culture was detected at birth (1%), and most positive cultures were found at 3 months of age; 15 excreters were found in total. Complement-fixation antibody was found in three out of 11 excreters and none of 39 nonexcreters. This study shows that acquisition of infection and virus shedding by babies were significantly lower in Seattle than in Japan.

There is evidence for intensive circulation of CMV in highly endemic areas. The respiratory route is suspected as one method of circulation. Olson *et al.* (1970) found CMV in the respiratory tract in 21.2% of 378 Thai children less than 4 years of age. Recovery rate was highest from infants less than 1 year of age. Forty percent of those with upper respiratory infection, 33% with pertussis, and 15% of well babies were respiratory carriers. Thirty-eight sera from 43 children who excreted virus had neutralizing antibodies. Of these, 37 showed IgG immunofluorescence, and 26 showed positive IgM immunofluorescence. This work suggests that CMV may cause or accentuate respiratory infection or that respiratory infection by other agents may predispose to CMV infection. Which event occurs, if either, is as yet unresolved. There is also evidence of shedding of CMV in normal tears (Cox *et al.*, 1975).

The transmission of CMV by transfusion of blood in neonates is discussed in Section 9.7.

9.2.4. Transmission in Newborn Nursery

Perinatal infection may be acquired by sick or immature neonates by as yet undefined routes of transmission outside of the mother. Using Hind III and EcoR-1 restriction endonculease mapping, Spector and Spector (1980) showed that monozygotic twins became postnatally infected at 6 and 9 weeks of life with different strains of CMV and that none was derived from the mother, who was seronegative until 6 months post-partum. The babies were hospitalized for 4 months because of premature rupture of membranes and cesarean birth.

Another nursery outbreak was documented by more conventional means (Gurevich and Cunha, 1981). Following admission to a neonatal intensive care unit of a female infant with fulminant congenital CMV disease who lived only 8 hr, four infant occupants became infected, of whom two died with disseminated infection. Two (one of whom succumbed) were twins. Because the mother of the twins was seronegative, congenital or perinatal infection transmitted by the mother could be ruled out.

Spector (1983) also isolated strains of CMV from three babies with identical restriction endonuclease fragments and suggested that two of them were infected in the nursery by the first. The method of transmission is unknown.

9.3. TRANSMISSION OF CMV IN DAY CARE CENTERS

Since infants and young children excrete CMV in their urine and respiratory tract over long periods of time, they may be sources of infection for other children and adults. However, until recently this has not been documented. Pass *et al.* (1984) found that about 80% of children cared for at day care centers acquired CMV infection, whereas only about 20% of children of similar socioeconomic background who were kept at home became infected. Excretion of CMV in saliva and urine of children of different ages was also documented by collecting samples on three occasions at 6-month intervals. Whereas fewer than 10% of children from 0 to 12 months of age excreted CMV in the urine or saliva, 60% to 80% of those who were 13 to 18 months of age excreted CMV in both urine and saliva. As children became older and learned to walk, they were able to have closer contact with one another, and the transmission of the virus may have been facilitated. Cytomegalovirus was recovered from toys mouthed by infants and toddlers. These may have been a source of infection, although this was not proven.

An interesting problem concerns the effect of these infected children on health care workers and their own families. Pass *et al.* (1986) followed longitudinally the serologic status of seronegative parents with children in day care centers. Fourteen out of 67 such parents acquired CMV infection, compared to none out of 31 controls. All 14 parents who seroconverted had a child who shed CMV in his saliva or urine. Among the day care group, acquisition of CMV occurred in 14 of 46 parents whose children shed CMV as compared with none of the 21 whose children did not excrete the virus. Adler (1986a,b) provided further proof using molecular epidemiology that infected children could transmit the virus to their parents. Nine of ten mothers of viruria children were seropositive, but only 12 of 33 mothers of nonviruria children at the day care center were seropositive. Three parents (a father and two pregnant mothers) were excreting CMV. When the DNA of the isolates was analyzed by restriction endonclease patterns, the viral DNA of the isolates from each parent was identical to that from the virus excreted by the child and from the virus excreted by other children at the day care center. In a prospective study, Adler (1988) further found that six out of 18 seronegative mothers became infected with

the same day care center strains 3 to 7 months after their children became infected. These studies and others (Spector and Spector, 1982; Dworsky *et al.*, 1984) document the intra-day-care-center and intrafamilial spread of CMV.

Although usually no morbidity with CMV is involved, Pass *et al.* (1987) showed that day care centers may be indirectly responsible for some symptomatic congenital CMV infections. Five families with infants who had congenital or perinatal CMV infections were described. Each had a child less than 3 years of age who attended a day care center and excreted CMV. In each family, isolates from the newborn, sibling, and mother were similar by DNA fragment polymorphism. The presumption is that CMV transmitted from a toddler to a seronegative pregnant mother may have caused congenital CMV infection.

Interestingly, similar studies in two Swedish day care centers showed that although the children had a high frequency of virus shedding, all isolates from 13 children were unique, suggesting that frequent horizontal transmission did not take place. The explanation may be that unlike relatively well-to-do Americans attending day care centers, about a third of Scandinavian children are infected perinatally (see Table 9.5). They bring their unique strains to the day care center and are relatively immune to superinfection.

Adler (1989) showed that American day care workers seem to be at risk in acquiring CMV infection from the children. Women who cared for children younger that 2 years were at significantly higher risk (46% seropositivity) than women who cared for older children (35%). The annual seroconversion rate of 202 initially seronegative caretakers (11%) was significantly higher than that for 229 seronegative hospital employees (2%). This study of 610 women in 34 centers is one of the few showing that the workplace may present risk for acquiring CMV infection (see Section 9.6).

9.4. CYTOMEGALOVIRUS INFECTION OF THE UTERINE CERVIX

The uterine cervix infected with CMV may be important as a source of infection not only during childbirth but also as a source of venereal infection later in life.

The frequency of isolation of CMV from the cervix of pregnant women varies, as does the prevalence of antibodies, with geographic locality, socioeconomic status, and sexual promiscuity. In 1967, Alexander recovered CMV from the cervix of 18 of 100 pregnant Chinese women in Taiwan but from none of 33 pregnant American women in the same area. No virus was cultured from the children at birth. Wentworth and Alexander (1971) recovered the virus from the cervix of only five of 258 women (2%) in Seattle.

Numazaki *et al.* (1970) first showed that CMV infection of uterine cervix changes during pregnancy. Montgomery *et al.* (1972) compared cervical CMV secretion rates in 71 pregnant Navaho Indians and 125 middle-class white and black pregnant women in Pittsburgh, Pennsylvania. Complement-fixation antibody was

found in 83% of 71 Navajos and 39% of 125 Pittsburgh women. Cytomegalovirus was recovered from the cervix in 8% and from the urine in 3%. It was recovered more frequently from the cervices of Navajo women (14%) than from black (5%) or Caucasian (4%) women. Cervical cultures were more frequently positive during the third trimester and in younger (<25 years) and primiparous women. It was pointed out that if the prevalence of antibody in the female population is taken into account to calculate cervical infection as a manifestation of reactivation, the rates for the Pittsburgh and Navajo populations are essentially the same (13.8% and 14.5%).

Stagno *et al.* (1975b) studied the cervical and urinary excretion of CMV in pregnant women in a highly immune low-socioeconomic black group in Alabama. This was a young group with a mean age of 20, a high rate of sexual activity with multiple partners, and a gonococcal infection rate of 10%. Comparison of 659 pregnant and 202 matched nonpregnant women showed identical overall cervical CMV excretion (9.5% and 9.4%, respectively). Eighty-nine percent of the pregnant group possessed antibodies by the FA test when admitted to the study. The rate of CMV secretion advanced significantly from the first to second and third trimesters (1.6%, 6.1%, and 11.1%), but cervical infection was for some reason actually reduced in early pregnancy when compared to the infection rate in nonpregnant women. Younger patients (15 years of age) had a higher rate than women over 30.

In a later study of 1101 pregnant and nonpregnant women by the same group (Knox *et al.*, 1979), pregnancy did not affect virus shedding in the urine or cervix, although in individual pregnant women shedding decreased in the first and second trimesters. Shedding also stopped after 31 years of age (see Section 9.1.2).

The summary of the three studies of cervical infection in 988 women in the three trimesters shows that women in late pregnancy have higher rates of cervical infection (Table 9.7). The rates increased from 1.5% in the first trimester to 13.5% in the third with an overall rate of 10%. A similar though less striking pattern was observed with respect to viruria.

The immunologic response of the excreters presents a contrast to renal transplant recipients who almost always show a serologic rise or seroconversion around the time of virus isolation (see Section 13.1). For example, out of 59 cervical shedders in the study of Stagno *et al.* (1975b), there was only one who seroconverted and six who had a serologic rise. All excreters had IF antibody titers when the study commenced. There was no serologic evidence for primary infection, and activation at the cervical site was not associated with a serologic rise. Evidence that infection with CMV was not limited to the cervix is that 14 (25%) had viruria and eight of 33 (24%) had virus in the colostrum. Schmitz *et al.* (1977) report that during pregnancy, specific IgM antibodies against CMV are detectable in 7% of pregnant women. Whether this is specific or meaningful has not been confirmed (see Section 11.3.1).

Griffiths *et al.* (1978b) undertook a longitudinal study of the serologic and virological status of 18 seropositive women infected with cytomegalovirus. They were followed for 18 to 66 months (mean 50.2 months) for viral excretion and serological changes. Cytomegalovirus was isolated from 58 out of 146 (39.7%)

TABLE 9.7
Cervical CMV Infection Rate According to Length of Gestation

Source	Infection rate (%) in trimester						Overall infection (%)	
	First		Second		Third			
Numazaki *et al.* (1970)	0	(0/30)	9.6	(6/62)	27.8	(17/61)	15	(23/153)
Montgomery *et al.* (1972)	2	(1/43)	7	(6/83)	12	(6/49)	7.4	(13/175)
Stagno *et al.* (1975b)	1.6	(3/183)	6.1	(22/359)	13.2	(42/317)	9.5	(63/659)
Total	1.5	(4/256)	6.7	(34/504)	13.5	(65/481)	10.1	(11/988)

cultures from various sites, mostly cervix. A total of 129 sera were obtained, and each was assayed in the late antigen, early antigen, anticomplement immunofluorescent, complement-fixation, and microneutralization tests. From three women virus was consistently reisolated. From three others, virus was never reisolated, and the remaining 12 women excreted virus intermittently. Interassay correlations were sought for each of the ten possible combinations of pairs of tests. The results of the ACIF, IFA-LA, neutralization, and CF tests were well correlated. The ACIF test of 109 sera of 18 women followed for 60 months showed a maximum variation of eightfold and a mean variation of less than twofold. The results of the early antigen tests did not correlate with the others. Secreting CMV in the cervix was not accompanied by any consistent change in the results of serologic tests.

Chandler *et al.* (1987) provided evidence that cervical infection occurs locally, and multiple infections with CMV in nonpregnant women may occur over time. Eight women attending a STD clinic and seven receiving routine prenatal care who had a positive CMV culture were serially cultured over a 6-week to 17-month period. Four of the eight women who attended the STD clinic were infected with more than one strain of CMV in the cervix, urine, or throat as identified by restriction endonuclease patterns. Two shed different strains at different times, and two shed different strains simultaneously from different sites. The women who were receiving prenatal care maintained identical strains.

We assume that cervical infection may be of two types. The first is the usual systemic infection accompanied by a serologic rise, some of which may result in persistent virus shedding in the cervix. The second is a local infection of the cervix and, occasionally, urine. The main characteristic is virus shedding without a serologic change. The precise pathogenesis of either type of infection is not clear. It is also unclear whether cervical infection of either type may reactivate. Possibly reactivation is associated with the hormonal changes of pregnancy.

9.5. SEXUAL TRANSMISSION OF CYTOMEGALOVIRUS

Sexual transmission of CMV probably represents the most important mode of horizontal transmission after puberty. Previously the evidence for sexual transmis-

sion was largely circumstantial. More direct evidence for sexual transmission is now available and the accumulated evidence for the role of homosexual sex in the transmission of CMV as a corollary of AIDS studies is particularly striking.

9.5.1. Heterosexual Transmission

The types of evidence supporting heterosexual transmission include the following. There is more CMV infection in sexually active women, and their uterine cervices are more often infected by CMV than in the less active. Cytomegalovirus mononucleosis may at times be transmitted after sexual contact. Identity of strains infecting sexual partners has been proven by restriction endonuclease patterns.

Jordan *et al.* (1973a) found that of 120 women examined because of suspected venereal disease, 16 (13.3%) had cervical CMV infection, and 41 (34.2%) had positive cervical culture for *N. gonorrhoeae*. No CMV was recovered from 76 women who had routine examinations, only two of whom (2.6%) had positive gonococcal cultures.

In a study of 531 females in a venereal disease clinic in England, Wilmott (1975) reported 6.6% cervical cultures positive for CMV and 5.3% positive for herpes simplex virus. These rates are higher than comparable rates in the general population. Twenty of 28 (71%) of those positive for CMV were on oral contraceptives, compared to 43% for the whole group. This may be a reflection of the sexual background of the patients, or oral contraceptives may activate CMV infection.

Chandler *et al.* (1985a,b) examined the association of CMV infection with indices of sexual activity of 347 women attending a sexually transmitted disease (STD) clinic. Stepwise multivariate logistic regression analysis showed that seropositivity was best associated with number of sex partners, early age of first intercourse, and being nonwhite.

Lamb and Stern (1966) reported a 31-year-old male who had CMV mononucleosis documented by viral isolation and a serologic rise. A 36-year-old roommate developed a similar febrile mononucleosis with 16% atypical lymphocytes and a serologic rise to CMV. No virus was isolated. The degree of contact between these two people is unknown. Chretien *et al.* (1977) provided epidemiologic evidence for venereal transmission of CMV in a university student community. The infected students were 24 to 26 years old. Two males with CMV mononucleosis proven by conversion of their CF titers and by viruria had sexual contact with a 24-year-old woman who, 4 and 8 weeks before onset of their illnesses, had a month-long febrile illness 3–4 months before contact with either man. She was seropositive, and CMV was isolated from her cervix. Another case was a female contact of one of the males. A tertiary case from the secondary case was also reported, but neither was proven. Interestingly, male and female roommates of the index cases who had no sexual contact with them remained well and seronegative.

More direct evidence of heterosexual transmission was provided by analysis of

CMV strains of sex partners (Handsfield *et al.*, 1985). Of 347 women attending a STD clinic, 63 referred a male partner. Cytomegalovirus was isolated from the semen or urine of four (22%) of 18 men whose partners shed CMV from the cervix or urine, compared to none of 42 whose partners were culture negative. DNA restriction typing of CMV isolates from three pairs of sexual partners showed two pairs to be infected with identical strains.

9.5.2. Homosexual Transmission

There is now epidemiologic as well as virological evidence that CMV may be transmitted by homosexual sex. First, there is overwhelming evidence that the practice of homosexuality is associated with acquisition of CMV infection. Drew *et al.* (1981) found that 94% of homosexuals attending a STD clinic in San Francisco were seropositive, whereas only 54% of the heterosexuals were seropositive. Greenberg *et al.* (1984) found that the corresponding rates in gay and heterosexual men attending a STD clinic in Houston were 98% and 43%. Doldi *et al.* (1985) found in Germany that all of 32 male homosexuals tested were seropositive but that only 30% of heterosexual controls were seropositive. Coutinho *et al.* (1984) found in Holland a group of 710 homosexuals who were only 70.6% seropositive, largely because they were preselected on the basis of being negative for hepatitis B markers. However, when 209 seronegative men were followed for 23 months, 27.3% seroconverted. This is a very high seroconversion rate compared to the results of large prospective studies on seronegative pregnant women, in which fewer than 1% develop primary CMV infection (see Table 11.2). Even more striking data were collected epidemiologically in San Francisco by Mintz *et al.* (1983). Seventy-one percent of seronegative men became infected with CMV in 14 months.

Infection with CMV is strongly correlated with certain sexual practices. Mintz *et al.* (1983) found that of seven different practices of the men, only passive anal–genital intercourse correlated significantly with the acquisition of CMV infection. Coutinho *et al.* (1984) and Collier *et al.* (1987) found that seropositivity correlated with duration of sexual activity, number of sex partners, history of syphilis, and anal intercourse. The acquisition of primary CMV infection was only correlated with a history of syphilis and anal intercourse.

Second, there is evidence that CMV infection is continuously active in sexually active gay men, as manifested by a high frequency of virus isolation and presence of CMV-specific IgM antibodies. Mintz *et al.* (1983) found that 34.6% of gay men shed CMV in the semen. The frequency of urinary shedding was much lower (7.7%), but this also rose to 30% if serial urine cultures were taken. In a study of men attending a Texas STD clinic, 18% of the homosexual men and only 4% of the heterosexual men shed CMV in the urine (Greenberg *et al.*, 1983).

Collier *et al.* (1987) prospectively studied CMV and HIV infection in Seattle, Washington. Cultures for CMV were obtained from semen, urine, throat, and rec-

tum. At the initial visit, the frequencies of positive cultures from these sites were 36%, 12%, 2%, and 1%. On serial sampling, CMV was isolated at some sites from 42% (38/91) of HIV-positive men and from 35% (12/34) of HIV-negative gay men. Shedding was much more constant in semen. Among CMV shedders in semen, the mean duration of shedding was 22 months, and it was 9 months in urine. Mintz *et al.* (1983) reported that in 95% of IgG-CMV-positive gay men, CMV-specific IgM was not short-lived but remained intermittently detectable.

Shedding in both semen and urine was inversely proportional to age. Others have also found that younger men were more often virus-positive (Biggar *et al.*, 1983). This is reminiscent of uterine CMV shedding, which is also more frequent in younger women. The reason for continued infective activity of CMV in gay men is unclear. There are two possibilities, which are not mutually exclusive. First, there may be continuing active infection or reactivation. This is supported by finding IgM antibodies during follow-up. In patients who are HIV positive, the acquired immunodeficiency may predispose to persistent CMV infection. However, there is evidence that persistent CMV infection occurs even in HIV-negative men (Collier *et al.*, 1987). The second possibility is that there is constant reinfection. So Spector *et al.* (1984b) used restriction endonuclease patterns and identified in two gay men with AIDS different strains of CMV from buffy coat, lung, and testes in one case and urine and lung in the other. This theory is also supported by documented reinfection in sexually active women (see Section 9.4). Taylor *et al.* (1988) also found two out of 20 promiscuous homosexual males showed evidence of reinfection. But, generally, CMV isolated from AIDS patients did not show major differences in genome structure.

The scientific basis for venereal transmission of CMV among males may be the presence of CMV in semen. Lang and Kummer (1972, 1975) first showed that CMV may be present in normal heterosexuals after an infection. Urine and semen samples obtained within a 14-month period from a 23-year-old male convalescing from CMV mononucleosis were repeatedly positive. The titers in the urine samples were between 2 and 4 log TCD_{50}/ml, whereas titers in semen were between 4 and 8 log TCD_{50}/ml. The significantly higher titers in semen suggested that the virus was not a contaminant from the urine. By autoradiographic studies, increased foci of DNA synthesis, presumably CMV synthesis, was demonstrated in heads of spermatozoa (Pagano, 1975), although in semen specimens, the virus was found primarily in the extracellular phase. The persistence of CMV in high titers also suggests that in the reproductive tract this virus may escape the immunologic defenses of the body. Cytomegalovirus was also isolated from the cervix of one sexual contact of this patient. What is not known is whether semen or other parts of the male genital tract may be infected locally.

In a survey, Lang and Kummer (1975) also found CMV in the semen of two out of 185 men seeking a fertility evaluation (about 1%), one out of ten patients being treated in a venereal disease clinic, and three out of 54 students and blood donors. The CMV antibody titers were significantly higher (mean 1 : 180) in the positive

patients than in those who had no virus in the semen. Parallel specimens of urine and blood, when available, were negative for the virus.

9.6. OTHER MECHANISMS OF TRANSMISSION, AND TRANSMISSION IN THE WORKPLACE

There is no evidence that the high frequency of CMV infection in transplant patients is related to interpersonal contact or a nonhuman source. Supposed "epidemics" of CMV infection in renal transplant populations (Coulson *et al.*, 1974) have not been confirmed. The human source for primary infection in this group seems to be the infected transplanted organ or the infected patient (see Section 13.1.1).

Pregnant women who have never been exposed to CMV before are at risk for developing primary infection, which may be transmitted to the baby. So far, two sources of infection have been identified. Cytomegalovirus may be sexually transmitted, or it may be transmitted in the family setting from infected infants and toddlers. Whether infection may be acquired at the workplace has not been conclusively proven. The risk may be present, but it is too small to be readily measured. We have already mentioned above (Section 9.3) the risk of seronegative pregnant mother acquiring CMV from a child in a day care center.

Some studies have explored the possibility of human-to-human transmission outside of perinatal and sexual contact. Yeager (1975) followed seroconversion and CMV antibody rises in hospital employees for a mean period of 15–17 months. She used the IHA and CF antibody tests. No rises were observed in seropositive patients. There were five seroconversions among 65 regular and neonatal ward nurses. Nurses in the neonatal ward and the regular wards had 4.1% and 7.7% conversion per year. No conversions were observed among 27 hospital employees with no patient contact. In a similar study, Haneberg *et al.* (1980) studied the prevalence of CMV antibodies in personnel of a children's hospital in Norway. Those at "high risk," that is, nurses or aides in infant units and laboratory technicians, had a 77% seropositivity, whereas 39% of the others were seropositive. The difference was more striking in younger workers. They also noted that nurses in the newborn nursery frequently kissed the babies. They suggest that intimate oral contact may be needed for transmission of CMV in these settings.

More recently, a number of studies have documented the spread of CMV among neonates using molecular markers in the newborn nurseries. However, attempts to show that pediatric health care workers can also be infected in the workplace have been largely unsuccessful.

Spector (1983) showed over a 4-month period in a pediatric intensive care unit that CMV isolates from three babies had identical restriction endonuclease patterns, indicating that they had been infected with the same strain of CMV. In a case of a CMV-negative pregnant nurse who developed CMV infection after caring for a

symptomatically infected baby and elected to terminate her pregnancy, Yow *et al.* (1982) showed that the isolates from the patient and from the abortus were different.

Dworsky *et al.* (1983) first determined that the prevalence of CMV antibody was the same in groups of medical students and house staff, nurses and physicians, and in control groups of pregnant and nonpregnant women in the community. The health care workers cared for newborns and premature infants. Then they showed that after exposure to infected babies for 2 years, the attack rates of CMV infection in the health care worker were not higher than the control population in the community. Similar results were found in Sweden (Ahlfors *et al.*, 1981b).

Balfour and Balfour (1986) enrolled four study groups, i.e., transplant or hemodialysis nurses, 204 neonatal intensive care nurses, 225 student nurses, and 251 blood donor controls, in an even more extensive prospective study. The prevalence of CMV antibody in these 943 subjects was 33.7% and did not differ significantly among the four study groups. During a 5-year follow up, 16 subjects experienced primary CMV infections. The rate of seroconversion during the study, established by observing 519 seronegative subjects for 10,420 person-months, was 1.84% per year and did not differ significantly among the four study groups. The proportions of patients with CMV infection (1.1% to 11.9%) or CMV diseases (0.5% to 3.4%) that were cared for at the workplace were not related to seroconversion in the nurses. The relatively slow rate of acquisition of CMV in susceptible adults in general suggested that it requires prolonged, intimate contact. Nurses and nursing students were not at greater risk than their peers in the community.

There is also no evidence that the renal dialysis unit presents a risk of CMV infection to the patient or personnel (Fiala *et al.*, 1975; Tolkoff-Rubin *et al.*, 1978; Betts *et al.*, 1979). Betts *et al.* (1979) found that although eight of 85 hemodialysis patients shed virus at one or more sites (most frequently from the mouth), no patient or any of the 49 staff members seroconverted during the 24-month study. Ikram (1981) measured CF antibodies in 983 dialysis patients and 238 staff members. Antibody levels were significantly higher in patients. It was thought that this might be a result of increased infection caused by transfusions (see section 9.7.1).

The closest example of direct demonstration of acquisition of CMV at a workplace was reported by Adler (1988, 1989). In a 26-month prospective molecular epidemiologic study of the spread of strains isolated from children in a day care center, four fathers, nine mothers, and two caretakers were found to shed the same CMV strains. This implied that the caretakers may have acquired CMV in the day care center. This conclusions was supported by serologic prevalence and seroconversion data in a study of 610 female day care workers (Adler, 1989; Section 9.3).

The present view is that no healthy health care worker, including seronegative pregnant nurses, need be excluded from caring for patients who shed CMV. Nor is routine screening for CMV antibodies advisable. Young *et al.* (1983) reported an attempt to screen women of childbearing age who worked in "high-risk areas" in order to exclude those who were seronegative from caring for infected babies. "It was a disaster and was withdrawn after 18 months" because it led to a sense of insecurity among the staff and generated all sorts of public relations, psychological,

and management problems. Our considered opinion is that all staff working with infected patients, whether they are infants or adults, should observe simple measures of hygiene such as handwashing (Onorato *et al.*, 1985). Theoretically this should be covered by the "universal precautions" now practiced in most American hospitals. How these views might be modified if more data supporting transmission of infection are obtained remains to be seen.

9.7. TRANSMISSION OF CYTOMEGALOVIRUS BY BLOOD

The mononucleosis syndrome has been observed after extracorporeal perfusion, and infected blood was rapidly imputed to be its cause. Such a case was one of the first demonstrations that CMV could cause disease in an immunocompetent adult. Kaariainen *et al.* (1966a) first noted rises in the CF and neutralizing antibodies, and in one case virus was isolated from the urine of a patient. Stulberg *et al.* (1966) isolated CMV from lymph nodes and blood at postmortem, and Harnden *et al.* (1967) first isolated it from peripheral blood. The demonstration of viremia made transmission by blood a reasonable diagnosis. Cytomegalovirus infection has also been documented in infants of seronegative mothers who received intrauterine transfusions of Rh disease (King-Lewis and Gardner, 1969).

Lang *et al.* (1968) reported four cases of CMV mononucleosis out of 131 cases of open heart surgery employing cardiopulmonary bypass at the Massachusetts General Hospital (MGH) (3%). Virus was recovered from the urine of all four patients and from the blood or throats of three. In a prospective study, Lang and Hanshaw (1969) described four symptomatic primary cases of CMV infection from the MGH. Leukocyte-rich plasma or washed leukocytes from these patients yielded CMV between 35 and 48 days after surgery. Besides conversion of CF titers, they all developed IgM antibodies. One donor of a positive case had viruria.

Foster and Jack (1968) showed that the virus may be isolated by inoculation of washed peripheral leukocytes on susceptible fibroblast cultures. Plasma did not yield any virus. Subsequently, buffy coat cultures for CMV have become a routine laboratory procedure. Their case was a 22-year-old woman who had developed postpartum sepsis with liver failure. She received 29 units of blood, 19 of which were less that 24 hr old. Complement-fixation titers rose from $<1:8$ to $1:512$ about 5 weeks after the first of the transfusions. Viruria was present when viremia had ceased. This was clearly a case of primary symptomatic posttransfusion CMV mononucleosis.

9.7.1. Prospective Studies of Cytomegalovirus Infections Transmitted by Blood

Early reports of cytomegalovirus mononucleosis after transfusions, as described, concentrated on the clinical or laboratory presentations of patients. Since

then, a number of prospective reports have been published that together provide some indication of the risk of infection after transfusions (Table 9.8). A number of these provide data on the risk of blood. The immune (serologic) status of the patient is also a factor in the risk of infection. I have included only those studies which gave the pretransfusion serologic state.

Some of the early studies, for reasons that are not entirely clear, show a remarkably high infection rate. Paloheimo *et al.* (1968) studied prospectively 63 patients who had open heart surgery with extracorporeal circulation by serology. The mean number of units of blood transfused per patient was 7.6. All patients received some fresh blood. The primary infection rate was 59% (ten out of 17 seronegative patients), and 25% (nine out of 36 seropositive patients) were secondarily infected. Seronegative patients were at greater risk. It is, however, to be noted that not all seropositive individuals were protected. Serologic changes occurred between 2 and 5 months after surgery. Out of 19 infected patients, only one was symptomatic. She had a primary infection with febrile mononucleosis, viruria, and mild hepatitis.

Embil *et al.* (1968) studied prospectively 16 children who had open heart surgery and who received fresh blood. This study complements that of Paloheimo *et al.* (1968) and suggests that risk of infection in children is similar to that in adults when large amounts of fresh blood are used during surgery. Symptomatic infection is probably equally low, although meaningful comparison is impossible.

Foster and Jack (1969) followed prospectively nine patients after open heart surgery, of whom only one was seronegative before operation. Six seropositive patients developed posttransfusion mononucleosis (defined as 3% or more atypical lymphocytes in peripheral blood). These are the highest rates I have found for CMV infection after transfusions and for infection of the secondary or reactivation type (67% and 75%), but it is not clear that CMV alone was responsible for the clinical syndromes.

Henle *et al.* (1970) studied by CF serology CMV infection in 152 patients after open heart surgery and extracorporeal circulation. The rates of infection in patients in the two cities were very similar. The number of units transfused was shown to be related to infection. In Philadelphia, 20 responses were seen among 49 patients exposed to 10 to 14 units, and only two out of 18 who received 2 to 4 units. Four out of 17 cases of primary infection from Philadelphia had the postperfusion syndrome. Secondary cases were not symptomatic. In Helsinki, 19 out of 26 seronegative patients became infected. They received a mean of seven units from 182 donors. Assuming that the 19 cases received virus from transfused blood, the minimum virus carrier rate among donors was 10.5% (19/182).

Prince *et al.* (1971) studied 152 patients transfused at the New York Hospital between 1966 and 1969 by following their CF titers (AD169). Of the total, 30 (20%) had serologic evidence of infection. In this study, the pretransfusion antibody status had no effect on risk of infection. Eighteen of 93 seronegative patients converted (19%). The infection rate for the 59 seropositive subjects was 22%. The infection rates for 32 patients who received fresh blood and 56 patients who re-

TABLE 9.8
Incidence of Primary and Secondary CMV Infection after Transfusion

Author	Total patients			Seronegative recipients			Seropositive recipients			Mean units	Symptomatic[c]
	No. infected			No. infected			No. infected				
Kaariainen *et al.* (1966a,b)	21	9	(43%)	6	6	(100%)	15	3	(20%)	NG	1 P
Paloheimo *et al.* (1968)	63	19	(30%)	17	10	(59%)	36	9	(25%)	7.6	1 P
Embil *et al.* (1968)[a]	16	7	(44%)	15	6	(40%)	1	1	(100%)	NG	5 P
Foster and Jack (1969)	9	6	(67%)	1	0	(0%)	8	6	(75%)	NG	6 P
Henle *et al.* (1970)	152	53	(35%)	61	36	(59%)	91	17	(19%)	7	4 P
Prince *et al.* (1971)	152	30	(20%)	93	17	(18%)	59	13	(22%)	NG	NG
Caul *et al.* (1971)	55	21	(38%)	21	7	(33%)	34	14	(41%)	8	3P, 3S
Luthardt *et al.* (1971)[b]	73	12	(16%)	35	8	(23%)	38	4	(11%)	NG	NG
MRC Report (1974)[d]	712	37	(5%)	270	24	(9%)	442	13	(3%)	4	19
Monif *et al.* (1976)	207	19	(9%)	140	16	(11%)	67	3	(4%)	4.3	NG
Armstrong *et al.* (1976)[a]	119	15	(13%)	93	9	(10%)	26	6	(23%)	3.9	None
Lang *et al.* (1977)	10	5	(50%)	6	4	(67%)	4	1	(25%)	19	NG
Tolkoff-Rubin *et al.* (1978)	16	3	(19%)	3	3	(100%)	13	0	(0%)	>3	3
Bayer (1977)	159	5	(3%)	30	1	(3%)	129	4	(3%)	3.2	None
Kumar *et al.* (1980)[b]	29	5	(17%)	10	2	(20%)	19	3	(16%)	NG	None
Alter *et al.* (1982)	107	22	(21%)	29	14	(48%)	78	8	(10%)	9	1 P
Adler *et al.* (1985)	79	12	(15%)	0			79	12	(15%)	2.4–3.2	None
Wilhelm *et al.* (1986)	595	7	(1.2%)	595	7	(1.2%)	0			2.6–2.9	None
Preiksaitis *et al.* (1988)	637	6	(0.9%)	637	6	(0.9%)	0			NG	4 P
Total	3211	293	(9%)	2062	176	(8.5%)					

[a]Children.
[b]Neonates.
[c]Symptomatic means detectable hematologic or clinical abnormalities or secondary infections; NG, not given; P, primary infection; S, secondary or reactivation infection.
[d]MRC, Medical Research Council.

ceived stored blood were 22% and 20%, respectively. Earlier, Stevens *et al.* (1970) found that infection in 41 patients who underwent aggressive surgery for tumors was related to number of units of blood transfused irrespective of whether the blood was fresh or stored. Those who received more than 15 units, 11–15 units, 6–10 units, or 1–5 units had the following rates of infection: 63%, 50%, 36%, and 6%. Most of their patients were seropositive before their operations. These two studies discredited the earlier notion that only "fresh" blood was risky.

A report to the British Medical Research Council Blood Transfusion Research Committee by the MRC Working Party of Post-transfusion Hepatitis (1974) differed from the results of Prince *et al.* (1971) concerning the effect of the serologic status before transfusion. They used 712 subjects, of whom 270 (38%) were seronegative. The latter received a mean of 4.4 units per person. Twenty-four seroconverted (9%). Thirteen of 442 seropositive transfusion recipients showed significant titer rises (2.9%). This group had received a mean of 3.6 units per person. These booster responses occurred only in patients with low antibody titers. They were not found in patients with initial titer of 1 : 128 and more (please see below work by Adler *et al.*, 1985). There was agreement that stored blood was also risky.

Armstrong *et al.* (1976) studied 119 children whose average age was 7.6 years. Of the 93 seronegative subjects, 13% seroconverted following open heart surgery after receiving an average of 3.9 units. There were 26 seropositive children, of whom four developed viruria and two developed serologic elevations postoperatively. It is difficult to diagnose reactivation of infection on the basis of viruria alone in children because they are often asymptomatically viruric. No ill effects of infection were found in any child. This study does demonstrate the frequency of primary infection in children, which is consistent with infection in adults but probably lower than in neonates. Of greater importance is the fact that no clinical symptoms could be attributed to any CMV infection. It appears that premature neonates constitute a uniquely susceptible group and that normal children and adults have considerable resistance to the disease if not infection by CMV (see below).

Two more recent studies, one from Holland by Wilhelm *et al.* (1986) and one by Preiksaitis *et al.* (1988) from Canada, suggest a slightly different picture from the previous studies. Both were concerned with the risk of transfusing seronegative recipients with blood from seropositive donors. Unlike other studies summarized in Table 9.8, both found the risk to be extremely low, 1% per unit transfused or less. Wilhelm *et al.* (1986) followed 595 seronegative recipients by periodically assaying serum samples by both CF and EIA tests. Seven (1.2%) were discovered to seroconvert, and none were symptomatic. To be noted is the fact that they also followed a group of controls consisting of 1425 donors who did not receive any transfusions. Eight of this group (0.56%) seroconverted at the same time. Although the transfused recipients had a higher rate of infection (1.2% versus 0.56%), the difference was not significant. Preiksaitis *et al.* (1988) studied 637 transfusion recipients who were not immunosuppressed but who included 114 neonates. Only six (0.9%) developed CMV infection, of whom four were symptomatic. No differences in risk among patients with different diseases were observed. Infected patients received a

significantly larger number of units of blood and plasma. For example, the mean number of whole blood units received was 50 in infected patients versus 6.2 in controls. These results represented a risk per unit of complete blood transfused of 0.14% or about 0.38% per unit of seropositive blood. This is about tenfold less than the findings from previous studies (see Table 9.8). Another interesting finding was that patients exposed to more than 30 donors had a higher risk of acquiring CMV infection than would be predicted from random distribution.

Adler *et al.* (1985) looked at a different problem. They tried to determine whether reinfection or reactivation of an endogenous infection was responsible for posttransfusion CMV infections in seropositive recipients of blood transfusions. They found that 12 out of 79 seropositive recipients became infected, as demonstrated by a fourfold or greater rise in IgG antibodies to CMV 8 to 12 weeks postoperatively. All subjects were cardiac surgery patients.

Adler then tried to determine whether patients who received blood from seropositive and seronegative donors had any difference in infection rate. They found that five of 46 patients who received only seronegative blood and seven out of 48 patients who received seropositive blood became infected. Thus, there was no difference in these rates, which suggests that the infection was largely a result of reactivation rather than reinfection.

Whether the extremely low rate of infection transmitted by seropositive donors to nonimmunosuppressed recipients (that is, excluding those who are immunosuppressed and premature newborns) in recent studies is real or not remains to be determined. That it has been shown in two studies in 1986 and 1988 in two different localities in the Western world suggests that it may be true. One reason may be that this is a more definitive study because of its large numbers, since most of the studies summarized in Table 9.8 are smaller. Other reasons for these differences are that the studies may not be strictly comparable. Open heart surgery patients may be prone to reactivation of CMV. The way blood is prepared, for example, leukocyte depletion, may be a factor. Possibly in order for seropositive blood to transmit the infection, additional biological factors not usually tested for are needed. One such factor may be active (as opposed to latent) infection as evidenced by the presence of CMV-specific IgM antibody. It is possible that the number of potentially infectious cells in circulation is higher in a subject who is actively infected. Finally, the number of recently infected subjects may have truly decreased with the onset of more rigorous screening of blood in the Western world following the development of the AIDS epidemic (see below).

9.7.2. Risk of CMV Infection from Transfusions in Neonates

Monif *et al.* (1976) conducted a prospective study of the effect of the antibody status of transfused blood on infection as determined by complement-fixation serology in both seronegative and seropositive recipients (Table 9.9). Eight hundred ninety-seven units were given to 207 recipients (mean 4.3 units per patient). As can

TABLE 9.9
Effect of Donor and Recipient CMV Serology on Acquisition of Posttransfusion CMV Infection[a]

Recipient status	Donor seronegative			Donor seropositive			Total patients	Total infected
		Infected			Infected			
	Total	No.	%	Total	No.	%		
Antibody (−)	86	3	3%	54	13	24%	140	11%
Antibody (+)	43	0	0%	24	3	13%	67	4%
Total	129	3	2%	78	16	21%	207	9%

[a]Data from Monif *et al.* (1976).

be seen from the table, the highest rate of infection occurred in antibody-negative recipients who received seropositive blood. No antibody-positive recipient who received seronegative blood became infected. Three susceptible recipients became infected after receiving seronegative blood, perhaps because the CF test used was not sensitive enough to detect low titers in donated blood. None of 43 antibody-positive recipients, presumably latently infected, became infected after transfusions.

This interesting study supports the idea that seropositive blood is latently infected and that seropositive recipients have increased resistance but not absolute immunity.

Concerning the risk of transfusion in neonates, Luthardt *et al.* (1971) were the first to provide significant data (Table 9.10). One hundred twenty-nine exchange transfusions in 91 neonates with hyperbilirubinemia at the University of Freiburg were prospectively investigated. Specimens for CF tests were obtained from all mothers and babies and from infants 2–4 and 6–9 months later. Seventy-three infants completed all follow-up studies. Blood was used within 3 hr of donation. Viruria was demonstrated in nine out of 12 infected babies. The presence of passively transferred antibodies in the baby's initial serum had a clear protective effect

TABLE 9.10
Frequency of CMV Infection after Exchange Transfusion in Neonates[a]

CF reaction in		Number of children	Number showing infection (serologic rise)	
Child	Donor		No.	%
+	0	25	2	8
0	+	15	8	53
+	+	13	2	15
0	0	20	0	0
Total		73	12	16

[a]Data from Luthardt *et al.* (1971).

against infection. Seronegative babies were at greater risk when they received blood from seropositive recipients. Interestingly, no infection occurred when seronegative babies were exchange transfused with blood from seronegative recipients. These data support the notion that CMV was transmitted by the blood of seropositive donors. The reason antibodies from these seropositive donors were not as protective as passively transfused antibodies from the mother probably lies with differences in quantity of antibodies transferred. An estimate was made that 50% of seropositive donors could transmit the virus.

The infected babies had no definite clinical symptoms. A criticism of this paper, which is difficult to overcome in such studies on neonates, is that transmission by blood was not completely proven. Of 12 infected infants, nine were not viruric at birth. But that does not rule out perinatal acquisition of CMV by means other than blood. Eight were CF negative and not viruric at birth but became viruric later. They all had seropositive donors.

A more definitive study was completed by Yeager *et al.* (1981). The results are given in Table 9.11. None of 90 infants of seronegative mothers who were presumably seronegative and susceptible to primary infection became infected if they received blood from seronegative donors. Ten became infected if they received blood from seropositive donors. All ten received more than 50 ml packed red cells (10/41 or 24%). Cytomegalovirus infection occurred with about the same frequency in neonates of seropositive mothers irrespective of whether they received blood from seropositive or seronegative donors (15% and 17.6%, respectively). These infections were asymptomatic, whereas 50% of the ten primary infections were fatal or serious infections (see Section 12.2.2). When only blood from seronegative donors was transfused, CMV infection of infants of seronegative mothers was eliminated. This work proves that (1) symptomatic primary infection may be transmitted by blood, (2) the serologic status is a valid indicator of both immunity and latent infection, and (3) for a susceptible human neonate, the infecting dose of blood is between 50 and 100 ml, a remarkably low figure.

TABLE 9.11
Frequency of CMV infection in Neonates after Transfusions According to Antibody Status of Mothers and Donors[a]

IHA reaction in[b]		Number of children	Number showing infection (virus shedding)	
Mother	Donor		No.	%
+	0	131	23	17.6
0	+	74	10	13.5
+	+	60	9	15.0
0	0	90	0	0.0
Total		355	42	11.8

[a]Data from Yeager *et al.* (1981).
[b]IHA, indirect hemagglutination assay.

The conclusions from studies of the risk and the nature of CMV in blood transfusions are as follows: (1) The risk of infection by CMV for immunocompetent adults or children is 3% per unit transfused or less. The risk of CMV disease is significantly less. (2) The risk of infection and disease is significantly higher for seronegative recipients, especially immature neonates and bone marrow transplant recipients. Data are inadequate for other immunosuppressed groups except for marrow transplant recipients (see Section 13.1.1). (3) A number of seropositive recipients will become infected, and it is unclear whether infection is *de novo* or reactivated; this type of infection may be more common after open heart surgery. (4) The risk for infection is greater if the blood comes from seropositive donors who are viruric or have other evidence of active infection. (5) The risk of infection is too small to exclude seropositive donors except in certain cases such as exchange transfusions for neonates. In this case, it is probably desirable to use only seronegative donors for seronegative recipients. (6) The precise conditions under which latently infected donors will produce active infections are still unknown.

9.7.3. Nature of Cytomegalovirus in Blood of Donors

The epidemiologic evidence is strong that seropositive blood donors can transmit CMV. Such evidence is similar to the evidence that CMV may be transmitted by transplanted kidneys and probably other organs of seropositive donors (see Section 13.1). What is unclear is why there seems to be such variability in the transmission by blood (Table 9.8).

The basic assumption is that asymptomatic donors can transmit CMV because their cells harbor CMV. Direct evidence for this assumption is rare. Tolpin *et al.* (1985) reported that an isolate from a transfused infant was identified by restriction endonuclease analysis to a urine isolate from a donor. The only available positive report of direct isolation of CMV from donated blood is that of Diosi *et al.* (1969), who found CMV in units of blood from two asymptomatic donors out of 35 tested. Since then, at least 1483 units of blood from volunteer donors in the United States and the United Kingdom were tested, and no CMV was detected by direct coculture (see Table 9.12). We can conclude from this that there is ordinarily no CMV viremia in normal, asymptomatic adults. Very likely Diosi's two cases represented individuals who were undergoing an acute infection with lytic virus infection in blood cells.

There is no doubt that viremia persists for some time following acute infections, such as CMV mononucleosis, even in normal subjects. Rinaldo *et al.* (1979a) found that viremia was demonstrable 49 and 93 days after onset of illness in two of their 14 patients, at times when they were clinically asymptomatic and conceivably could have donated blood.

Similar comments may be made concerning viruria. Kane *et al.* (1975) identified seven (3%) viruric donors out of 223. All three patients who received blood from these viruric donors developed serologic evidence of CMV infection. One also

developed viruria, but none was symptomatic. Hence, viruria is also rare in asymptomatic subjects, but when it occurs, it probably represents active infection. One important possible conclusion from these studies is that not all seropositive units of blood are equally risky. Perhaps those from donors with active infection are more infectious because they have more infected cells or more cells that are activable after transfusion. It is quite possible that with the elimination of at-risk persons for HIV infections from the donor pool by voluntary exclusion and antibody testing, the number of donors with active CMV infection has been reduced since 1984.

Most methods of virus detection in blood cells involved cocultivation of donor white cells with permissive cell cultures. This should allow for reactivation and replication of some latently infecting viruses, but perhaps not the more restrictive forms. Pagano and Huang (1974; Pagano, 1975), using whole genome probes, showed that buffy coat cells of a small number of normal donors (one out of three) had a few (6.5) genome equivalents of CMV DNA as demonstrated by cRNA–DNA membrane hybridization.

The possible explanation for past failures came when appropriately specific and sensitive probes were used (see also Section 5.5.3). First, Rice *et al.* (1984) found that fresh clinical isolates of CMV could infect human lymphocytes of T- and B-cell lineage, natural killer cells, and monocytes. However, virus expression was limited to the synthesis of immediate early antigen, and no mature virions were synthetized. Next Schrier *et al.* (1985) used as a probe the Eco RIJ fragment of CMV DNA containing the coding sequences for immediate early antigen in *in situ* hybridization. Examination of eight individuals who were seropositive for CMV revealed two whose peripheral mononuclear cells contained specific viral RNA. Such cells may be the source of the infection in blood transfusions, since transfused lymphocytes are known to circulate after blood transfusions (Schechter *et al.*, (1977). However, it is unclear under what circumstances such abortively infected cells are reactivated to form complete viruses. Possibly the number of such infected cells, the stage of infection, as well as the type of cell infected play a role. Lang (1972) suggested in 1972 that an *in vivo* mixed-lymphocyte reaction might stimulate reactivation of CMV. Such reactions might take place after transfusions. There is no evidence to support this theory. Conversely, Chou *et al.* (1987a, b) made the interesting observation in seronegative recipients that three out of eight (38%) who received blood from HLA-identical donors became infected, whereas only one (3%) of 32 recipients seroconverted. This suggests that infected cells may survive longer in HLA-compatible recipients and may be more infectious in such recipients. The role of histocompatibility in reactivation of CMV is clearly also of interest for effective transmission of CMV from donor organs after transplantation. In CMV viremia proven by routine cultural methods, it has now also been shown by hybridization methods, that the polymorphonuclear leukocytes are primarily infected (Saltzman *et al.*, 1988).

Another factor determining the infectivity of transfused cells is the passive transfer of immune factors. Beneke *et al.* (1984) studied the IgG and IgM CMV antibody titers of donors of 28 patients who developed a CMV infection and 23 who

TABLE 9.12
Attempts to Isolate CMV from Donated Blood or Blood Donors

Authors	Units tested	Virus isolation			Location
		Sample	No. (+)	%	
Diosi *et al.* (1969)	35	Blood	2	6	Timisoara, Rumania
Wentworth and Alexander (1971)	300	Blood	0	0	Seattle, Washington
Perham *et al.* (1971b)	195	Blood	0	0	Bristol, U.K.
Mirkovic *et al.* (1971)	290	Blood	0	0	Houston, Texas
Kane *et al.* (1975)	223	Blood	0	0	Kansas City, Missouri
		Urine	7	3	
Bayer and Tegtmeier (1976)[a]	120	Blood	0	0	Kansas City, Missouri
Armstrong *et al.* (1976)	320	Blood	0	0	Pittsburgh, Pennsylvania
Total	1483	Blood	2	0.13	

[a]Used technique of Diosi *et al.* (1969).

did not. Donors of the infected group had lower (1 : 654 by IHA) titers than donors of the uninfected group (1 : 1360). This suggested that passive transfer of high-titered antibody may be protective. This may be an important factor, particularly in immunosuppressed subjects. The possible role of transfer of immune cells is unknown. Seven (1.3%) of the 529 blood donors had CMV-specific IgM antibody titers $\geq$ 1 : 16, and all seven were associated with development of infection in the recipient. However, exclusion of IgM-antibody-containing blood would only have prevented 43% of CMV infections. In summary, recent virological evidence supports the serologic evidence that blood from seropositive donors may be infectious. However, there is probably a complex set of circumstances, largely undefined, that determine whether or not virus transmission and/or disease takes place. These relate to the immune status of the host, the nature of the transfused virus-infected leukocyte, and factors that allow latently infected leukocytes to reactivate in the recipient.

9.7.4. Methods to Eliminate CMV from Blood

Tolkoff-Rubin *et al.* (1978) showed in a study in Boston that patients who had received more than 3 units of conventional blood were 82% seropositive (13/16), whereas those who had received no blood or only frozen red cells thawed and deglycerolized by the Huggins technique of cytoagglomeration were only 66% (42/64) seropositive ($P < 0.01$). More convincingly, none of 21 seronegative recipients seroconverted after receiving a total of 157 units of frozen blood, whereas all three seronegative patients who received conventional blood seroconverted and excreted virus.

It is thought that freezing destroys the viability of leukocytes, which may be essential for carriage of the virus. Living mononuclear cells or polymorphonuclear leukocytes are thought to carry the virus (Fiala *et al.*, 1975; Rinaldo *et al.*, 1979a).

Brady *et al.* (1984) studied the effect of frozen, deglycerolized red blood cells. One hundred six seronegative infants received 313 units of deglycerolized CMV-positive blood and 296 units of CMV-negative blood. When evaluated 3 months after their transfusions, none developed serologic conversion or evidence of viruria. This study showed that freezing and deglycerolization could prevent transmission of CMV.

In view of the high cost of red-cell-freezing technology, attempts were made to achieve the same objective by depleting leukocytes by centrifugation, saline washes, or filtration. In one study, 89% of the leukocytes were removed, but CMV infection was still observed in six (11%) of the 54 seronegative neonates transfused, and four became symptomatic (Demmler *et al.*, 1986). Luban *et al.* (1987) reported that of 76 seronegative infants who received at least one washed transfusion from a seropositive donor, one seroconverted. Although this rate of infection (<1%) is significantly less than that seen in some other studies, it should be apparent that such conclusions with historical controls are notoriously difficult to evaluate.

Gilbert *et al.* (1989) conducted a multicentered study in Australia to determine the effect of removal of leukocytes from blood by means of "Imugard IG500" (Terumo) cotton wool filters. Only babies whose mothers were seronegative were enrolled. Nine (21%) of 42 infants who received unfiltered seropositive blood were the only infected. These results suggest that transfusion-acquired CMV infection is preventable by filtration. This may be a more practical and cost-effective method than others, including the use of only seronegative blood for newborn infants.

In view of the fact that IgM antibodies against CMV indicate a recent and perhaps active CMV infection, and circumstantial evidence indicates that donors with such infection may transmit CMV more readily, exclusion of IgM-positive donors has been suggested as a possible way to screen for CMV. Lamberson *et al.* (1988) found that seven out of 222 seronegative infants developed CMV infection after transfusions, and all seven received blood that was both ELISA- and IgM-positive (IFA) for CMV antibodies. However, in a prospective study in which IgM-positive blood was excluded, one infection still occurred in 141 recipients. Therefore, screening reduced but did not entirely prevent CMV infections.

9.7.5. Transmission of CMV by Leukocyte Transfusions

Leukocyte transfusions have been frequently used in neutropenic patients who are at great risk for many types of infections, although their efficacy has not been proven (Rosenheim *et al.*, 1980). From what we know about the ability of blood to transmit CMV, and the identification of leukocytes as the source of virus (Rinaldo *et al.*, 1977; Schrier *et al.*, 1985), such transfusions might be expected to be particularly hazardous.

Winston *et al.* (1980) observed more CMV infections in marrow transplant recipients and newly diagnosed acute leukemia patients who had received a large

number of leukocyte transfusions. A mean 23.1 units (about 10^{10} leukocytes per unit) was given prophylactically to 31 patients, of whom 19 (61%) developed CMV infection. Seven out of 27 (30%) control patients who received no leukocyte transfusions or only therapeutic transfusions (mean 3.8 units) became infected. Twelve of the 19 infected in the treated group and four of the seven infected in the control group were symptomatic. The difference in frequency of infection was only significant for the seronegative subjects and for the marrow recipients, which suggests that these two groups of patients were at greater risk.

Similar results were obtained by Hersman *et al.* (1982) from Seattle. Data from 387 patients receiving marrow transplants from HLA-matched siblings were analyzed. Overall incidence of CMV infection was not increased by granulocyte transfusions. Although seronegative recipients of granulocytes from seropositive donors had significantly more infection, deaths from interstitial pneumonia were not significantly different.

9.8. REACTIVATION OF CMV IN IMMUNOCOMPETENT SUBJECTS

We have discussed several situations in which CMV remains active in otherwise healthy immunocompetent subjects (see Sections 9.4 and 9.5). Cytomegalovirus may be found in the uterine cervix, urine, and breast milk of women. It is routinely found in the semen and urine of homosexually active men. We have noted that antibody titers including IgM titers may be elevated in the male homosexual population. No clinical syndrome has been ascribed to these virologically active states. In this respect, other herpesviruses also provide analogies. Oral and genital herpes are known to reactivate both silently and symptomatically (Corey and Speer, 1986). Herpes zoster represents the clinically apparent reactivation of latent VZ virus in dorsal ganglia. It is well known that the frequency of herpes zoster increases with age (Hope-Simpson, 1965). There is no clear description of EBV reactivation. However, the fact that it may be cultured normally from the saliva of seropositive subjects (Evans and Niederman, 1982) suggests that there may be constant reactivation or persistent infection by this virus. Glaser *et al.* (1985) reported that antibody titers against EBV increase with age and suggest that the virus is more active as one ages. Whether this is related to the decline of immunity with age is unknown.

Musiani *et al.* (1988) compared IgG antibody titers against immediate early, early, and late antigens (see Chapter 1) in an old and young population. The old population consisted of 69 geriatric patients aged 70 to 92 (mean age 79), and the young consisted of 67 sex-matched blood donors aged from 25 to 38 (mean 33).

An immunoassay using acetone-fixed cells that expressed the required antigen was used. Cells infected for only 1 hr expressed immediate early (IE) antigen. Early antigen (EA) was expressed by infection in the presence of cytarabine. Cells containing late antigen (LA) were obtained after incubation for 72 hr after infection.

The IgM titers were determined using sera that were preabsorbed with staphylococcal protein A to remove IgG.

All of the geriatric group had antibodies against CMV, and 82% of the control population were seropositive. The mean titers of antibodies against LA were 1 : 245 in the geriatric group and 1 : 79 in the control group ($P < 0.001$). Titers against IE and EA were also significantly elevated. In the elderly group 15% had IgM antibodies greater than 1 : 20, and 11% had such titers in the control group. Although the differences in IgM titers were not significant, the differences in IgG titers were and were thought to indicate a higher frequency of reactivation of CMV infection in the elderly population.

McVay and Adler (1989) used immunoblot (Western blot) to analyze IgG and IgM antibodies in the sera of 43 surgical patients, 31 patients with solid tumors, and 124 healthy individuals. Logistic regression analysis revealed that the presence of IgM antibodies to CMV proteins correlated with increasing age ($P < 0.0001$), the female sex ($P < 0.005$), and with the IgG antibody titer against CMV ($P < 0.03$).

The frequency of IgM positivity among the IgG-seropositive groups increased from 15% for those less than 20 years of age to 63% for those older than 60. The IgG antibody titers on 197 samples were also specifically correlated with age, sex, and IgM positivity.

Taken together these data suggest that CMV reactivation in immunocompetent seropositive individuals occurs frequently, especially in women, and increases linearly with age.

10

Pathology of Cytomegalovirus Infection

10.1. CHARACTERISTIC CYTOLOGIC REACTION

The history and practice of the pathology of CMV infection has until recently been dominated by cytomegaly, the large giant cells with type A intranuclear inclusion (Cowdry, 1934). The classical pathology of C.I.D. is well described in standard texts, particularly Seifert and Oehme (1957), and is not repeated here in detail. In acute neonatal and adult infections, CMV has been detected by morphological means in a wide variety of organs, including the salivary glands, lung, liver, pancreas, kidney, eye, ear, placenta, alimentary tract, heart, ovaries, anterior pituitary gland, adrenal gland, thyroid, brain, and skin (Seifert and Ohme, 1957; Rosen and Hajdu, 1971; Stagno *et al.*, 1977c).

The evidence that these large cells represent lytic virus infection comes from virological studies in cell culture, where the typical changes can be observed in infected monolayers. Both intranuclear and cytoplasmic inclusions have been shown to contain virions or nucleocapsids and virus-specific antigens by electron microscopy (see Section 4.3).

We now know that cells may be abortively or latently infected in tissues without producing the typical cytomegaly and hence may be unrecognized in a tissue section (see Section 10.2).

A frequent and interesting unresolved problem is the identity of the cytomegalic cells. They are frequently associated with ductal epithelium and have thus been assumed to represent epithelial cells. It is paradoxical that epithelial cells in culture are not usually permissive for CMV and that, typically, fibroblasts are most susceptible. However, infected fibroblasts are not commonly seen in tissue sections, at least not as far as can be determined on morphological grounds. We now know that such cells and other highly differentiated cells may be abortively infected (see Section

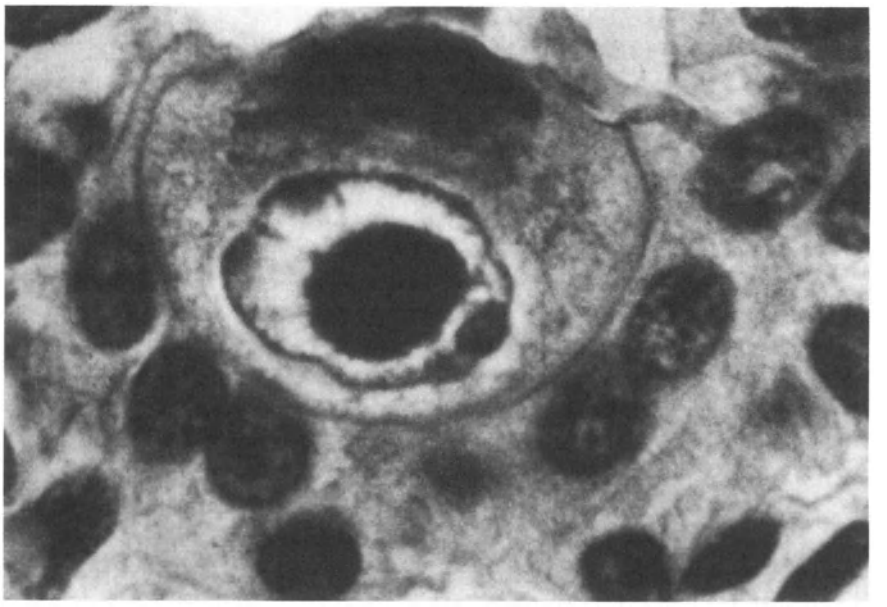

Figure 10.1. A giant cell in the alveolus of a patient with interstitial pneumonia caused by cytomegalovirus. An inclusion occupies the entire nucleus except for a perinuclear halo. Less well defined increased cytoplasmic inclusions may also be seen. (Courtesy of R. L. Myerowitz.)

10.2). The reason for these striking differences between what is observed in tissues and in cell cultures is unknown.

The typical giant cells are two to four times larger than normal surrounding cells (25–40 μm); the round-oval 10 to 15-μm large nucleus is frequently eccentrically displaced toward one end of the cell. The inclusion itself, 8–10 μm, may also be eccentrically placed, presenting an "owl's eye"-like appearance. It is large and characteristically surrounded by a clear halo, which extends to the nuclear membrane. The latter is characterized by clumped chromatin, occasionally in polar-body-like aggregates (Fig. 10.1).

Occasionally, less well defined but characteristic granular inclusions may be seen in the cytoplasm. These are 0.3 to 0.5-μm ill-defined basophilic granules occasionally interspersed with vacuoles and, in the parotid, directed toward the luminal side (Seifert and Oehme, 1957). These inclusions are very important in distinguishing CMV from other DNA viruses, particularly herpes simplex and varicella–zoster viruses.

A cellular infiltrate is frequently but not always seen in the vicinity of the giant cells. Infiltrates consist of plasma cells, lymphocytes, reticulum cells, and, in the case of neonates, hemopoietic elements. In the secretory organs, particularly the salivary glands, there may be signs of secretory disturbance such as concentration of

mucus, hypersecretion, and cyst formation of the ducts. This has been characterized as "dyschylia" (Seifert and Oehme, 1957).

10.2. NONMORPHOLOGICAL METHODS OF DETECTION

It became apparent after the discovery of methods of culturing the virus that identification of morphological cytologic changes in tissues was not the most sensitive method for the detection of CMV. It is not uncommon to be able to culture the virus when no morphological changes are found (Smith *et al.*, 1975).

With the availability of highly specific polyclonal and monoclonal antibodies and development of methods to detect corresponding antigens in tissue sections using immunoperoxidase or other enzyme method, CMV can now be more precisely localized in tissue sections. This method may also lead to more specific diagnosis. For example, the specific diagnosis of CMV hepatitis in biopsies and sections of the liver utilized a specific polyclonal antibody and the avidin–biotin peroxidase method (Bronsther *et al.*, 1988). Sensitive and specific methods of diagnosing CMV pneumonia and specific organ pathology include tissue immunofluorescence, immunoperoxidase stain with monoclonal antibodies against immediate early antigen, and nucleic hybridization (Hackman *et al.*, 1985) (see also Sections 5.2–5.4).

Abortive infection by CMV may be demonstrated in peripheral leukocytes using monoclonal antibody to immediate early antigen or *in situ* hybridization using a gene probe of this region (Einhorn and Ost, 1984; Rice *et al.*, 1984; Saltzman *et al.*, 1988). This discovery promises to extend the horizon of pathological study beyond cells producing complete lytic virus. Myerson *et al.* (1984), in two cases of disseminated CMV infection in bone marrow recipients, compared the distribution of CMV infection by morphological changes and by *in situ* hybridization. They used a biotinylated probe that constituted about 56% of the total genome. Besides showing CMV genes in cytomegalic cells, this method detected infection in many normal-appearing cells. Interestingly, many types of cells were involved by hybridization but not by cytomegaly. These included highly specialized cells such as cardiac myocytes, hepatocytes, cells in the renal glomerulus and tubules, adrenal cortex, and medulla, fallopian tube submucosa, myometrium, endometrial, stromal, and glandular cells of the uterus, and reticular cells of spleen and lymph node. Hence, it is clear that virus infection is much more widespread than anticipated. Bale *et al.* (1989) have applied hybridization methods to tissues of congenitally infected infants. Tissue pathology may also occur in the absence of viral genomes, for example, in the brain. "Clearance" of infected cells may have occurred.

Toorkey and Carrigan (1989) described the presence of immediate early antigen (68 kDa) detected by monoclonal antibodies in tissues of healthy seropositive individuals who died of trauma. Normal-appearing tissue cells found to contain antigen were found in the brain, kidney, spleen, lung, and liver. No productive infection was found.

The amplification of CMV nucleic acid, which is present in small amounts in tissues, by polymerase chain reaction may provide yet another sensitive method to detect CMV infection in tissues (see Section 5.3.1).

It is clear that both lytic and abortive or latent infection can exist in tissues without inflammation or cell destruction. On the other hand, foci of the infected cells may form the center of inflammation of necrosis. A major unsolved problem in understanding the pathogenesis of CMV infection is at what point are inflammation, necrosis, and disease of a tissue produced, since clinical manifestations are associated with such changes.

10.3. PATHOLOGY OF SPECIAL ORGANS

The *salivary gland* is the site of isolated localization of CMV as revealed in autopsy series of children. Farber and Wolbach (1932) first pointed out in the United States that this is a frequent occurrence in unselected autopsy cases. A comparison with other series in various parts of the world shows that the rate of salivary gland infection varies from 8% to 32% (Table 10.1). The most frequently afflicted gland is the parotid gland. The submandibular gland is less often affected, and the sublingual gland is rarely infected (Seifert and Oehme, 1957). Cytomegaly is almost always exclusively observed in the ductal epithelium. The serosal end portions are less often involved, and the mucosal cells never are. This is different from the effect of murine CMV, which affects the acinar cells but is the same type of pathology seen in infection by guinea pig CMV (Jackson, 1920; Fong *et al.*, 1980).

Interestingly, ten Bensel and St. Geme (1968) were not able to find any evidence of CMV infection in the submandibular and parotid glands of 100 autopsy subjects over 10 years of age in Minnesota. The gland had no cytomegalic cells, and no CMV was cultured after homogenates of the gland were inoculated in human fibroblast cultures. Very possibly, as in the mouse, hCMV does not persist in the

TABLE 10.1
Frequency of CMV Infection in Salivary Glands of Children in Autopsy Series[a]

Author	Place	Number of cases examined	Number positive	Percent positive
Löwenstein (1907)	Bonn	36	4	11
Farber and Wolbach (1932)	Boston	183	22	12
McCordock and Smith (1934)	St. Louis	60	6	10
Kuttner and Wang (1934)	Peking	49	4	8
Prawirohardjo (1938)	Indonesia	53	17	32
Potenza *et al.* (1954)	Venezuela	84	15	18
Haraszti (1955)	Hungary	100	22	22
Vortel (1956)	Czechoslovakia	279	51	18
Seifert (1957)	Leipzig	822	76	9

[a] Adapted after Seifert and Oehme (1957). Children were neonates to 14 years old.

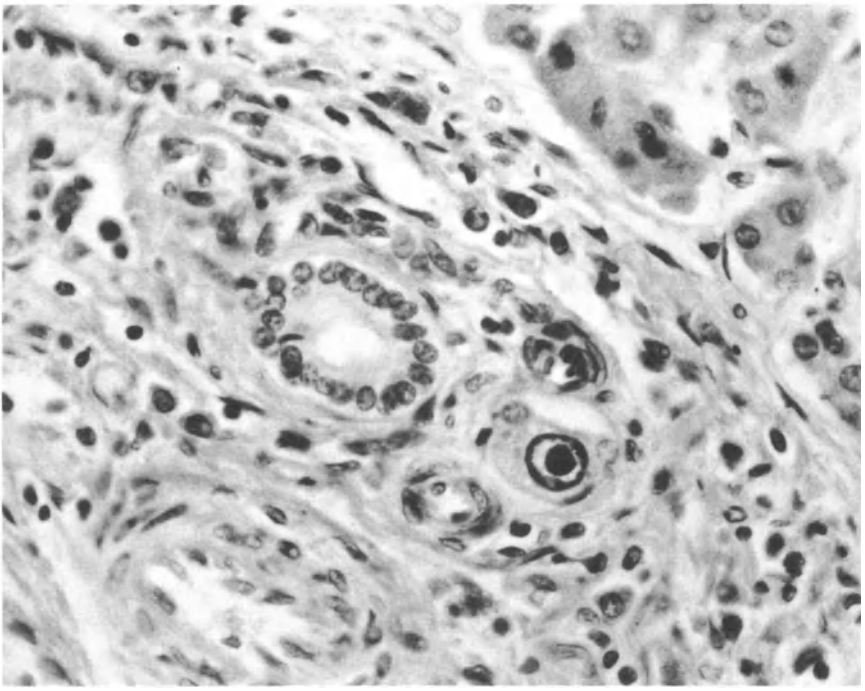

Figure 10.2. A section of liver from a 2½-year-old male who died of C.I.D. Parenchymal cells may be seen at right top of upper picture, and a bile duct is in the center. Nuclear inclusion with halo is in a large cell associated with ductal epithelium (×433). (Courtesy of Dr. E. J. Yunis, Children's Hospital, Pittsburgh.)

salivary gland for life, at least not in a readily apparent form. It was more readily recovered in infants because they were infected relatively recently.

In the *intestines,* the presence of giant cells may be obscured by an enterocolitis. When present, they are in the mucous epithelium of the lower intestines or in the endothelium of the submucosal endothelial cells. Vasculitis in the submucosa may be the basis for ulcerations caused by CMV almost anywhere along the gastrointestinal tract.

In the *liver* of the newborn, there may be areas of extramedullary hematopoiesis and round cell infiltration of the portal triads, particularly in cases of C.I.D. Transitional stages to varying degrees of cirrhosis may occur. Giant cells are most often seen in the bile ductal epithelium, less commonly in the capillary endothelium or portal areas, and almost never in the parenchymal cells (Fig. 10.2). It may be quite difficult to differentiate C.I.D. in the liver from erythroblastosis fetalis except for the presence of cytomegalic cells.

In cases of benign hepatitis or hepatitis associated with CMV mononucleosis in children and adults, when liver biopsies have been available, foci of mononuclear

infiltrates and at times of small noncaseating granulomas, usually without evidence of cytomegaly, can be observed. They consist of loosely arranged nests of epithelioid cells and scattered lymphocytes. The architecture of the liver is well preserved, but there may also be areas of mononuclear inflammation, particularly in the areas of portal triads (Bonkowsky *et al.*, 1975). Inclusion-containing cells may not be a prominent feature. They are more common in disseminated disease.

Reller (1973) and Bonkowsky *et al.* (1975) reported finding in CMV hepatitis intralobular epithelioid granulomas typically not accompanied by inclusion bodies in cases of CMV mononucleosis. In another similar case in a homosexual male with HIV infection but without evidence of AIDS, besides such granulomas, several were of the "ring" type characteristically seen in Q fever (Ende *et al.*, 1957). They possessed a central vacuole resembling lipid, surrounded by a distinct eosinophilic fibrinoid ring (Lobdell *et al.*, 1987).

Cytomegalovirus hepatitis is fairly common in transplant recipients, particularly after liver transplantation. Pathologically, it has been clearly distinguished from rejection reactions (Demetris *et al.*, 1985; see Section 13.3.3).

In the *lungs,* giant cells are seen primarily in the alveolar and bronchial epithelium and less often in the mucous membrane of the trachea or tracheobronchial tree. Next to the kidneys, the lung is the most common site of pathology in acquired CMV infection. It may be the site of focal or disseminated infection.

The *pancreas* is frequently involved in congenital disease (Fig. 10.3) (Seifert and Oehme, 1957). As in the salivary gland, inclusions may be seen in the ductal epithelium.

Type I virus infection of the *islets of Langerhans* may be related to the development of diabetes mellitus (see also Sections 11.4 and 12.4.10), although direct evidence is still lacking. The pancreas, including the islets, is frequently involved in congenital infection (Gajl-Peczalska, 1967; Jenson *et al.*, 1980). Twenty out of 45 cases of children who died of disseminated CMV infection had characteristic inclusion bodies in islet cells (Jenson *et al.*, 1980). Numazaki *et al.* (1988) infected cultures of fetal islets of Langerhans with CMV. Four days later, cells rounded up and floated off the monolayer. Cytomegalovirus was recovered, and late antigen was detected in infected cells. However, β cells were not infected, and insulin production was not affected. There is now indirect though unconfirmed evidence that type 1 diabetes is associated with a higher frequency of inapparent infection of white blood cells as determined by DNA–DNA hybridization (Pak *et al.*, 1988; see Section 12.4.10).

Involvement of *ovaries* by CMV in normal or opportunistically infected patients has been rare. The corpora lutea of ovaries are infected in experimental murine CMV infections in mice (Mims and Gould, 1979; see Section 16.3). However, subclinical infection may be difficult to detect in humans. Subietas *et al.* (1977) reported three cases of CMV oophoritis, two of whom were misdiagnosed as having neoplasms. One case was assumed to be primary infection in the ovary. Kleinman et al. (1984) described the infection of endometrial cells with human CMV.

LiVolsi and Merino (1979) described a 61-year-old woman who died of immu-

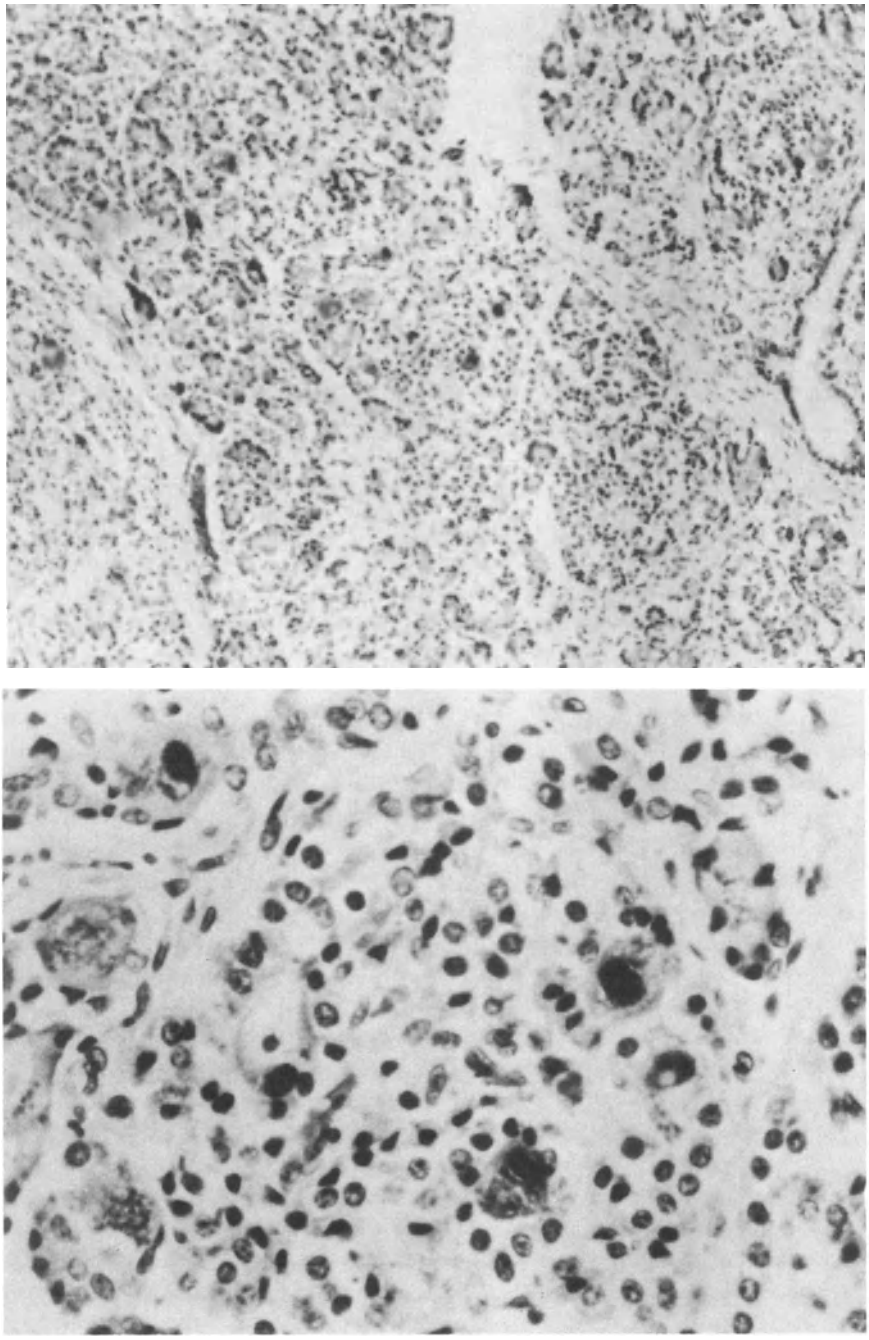

Figure 10.3. Pancreas from a $2\frac{1}{2}$-year-old male infant who died of C.I.D. Top picture ×133; bottom picture ×433. Cytomegalic cells are related to ductal epithelium. (Courtesy of Dr. E. J. Yunis, Children's Hospital, Pittsburgh.)

noblastic B-cell sarcoma. The right ovary contained a 1-cm nodule that resembled a fibrothecoma. At one end of the mass consisting of fibroblasts were large 25- to 40-μm cells with intranuclear inclusions. Typical herpesvirus particles were seen by electron microscopy.

The ovaries of eight cases with postmortem diagnoses of CMV infection were examined by Subietas *et al.* (1977). Three infections of the ovaries were detected by histological examination. The most characteristic feature was multifocal cortical necrosis with neutrophils in variable numbers and nuclear debris. Clusters of giant cells with intranuclear inclusions and occasionally bipolar cytoplasmic inclusions were seen. The capsule and medulla of the ovaries were spared. There was no vasculitis to account for the topography of the lesions. All patients with ovarian lesions were postmenopausal, whereas the five without ovarian lesions were premenopausal. It was postulated that vascular insufficiency and viral cell surface antigens triggered an acute inflammatory response and necrosis (Subietas *et al.*, 1977).

The *involvement of skin* is now well recognized in all forms of CMV disease (see also Sections 12.4.8 and 13.3.7). In congenital infections, petechial and hemorrhagic–purpuric skin rashes have been described. One case had vesicles with fluid from which CMV was cultured (Blatt *et al.*, 1978). Vesicles are uncommon in CMV infections, although they are common in other infections caused by other herpesviruses such as herpes simplex and varicella–zoster viruses. The pathogenesis of the rash in congenital infection is not well understood except when it is clearly a result of a hemorrhagic diathesis. Less commonly, dermal erythropoiesis may develop in bluish, infiltrated, purpuric papules. This may represent vestigial early development (Brough *et al.*, 1967).

Rubelliform or maculopapular rashes may occur in normal patients with CMV mononucleosis with or without administration of ampicillin (Klemola, 1970) (see also Section 12.4.8). Muller-Stamou *et al.* (1974) described an unusual 40-year-old man who developed vesicular lesions and a generalized epidermolysis 8 weeks after onset of hepatitis. He was found to have viruria and viremia caused by CMV.

Minars *et al.* (1977) described a 24-year-old renal transplant patient who was admitted 10 months after renal transplantation with punched-out necrotic ulcers of the skin. She also had pneumonitis, decreased visual acuity, and eventually died of a GI hemorrhage. Disseminated CMV was found at autopsy. The skin showed inclusion bodies and viral particles characteristic of CMV. There were severe perivascular infiltrates that included cells with typical inclusions and involved arterioles, venules, and capillaries. The type of vasculitis is similar to what has been found in gastrointestinal ulcerations. Skin involvement may be striking in CMV infection in AIDS patients (Section 13.3.7).

Lee (1989) reported on the pathological findings in three patients with CMV culled from 22 skin biopsies obtained from 1610 patients. An analysis of a total of 21 cases in the literature reviewed by her showed that five had AIDS, six were organ recipients, and three had hematologic malignancies. Mixed infections were common, particularly in AIDS patients. The cutaneous manifestations varied and in-

cluded skin ulcers (7), necrotic papules (3), skin wounds (3), maculopapular eruptions (7), and hyperpigmented and indurated skin. Microscopically CMV was identified by intranuclear inclusions or by the presence of CMV antigens as determined by the immunoperoxidase test. Cytomegalic cells were present in the endothelium and perivascularly. Palisaded histological infiltrations and fibrosing granulation tissue may be seen in the dermis.

The bones may also be involved in congenital infection. The earlier reviews of Wyatt *et al.* (1950) and Medearis (1957) mentioned cases of C.I.D. with bone changes similar to "rickets," "syphilis," and "atrophy of the cortex and spongiosa." Mercer *et al.* (1953) and Seifert and Oehme (1957) described infants with sclerotic shafts and zones of diminished density at the ends of long bones. The changes illustrated were nonspecific and now would be accepted as patterns of normal ossification in the newborn (Graham *et al.*, 1970).

A more recent contribution by Sacrez *et al.* (1960) described a 2500-g infant with congenital C.I.D. whose femoral metaphyses showed irregularity and decreased density. Graham *et al.* (1970) pointed out that the changes are similar to those seen in congenital rubella. Graham *et al.* (1970) added a case of C.I.D. with positive urine and throat cultures at birth. The femur showed an osseous pattern disrupted by ovoid and linear lucent regions between zones of slcerosis, more marked in the distal metaphyses ("celery stalking"). There were no periosteal changes. These changes were apparent at birth, improved by 10 days, and completely healed by 5 weeks, although the baby died at 66 days of age. Similar cases were reported by Merten and Goodling (1970) and McCandless *et al.* (1975). The x-ray changes were considered by the latter to be a disturbance of endochondral bone formation and not the result of viral osteomyelitis.

Evidence of direct invasion of bone by CMV is rare. Ward *et al.* (1965) described a 48-year-old female with ulcerative colitis who was found at postmortem to have disseminated CMV infection that involved the epiglottis and the temporal bone.

In the kidneys, giant cells are characteristically associated with an interstitial cellular infiltrate. Striking clusters of giant cells may fill the proximal tubules near the cortical areas. They may also be present in Henle's loop or in the collecting tubules (Fig. 10.4) but rarely in the glomeruli. Immune complexes deposited in glomeruli have been described recently (see Section 10.6). The kidneys are probably the most common site of infection in congenital, perinatal, and adult CMV infection, as almost all forms of CMV infection are associated with viruria. Persistent viruria is not associated with any known disturbance of renal function.

Although pathological evidence of tubular–interstitial involvement in the kidney is common after CMV infection, signs of renal failure as a result of such involvement have been conspicuously absent. Cameron *et al.* (1982) described a 34-year-old female who developed renal failure 21 months after transplantation. She had fever, pancytopenia, CMV viruria, and serologic evidence of infection. Renal biopsy showed severe tubulointerstitial changes with intranuclear inclusion bodies. There were no signs of rejection. Shorr *et al.* (1987) described a similar case with

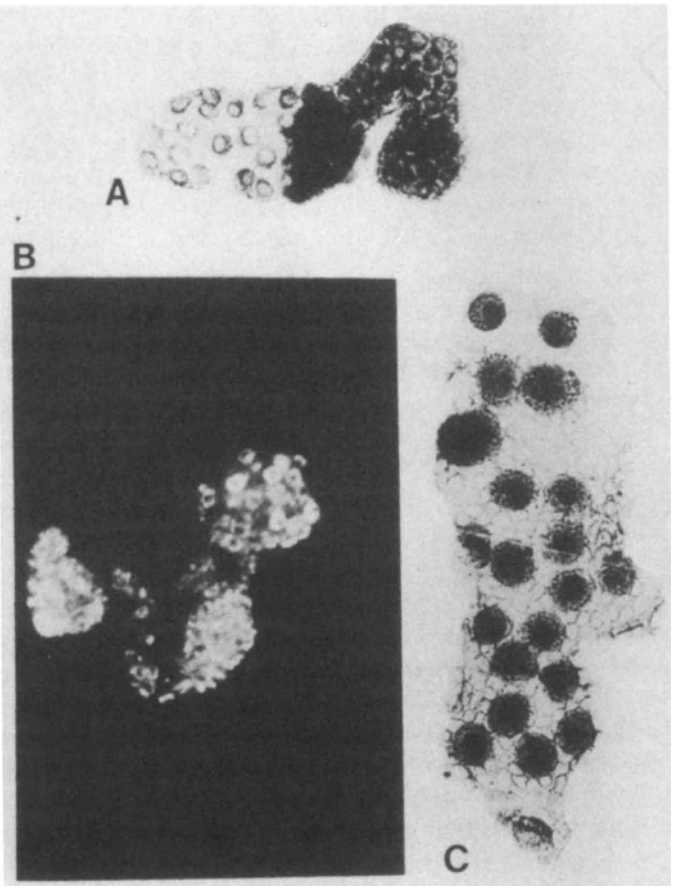

Figure 10.4. A and C are mosaic photomicrographs of stained fragments of distal convoluted tubules from a case of C.I.D. dissected by the Oliver technique for microdissection of the nephron. The cytomegalic inclusions are best shown in tubule of A, where a portion of wall has been turned back, and in C, which is a single-layered fragment. In B, an unstained fragment of a distal convoluted tubule is shown suspended in water. Many of the inclusions appear refractile by incident light at an acute angle (A, ×152; B, ×143; C, ×314). No inclusions were found in the proximal tubules. (A is on the top, B on left, C on right.) (From Fetterman *et al.*, 1968. Reproduced by permission of Dr. Fetterman and the editors of *Archives of Pathology*.)

hemorrhagic cystitis in a bone marrow recipient. The kidney biopsy sample showed tubulointerstitial nephritis with focal necrosis of tubules and positive CMV immunofluorescence in tubular epithelium. Viral cultures for JC and BK viruses, common opportunistic viruses associated with hemorrhagic cystitis (Arthur *et al.*, 1988), were absent.

Heieren *et al.* (1988) assayed human fetal kidney cortex cell cultures for their ability support CMV infection. Virus-specific immunofluorescence and *in situ* hy-

bridization documented the presence of CMV-specific proteins and nucleic acid in purified kidney mesangial cells. The hypothesis is that when CMV infects the kidney, it first infects the mesangium either by immune complex deposition or by macrophage migration. Trafficking of virus from the mesangium to the tubules leads to tubular infection and viruria.

A distinct glomerulopathy characterized by enlargement or necrosis of endothelial cells, accumulation of mononuclear cells, and fibrillar material in glomerular capillaries has been reported to occur in renal transplant patients with CMV viremia. Unlike rejection, renal function improved with reduction of immunosuppression (Richardson *et al.*, 1981) (see also Section 13.1.4).

However, other workers failed to detect CMV infection in the glomeruli of transplant recipients (Herrera *et al.*, 1986). Specimens were studied from (1) six autopsies of bone marrow recipients who died with disseminated CMV infection, (2) 15 autopsies of children who died of disseminated CMV infection, and (3) seven out of 78 kidney biopsies from renal transplant recipients who showed suspected CMV glomerulopathy not associated with acute rejection. The first two groups, as would be expected from previous pathological studies,showed no evidence of glomerulitis associated with CMV, although tubular lesions were common. In the third group, diffuse glomerulopathy characterized by enlargement and necrosis of endothelial cells and accumulation of fibrillary material in capillary spaces was thought to be consistent with the CMV glomerulopathy described by Richardson *et al.* (1981). However, no CMV antigens were demonstrated by immunofluorescence, and no viral particles were demonstrated by electron microscopy. No immune complexes were demonstrated either. The lesions were thought to represent transplant glomerulopathy. Similar lesions may be found in hyperacute rejection. The authors concluded that this glomerulopathy may represent a peculiar rejection phenomenon that may have been triggered by systemic CMV infection.

10.4. CENTRAL NERVOUS SYSTEM

The brain is typically involved in C.I.D. (see also Section 11.4.1). Grossly, there may be hydrocephalus, periventricular calcification, focal softening, hemorrhages, astrocytic proliferation, perivascular inflammatory infiltration, and ependymal granulations (Haymaker *et al.*, 1954). Functional disturbance may be severe. Diezel (1954) first reported the association of cytomegalic inclusion disease with microgyria or micropolygyria. Microgyria is a true cerebral malformation characterized by an excess of small, abnormally formed gyri (Crome, 1952). Histological and embryological considerations suggest that the cause of this condition operates before the sixth month of fetal development. Crome and France (1959) described a baby with presumptive C.I.D. who died 31 hr after birth. Its 25-year-old mother had glandular fever but a negative Paul–Bunnell test. The brain showed polymicrogyria but no definite inclusions.

Microgyria is a common feature in the brain of a congenitally CMV-infected

baby. Dias *et al.* (1984) postulate that CMV microgyria is a result of insufficiency of cerebral blood supply and is not caused by a disturbance of neurogenesis or histogenesis resulting from the direct viral effects on germinal cells. The way in which CMV causes ischemia is not known. One possibility is by thrombotic vasculitis such as described by Bernischke *et al.* (1974) (Section 10.5) in the placenta. Another possibility is perfusion failure caused by transient systemic hypotension.

The brain may also be involved in adult CMV infection (see Sections 12.4.5 and 13.3.6). Self-limited encephalitis has been described in adults, but no histological examination has been reported. On the other hand, the brain, the spinal cord, and peripheral ganglia can all be involved in CMV infection in AIDS (see below).

10.4.1. Neuropathology of CMV Infection in AIDS Patients

More is known about the pathology of CMV in the nervous system in AIDS patients than its clinical manifestations (Section 13.3.6). Morgello *et al.* (1987) studied 107 autopsied cases of AIDS and found 30 cases (18%) with involvement by CMV by histological criteria.

Similarly, Vinters *et al.* (1989) at UCLA found CMV infection of the nervous system in about 20% of AIDS patients, and over half had evidence of other systemic involvement by CMV (see Section 13.2). Most patients with nervous system involvement, however, were not diagnosed before death. For example, in Vinters' series of CMV infections in the brain, 27 imaging studies were done during life: 19 were thought to be normal, and eight showed nonspecific cerebral atrophy. There was one clear case of necrotizing meningoradiculitis, which presented clinically as ascending paralysis. No doubt further studies will enable us to diagnose some CNS syndromes caused by CMV with greater precision, but studies so far suggest that CMV may often localize in the nervous system without significant clinical sequelae. This is entirely consistent with the behavior of CMV in other organ systems.

Before the important role of HIV in producing the AIDS dementia complex was recognized (Navia *et al.*, 1986a,b), a "subacute encephalitis" was wrongly assumed to be caused entirely by CMV infection even though isolation of CMV from the brain was rare (Snider *et al.*, 1983; Nielson *et al.*, 1984) (see Section 13.3.6). De la Monte *et al.* (1987) recovered HIV from the neural tissue of ten of 11 patients with subacute encephalitis, and in seven it was the only pathogen isolated.

At this time it appears that both HIV and CMV, singly or in combination, may produce encephalitis other than typical subacute encephalitis. Wiley and Nelson (1988) studied the brains of 93 AIDS patients at UCSD. Neuropathological changes consistent with viral encephalitis were present in 54 brains (58%). An HIV antigen (gp 41) was detected in 37. A CMV antigen was detected by immunochemical methods in 31, of whom 22 also had HIV. It was postulated that the coinfected cell may have been the site of an *in vivo* interaction (see Section 13.2.1).. Petito *et al.* (1986) described CMV encephalitis in 26% (40/153) of autopsied AIDS patients in New York City.

The pathological lesions of AIDS dementia and CMV encephalopathy are easier to distinguish than the clinical diagnosis. Cytomegalovirus encephalopathy is particularly difficult to diagnose clinically. Pathologically, both HIV and CMV produce characteristic microglial nodules. The former are poorly circumscribed collections of monocytes with small amounts of foamy cytoplasm, characteristic multinucleated giant cells, and sparse lymphocyte infiltrates. Multinucleated giant cells are pathognomonic of HIV infection and probably represent cells fused by HIV. They commonly involve subcortical white matter in the cerebrum and cerebellum (Petitio *et al.*, 1986; Navia *et al.*, 1986b). Microglial nodules are also a hallmark of CMV encephalopathy. They are more densely cellular than HIV-related nodules and are composed of macrophages, rod cells, or both. Inclusion-bearing cells were present in only 6.5% of nodules but were pathognomonic when present. The nodules have a predilection for gray matter in the brainstem, basal ganglia, cerebellum, and cerebrum (Morgello *et al.*, 1987).

Morgello *et al.* (1987), in a study of 107 autopsies of cases of AIDS, found 30 cases with CMV involvement (28%). Five major neuropathological lesions were identified. Besides microglial nodules, isolated inclusion-bearing cells, focal parenchymal necrosis, necrotizing ventriculoencephalitis, and necrotizing radiculomyelitis were seen.

Wiley *et al.* (1986) used a DNA probe containing the genes for the immediate early (IE) antigen and stained for IE antigen to further elucidate the pathogenesis of lesions in the central nervous system (CNS) of an AIDS patient who died of fulminant CNS and systemic CMV infection. Often morphologically normal cells proved to be DNA or antigen positive. The periventicular tissue showed strikingly positive rimming, suggesting complete seeding of the ependymal and subependymal regions. One suspects that there were uniform ventriculofugal cells from the ventricular walls to the brain parenchyma. This picture of venticuloencephalitis is similar to what one sees in the brain of the child with cytomegalic inclusion disease, where the most extensive damage is a necrotizing encephalitis of deep ventricular structures (Hanshaw, 1971).

In terms of frequency of nervous system lesions in a group of 30 patients, isolated inclusion-bearing cells were seen in half of the patients (15/30). Cytomegalovirus inclusions were identified in capillary endothelial cells, astrocytes, and neurons. There was a diffuse ependymal, subpial, and periventicular distribution of such cells. Focal parenchymal necrosis, necrotizing ventriculoencephalitis (Wiley *et al.*, 1986), and radioculomyelitis were seen less frequently, in three or four patients. No consistent identifiable clinical syndrome caused by CMV emerged from this pathological study except for focal neurological deficits of necrotizing radioculomyelitis (Moskowitz *et al.*, 1984; Behar *et al.*, 1987; see also Section 13.3).

Grafe and Wiley (1989) studied the spinal cords, nerve roots, and peripheral nerves of 27 AIDS patients. Vacuolar myelopathy was seen in 31%, but HIV antigens were found in only 11% of the spinal cords. Cytomegalovirus antigens were not found either. Focal nerve root or peripheral nerve inflammation was seen

in seven patients, of whom four had CMV but not HIV antigens. Cytomegalovirus appears to be an important cause of peripheral neuropathy in AIDS and Gullain–Barré-like syndromes (see Sections 12.4.5.2 and 13.3.6).

10.4.2. Cytomegalovirus Infection of the Inner Ear

Neurosensory deafness is an important late sequela of congenital CMV infection that is frequently undetected at birth. Hence, there is great interest in the pathology of CMV infection in the middle ear. We have numerous descriptions of ear pathology in C.I.D.

The importance of neurosensory deafness as a subtle sequela of congenital CMV infection is described in Section 11.4.3. Cytomegalovirus has been recovered from the inner ear fluid of a clinically deaf infant who died of symptomatic CMV (Davis *et al.*, 1981) as well as from a 5-month-old infant with probable congenital infection but no evidence of hearing impairment (Davis *et al.*, 1987). Cytomegalovirus inclusions were identified in cells lining the cochlear duct (Davis *et al.*, 1969).

Viruses implicated clinically in inner ear disorders include rubella, rubeola, mumps, influenza, varicella–zoster, EBV, CMV, poliovirus, variola, adenovirus, and parainfluenza (see Strauss and Davis, 1973). However, except for CMV, no other virus has actually been identified in the human inner ear. There are two types of viral involvement of the inner ear. One is an endolymphatic labyrinthitis secondary to viremia, perhaps transplacental viremia, which infects and injures the stria vascularis. This is seen after rubella, mumps, rubeola, and CMV infections in infants. The second is a perilymphatic infection secondary to meningeal, perineural, and endoneural spread. It is characteristic of labyrinthine extension of herpes zoster.

Myers and Stool (1968) described a 3-week-old infant who died of neonatal CMV disease who had inclusions in the epithelial cells of the cochlea, stria vascularis, Reissner's membrane, limbus spiralis, saccule, utricle, and semicircular canal. No inclusions were seen in the organ of Corti, cristae, or ganglion.

Davis (1969) reported incidental CMV labyrinthitis in a 6-week-old black male who died of pneumococcal meningitis. Cytomegaly was found in the tubular cells of the kidneys and in the submandibular salivary glands. Cytoplasmic inclusions were found in the epithelial cells lining the right cochlea, i.e., the stria vascularis and Reissner's membrane, and also in the columnar epithelium of both semicircular canals. No inclusions were found in organ of Corti, spiral ganglion, and nerve. From a female baby with C.I.D., CMV was isolated from a culture of the perilymph as well as from other organs (Davis *et al.*, 1979). Cytomegalovirus has been recovered from the inner ear fluid of a clinically deaf infant who died of symptomatic CMV (Davis *et al.*, 1981) as well as from a 5-month-old infant with probable congenital infection but no evidence of hearing impairment (Davis *et al.*, 1987). Cytomegalovirus inclusions were identified in cells lining the cochlear duct (Davis, 1969). Recently, a model of guinea pig CMV labyrinthitis has been described

following congenital infection that is quite similar to the human counterpart (Harris *et al.*, 1984; Woolf and Harris, 1986; see Section 15.3.3), but a clear description of the late sequelae of CMV infection in the inner ear is still not available.

10.5 PLACENTAL PATHOLOGY IN CYTOMEGALIC INCLUSION DISEASE

Bernischke *et al.* (1974) described five cases of C.I.D. of varying degrees of severity that were accompanied by different types of placental pathology.

An abortus 7 weeks of age was an example of infection that occurred during the fourth week of pregnancy documented by CMV mononucleosis in the mother. There were inclusions in the brain, liver, pancreas, lung, and skin. As is characteristic of infection in early fetal life, there was little inflammatory reaction in any of these tissues, and no plasma cell infiltration in the placenta. Inclusions in the placenta were difficult to find. Davis *et al.* (1971) described a similar case. The mother suffered from CMV mononucleosis between the fourth and 14th week of pregnancy. the baby was aborted and examined at 22 weeks of age and showed only scattered cytomegalic cells. The placenta was free of pathology. At this early stage, it was thought that no inflammatory response could be mounted.

Later in the pregnancy, two types of responses may occur. The child may have clinical C.I.D., and the placenta may show diffuse inflammation, villitis, and plasma cell infiltration. Inclusion bodies may still not be evident. Then, there are many cases of inapparent congenital infections characterized only by viruria but no recognizable lesions in the placenta.

10.6. IMMUNOPATHOLOGY IN HUMAN CYTOMEGALOVIRUS INFECTION

Relatively little work has been done on the role of immunopathology in the pathogenesis of CMV infections. One might expect immunopathological reactions in a chronic and persistent virus infection, as indeed has been found in murine CMV (Olding *et al.*, 1976; Mims and Gould, 1979). Stagno *et al.* (1977a) studied immune complex formation in 29 cases infected *in utero* and two perinatally infected babies. Compared to uninfected controls, sera from these patients had more (34%) anti-complementary effect, and 45% had circulating immune complex demonstrated by reaction on Raji cells.

Sera from two congenital subclinically infected infants had 12–16 S immune complexes, whereas sera from four clinically infected infants had heavier complexes (18–22 S). Three infants with heavy complexes died. Their renal glomeruli had deposits of IgG distributed along the glomerular basement membrane in a lumpy-bumpy pattern typical of immune complexes. Tubular cells also showed specific

fluorescence for virus antigen but not trapped antigen–antibody complexes. The glomerular fluorescence also disappeared after acid treatment, which dissociated the complexes. Cytomegalovirus antigen was not demonstrated in the glomeruli.

It was suggested that excess antigenic stimulus in congenital infection is important in the pathogenesis of immune complex deposition. These babies excrete significantly more virus in the first 6 months (Stagno *et al.*, 1975a). Typically, complexes found in the presence of antigen excesses are large (> 19 S) and may be injurious. It is possible that aggregated IgG or anti-B-lymphocyte antibody may have produced a false-positive Raji cell test. But aggregated IgG is not acid dissociable, and the anti-B-cell antibody cannot explain the anticomplementary effect observed when sera were tested.

Ozawa and Stewart (1979) examined the kidneys of a patient who was on steroids for a dermatitis and who developed hematuria and proteinuria in association with CMV pneumonitis. Mesangial proliferative glomerulonephritis was revealed by light microscopy. The mesangium contained granular deposits of IgG, IgA, C3, and C4 by immunofluorescence. Cytomegalovirus antigen was found in the same location, and antibodies against CMV could be eluted from the glomeruli.

Kantor *et al.* (1970) described one case of postperfusion mononucleosis in an adult who developed hematuria and proteinuria. Biopsy of the kidney showed mesangial thickening and thickening of the basement membrane by both light and electron microscopy.

These findings taken together suggest that CMV immune complex glomerulonephritis is a definite entity in both congenital and acquired infections. Further study should revel the conditions under which it occurs.

11

Congenital and Perinatal Human Cytomegalovirus Infections

11.1. INTRODUCTION

One can define operationally a number of distinct neonatal infections, although there is still lack of consensus regarding terminology. An infection is a virological event documented by isolation of virus or by serologic evidence. More often than not it is asymptomatic, subclinical, or inapparent. When it is accompanied by manifestation of disease, we speak of "symptomatic" infection or infection with disease.

Infections detected within the first few months of life, irrespective of whether they were contracted in the uterus or outside, are called "neonatal infections." A neonatal infection is called "congenital" if it was transmitted transplacentally or within the uterus. If a neonatal infection is caused during birth by a virus in the birth canal or after birth by virus from the mother (such as urine, milk, or respiratory secretions), we speak of "perinatal infection" (Levinsohn *et al.*, 1969; Numazaki *et al.*, 1970). Perinatal infections defined in this manner were called "natal" by the Alabama group (Reynolds *et al.*, 1973). We do not use "perinatal infections" as a generic term but to designate a specific type of neonatal infection. If a generic term to include congenital and perinatal infections is needed, "neonatal infection" is proposed.

The operational definition of perinatal infection in terms of viral isolation is that virus cannot be isolated at birth but is isolated within the first few months of life. Possibly, as we understand better the causes of perinatal infection, further subdivisions based on the mechanism of transmission or type of morbidity produced may be necessary. At this time, nothing definite is known about either. There is, of

course, a point at which we should no longer speak of "perinatal" infection but of infant or early childhood infection. Tentatively, a newly discovered CMV infection beyond 6 months of age will be considered the dividing line. Practically, it is often difficult to prove whether an infection at that age is a *de novo* infection or one that originated around the time of birth.

11.2. LABORATORY DIAGNOSIS OF CONGENITAL AND PERINATAL INFECTIONS

The most reliable test for diagnosing either congenital or perinatal infection is isolation of the virus, usually from the urine or throat. Viruria demonstrated at birth or within the first week of life is generally considered sufficient proof of congenital infection. The first appearance of viruria between 3 and 5 weeks after birth indicates perinatal infection if virus was not isolated at birth. Negative cultures in a newborn rule out a congenital infection. One cannot distinguish congenital from perinatal infection if a positive culture is obtained for the first time in a child over a month old. In the absence of immediate postnatal cultures, one must resort to serologic means of diagnosis.

Serologic diagnosis may be difficult in infants because of the complication of transferred antibodies from the mother and because one must distinguish between two possible modes of infection, congenital and perinatal.

The presence of specific IgM antibodies against CMV in cord blood or at birth is presumptive but not conclusive evidence for congenital infection. Not only is this test relatively insensitive, hence producing false negatives, but if rheumatoid factor is present, it may also produce a false positive result (see Section 6.9.1). Stagno *et al.* (1980) reported that 63% of congenitally infected babies born of mothers with primary CMV infection had specific IgM antibody immunofluorescence (IF) in cord blood, whereas only 21% of congenitally infected babies born of "immune" or seropositive mothers had CMV-specific IgM antibodies. More recently cord sera were tested by the ELISA and RIA tests for CMV IgM. The sensitivities of the ELISA and RIA tests for cord serum CMV IgM of congenitally infected babies born after primary infection were 72% and 80%. Cord serum from symptomatically infected babies tested 91% and 100% (Stagno *et al.*, 1985). Thus, the ELISA and RIA assays for CMV-specific IgM in cord serum are improved over the IF assay. Similar conclusions were arrived at in the testing of maternal serum (see below).

The presence of IgG antibodies at birth obviously is not diagnostic of infection because they may be transferred from the mother. Persistence of IgG antibodies during early life, either of the CF type or IFA antibodies against late antigen, is characteristic of congenital infection. But a single positive test does not differentiate between congenital and perinatal infection unless the mother is found to be seronegative. In that case, the baby may be assumed to be postnatally infected.

The IgG antibodies will decline with catabolism of maternal antibodies within the first month after the birth of an uninfected child. In the congenitally infected,

CMV-specific IgG may remain stationary, decline, or rise. In the perinatally infected child, CF antibodies may disappear as in the uninfected child and then rise. The demonstration of such a pattern allows one to diagnose perinatal infection. However, such a pattern is not always seen, and the CF test may not yield an interpretable result (Stagno *et al.*, 1975a).

The IFA test for early antigen (EA) has been proposed to be helpful diagnosing perinatal infection (Stagno *et al.*, 1975a), but it too is not entirely reliable. In summary, serologic diagnosis of different types of neonatal infection leaves a great deal to be desired (see also Section 6.9.1).

11.3. FREQUENCY OF CONGENITAL INFECTION

The results of a considerable number of surveys provide a good indication of the frequency of asymptomatic and symptomatic congenital infections. Those from 1958 to 1986 are summarized in Table 11.1. Two methods of detecting CMV infection were used: virus isolation from urine or throat or measurement of IgM. The populations studied were unselected newborns, babies born of mothers who were initially seronegative, or babies born of seropositive mothers.

The frequency of congenital infections in these studies varied from 0.27 to 2.26 per 100 births. In studies using virus isolation as the diagnostic criterion, the frequency was 0.68%, compared to 0.43% in studies where specific IgM antibodies were measured. Measuring CMV-specific IgM (0.61%) was more sensitive than measuring elevated nonspecific IgM (0.24%). However, either IgM method was less sensitive than the virological methods (Hanshaw, 1969), particularly the IF test used in most studies summarized in the table.

In Sapporo, Japan, only 6.2% of 1233 pregnant women were seronegative (Kamada *et al.*, 1983). In this highly immune population, 0.5% (11 of 2070) of newborns were found to have congenital CMV as determined by viruria. None was symptomatic. A similar study was conducted from 1977 to 1982 in Malmo, Sweden, where the seropositivity rate of pregnant women was 72% (Ahlfors *et al.*, 1984). The frequency of congenital infections was the same as in Japan, although more were symptomatic in Sweden. In the Brazilian series (Pannuti *et al.*, 1985), the seroprevalence of middle- and low-income mothers was, respectively, 67% and 84%. Their children were tested separately for congenital infections. They were less frequent in the middle-income group, but the numbers were too small to attain significance. As in the Japanese series, the Brazilian study may not have had enough infected babies for the determination of the number of symptomatically infected infants. There appear to have been more symptomatic infections in the United Kingdom than in Japan, Sweden, or Brazil. These international series are consistent with the idea that symptomatic congenital infections are less common when the prevalence of immune mothers is high. But since the studies are not strictly comparable, rigorous proof of this statement is lacking.

The Alabama group has provided separate data for congenital infection in

TABLE 11.1
Frequency of Congenital CMV Infection[a]

Author	Place	Type of test	No. positive/ No. tested	Percentage positive	Symptomatic at birth	Patient population studied
Rowe *et al.* (1956)	USA	Viruria	0/108	0.00	0	Unselected newborns
Stern (1968)	USA	Viruria	3/118	2.54	3	Unselected newborns
Levinsohn *et al.* (1969)	USA	Viruria	1/93	1.08	0	Unselected newborns
	USA	Throat	0/98	0.00	0	Unselected newborns
Starr and Gold (1969)	USA	Viruria	8/507	1.58	1	Unselected newborns
Birnbaum *et al.* (1969)	USA	Viruria	5/545	0.55	3	Unselected newborns
Hanshaw (1969)	USA	Viruria	7/685	1.02	3	Unselected newborns
Collaborative Study (1970)	USA	Viruria and throat	13/3182	0.40	0	Unselected newborns
Stern and Tucker (1973)	UK	Viruria	5/1040	0.48	1	Seronegative mothers
Gold and Nankervis II (1976)	USA	Viruria	8/3000	0.27	4	Seronegative mothers
Monif *et al.* (1972)	USA	IgM	22/9100	0.24	0	Seronegative mothers
Leinikki *et al.* (1972)	Finland	Viruria	5/221	2.26	0	Normal newborns
Reynolds *et al.* (1974)	USA	IgM (>19 mg/100 ml)	22/9100	0.24	0	Unselected newborns
Hanshaw *et al.* (1976)	USA	IgM (specific)	53/8644	0.61	2	Unselected newborns
Mason *et al.* (1976)	USA	Viruria	9/953	0.94	0	Primaparous mothers
Granström *et al.* (1977)	Finland	Viruria	3/148	2.03	0	Normal newborns
Kamada *et al.*(1983)	Japan	Viruria	11/2070	0.53	0	Unselected newborns
Ahlfors *et al.* (1984)	Sweden	Viruria	50/10328	0.48	9	Unselected newborns
Pannuti *et al.* (1985)	Brazil	Viruria	7/1026	0.68	0	Middle and low income
Pannuti *et al.* (1985)	Brazil	IgM	5/1744	0.29	0	Middle and low income
Peckham *et al.* (1983)	UK	Throat	42/14789	0.28	17	Unselected newborns
Stagno *et al.* (1982a)	USA	Viruria	20/2330	0.86	0	Seropositive mothers
Stagno *et al.* (1986)	USA	Viruria	37/5199	0.71	8	Seronegative mothers

[a]Congenital infection by viruria: 126/18419 = 0.68%. Congenital infection by IgM: 79/18,408 = 0.43%. Rate of symptomatic infection among those infected: total, 47/247 = 19.0%; viruric, 24/126 = 19%; IgM, 6/79 = 7.6%; throat swab, 42/14,887 = 0.28%.

seronegative and seropositive mothers (Stagno *et al.*, 1977b, 1982a, 1986). Contrary to prior belief, they showed clearly that babies of both seropositive and seronegative mothers could be congenitally infected. As shown in Table 11.1, the frequency of congenital infections was actually higher in "immune" seropositive mothers than in seronegative mothers. However, none of the congenital infections in seropositive mothers was symptomatic. This confirms the previous important finding that symptomatic congenital infections are, practically speaking, only important after primary or *de novo* infections in seronegative mothers.

Stagno *et al.* (1982a, 1986) also provided important data on the relationship between congenital infection and socioeconomic factors. They compared a middle- to upper-income white population with a low-income black population. Significantly more congenital infection occurred among blacks than middle-income whites (1.6% versus 0.6%). Primary infection in seronegative mothers was also higher in the lower socioeconomic group. We still do not know if blacks have more symptomatically infected babies, since the numbers are still too small to detect a statistically significant difference.

The study of Peckham *et al.* (1983) in a number of respects complemented that of Stagno *et al.* (1982a). Forty-two congenitally infected neonates monitored by throat cultures were detected by following 14,789 pregnant women. Seven were offspring of "immune" mothers, including one severely handicapped one. Twenty-eight (67%) of the infected infants were born to mothers who experienced primary infections. In the remaining seven, the type of maternal infection could not be determined. The findings confirm the results from Alabama that immune mothers can bear congenitally infected babies, although one would have expected proportionately a great number of such infections.

A summary of all the studies in Table 11.1 shows that 19% of congenitally infected babies were symptomatic at birth. Where looked at, symptomatic infections were largely a result of primary maternal infections. An unknown number of congenitally infected infants may develop late sequelae (see Section 11.4.3), but it is clear that by far the majority of babies with congenital CMV remain clinically normal. Except for the previous comments about the Alabama studies, it is difficult to say from the data from other parts of the world whether the frequency of congenital infection varies geographically or with socioeconomic conditions. To be certain of any real differences in the frequencies of infections, even larger groups have to be compared, and methods of diagnosis and data collection would have to be standardized. There are essentially no comparable data on differences in *symptomatic* congenital infection. In some countries, one does not even know if the problem exists

11.3.1. Immunoglobulin M Studies in Pregnant Mothers

If most symptomatic congenital infections are caused by primary maternal infection, then detection of such infection becomes important. Serial serologic tests

are cumbersome and time consuming, and required samples are often not available. A single screening test would be highly desirable. The specific IgM antibody test has been studied in this regard. Earlier reports using immunofluorescent (IF) tests left the utility of the IgM test in pregnancy in doubt, but results using radioimmunoassay (RIA) are more promising.

Schmitz *et al.* (1977) looked for IgM antibodies to CMV by IF in 629 pregnant women and found them in about 7%, with equal distribution in the three trimesters. For comparison, only 2.6% of 225 nonpregnant women controls were positive. Fifteen of 195 pregnant women who had two or more serial specimens at 3-month intervals were positive. Twelve represented reactivation or reinfections because they had a prior serum sample positive for CF antibodies. Hence, the presence of IgM by IF did not necessarily denote a primary infection (see Section 6.10.1). Three of the 12 represented primary infection. Unfortunately, no correlative virological studies were done, so we do not know which of these were true infections. Because no independent evidence of infections was given, the sensitivity of the IgM test could not be determined.

Umbilical cord serum from 17 of 57 babies whose mothers had CMV IgM antibodies was tested, and five were positive (29%). This contrasts with one out of 54 neonates whose mothers were IgM negative during pregnancy (1.9%). It is not stated how many of the five represented primary infections in the mother, but at least one came from a mother with reactivation infection. All five neonates were asymptomatic. These data suggest that detection of CMV IgM in the mother is another risk factor for fetal infection, but its precise meaning was not clarified.

DeSilva *et al.* (1977) followed 1065 unmarried pregnant women in Manchester, England and identified 24 with specific IgM antibodies by IF in their sera; of these, 17 were followed to term. As in the report of Schmitz *et al.* (1977), separate objective definitions of primary and secondary CMV infection were inadequate, and the sensitivy and specificity of the test could not be determined.

Griffiths *et al.* (1982) used groups of sera from pregnant women in Alabama with defined infections to evaluate the CMV IgM by radioimmunoassay (RIA) as well as by IF. Specific IgM antibodies by RIA were detected in 55% (16 out of 29) of pregnant women with known primary infection proven by seroconversion. The IgM test by IF was 62% (18 out of 29) sensitive. The poor sensitivity of the IgM test either by RIA or IF resulted from the transient nature of IgM, as most sera within 16 weeks of seroconversion were positive but later sera were frequently not positive. The IgM test by RIA did not yield any false positives. Some seropositive women who transmitted CMV to their offspring or who were shedding CMV from their cervices tested negative. In contrast, as suspected from earlier reports, the IgM test by IF yielded 18% (19 out of 104) false positives from such individuals. The reason for this lack of specificity of the IF test is unclear, but it could not be entirely corrected by excluding the rheumatoid factor. The ELISA test has also been shown to be comparable to the RIA test (Stagno *et al.*, 1985).

In conclusion, it appears that the CMV IgM antibody test is not very sensitive but quite specific to detect primary infection during pregnancy if the proper test

(RIA or ELISA) is used. False negatives (lack of sensitivity) is a problem that cannot be overcome by single serum tests. The most sensitive test is still seroconversion, which requires at least two samples. It would seem desirable to collect sera on all pregnant women as part of routine prenatal care to help diagnose primary CMV infection. The verdict is not yet in on the other IgM tests, such as immunoperoxidase staining, indirect HA, and latex agglutination.

11.3.2. Congenital Infection and Time of Maternal Infection

In most congenital infections the mother is infected *de novo,* and certain periods during pregnancy are more vulnerable than others. The gestational age of the conceptus at the time of infection profoundly affects the frequency as well as morbidity of intrauterine infections. For example, infection during the first trimester by rubella virus is associated with a high probability of infection and birth defects. In the case of intrauterine infection with *Toxoplasma,* the frequency of infection paradoxically increases with gestational age so that it is highest in the third trimester. However, infection in the first trimester still produces the greatest probability of symptomatic damage (Desmonts and Couvreur, 1974).

What is the precise risk of primary CMV infection? Is there a particular time during pregnancy when the outcome of primary infection is more hazardous that at others? The picture is still not as clear in the case of CMV as in other congenital infections. Some data are summarized in Table 11.2. Monif *et al.* (1972) followed 664 pregnant women with a CF titer of less than 1 : 8 at bimonthly intervals. Two women were diagnosed to have primary infections in the second trimester. Both delivered infected infants. Two other women seroconverted during the third trimester. Both mothers produced infants who had elevated IgM in their cord serum and viruria at birth but were otherwise normal. In this study, there was 100% correlation between primary CMV infection in the mother and congenital infection in the baby. Infection was more serious in the second trimester than in the third.

Stern and Tucker (1973) studied 1040 pregnant women in London and found that 56% of the white and 90% of the Asian women were seropositive. Primary infection, detected by the CF test, occurred in eight (3%) of 254 white and three (19%) of the 16 Asian women. About 46% of their offspring (five of 11) had viruria at birth. One mentally retarded child was infected during the second trimester. Gold and Nankervis (1976) identified eight primary infections among more than 3000 pregnant women. Four infected babies were produced. All were viruric but clinically normal.

The largest and most informative study is of the cumulative data from 1978 to 1984 of more than 16,000 pregnancies from Alabama (Stagno *et al.,* 1986). The probability of developing congenital infections among mothers with primary infection was found to be 46–60% in each of three trimesters in 5199 seronegative pregnant women (Table 11.2). Both IgG and IgM antibodies were used to time the occurrence of primary infection. Out of 37 congenitally infected babies there were

TABLE 11.2
Risk of Congenital CMV Infection following Primary Infection of the Mother during Pregnancy

Author	Number of pregnancies	Trimester	Primary CMV infection	Congenital CMV infection		Stigmata	Congenital/ primary
				No.	%		
Monif *et al.* (1972)	664	1	0	0		0	
		2	2	2	100%	2	4/4 (100%)
		3	2	2	100%	0	
Stern and Tucker (1973)	1,040	1	2	2	100%		
		2	4	2	50%	1	5/11 (46%)
		3	5	5	20%	0	
Gold and Nankervis (1976)	≥3,000	1	1	0	0%	0	
		2	4	1	25%	0	4/8 (50%)
		3	3	3	100%	0	
Stagno *et al.* (1986)	14,818 (T)	1	33	17	51%	7	
	5,199 (SN)	2	10	6	60%	1	37/69 (54%)
		3	26	14	46%	0	
Total	19,522		92[b]	50[c]		11/50 (22%)	50/92 (54%)

[a] Primary infections in mothers were detected by antibody rise. All were asymptomatic. Monif *et al.* (1972) detected infection in neonates by measuring IgM against CMV in cord serum. The other authors measured viruria at birth. Stigmata consisted of clinically recognizable abnormalities at birth or during follow-up.
[b] Rate of primary CMV infection in all pregnant mothers was 92/19,522 = 0.47%. Primary infections in seronegative mothers was 59/5199 = 13.3% (Stagno *et al.* 1986). T, total; SN, seronegative mothers.

eight symptomatic babies, and seven were infected during the first trimester. Although the proportion of primary infections resulting in congenital infections was similar in each trimester, the babies who were infected in the second and third trimesters remained mostly asymptomatic. Britt and Vulger (1990) found no deficiencies in antibody responses to specific CMV proteins (see Chapter 3) and suggested that early acquisition of virus during pregnancy is the main factor in severity of congenital infections.

A summary of all reports (Table 11.2) indicates that intrauterine infection following primary CMV infection in the mother is high (50 of 92, 54%). The rate of primary infection of the mother will clearly vary with the immune status of the population, but in this series it was 0.47%. Interestingly, although populations with a low proportion of seronegative individuals are at lesser risk for developing primary infections, individuals in lower socioeconomic groups have a greater probability of developing primary infection (Stagno *et al.*, 1986). One may therefore expect this rate to be fairly constant. The rate of intrauterine CMV infection (50 of 19,552 pregnancies) was 0.26%, which is consistent with data collected by other methods (Table 11.1). Eleven out of 50 (22%) of the infected babies had clinically recognizable disease, whereas it was 19% in data collected in Table 11.1, which do not specify what type of infection the mother had. If we accept the Alabama data that the chance of a primary maternal infection resulting in a congenitally infected baby is 51%, and the chance that such a baby being symptomatically infected is 41% (7/17), then the chance of a maternal primary infection resulting in a symptomatically infected baby the first trimester is 21%. The risk of infection seems significantly lower later, particularly during the third trimester. We are dealing with two populations of congenital infections: one population consists of babies born of immune mothers, and another of babies from mothers with primary infection during pregnancy. Clinically significant disease probably occurs primarily in the latter population. It is not known whether secondary infection, i.e., reactivation or reinfection, at particular times during pregnancy is correlated with congenital infection.

11.3.3. Congenital Infection in Immune Mothers or during Consecutive Pregnancies

Now that we know immune mothers may also give birth to congenitally infected babies (Stagno *et al.*, 1977b, 1986; Peckham *et al.*, 1983), it should not surprise us, as it once did, to discover that congenital infections may occur in consecutive pregnancies.

Embil *et al.* (1970) first described two consecutive children born of an 18-year-old Caucasian. The first had disseminated C.I.D. and died at age 30 days. The second, born within a year, had viruria at birth for $8\frac{1}{2}$ months and viremia at 5 months of age but was otherwise asymptomatic.

Stagno *et al.* (1973) described congenital CMV infection in two siblings born 3 years apart. The older child had viruria at birth, severe growth retardation,

hepatosplenomegaly, and later severe psychomotor retardation. The second infant had viruria at birth but was otherwise normal. A similar set of cases were described by Krech *et al.* (1971c).

A number of other studies suggest that congenital infection can be transmitted by immune mothers. Nankervis *et al.* (1973) found that 12 out of 124 pregnant women who were CMV excreters and, hence, presumably "immune" delivered congenitally infected but asymptomatic babies. Others have found congenitally infected babies in immune mothers in individual cases (Ahlfors *et al.*, 1981a; Chiba *et al.*, 1984) or in population studies (Peckham *et al.*, 1983).

Huang *et al.* (1980a) demonstrated by restriction endonuclease analysis that the CMV isolates carried by a seropositive "immune mother and her congenitally infected baby were identical," showing that transplacental infection can occur in immune mothers.

Although the evidence is that congenital infections arising from immune mothers are usually asymptomatic, there are exceptions. Cytomegalic inclusion disease or symptomatic disease in babies whose mothers were "immune" or seropositive prior to being pregnant has been documented. Ahlfors *et al.* (1981a) report such a neonate with hepatosplenomegaly and petechiae at birth who survived but later was found to have bialateral deafness. Cytomegalovirus was cultured from urine 1 week after birth, and the mother was seropositive 2 years prior to pregnancy. Chiba *et al.* (1984) report a similar symptomatic case with fatal outcome, but the mother's seropositivity was less well documented. Symptomatic cases reported in the series of Peckham *et al.* (1983) have already been mentioned (see Section 11.3).

11.4. CLINICAL MANIFESTATIONS OF CONGENITAL INFECTIONS

The picture of symptomatic congenital infection, cytomegalic inclusion disease, is well known and has been repeatedly described in past reviews (Hanshaw, 1968; Krech *et al.*, 1971a; Weller, 1971). What is new has been a gradual awareness that C.I.D. is but one extreme of a spectrum of manifestations of congenital infections. Many of these are largely asymptomatic, some have only one or a few of the features of C.I.D., and a number will have other symptoms including late sequelae.

With respect to the classical manifestations of C.I.D., the review of Medearis (1957) encompassing the historic literature may serve as an example. He reviewed 132 cases of newborns who had cytomegalic inclusions in organs other than the salivary gland. Forty-two patients (32%) were considered to have the typical neonatal disease; i.e., their disease was evident at birth or shortly thereafter, and they died of the disease. All except one had inclusions in three or more viscera. This mortality obviously does not apply to the experience of more modern centers (see below). They were jaundiced and had hepatosplenomegaly and frequently a hemorrhagic diathesis. Thrombocytopenia was very common. A typical case is a premature infant noted to be icteric within the first day of life. Hepatosplenomegaly is

found by abdominal palpation, and within hours cutaneous petechiae appear. There may be cerebral calcifications. Central nervous system depression and respiratory distress indicative of pulmonary disease progress within hours or days, and the patient dies. Patients who survived longer generally had less organ involvement.

In terms of organ involvement, 25 cases had some form of central nervous system disease. Fourteen had meningoencephalitis, of whom 13 presented the typical neonatal syndrome. Eighty-four (64%) of the 132 patients had pulmonary disease clinically or at necropsy. These cases had no other clinical organ involvement but had inclusions in the salivary gland, lung, or kidney. Pulmonary disease was often associated with other conditions such as pertussis (31 cases).

Pass *et al.* (1980) summarized the Alabama experience with 34 symptomatically infected neonates who were prospectively followed for a mean of 4 years. Petechiae, hepatosplenomegaly, and jaundice were also the most common presentations. Others were microcephaly (50%) prematurity (34%), inguinal hernia (26%), and chorioretinitis (12%). As opposed to asymptomatic congenitally infected babies, those with symptomatic infection were more likely to have specific IgM antibodies (84%). Ten patients died, and eventually two out of 23 survivors had central nervous system or auditory handicaps. Eight (35%) had neuromuscular disorders, and seven (30%) had moderate to severe unilateral or bilateral nerve deafness.

The following is a case of congenital C.I.D. from the files of the Children's Hospital of Pittsburgh:

> D.B. was born of a 16-year-old primiparous black girl after $8\frac{1}{2}$ months of gestation. His birth weight was 4 lb 7 oz. He was jaundiced at birth and spent 5 weeks in the premature nursery with a diagnosis of neonatal hepatitis before discharge. Shortly thereafter, he was readmitted because of nosebleeds. He was febrile and had hepatosplenomegaly. Before death at the age of $2\frac{1}{2}$ months, his blood culture and cerebrospinal fluid grew out *Hemophilus influenza*.
>
> At postmortem examination, there were calcified deposits in the subependymal areas and some gliosis of the ependyma. Cytomegalic inclusions were found in the parotid, pancreas, kidney, liver, and lungs. There was a CMV pneumonitis, hepatitis, and marked extramedullary hematopoiesis. The terminal event was bacterial meningitis.

This appears to be a bona fide case of intrauterine CMV infection that illustrates other characteristic features. Not all the stigmata of C.I.D. need be present at birth, and complicating infections are not unusual.

The clinical course of children who are asymptomatic or mildly symptomatic at birth varies a great deal. Most infected children survive without any known sequelae, even though these are now being detected, including a 15% frequency of hearing loss (see Section 11.4.3). Others may present with chronic gastroenteritis, recurrent papular and petechial rashes, or persistent pneumonitis. Pneumonitis may present later in the neonate with an asymptomatic interlude. It may be difficult to distinguish such cases from pneumonitis caused by perinatal infection. A case of this type is presented below (see Section 11.5).

A number of children with CMV infection will continue to have or appear later

with hepatosplenomegaly and hepatitis. McCracken *et al.* (1969) followed 20 infants with hepatitis, 18 of whom had congenital infection, for periods up to 9 years. Eighteen of the 20 infants had hepatomegaly, and ten had abnormal liver function tests up to 21 months after birth. The result of liver biopsies ranged from normal tissue, extramedullary hematopoiesis, and cholangiitis to portal fibrosis. Serial studies demonstrated reversibility and improvement of hepatitis. Hanshaw *et al.* (1965) found hepatomegaly among 20 institutionalized but asymptomatic viruric children aged 0 to 19. Abnormal liver function was six times more frequent in this group than in controls. Also, among 23 children with unexplained hepatomegaly or chronic liver disease, CMV was isolated from nine. This study suggests that liver disease, at least in institutionalized children, may be caused by CMV. As in the case of pneumonitis, however, unless such children are followed from infancy, we cannot distinguish late manifestations of congenital infection from acquired CMV disease.

In view of the frequent involvement of the liver in CMV infection, the question arises whether it causes irreversible liver disease. Liver disease in adults and in immunosuppressed subjects is discussed in Section 12.4.2 and 13.3.3. In the neonate, at least some of the hepatosplenomegaly may be attributed to extramedullar hematopoiesis and is reversible. More important, McCracken *et al.* (1969) followed 20 infants with CMV infection for up to 9 years, and Berenberg and Nankervis (1970) followed 12 children from 3 to 12 years without finding any evidence of irreversible liver damage.

There are occasional indications of the contrary. Dresler and Linder (1978) described a 4-year-old girl who died of esophageal and variceal bleeding caused by "noncirrhotic portal fibrosis." She was born with a petachial rash, hepatosplenomegaly, and jaundice; on the fourth day of life, cytomegalic cells were detected in her urine, all manifestations of congenital CMV infection. The first episode of hematemesis developed at 16 months of age. At postmortem examination, the liver showed focal subendothelial fibrosis, portal vein radicals, mild portal tract fibrosis, and some accumulation of collagen in the space of Disse, findings consistent with noncirrhotic portal fibrosis. Although the etiology of this condition is unknown (Sama *et al.*, 1971), it is rarely seen in persons less than 20 years of age in North America. This case suggests that occasionally CMV may be a cause of this condition. A similar case was described by Reily *et al.* (1979).

In conclusion, hepatitis is almost always a part of symptomatic congenital or acquired disease, even more so than pneumonitis. It does not usually cause liver failure or irreversible hepatitis.

A less common complication of congenital infection is diabetes mellitus. This has been described in a 13-month-old child (Ward *et al.*, 1979). Whether diabetes can occur as a late complication of inapparent neonatal infection has not been adequately studied (for diabetes in acquired infection, see Sections 10.3 and 12.4.10).

Stagno *et al.* (1982a) studied 118 patients with congenital CMV infection documented by viruria. Twenty-five were symptomatic, and 93 had subclinical

infections. All were examined when they were older than 18 months. Tooth defects occurred in 40% (10/25) of the symptomatically infected and in 5.4% (5/93) of those asymptomatically infected. The defect was characterized by yellow discoloration, opaque or absent enamel, and hypocalcification associated with fractured borders. Rampant dental caries was frequent. Enamel hypoplasia may come from a defect in amelogenesis. Dental care is clearly important not only for symptomatic but also for asymptomatically infected children.

11.4.1. Congenital Infection and the Central Nervous System

The central nervous system (CNS) can be involved in a number of different ways. Extensive and severe involvement is part of classical C.I.D. Subtle long-term effects on the CNS are described below (Section 11.4.3). The severely brain-damaged infant may have periventricular calcifications, microcephaly, seizures, and spastic quadriplegia (Hanshaw, 1966a). Some of the manifestations may be irreversible and persist; these include mental retardation, microcepaly, hydrocephalus, epilepsy, cerebral palsies, and optic atrophy. Weller and Hanshaw (1962) found 14 out of 17 infants with congenital CMV infection to have microcephaly. Pass *et al.* (1980) also find that microcephaly is common in the symptomatically infected. Any evidence of CNS damage at birth is a poor augur for normal mental development. Other manifestations may only become apparent with growth and development. Retardation in mental and motor functions are examples.

In a series of 18 patients with congenital infection, McCracken *et al.* (1969) found some evidence of CNS disease in nine during the first 6 months of life. Seven had microcephaly (see Fig. 11.1). An eighth infant was noted to be microcephalic during the second year of life. Five of the eight had intracranial calcifications, which were periventricular in three and generalized in two. Four were deaf. Fourteen infants were followed up to 9 years. Four were normal at 7 and 8 years of age. Seven showed severe mental and developmental retardation, of whom one died at 9 months and the other at $4\frac{3}{4}$ years of age.

Berenberg and Nankervis (1970) noted chorioretinitis in four out of 12 patients with C.I.D. This manifestation is usually present on initial examination if at all. Out of 12 patients followed, only three developed normal intelligence, all the remaining having mild to severe mental retardation (Fig. 11.2).

Cytomegalic inclusion disease is often associated with microcephaly. Still, most cases of microcephaly in our institutions are of unknown etiology. Other known causes of microcephaly in our institutions are infections by *Toxoplasma* or rubella, irradiation, aminoacidurias, Fanconi's anemia, cortical atrophy, and chromosomal disorders (Baron *et al.*, 1969). Hanshaw (1966b) thought that CMV infection might be a significant cause of those cases of unknown etiology. In a test of this hypothesis, a group of 41 institutionalized microcephalic children 5 months to 8 years of age in Rochester, New York screened for CF antibodies against CMV. Forty-four percent were positive. By comparison, only 4% of a group of nor-

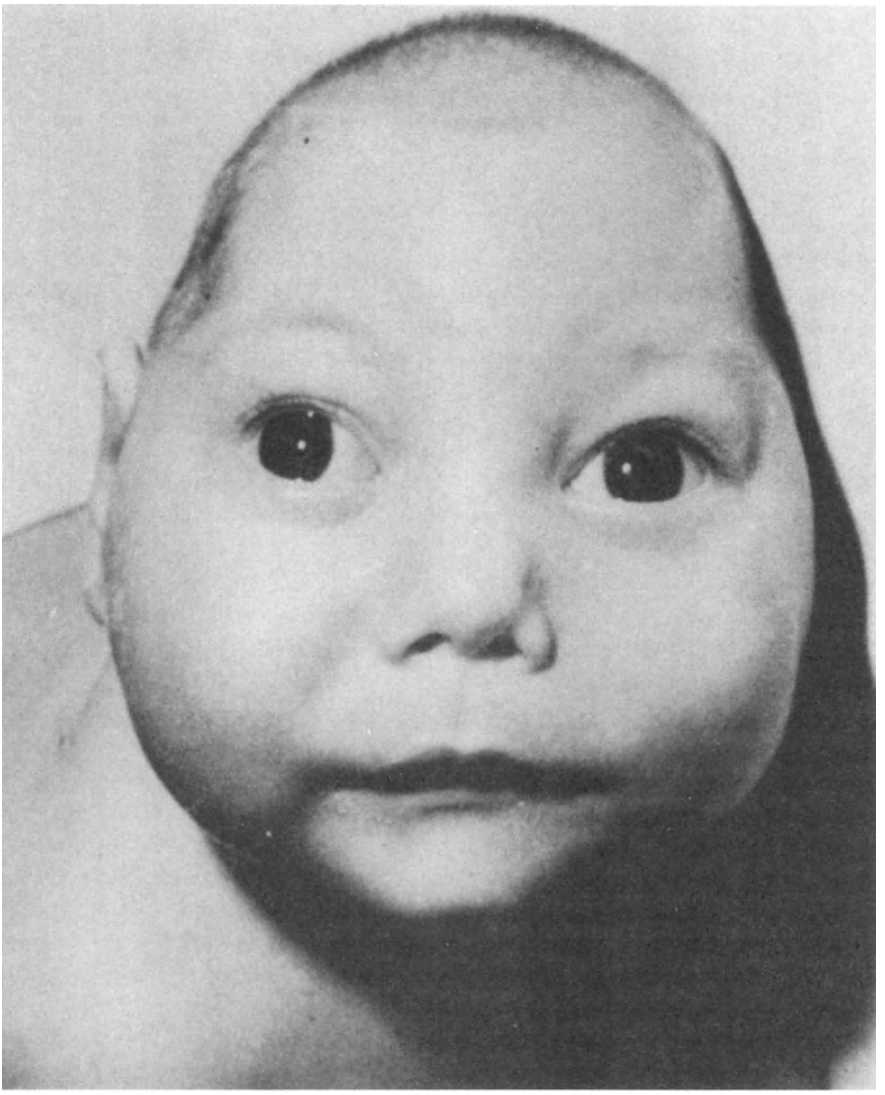

Figure 11.1. A 7½-month-old infant with congenital CMV infection and marked microcephaly. (H. T. Wright in A. S. Kaplan, *Herpesviruses*. Reproduced by permission of Dr. Wright and Academic Press.)

mocephalic control children were seropositive. Other stigmata of possible previous neural invasion by CMV were found in 18 of the seropositive microcephalic children, i.e., periventricular calcifications (four), microphthalmia (two), chorioretinitis (one), spastic diplegia or quadriplegia (one), and prematurity (eight).

Baron *et al.* (1969) conducted a similar study in Pittsburgh but could show no association between CMV infection and microcephaly. Eight of 62 (13%) micro-

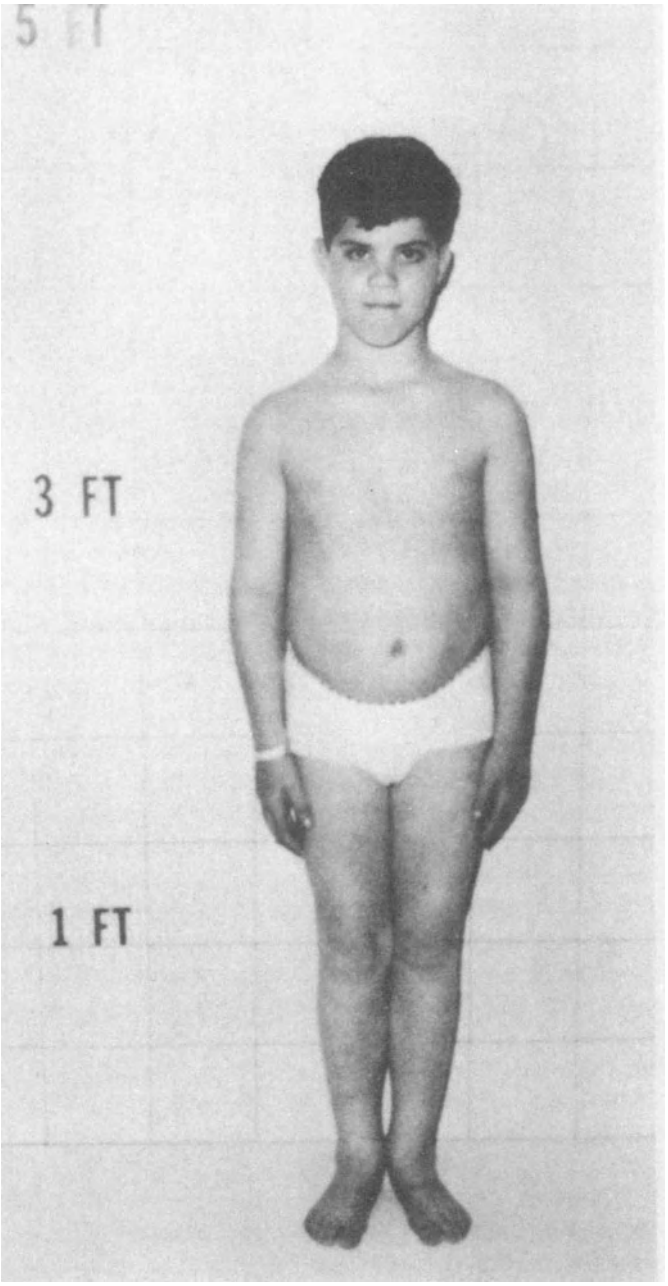

Figure 11.2. A 10-year-old boy with congenital CMV infection and hepatosplenomegaly at birth. He now has congenital heart disease, scoliosis, defective vision, and an abnormal electroencephalogram but is doing well. (H. T. Wright in A. S. Kaplan, *Herpesviruses*. Reproduced by permission of Dr. Wright and Academic Press.)

cephalic children from 5 months to 5 years of age had CF antibodies. In a control group, four out of 26 (15%) mentally retarded normocephalic children were seropositive. No association of microcephaly with rubella, herpes simplex, or *Toxoplasma* was found either.

Ahlfors *et al.* (1986) studied 14,724 newborns in Malmo, Sweden between 1977 and 1983 for congenital CMV infection by isolation of CMV during the first week of life, and they were followed for microcephaly. None of 56 congenitally infected infants had microcephaly defined by head circumference smaller than 3 S.D. below the mean for age and sex. However two infants did have a head circumference of -2 S.D. Out of 10,000 children living in the city, 12 microicephalics were found. In one of them, evidence of primary CMV infection was found in the mother. A probable cause for microcephaly unrelated to CMV was found in ten of the 12 cases.

It is probable that a certain proportion of surviving microencephalic children suffer from congenital CMV infection. What this proportion actually is remains to be determined, and it may vary from region to region. However, it is probably small.

11.4.2. Congenital Infection and Fetal Development

Cytomegalic inclusion disease may affect the development of other organs besides the central nervous system. In general, CMV, unlike rebella virus, is not noted for producing striking congenital abnormalities. Naeye (1967) studied the state of maturity and development of 12 cases of C.I.D. based on the presence of cytomegalic inclusions in three to five organs. The following calculations are based on his data. Gestational age of affected children varied from 20 to 40 weeks with a mean of 36.2 ± 3.4 weeks. The body weight as percentage of expected gestational body weight was $82 \pm 17\%$. Except for two, all babies were below expected weight and were slightly premature. The spleens of affected children were consistently larger than controls but had subnormal numbers of lymphocytes. They usually showed evidence of extensive hematopoiesis and erythrocyte congestion. The thymus was usually subnormal. The weights of brain, liver, kidney, and adrenal glands were often abnormal but bore no consistent relationship to body weight.

Gross malformations were not noted in any organ except the brain. Abnormalities noted in the brain were microcephalus (one), hydrocephalus (two), periventricular calcifications (six), cerebral necrosis and inflammation (six), and ventricular (one) and subarachnoid hemorrhage (one).

The number of cells was subnormal in most organs except in the spleen and liver. In ascending order, cell counts were below controls in the brain, thymus, pancreatic acini and islets, glomeruli, and myocardium.

Rarely, congenital CMV infection produces other congenital malformations and teratogenesis. McCracken *et al.* (1969), in a survey of 20 patients with neonatal CMV, found pulmonary valvular stenosis in one. McAllister *et al.* (1964) described

a case of pulmonary and mitral valve stenosis diagnosed by cineangiogram and cardiac catheterization. Benyesh-Melnick *et al.* (1964), searching for viruses in patients with congenital heart malformations, found a male infant with mitral stenosis and atrial septal defect from whose kidneys CMV was cultured at postmortem.

McCracken *et al.* (1969) noted that five out of 18 cases of C.I.D. had structural abnormalities of the derivatives of the first embryonic arch. One patient had a high arched palate, micrognathia, left facial weakness, and deafness. Late onset of deafness and retarded intelligence is discussed below.

Lang (1966) noted a high incidence of inguinal hernias in males with C.I.D. (11 out of 14). This is also borne out in a more recent series (Pass *et al.*, 1980). This phenomenon could not be explained by prematurity of the infants or hepatosplenomegaly. Bilateral diaphragmatic eventrations have also been noted (Wayne *et al.*, 1973; Becroft, 1979).

Stagno *et al.* (1982b; Reynolds *et al.*, 1986) noted a defect in amelogenesis (enamel) that affects mainly primary dentition. Teeth showed fractured borders and opaque and hypocalcified enamel.

Byrne *et al.* (1987) reported a striking case of cyclopia in a male infant with histological evidence of disseminated CMV infection autopsied 6 days after birth. There was a single orbital opening and a single eye globe with two anterior segments.

In conclusion, infants with C.I.D. may have a number of developmental abnormalities. Usually the virus does not affect critical organ development except in the central nervous system.

The following case of C.I.D. and thymic aplasia is from the files of the Pittsburgh Children's Hospital. It suggests a novel association of CMV with a developmental abnormality.

> A.A.J. was a full-term baby boy born of a primiparous 27-year-old white woman. He was discharged 5 days after birth and was apparently well until around 3 weeks of age, when he developed a dry cough. Upon admission to the hospital, a right middle lobe infiltrate was found. He was found to have in the serum nondetectable levels of IgA, IgM, and an IgG level of 175 mg/dl (normal >300 mg/dl). His downhill 42-day hospital course was later complicated by hypoglycemia and convulsions.
>
> At postmortem, there was an absence of plasma cells in the hypoplastic lymphoid tissues. The thymus was hypoplastic. There were severe chronic pneumonia and inclusions in the liver, spleen, pancreas, adrenal glands, kidneys, and lymph nodes. In addition, there were focal calcifications in the brain and an inclusion in an endothelial cell of the olive of the medulla. Inclusions were also found in the heart and in the paratracheal, hilar, and paraaortic lymph nodes.

Very likely this patient had intrauterine CMV infection in view of the relatively early onset of disease, the widespread nature of the infection, and changes in the brain suggestive of a chronic infection. In addition, he had thymic aplasia with hypogammaglobulinemia. The relationship of this immunologic deficiency to CMV is interesting to speculate on. The two may be unrelated. Or CMV caused the immunologic deficiency by invasion of the thymus and lymphoid tissue. Support for this possibility may be found in murine infections (see Section 16.3). The third

possibility is that the immunologic deficiency conditioned the CMV disease. Is it possible that cell-mediated immunity, defective *in utero*, permitted a generalized disease to develop, whereas it would have otherwise been contained? This may be an important cofactor accounting in some cases for the development of symptomatic CMV disease.

11.4.3. Subtle Sequelae of Congenital Infections

A number of possible late sequelae of congenital infections have been under investigation.

Kumar *et al.* (1973) followed a group of 15 congenitally infected infants who had no stigmata of infection at birth. The intelligence quotient (IQ) of the infected children was 85.2, which was disturbingly low. The IQ of a control group was only 86.5. They concluded that no significant abnormalities could be detected in the infected group.

Reynolds *et al.* (1974) followed 22 cases of asymptomatic congenital CMV infections among 267 neonates with elevated IgM levels ($\geq$ 15 mg/100 ml) discovered in the course of the screening 9100 live births (Table 11.1). Viruria persisted in these patients until the fourth year. Virus shedding from the throat usually ceased after 1–2 years. Some degree of sensorineural hearing loss, mostly unilateral, was detected in nine of 16 tested as opposed to two of 12 controls. General health and development were good in 18 infected children except for one whose head circumference was small. The mean IQ of those infected was 92, compared with 100 in controls. As in the report of Kumar *et al.* (1973), the IQ of infected children was not significantly lower.

In a group of neonates from higher socioeconomic strata, Hanshaw *et al.* (1976) identified 53 with IgM antibodies against CMV in cord blood. One was stillborn, and one had clinical disease and died at 48 hr of age. Forty-four were evaluated at 3.5–7 years of age. The group mean IQ was 103, which was significantly lower than in matched controls (112, $P < 0.025$). The predicted school failure rate, based on IQ, behavioral, neurological, and auditory data, was 2.7 times that of controls matched for socioeconomic status. Saigal *et al.* (1982) followed prospectively 64 congenitally infected Canadian children and matched controls. The Stanford–Binet intelligence test revealed normal scores at 3 and 5 years of age for both CMV-infected and normal subjects. So far there is no conclusive evidence that CMV affects intelligence apart from its other effects on the central nervous system.

Hanshaw *et al.* (1976) also reported that five of 40 (13%) infants with asymptomatic congenital CMV infection had bilateral hearing loss, and three had profound deafness. These data and those of Reynolds *et al.* (1974) suggested that some of the estimated one in 1000 cases of unexplained profound deafness in American children may be caused by congenital CMV infection.

Stagno *et al.* (1977c) and Pass *et al.* (1980) supplied data from Alabama on the incidence of sensorial hearing loss in neonatal CMV infection (see Table 11.3).

TABLE 11.3
Incidence of Auditory Defects in Congenital CMV Infection

	Type of congenital infection			
	Symptomatic		Subclinical	
Auditory defect	*a*	*b*	*a*	*b*
Bilateral				
Profound	3	1	2	2
Moderate	24	0	3	1
Unilateral				
Profound	1	0	2	2
Moderate	2	0	0	1
Defect/ infected[c]	10/31 (32%)	1/4 (25%)	7/51 (14%)	6/37 (16%)
	11/35 (32%)		13/88 (15%)	
Defect/ all infections	22/140 (16%)			

[a]Data from Stagno *et al.* (1977c) and Pass *et al.* (1980).
[b]Data from Saigal *et al.* (1982).
[c]No. patients with hearing defect/No. with symptomatic infections.

Children with three classes of CMV infection were studied. Stagno *et al.* (1977c) and Pass *et al.* (1980) together described 82 patients with congenital infection, 31 of whom were symptomatic at birth. Twenty-one had perinatal CMV infection, none of whom developed hearing loss (not shown in table). Ten of the 31 (32%) infected and symptomatic infants had auditory defects, whereas seven of the 51 (14%) subclinically infected had auditory defects. These were not detected at birth but later. Thus, the two Alabama reports yield a total of ten patients with auditory defects out of 31 with symptomatic infections (32%).

Saigal *et al.* (1982) (Table 11.3) from Hamilton, Ontario provided a prospective controlled study of the magnitude of hearing loss in congenital CMV infection. They identified by neonatal urine culture 64 infected children out of 15,212 unselective births. Four had C.I.D. Matched controls were selected for 46 infected children. Seven (17%) of 41 infected children had varying degrees of sensorineural hearing loss, whereas none of the controls had this type of deafness. Only one of the seven had symptomatic infection at birth; the others were thought to be asymptomatic. As shown in the table, one out of four (25%) symptomatically infected and six out of 37 subclinically infected (16%) had hearing defects. Summarizing the data of the three authors described in Table 11.3, a third of symptomatically infected have neurosensory hearing loss, and 15% of those who are asymptomatic at birth are found, usually some time after birth, to suffer from hearing loss.

At present it is still unclear how often sensorineural deafness is progressive. All cases in a series on symptomatic patients were apparent by 2 years of age (Pass *et al.*, 1980), but Stagno *et al.* (1977c) reported a child who was asymptomatic at birth and developed profound bilateral deafness after 2 years of age. None of the

Canadian patients became impaired as they got older. However, impairment may be difficult to detect early in life, and serial studies using evoked potential audiometry are desirable.

There are only a few pathological studies of the inner ear in congenital infection. Two patients showed evidence of virus in the cells of the organs of Corti and neurons of the spiral ganglia (Stagno *et al.*, 1977c). Rare cells with typical intranuclear inclusions were seen in the vestibular (Reissner's) membrane and stria vascularis of the cochlea (see also Section 10.3). A third patient studied postmortem had disseminated symptomatic disease but no evidence of viral infection in the inner ear. Strauss (1985) did not find any evidence of CMV endolabyrinthitis in 52 pairs of temporal bones of children with congenital CMV infection but in separate functional studies did find evidence of vestibular deficits as well as sensorineural hearing loss.

The hearing problem is insidious in nature; it is difficult to detect and may be progressive. Hearing loss is more progressive in the symptomatically infected, but it may be present in otherwise asymptomatic babies, and estimates so far may be minimal. The pathogenesis of these changes is unclear. Possibly an immune mechanism underlies the development of hearing loss.

In contrast, the pathological condition of the eye corresponded roughly to the severity of infection. It was found in seven of 43 patients with congenital CMV infection. Five of the eight patients (63%) who were symptomatically infected had lesions. None with perinatal infections had pathological conditions of the eye (Stagno *et al.*, 1977c).

11.5. CLINICAL MANIFESTATIONS OF PERINATAL INFECTION

A clearly defined perinatal infection that may be symptomatic or even end in death is CMV infection transmitted to the immature neonate by transfusions. This is discussed in Section 12.2.2, as is CMV infection acquired later in childhood. Here we deal with clinical aspects of perinatal infections discovered by routine screening of urine and/or throat cultures for CMV. We have already discussed the epidemiology of these infections and the possible role of the infected uterine cervix, infected milk and colostrum, and possibly other maternal reservoirs in the transmission of perinatal infection (see Section 9.2).

Kumar *et al.* (1975) described 21 infants of virus-excreting mothers who acquired CMV infection in infancy. Seven were symptomatic and developed pneumonitis, lymphadenopathy, hepatosplenomegaly, and rash. They seemed to develop normally. In contrast, of 96 patients who perinatally acquired CMV infection discovered by postnatal screening and followed by the Alabama group (Whitley *et al.*, 1976), only one developed protracted pneumonitis with lower respiratory obstruction beginning at 1 month of age. After a 5-day hospital course, the child was readmitted at 12 weeks with an exacerbation of respiratory distress. Her mother was a proven cervical carrier before parturition (36 weeks). The baby's urine was nega-

tive at birth, but both urine and throat culture became positive at 4 weeks and remained positive up to 24 weeks. Her CMV IgG EA assay rose from < 8 to 256, which is typical for perinatal infection. A second patient who was not followed before 4 weeks of age had otherwise almost identical clinical and laboratory findings. Cytomegalovirus was also isolated by lung biopsy. He had normal numbers of B and T cells, which responded normally with thymidine uptake following CMV-specific blastogenic stimulation. This is in contrast to congenitally infected children, whose reaction is frequently depressed (see Section 7.3.3).

The following case from the Pittsburgh Children's Hospital illustrates some of the problems of CMV pneumonitis in the newborn (Fig. 11.3). When a baby without any stigmata of congenital infection gets CMV pneumonitis in the newborn period, it is not always possible to determine whether one is dealing with neonatal or perinatal infection.

> M.S. was a full-term baby born of an 18-year-old primipara black woman. He did well until 3 weeks of age, when he developed a persistent cough with the x-ray appearance of interstitial pneumonia. Urine cultures were positive for CMV. He became worse, and at 2 months of age, a lung biopsy of the right middle lobe and right lower lobe was done. It showed marked fibrosis, swollen alveolar cells, and dense infiltrates of lymphocytes and

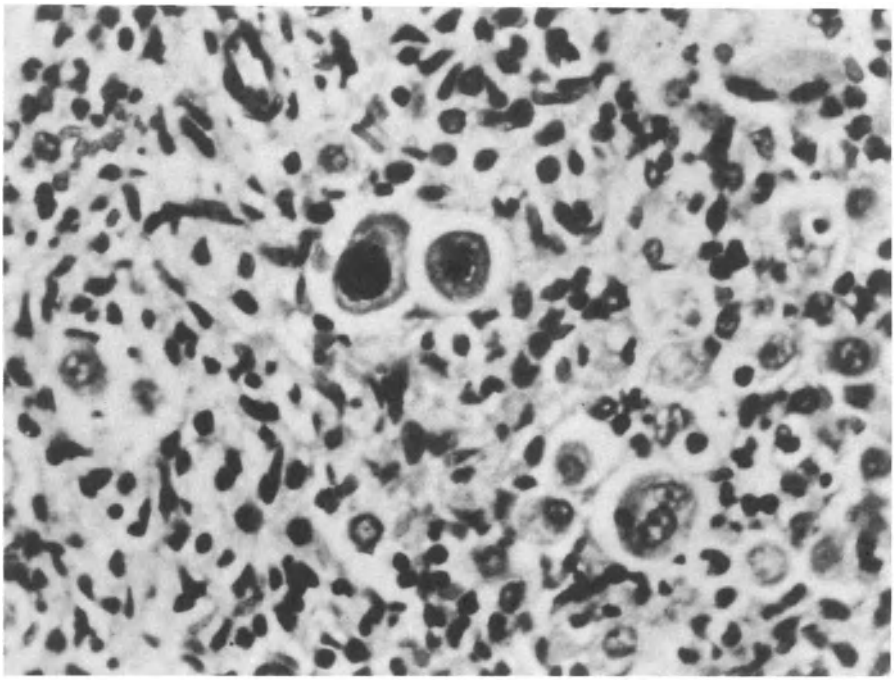

Figure 11.3. Lung biopsy of a 7-week-old boy with respiratory distress, which shows interstitial pneumonia and cytomegalic cells. It was positive by culture for CMV (×433). It is unknown whether patient was infected *in utero* or perinatally. (Courtesy of Dr. E. J. Yunis, Children's Hospital, Pittsburgh.)

plasma cells. Many large cells contained nuclear and cytoplasmic inclusions. Electron micrographs showed mature and immature viral particles consistent with CMV. Viral cultures of the biopsy, urine, and throat were positive for CMV. After a stormy period during which 100% oxygen and artificial respiration were required, the patient gradually improved and was discharged at 5 months of age.

This patient had typical interstitial CMV pneumonitis. In this case, as in many similar ones, no neonatal specimens were taken. The pneumonia may have been a result of perinatal infection, but congenital infection cannot be ruled out.

Two other aspects of the problem of perinatal CMV infection have been looked at. It may produce disease in immature infants, or it may produce other unforseen sequelae later in life.

Brasfield *et al.* (1987) studied the etiology of 205 infants less than 3 months of age who were hospitalized for pneumonitis. Twenty percent (42/203) were infected with CMV. Although this is significantly higher than controls (3%), it is not clear that these cases were caused by perinatal CMV infections.

Paryani *et al.* (1985) compared 55 premature or term infants sick with perinatal CMV infections and 55 controls. They were evaluated at 3 years of age. Infants with onset of CMV excretion during the first 2 years of life had more neuromuscular disability (3/13).

Sawyer *et al.* (1987) studied 32 perinatally infected infants who weighed less than 2000 g and found that 75% (24) developed bronchopulmonary dysplasia, a common chronic complication of sick premature infants, while fewer controls (38%, 12/32) were significantly afflicted.

Using 200 Finnish mother–infant pairs originally used for the study of perinatal infection, Leinikki *et al.* (1978) and Granström and Leinikki (1978) sought illnesses associated with perinatal cytomegalovirus infection during the first 2 years of life. A prospective study was performed on 147 children during their first year of life. Forty-eight infants were infected with CMV perinatally. Data on clinical aspects of infections during the second year were available from a total of 108 infants, and serum samples were assayed for viral antibodies at the age of 24 months from 111. No specific symptoms or signs could be attributed to perinatal infection. None of the manifestations, such as hepatitis, previously described in connection with CMV infection in childhood was observed. Urinary tract infections were, however, noticed more frequently in the group of perinatally infected children (4/48 as opposed to 1/91 in uninfected). Judging from the prevalence of illnesses and the frequency of elevated antibody titers against different agents, perinatal CMV infection did not seem to influence the general morbidity of a child. In general, the frequency and distribution of illnesses were the same as previously reported by Finnish authors. Breast feeding did not protect against infection. The children of CMV-seronegative mothers were not more resistant to other infections, although all of them escaped perinatal CMV infection.

Granström et al. (1980), in another study of 148 Finnish infants, found 33% perinatally infected before 6 months of age. Perinatally infected children were more

often delayed in speech than noninfected children at the age of 2, but results of psychological tests at 5 years of age were not different.

From the above, it is clear that perinatal infection may cause symptomatic infection such as pneumonitis, but it is a fairly rare event, probably less than 1%. One can assume that the normal neonate, unlike the embryo, is quite resistant to CMV disease, as are normal children and adults. The situation may be quite different if the neonate is immature. This is best shown in primary infections acquired after blood transfusions (see Sections 9.7.1 and 12.2.2), which are frequently given to immature infants. Other subtle sequelae of perinatal infection may still be discovered and detected after long-term follow-up.

12

Acquired Cytomegalovirus Infection in Immunocompetent Patients

12.1. INTRODUCTION

Acquired cytomegalovirus infection may be asymptomatic or produce a mononucleosis syndrome. Complications of the latter include pneumonitis, hepatitis, or involvement of the nervous system.

The mononucleosis syndrome was classified clinically by Tidy (1934) into three types. The pharyngeal type is characterized by exudative pharyngitis usually accompanied by lymphadenopathy. The glandular type has lymphadenopathy without pharyngitis or tonsillitis. Fever dominates the picture in the typhoidal type, and severe pharyngitis or enlarged lymph nodes are absent.

Mononucleosis or glandular fever is a clinical and hematologic syndrome with many causes. The heterophile agglutination (Paul–Bunnell) test successfully diagnoses a significant proportion of those cases caused by EB virus. This leaves the problem of heterophile-negative mononucleosis, which may represent a missed EB virus infection or some other viral or nonviral infections. Candidate agents include *Toxoplasma;* rubella, herpes simplex, and adenoviruses (Evans, 1972).

Klemola (1973) summarized the Finnish experience with 494 patients with spontaneous mononucleosis from 1965 to 1972. They had febrile illnesses and relative (> 50% of white cells) or absolute lymphocytosis with > 10% atypical lymphocytes lasting for at least 10 days. Three hundred ninety or 70% were heterophile positive, and 104 (21%) were heterophile negative. Of the latter, 73 patients over 15 years old were studied, and 33 (45%) had CMV mononucleosis. Assuming that the 33 represented all those infected with CMV, then 6.6% of all cases of mononucleosis syndromes were caused by CMV.

A similar picture emerges from a study of 398 patients in Minnesota (Horwitz *et al.*, 1977); 80% were heterophile positive, which is higher than the Finnish study, perhaps a reflection of the fact that the more sensitive heterophile agglutination test using horse red cells was used. Of the 43 patients in the heterophile-negative group, 70% (30) were infected with CMV. Hence, 7.5% of all patients with mononucleosis were infected with CMV.

In both studies, no cases of CMV mononucleosis occurred below the age of 15, although 20% of heterophile-positive mononucleosis cases occurred below that age, which indicates that CMV attacks an older age group.

12.2. CLINICAL ASPECTS OF CYTOMEGALOVIRUS MONONUCLEOSIS

The cardinal features of CMV mononucleosis were clearly described in the first article on the subject (Klemola and Kaariainen, 1965). From February to April, 1965, nine patients with classical EB virus mononucleosis with positive heterophile agglutinins were admitted to the Aurora Hospital. At the same time, five patients with mononucleosis and negative heterophile agglutinins were admitted. They were 23–33 years old except for a 1-year-old female child. Every patient with EB virus mononucleosis had the pharyngeal type of disease, tonsillitis and enlargement of lymph nodes, but none of those with CMV mononucleosis had tonsillitis. Only one had transient lymphadenopathy. Their disease was the typhoidal type. Fever lasted for 2–5 weeks, reaching high levels in three cases. Headache and myalgia were uniform complaints. The general condition of the patients was good. Clinical complications were seen in the one child: central vestibular damage, pneumonia, and arthritis. Atypical lymphocytosis varied from 15% to 30%. All patients had mild hepatitis with moderate elevations of SGOT and positive thymol turbidity but no bilirubinemia. There was a fourfold or greater elevation of cold agglutinins. Later, as more patients accumulated, this clinical description was confirmed (Klemola *et al.*, 1970).

By screening undiagnosed febrile illnesses in an infectious disease department in 1965–1967 in Sweden, Carlström *et al.* (1968) diagnosed 11 cases of CMV mononucleosis by serology. In contrast to the Finnish work based on cases with heterophile-negative mononucleosis, six of the 11 had pharyngitis or tonsillitis, four had marked splenomegaly, and two had bronchopneumonia. Gastroenteritis and abdominal pain were seen in three, and vaginitis in two. Only atypical lymphocytes were found in five. One had a peripheral white cell count of 20,000 with a shift to the left. Klemola (1973) suggests that there may have been another concurrent viral illness to account for the pharyngitis in these cases. Sterner *et al.* (1970) extended the Swedish series with a further report of 17 cases. The most striking finding was high fever ($< 39°$) suggestive of septicemia, which lasted about 3 weeks. In nine out of 14 patients, fever was the sole physical abnormality. Three patients had leukocytosis, two had pharyngitis, three had pneumonia, one had myocarditis, and one

had meningoencephalitis. Only four patients had hepatomegaly, although all had abnormal liver function tests.

Klemola *et al.* (1967) state that in mild cases in which fever is of short duration, the clinical diagnosis may be very difficult because hematologic changes are often not found until relatively late, that is, 1–2 weeks after the beginning of the disease. The mean dates when maximum lymphocytosis and atypical lymphocytosis were reached were 15 ± 3 and 20 ± 13 days after onset of illness according to a study of eight cases by Levin *et al.* (1979).

Atypical lymphocytes are a feature in many virus infections. However, a feature common to both EB virus and CMV mononucleosis is that some atypical lymphocytes may persist for many months or even years after the illness (Klemola *et al.*,1967; Jordan *et al.*, 1973b; Klemola, 1973).

Pneumonitis was found in both series. In the 32 cases of the Finnish series (Klemola *et al.*, 1972), there were three cases of pneumonitis. The Swedish groups (Carlström *et al.*, 1968; Sterner *et al.*, 1970) noted five cases among 28 (frequencies: 9% and 19%). No fatalities were noted, in contrast to CMV pneumonia after transplantation (Section 13.3.2).

12.2.1. Cytomegalovirus Mononucleosis in Infants and Children

Klemola *et al.* (1967) stated that acquired CMV mononucleosis is uncommon in children. Certainly, mononucleosis is not a common finding among the large number of children who become infected in infancy or childhood. On the other hand, as with EB virus mononucleosis, CMV mononucleosis has been recognized in childhood.

Mononucleosis is also not a common finding in congenital or early postnatal CMV infection. It is not a part of the picture of C.I.D. Hanshaw (1964), in describing the involvement of the liver in childhood CMV infection, did not mention mononucleosis. Stern (1968) investigated 11 children aged 5–15 years with heterophile-negative mononucleosis. Cytomegalovirus was isolated from only one 6-year-old boy.

One of the first cases of CMV mononucleosis recognized in a child was a 1-year, 10-month-old female child (Klemola and Kaariainen, 1965). She was different from the four adult cases. Besides febrile mononucleosis, she had arthritis, pneumonitis, and a hepatitis that reappeared 4 months later associated with massive hepatosplenomegaly.

From Hong Kong, Lui and Chang (1972) described a 7½-month-old girl and a 4-month-old girl with 15% and 20% atypical lymphocytes. The first presented with eventually fatal persistent pneumonia, and the second with thrombocytopenic purpura. Both had lymphadenopathy, hepatosplenomegaly, and negative heterophile agglutinins. Cytomegalovirus was isolated from the urine of both cases but not from the mothers. The second case had a rise in CMV CF titer, and the mother was seropositive. Neither child was transfused, but it is impossible to tell if infection had been contracted before, during, or after birth.

From Japan, Nakao *et al.* (1967) reported CMV mononucleosis in a 2-year-old boy, a 1-year-old boy, and a 5-month-old girl. They had 15% to 18% atypical lymphocytes. Cytomegalovirus was isolated from the urine. Pharyngitis was noted in one patient. Umetsu *et al.* (1975) described a 3-week-old male who developed febrile mononucleosis. He had been exchange transfused on the fourth day of life because of hemophilia. At 6 months of age, he presented with icterus, hepatomegaly, hepatic dysfunction, 32% atypical lymphocytes, and 88% lymphocytosis. Heterophile agglutination was negative, but CMV IgM and IgG were both positive. Virus was recovered from the throat and urine. This may have been a case of transfusion mononucleosis.

12.2.2. Posttransfusion Cytomegalovirus Mononucleosis

The epidemiology of CMV mononucleosis transmitted by blood transfusions has already been discussed (Section 9.7). Here we review the clinical picture. In the normal subject, be he adult or child, it is usually indistinguishable from spontaneous mononucleosis.

In 1951, Wyatt *et al.* (1951) reported what probably was the first such recognized case, albeit in an immunosuppressed subject. Disseminated CMV infection occurred in a 20-year-old woman with a 7-year history of primary refractory anemia who had received 135 blood transfusions. At death, inclusions were found in the liver, adrenals, lung, and pancreas. The suggestion was made that the virus may have been transmitted by exogenous blood, although reactivated latent infection was thought more likely.

After extracorporeal cardiopulmonary bypass circulation for open heart surgery was introduced, a series of reports describing febrile illness called the "postperfusion" or "postpump" syndrome appeared (Kreel *et al.*, 1960; Wheeler *et al.*, 1962; Smith, 1964). Fever occurred between 21 and 34 days after surgery (mean 27 days). Splenomegaly, atypical lymphocytosis, lymphadenopathy, and occasional rash were considered part of the syndrome. The resemblance of this syndrome to EB virus mononucleosis was evident, but the heterophile agglutination reaction was usually negative. Some authors postulated an unknown virus infection; others suggested an autoimmune phenomenon or allogeneic reaction of either the graft-versus-host or host-versus-graft variety as a consequence of the large numbers of blood transfusions usually required for such operations (Foster, 1966; Lang, 1972). Kaariainen *et al.* (1966a) first described four cases of posttransfusion mononucleosis who had rises in CF or neutralization antibody titers against CMV and implicated CMV directly in the postperfusion syndrome. In one case, the virus was isolated from urine. The patients became febrile and developed 12–27% atypical lymphocytes 3–4 weeks after surgery. One patient had had a uterine curettage, and two had undergone heart surgery, one with and one without extracorporeal circulation. Fresh blood was used. The term "CMV mononucleosis" was first used in this paper.

The same group (Kaariainen *et al.*, 1966b) diagnosed such a case by viro-

logical isolation of CMV. A 20-year-old female had undergone an open heart operation for aortic stenosis and was perfused with blood from 14 donors. She developed fever 21 days later followed by a rash and 13% atypical lymphocytes. Inclusions were seen in the urinary sediment, and CMV was isolated from the urine. The CF titer against AD169 was still negative at 5 weeks but rose to 1 : 128 40 days after surgery and later reached 1 : 512. Kaariainen *et al.* (1966b) also followed 20 other patients with open heart surgery. Five who were initially seronegative became infected.

Cutforth *et al.* (1968) described a 15-year-old boy with crush injuries of both legs who received refrigerated blood for shock and subsequently was hemodialyzed. The machine was primed with fresh blood. Four weeks after admission, he developed fever, a transient rash, and a systolic murmur with T wave changes and ST depression in lateral chest leads suggestive of myocarditis. Cytomegalovirus inclusions were demonstrated in the urine sediment, and there was a serologic rise, although precise data were not given.

Lerner and Sampliner (1977) described three patients who sustained gunshot wounds and trauma that required multiple transfusions. Two of them developed typical CMV mononucleosis. These reports show that CMV mononucleosis, indistinguishable clinically from the spontaneous disease in normal subjects, is not limited to open heart surgery. It may present as fever or pyrexia of unknown origin. When it occurs in patients who have undergone surgery, it may be confused with surgical infections (such as abdominal abscess) that require drainage.

Symptomatic disease is particularly notable following blood transfusions in premature or compromised neonates. McCracken *et al.* (1969) described two infants, one preterm, who were thought to have symptomatic infection 39 and 58 days after birth, possibly secondary to transfusions. McEnery and Stern (1970) described a premature infant who received intrauterine blood transfusions and died with CMV infection, pneumonia, and hepatosplenomegaly. Yeager (1974) described six infants, three preterm, who probably acquired CMV infection postnatally. One had a febrile illness, two had splenomegaly, and two had hepatosplenomegaly. Pneumonia and thrombocytopenia were also noted.

While studying 51 small, sick preterm infants who had received multiple transfusions in a neonatal intensive care unit, Ballard *et al.* (1979) recognized a self-limited clinical syndrome. Fourteen out of 16 CMV-infected babies had respiratory deterioration, hepatosplenomegaly, a remarkable gray pallor, and both an atypical and absolute lymphocytosis. Most (13) had hepatosplenomegaly, and 12 appeared "septic." Three of the sickest infants died.

Yeager *et al.* (1981) studied 355 neonates who received postnatal transfusions (see Section 9.7.2) Cytomegalovirus infections developed in 10 infants of seronegative mothers and 32 infants of seropositive mothers. None of the latter group developed any disease attributable to CMV, except that nine had hospital stays longer than 28 days. Extensive morbidity was observed in the former group. Two of the ten infected babies born of seronegative mothers had fatal CMV disease. One had pneumonia, and the other widespread viral dissemination. Three had

serious signs of systemic infection such as pneumonia, hepatitis, anemia, and thrombocytopenia, and two died shortly after discharge. Hence, this group had a mortality of 40% and a morbidity of 50%. Very likely, placentally transferred antibody exerted a powerful protective effect against the serious effects of neonatally acquired CMV infection. As discussed above, these data suggest that seronegative neonates should not receive blood from seropositive donors, although more recent data present a less clear-cut picture (see Section 9.7.1).

12.3. LABORATORY DIAGNOSIS OF CYTOMEGALOVIRUS MONONUCLEOSIS

As in most virus diseases and in other types of CMV infection, the quickest laboratory methods for making the diagnosis of CMV mononucleosis are virological. Virus in the urine or at other sites is probably demonstrable at some time during the illness in all such patients. Kaariainen *et al.* (1966b) first diagnosed a case of transfusion mononucleosis by demonstrating viruria. Klemola *et al.* (1967) found viruria in three out of five cases. Jordan *et al.* (1973b) isolated virus from the urine of all nine cases reported. Virus was also isolated from oropharynx, stool, and leukocytes, but frequencies were not given. In the study of four posttransfusion and four community-acquired cases of mononucleosis by Levin *et al.* (1979), blood and urine cultures were taken two to four times from each patient. Both cultures were positive at some time in the course of each patient except one who remained free of viremia.

One possible complicating factor in virological diagnosis is the persistent virus excreter. Virus excretion in the urine, oropharynx, or other sites is uncommon in the normal adult, although it has been described (see Section 9.7.3 on urine carriers among blood donors, 9.5.2 on semen carriers in homosexuals, and 9.4 on cervical carriers). In the series cited above, Klemola *et al.* (1969) found that all three of his patients were still viruric after 2 years. Jordan *et al.* (1973b) followed their patients up to 1 year. Rinaldo *et al.* (1977, 1979a) found that in two of 14 patients, viremia was still demonstrable 49 and 93 days after onset of illness. These data indicate that although virus isolation in the proper clinical setting is presumptive evidence of an acute infection, the possibility of chronic virus excretion as a result of past infection exists. Definitive proof of infection still requires a serologic conversion or rise.

The discovery of CMV mononucleosis was based on the observation of a rise in the CF titer against CMV. To be noted is that CF titers may rise belatedly. ELISA and other tests are better in this regard (Section 6.10). The pattern noted on analysis of 13 patients was as follows (Klemola and Kaariainen, 1965; Klemola *et al.*, 1967). The first serum of five patients taken 8 to 10 days after onset of fever was still negative. When samples were taken from three other patients 6 to 17 days after onset, titers of 1:8 to 1:64 were found. The mean time from onset of fever to the time the highest titer was reached was 58.8 ± 10.8 days. The highest titers reached were 1:64 to 1:256. In two cases no antibodies had developed 14 and 20 days after

onset. In all 13 cases, 6 months to 1 year after illness, the titers had fallen to 1 : 4 and 1 : 64.

Other studies that did not rely on the CF test suggest that the infection may be missed if no other test is performed. Jordan *et al.* (1973b) demonstrated a CF titer rise in only one case out of nine when the first sample was taken 2 to 7 weeks after onset of illness. The IHA test was equally noncontributory. These authors relied on viral cultures. The IFA and ACIF tests for late antigen may become positive earlier than CF tests (see Section 6.2.4).

Schmitz and Haas (1973) also reported no rise in CF titers after 1 week of illness. They found specific IgM antibodies in 12 cases, which they thought was a more reliable test. Peak titers ranging from 1 : 32 to 1 : 1024 were found. Six preillness samples were all IgM negative. The IgM titers declined variably over several months. It was still present in five out of 12 patients over 8 months after onset of illness. No virological cultures were reported.

Out of 30 cases of CMV mononucleosis reported by Horwitz *et al.* (1977), 29 had peak CMV CF antibody titers $\geq$ 1 : 64. Out of six serum samples obtained before the illness, four were antibody negative, one had a titer of 1 : 32, and another 1 : 64. This supports the impression that most cases of CMV mononucleosis are primary infections but that reinfection or reactivation may account for some cases.

By and large, EB virus and CMV infections are distinct entities which are clearly distinguishable serologically except that patients with a positive heterophile agglutinin have positive anti-CMV IgM (see Section 6.10.1). Antibodies against EB virus do not cross with anti-CMV IgG. In fact, it has been noted that the proportion of sera from patients with EB virus mononucleosis positive for CMV CF antibodies is lower than what one would expect for the general population (Carlström *et al.*, 1968; Horwitz *et al.*, 1977). The reason for the crossing of IgM antibodies is not thought to be sharing of antigens by the two viruses but rather that one of the many nonspecific IgM antibodies stimulated during an EBV infection might react with an antigen present on CMV-infected cells. These findings seem to pose a severe restriction on the utility of using the CMV-specific IgM as a diagnostic test without a concurrent test for EB virus infection. It also provides an added reason to do a heterophile agglutinin test on all cases of suspected CMV mononucleosis before specific IgM tests for CMV are done. Other limitations of the IgM test are described in Section 6.10.1.

Horwitz *et al.* (1977) also noted that sera from six cases of CMV mononucleosis contained anti-D or anti-R to the early antigen of EB virus. This does not usually occur in subjects not infected with EB virus and raises the question of whether a latent EBV infection was activated by CMV. That possibility is also raised by a case of CMV mononucleosis who demonstrated a fourfold rise of antibody to EBV capsid antigen and early development of antibody to antinuclear antigen and to R.

In summary, the CF, FA, and ELISA tests for IgG antibody and the IgM tests are all useful, but each has its limitations (see also Section 6.11).

In addition to blood, urine, and respiratory tract, the virus has been recovered from the liver. In two cases, needle biopsies of the liver were performed 10 days and 3 weeks after onset of illness (Klemola *et al.*, 1969). No inclusions were seen, but the biopsy yielded CMV. The patient also had viruria. The serologic responses and responses of cell-mediated immunity in these patients are described in Section 12.2 and Chapter 7.

Klemola *et al.* (1969) studied nonspecific immunologic abnormalities in CMV mononucleosis. All five cases showed by immunoelectrophoresis a transient polyclonal increase in immunoglobulins, especially of IgM. In three cases, transient IgM–IgG–IgA cryoglobulinemia was found (Wager *et al.*, 1968). Cold agglutinins were frequently significantly elevated. In four out of five patients examined, titers from 1 : 32 to 1 : 512 were attained (Klemola *et al.*, 1967). In another study of five patients, two had cold agglutinin titers of 1 : 64 and 1 : 2000. Three had titers $\leq$ 1 : 16 (Klemola *et al.*, 1969). Jordan *et al.* (1973b) reported that three out of nine cases had cold agglutinins (1 : 32 to 1 : 224). Cryoprecipitates were found in two of them. Antinuclear antibodies and rheumatoid factors were absent in four patients tested. These were found in a case of postperfusion mononucleosis (Kantor *et al.*, 1970).

As already discussed, CMV mononucleosis, like many other types of CMV infection, is immunosuppressive against both specific and nonspecific cellular immunity. The response of lymphocytes to concanavalin A and pokeweed antigen is suppressed during the symptomatic phase of the disease. Community-acquired and transfusion mononucleosis are similar in this respect, except that in the latter condition, the mitogenic response to PHA is also suppressed (see Section 7.3.1).

12.4. ORGAN SYSTEMS INVOLVED IN ACQUIRED CYTOMEGALOVIRUS INFECTION

12.4.1. Blood Leukocytes and Lung

The mononucleosis syndrome, characterized by the presence of relative lymphocytosis and atypical lymphocytes, represents a point, perhaps a patch, in the spectrum of morbidity produced by CMV infections. It is frequently but not always present even when the virus symptomatically involves other organ systems. Symptomatic infection of an immature or immunosuppressed host characteristically does not produce the mononucleosis syndrome. As already stated (Section 12.2.1), mononucleosis is not a part of C.I.D., and mononucleosis may be absent or minimal in symptomatic CMV infections of transplant patients. In EB virus mononucleosis, the atypical lymphocytes are thought to represent a T-cell response, presumably a response of an immunocompetent subject (Sheldon *et al.*, 1973). Similar studies have not been reported in CMV mononucleosis. It is possible that when it is absent

in a person who has a symptomatic infection, a normal response may be deficient or absent.

In the following, we discuss various other organ systems that may be involved in CMV infections. Blood leukocytes and pneumonitis have already been discussed (see Section 12.2). The lung is the most common symptomatically involved organ in both normal and compromised subjects who acquire infection. Other organs may become involved in infections of normal subjects. Some types of involvement may rarely be seen in normals and are almost always manifestations of disseminated infections or infection of immunosuppressed subjects. The lines cannot always be clearly drawn.

12.4.2. Hepatitis

Although abnormal liver functions are the rule in CMV mononucleosis, severe hepatitis and gross abnormalities of liver function are unusual.

Lamb and Stern (1966) reported a 31-year-old man who presented with severe jaundice and hepatomegaly. The jaundice subsided in 2 weeks, but fluctuating fever between 100 and 103°F persisted for 3 weeks. Prolonged fever was the first clinical indication that one was not dealing with hepatitis A or B. The patient developed 34% atypical lymphocytosis and a rise of CMV CF titer from 1 : 8 to 1 : 2048 between the 16th and 24th day of illness. Liver biopsy revealed no cytomegaly or virus by culture. Throat and urine cultures were positive. Urine cultures were still positive 3 months after onset of illness.

Liver involvement in neonates and children with CMV infection without apparent mononucleosis was described by Hanshaw *et al.* (1965) (see Section 11.4). It was suggested that chronic liver disease could result, but this has not been subsequently borne out. There is no evidence that any form of chronic hepatitis in adults can be traced to CMV (Toghill *et al.*, 1967; for hepatitis in the immunosuppressed, see Section 13.3.3). There may be a difference between liver involvement in CMV mononucleosis in children and in adults. Whereas hepatitis is minimal and transient in adults, it may be more severe in children. Klemola *et al.* (1969) reported that a 1-year-old female child, one of the original cases of CMV mononucleosis reported (Klemola *et al.*, 1967; Klemola and Kaariainen, 1965), developed massive hepatosplenomegaly and another febrile episode 4 months after apparent recovery from primary CMV mononucleosis. One year later, the liver was still enlarged, but liver functions were normal. Nevertheless, irreversible hepatitis has not been described in either age group.

Oill *et al.* (1977) reported a 35-year-old male who developed migratory polyarthralgia, vesicular pharyngitis, and severe hepatitis. The SGOT and SGPT levels reached 300 and 700, respectively, 10 days after onset of illness. This degree of enzyme elevation is unusual in CMV mononucleosis (Klemola *et al.*, 1967; Jordan *et al.*, 1973b), even in cases whose primary presentation was hepatitis (Reller,

1973). The patient, however, as in other cases of CMV mononucleosis, did not have bilirubinemia, and enzyme levels were normal 6 weeks after onset of illness.

In patients with fever and hepatitis, a liver biopsy yielding granulomata ordinarily suggests tuberculosis or other granulomatous diseases. Cytomegalovirus, however, may also be a cause (Bonkowsky *et al.*, 1975; see Section 10.2).

12.4.3. Hemolytic Anemia and Thrombocytopenia

These are frequent and often severe manifestations in congenital CMV disease, but they may also occur as a complication of CMV mononucleosis in adults. Zuelzer *et al.* (1966) believed that some cases of acquired hemolytic anemia in children may be caused by CMV. They describe an association between episodes of lymphadenitis, presumably caused by CMV, and periods of hemolysis in 22 children with acquired hemolytic anemia aged from birth to 12 years. In 18 of them, the direct antiglobulin tests were positive.

In two adult cases of hepatitis caused by CMV, Toghill *et al.* (1967) observed episodes of hemolysis, one of which was severe enough to require transfusions.

Coombs (1968) described a 45-year-old man with severe acquired Coombs-positive hemolytic anemia who was treated with transfusions, steroids, and splenectomy. The patient died of a febrile illness after the operation. Inclusion bodies were found in the lung and other organs, suggestive of disseminated infections. However, the precise etiologic relationship of the hemolytic anemia to the CMV infection was not demonstrated in this case or in those of Zuelzer *et al.* (1966).

Transient reduction in hematocrit was noted by Kantor *et al.* (1970) in five out of ten patients with transfusion mononucleosis. Three were hemolyzing, two of whom had positive direct Coombs' tests, and one had elevated cold agglutinins.

Fiala *et al.* (1973) presented a convincing association between CMV mononucleosis, and thrombocytopenia. An 18-year-old man had mononucleosis, lymphadenopathy, atypical lymphocytes, and elevated SGOT levels. Persistent viremia, IgM, and high IgG IFA antibodies were found. He had petechiae, confluent purpura over his extremities, and a platelet count of $2000/mm^3$. It responded to prednisone after 46 days. Megakarocytes were not decreased.

Chanarin and Walford (1973) described a 33-year-old man with CMV mononucleosis who presented with generalized purpura and bleeding gingiva. His platelet count was $5000/mm^3$, hemoglobulin was 3.6 g/100 ml, and reticulocyte count was 1.2%. There was serologic evidence of CMV infection and viruria. The patient recovered after treatment with prednisone. Harris *et al.* (1975) described a 26-year-old man with CMV infection who, in addition to thrombocytopenia and purpura, had decreased red cell life-span and elevated reticulocyte count. Thrombocytopenia may also persist after the acute illness and in one case required splenectomy (Sahud and Bachelor, 1978).

These cases together suggest that CMV infection may be associated with transient thrombocytopenia and hemolytic anemia.

12.4.4. Myocarditis

Myocarditis was described in earlier articles on CMV mononucleosis, although it has not been a prominent or serious complication in recent descriptions. Klemola *et al.* (1967) found three cases among eight who developed reversible ECG changes consisting mostly of T-wave inversions. No other manifestation of myocarditis was described. Sterner *et al.* (1970) did repeated ECGs on all of their 17 cases from Sweden, and two were found to have myocarditis. There were typical depressed or inverted T waves in several leads, which reversed without clinical manifestations or treatment. A 60-year-old female patient of Wilson *et al.* (1972) was more symptomatic. Without any previous history of cardiac abnormalities, she developed elevation of jugular venous pressure, fine rales at both lung bases, a gallop rhythm at 130 per minute, and pansystolic and middiastolic murmurs at the apex. The ECG showed sinus tachycardia, left bundle-branch block, and left ventricular hypertrophy. The chest x ray showed cardiomegaly and pulmonary congestion. Her cardiac abnormalities and failure were still evident 5 months after onset of illness. An elevation of CMV CF titer from 1 : 96 to 1 : 384 was noted, although no virus was cultured. The suspicion was that mitral incompetence developed as the result of CMV myocarditis.

12.4.5. The Nervous System in Acquired Cytomegalovirus Infection

The most devastating component of C.I.D. is its destructive effect on the central nervous system. Accessory organs of that system such as the eye and ear are also frequently involved. However, in postnatal life, the affinity of CMV for the nervous system in the immunocompetent subject is much reduced. It is only recently that cases of direct invasion of the central nervous system in the normal subject have been reported. Many cases were circumstantial, but in view of the frequent involvement of CMV in the nervous system of the AIDS patients (Section 13.3.6), they are probably real. The most common instance of central and peripheral nervous system involvement, the Guillain–Barré syndrome, may not represent direct viral invasion but may be an example of immunopathology.

12.4.5.1. Meningoencephalitis and Encephalitis

As in the case of infection with other herpesviruses (herpes simplex type 2, EB virus, and varicella–zoster virus), meningoencephalitis may occur as the presenting picture or as a complication of CMV mononucleosis. Klemola *et al.* (1967) described a case of mild meningoencephalitis in their first series. Cytomegalovirus encephalitis is well known in congenital infections, but it is rare in CMV infection in adults, even in the immunosuppressed host. Dorfman (1973) described a case on the basis of histological examination but provided no virological evidence.

Chin *et al.* (1973) reported a 31-year-old man in previous good health who developed presumed CMV meningoencephalitis, but no virus could be isolated from cerebrospinal fluid (CSF). Another case of meningoencephalitis was described by Perham *et al.* (1971a) on the basis of an increasing titer from < 1 : 8 to 1 : 128. No virus was isolated from urine. Yanagisawa *et al.* (1975) described a patient with possible dual infection with HSV and CMV. The diagnosis was made after death on the basis of large CMV inclusion cells in the brain.

Two cases with convincing laboratory evidence were described by Phillips *et al.* (1977). The first was an afebrile 52-year-old male physician admitted with severe bifrontal headaches, nausea, and lethargy. Cytomegalovirus was isolated from both urine and CSF. The CF titer was 1 : 16. The second case was a 20-year-old woman who had a 4-day history of headaches, confusion, grand mal seizures, and a temperature of 38.5°C. Babinski reflexes were present bilaterally, and there was a questionable right facial weakness. The CSF showed 32 red cells, two polymorphonuclear leukocytes, and 16 lymphocytes per mm^3. Glucose and protein levels were normal. A left temporal lobe biopsy showed microglial and astrocytic proliferation with satellitosis of neurons and focal neuronal degeneration. No inclusions were seen. Cultures taken from the spinal fluid, urine, and brain were all positive for CMV. No CF or neutralizing antibodies were detected. Both patients were treated with ara-A and recovered. The clinical picture of the second case was similar to HSV encephalitis.

None of the five patients with CMV meningoencephalitis described above were immunosuppressed. Taken together, these studies show that CMV infection of the central nervous system may occur in normal adults.

12.4.5.2. Guillain–Barré Syndrome

Cytomegalovirus polyradiculopathy is now a proven complication of AIDS (Section 13.3.6), which makes its occasional occurrence in immunocompetent subjects more credible. Klemola *et al.* (1967) first described a case of Guillain–Barré syndrome with CMV mononucleosis:

> A 27-year-old man had fever for 4 days. Then, motor and sensory deficits started distally and progressed proximally in the extremities. Three weeks later, he had symmetrical paresis of the Vth and VIIth nerves, moderate difficulty swallowing and breathing (involvement of XIth and XIIth nerves), marked paresis of upper and lower extremities, and a level of sensory loss reaching the chest. Recovery began 1 month after onset and was almost complete in 6 months. The CSF protein rose to 166 mg per dl. At the height of his illness, the CF titer was negative, but 6 weeks after onset it was 1 : 64. CMV was isolated from the urine, and cytomegalic cells were seen in the urine sediment.

Ironside and Tobin (1967) described a variant of the Guillain–Barré syndrome that is quite typical:

> A 41-year-old man had a 3-week history of pharyngitis, myalgia, and tingling of hands and feet. This progressed to weakness of the arms and legs, difficulty in speaking and inability to close his eyes. He had complete bilateral facial nerve palsy, weakness in all

limbs, and stocking-and-glove anaesthesia. He had 20% atypical mononuclear cells, and CSF protein was 400 mg per dl. Sensation recovered first, but 6 months later he was still weak. The CF titers were 1 : 128. CMV was isolated from the throat but not urine.

There are also reports of atypical involvement of the peripheral nervous system. Duchowny *et al* (1979) described a 59-year-old man with fever, atypical lymphocytes, and slightly abnormal liver function tests. He developed pain in both shoulders and wasting of the left deltoid, pectoralis major, supraspinatus, and infraspinatus muscles. There was a rise in CF antibodies, and CMV was isolated from the urine. This example of brachial plexus neuropathy resolved over 6 months.

A report of nine cases from England (Leonard and Tobin, 1971) and 19 cases from Germany (Schmitz and Enders, 1977) provide a good clinical and laboratory basis for understanding the Guillain–Barré syndrome in CMV mononucleosis. Most cases began with signs and symptoms of an upper respiratory infection, fever, and progressive peripheral neuropathy. In addition to the neurological presentations of the above cases, headache, papilledema, diplopia, dysarthria, and urinary and fecal incontinence were noted. Involvement of the respiratory muscles was common, and care in the respiratory unit was needed for four of the nine British cases and five of the ten German cases, although assisted ventilation was unnecessary. The British group noted atypical lymphocytes in only four of their cases, whereas it was present in all of the German cases. Mild elevation of the liver enzymes, particularly SGPT, was seen in the latter; most values were less than 100, but one value was 324.

The British observed a CF antibody rise in six of their nine patients. The German series could demonstrate a rise in only two of their ten cases, even though the first specimens were collected within 1 week of onset of illness. All initial specimens were seropositive. The German group emphasized the utility of testing for specific IgM. This was demonstrated in all of their patients, and the initial specimen was positive in nine of them (see Section 6.9.1).

Recovery was usually complete in about 3 months. One of the British cases developed massive pulmonary embolism, and another secondary optic atrophy that did not impair vision. A third patient who died was atypical in many ways. A 21-year-old woman first developed signs of hepatitis 6 weeks before flaccid quadriplegia and required assisted ventilation. She then developed severe pancreatitis and secondary insulin-deficient diabetes before death. In view of the frequent involvement of the pancreas in C.I.D. (see Section 10.2) and the interest in the viral etiology of diabetes mellitus, it would have been of great interest to know if the diabetes was caused by viral invasion of the islet cells (Section 12.4.10).

What proportion of cases of Guillain–Barré syndrome are caused by CMV? There is no generally accepted answer to this question. In more and more cases, antecedent causes are being recognized. It is now clear that this syndrome can be a complication of viral immunization. Immunization with the U.S. inactivated influenza vaccine of 1976–77 was followed by the Guillain–Barré syndrome in about one case out of 100,000 (Schoenberger *et al.*, 1979). These are examples of an immunopathological reaction triggered by recent exposure to an exogenous agent

(Bell and McCormick, 1975). In addition, the syndrome has been suspected to follow acute infections of various types. This is now well established for at least two members of the herpesvirus group, EB virus and CMV. P. Dowling *et al.* (1977) measured CMV, measles, and adenovirus antibodies in sera of 92 cases and 120 controls. Thirty patients (33%) had CF titers against AD169 of 1 : 128 or higher. None of the 34 normal controls and only two of the 86 patients with other neurological diseases had such high titers. In 21 of 26 patients with available sera, a fourfold or more rise or fall in titer could be demonstrated. As in other studies, rises frequently could not be detected because samples could not be obtained early enough. In fact, 18 of the 21 cases had falling titers. Four CMV isolates were made. No significant elevation of antibodies to measles and adenovirus 7 was seen. No patient had a positive "mono-spot" test, and there were no further data on EB virus serology.

12.4.6. The Gastrointestinal Tract

Our understanding of the involvement of the gastrointestinal (GI) tract by CMV infection is still at the pathological–anatomic stage. Again, as in the case of the nervous system, the frequently proven involvement of CMV in the GI tract in transplant and AIDS patients makes it likely that such involvement may also occur in immunocompetent subjects. Cytomegalic inclusions have been identified in many parts of the GI tract, such as in mucosal ulcerations in the esophagus, stomach, duodenum, large and small intestines, and in the anal region (see Diethelm *et al.*, 1976). They are frequently unexpected, incidental pathological findings difficult to relate to disease in the patient. Virological or serologic confirmation of active infection may be lacking, and the nature of the pathological changes may be such that one often cannot ascribe any pathophysiological significance to them. The inclusion-containing cell is frequently not accompanied by significant inflammation or tissue destruction.

Some authors point to the possibility that CMV may cause vasculitis and severe GI hemorrhage. Rosen *et al.* (1973) thought that the virus may have a particular affinity for sites of preexisting ulceration in the intestines or other organs and that the virus itself does not cause such ulceration. Whether it produces other functional disturbance in the GI tract is unknown. Cytomegalovirus inclusions have been described in cases of ulcerative colitis, idiopathic steatorrhea, afferent loop syndrome, and ulcerations of stomach, esophagus, small intestine, and rectum.

In the following, each reported association is illustrated by case reports and comments.

There is now strong evidence that the transmission of CMV is associated with increased homosexual activity, particularly passive anal intercourse (see Section 9.5). With the marked increase of gastrointestinal disease caused by CMV in association with AIDS (see Section 13.3.5), it is possible that passive anal intercourse may be a direct cause of gastrointestinal infection and disease. Surawicz and Myer-

son (1988) noted two cases of biopsy-proven CMV colitis in immunocompetent individuals after passive anal intercourse. Both cases resolved spontaneously.

> One case was a 37-year-old woman who developed rectal pain and hematochezia after the first episode of rectal intercourse. A few days later, rectal biopsy showed cytomegalic inclusions in the lamina propria and submucosa.
>
> The second case was a 25-year-old bisexual male who developed hematochezia, constipation, and fever after passive anal intercourse. Two weeks later rectal biopsy showed a focal area of friable mucosa in the rectum. Typical CMV cells were found in lamina propria and submucosal infiltrate.

These two cases of self-limited CMV colitis in immunocompetent individuals suggest not only that this entity can exist without immunosuppression but also that very possibly the virus responsible for the colitis may be directly inoculated during passive anal intercourse. The virus could, of course, also arrive in the gut by other routes.

12.4.6.1. Gastrointestinal Ulceration and Bleeding

Diethelm *et al.* (1976) described the following case:

> A 45-year-old renal transplant recipient who developed gastric hemorrhage 68 days after transplant. Two weeks later, and after subtotal gastrectomy, the patient succumbed. CMV was isolated during life from the throat but not urine or blood.. At postmortem, chief cells containing inclusions were seen in the vicinity of submucosal gastric ulcers and in mucosal glands. There was remarkably little inflammation associated with these cells. Exfoliated cells were also seen in the lumina of the glands.

Bleeding may have been related to CMV infection, but it may also have been caused by steroids or renal failure.

Cytomegalic inclusion bodies have been found in the stomachs of more than 20 patients, mostly with gastric ulcers. Many of the reported cases have occurred following stress, including trauma, sepsis, hypoxia, and surgery. The inclusions were usually found in granulation tissue at the margin or base of the ulcer. Campbell *et al.* (1977) described a case of biopsy-proven CMV-infected gastric ulcer:

> A 29-year-old male with multiple fractures and lacerations received 9 units of blood. On the 22nd hospital day, the patient developed hematemesis, melena, and hypotension. Atypical lymphocytosis and an abnormal liver function test developed. The CF titer rose from 1 : 8 to 1 : 128 between 40 and 60 days after surgery. A gastric ulcer was revealed by gastroscopy, and a biopsy from the base and margin of the ulcer revealed cytomegalic cells. Cultures for CMV were positive.

This and related cases provide about as much proof as is possible under present circumstances that CMV infection may indeed be associated with gastrointestinal ulcerations. Were specific therapeutic measures against CMV infection available, they would probably have been administered to this patient. Under what circumstances and how frequently ulcerations develop in nonimmunosuppressed subjects require further study. Some form of stress such as surgery or trauma or underlying gastrointestinal pathology seems required. It is striking that gastrointestinal ulcera-

tions have not so far been described in spontaneous CMV mononucleosis without other medical complications.

Goodman and Porter (1973) described this case:

> A 40-year-old man with lymphoma treated by chemotherapy developed thrombocytopenia and had a massive colonic hemorrhage. Typical CMV inclusions were seen in the endothelium of the submucosa, arterioles, capillaries, and venules of the colon. A throat culture grew out Coxsackie B5, and a urine culture was positive for CMV. The CF antibody against CMV was 1 : 128. Specific immunofluorescent studies of tissues showed that the inclusins containing cells were positive for CMV.

This patient's bleeding might have resulted from vasculitis caused by CMV, which is an important pathogenetic process in the GI tract. But thrombocytopenia secondary to chemotherapy may have played a role.

Wolfe and Cherry (1973) described the following case:

> A 67-year-old man with multiple pelvic and tibial fractures following an automobile accident received one unit of blood. Two and one-half weeks and again 1 month after injury, he had severe cecal bleeding for which he received 18 units of blood. A right hemicolectomy was performed, but he died 9 days later. The urine was positive for CMV. The CF titer was negative 1 month after injury. Postmortem examination showed ulcers with inclusions in the esophagus, stomach, and colon, as well as salivary glands and adrenal medulla. Penetrating ulcers extending to the submucosa were seen in the cecum.

The authors consider this a case of cecal hemorrhage caused by CMV infection. It may have been transmitted by the one unit transfused on admission. The incubation period is too short for the infection to have developed from the 18 units transfused later. The negative CF titer suggests that this may have been a case of primary CMV infection with dissemination to the GI tract.

These cases in their aggregate, considered in addition to the syndrome of GI bleeding associated with severe CMV disease in transplant patients (see Section 13.3.5), suggest that CMV infection can indeed cause symptomatic disease in any part of the GI tract.

12.4.6.2. Acute Ulcerative Colitis and Cytomegalovirus

Powell *et al.* (1961) described for the first time a possible case of acute ulcerative colitis caused by CMV:

> An 18-year-old boy developed bloody diarrhea, which was not controlled by ACTH or corticosteroids. After 65 days of hospitalization, a subtotal colectomy was performed, and an ileostomy was established. The specimen showed typical changes of ulcerative colitis, but, in addition, cytomegalic inclusions were a constant feature in endothelial nuclei of dilated, engorged vessels and also among inflammatory cells at the base of the ulcers. No CF antibodies were found in the serum, but the patient became seropositive a year later. Attempts to grow the virus from the specimen were unsuccessful.

The authors thought CMV infection of the endothelium produced the angiitis and ulcerations. The young man apparently did have a primary CMV infection.

> An otherwise normal 41-year-old Japanese woman had an abortion at 6 months of pregnancy and then developed severe diarrhea, fever, and weight loss. Her serum protein was 2.2 g/100 ml. She expired 1 month after onset of symptoms. The jejunum and ileum were edematous and were covered with pseudomembranes and extensive ulcerations. Inclusions were found in the capillary endothelium of such ulcers. They were also found in the alveolar cells of the lung and in the liver (Tajima, 1974).

A similar case of "primary cytomegalic inclusion ulcer of the esophagus and stomach" was seen by the author in a 7-year-old girl. Twelve other cases were also described by the author, but they were secondary to another disease, usually a malignancy.

Tamura (1973) described the following case of acute ulcerative colitis:

> A 65-year-old white man with hypertension and arteriosclerotic heart disease was admitted following injury to his ankle. He developed abdominal pain and severe diarrhea with passage of bloody stools. Sigmoidoscopy and barium enema studies were consistent with idiopathic ulcerative colitis. To control the disease, subtotal colectomy was performed, but the patient died a week later. At postmortem, inclusion bodies of CMV were found in the entire GI tract, in the lung, and rarely in the liver. The esophagus showed a membrane, and small and large intestines were thickened with superficial ulcerations. Some inclusions were found in the entire small bowel, but more were found in the large bowel. They were, however, not as typical as in the lung.

On the basis of the acuteness of the disease, the large number of inclusions, and the presence of ulcers as well as inclusions in the large bowel, it was assumed that this was a case of primary CMV colitis.

Similar cases of presumed primary CMV colitis may be found in Wong and Warner (1962) and Evans and Williams (1968). The cases of Powell *et al.* (1961) and Nakoneczna and Kay (1967) are other possibilities. The latter was a case of disseminated CMV presenting with a gastrointestinal lesion. In conclusion, the case for CMV as a cause of ulcerative colitis seems weaker than for GI bleeding. Ulcerative colitis is a chronic relapsing illness often treated with immunosuppressive agents. Cytomegalovirus might be an opportunist under these conditions.

12.4.7. Conjunctivitis

Eye involvement in the form of chorioretinitis is seen in the congenital form of the disease and as a complication of systemic CMV infection in the immunosuppressed adult (see Section 13.3.4).

Chawla *et al.* (1976) recently found chorioretinitis in a previously healthy adult with CMV mononucleosis associated with keratoconjunctivitis sicca and superficial punctuate keratitis. No viral studies were done.

Weller (1971) suggests that tears may be a way of transmitting CMV. Cytomegalovirus was recovered from serial cultures of tears and conjunctival swabs obtained over a 9- to 15-month period in eight children with acute lymphatic leukemia among a group of 45 children with abnormal immune responses who were

chronic excreters of CMV in the urine or saliva (Cox *et al.*, 1975). None of these children had conjunctivitis.

Garau *et al.* (1977) reported the following case in a previously well person:

> A 31-year-old male had nonpurulent catarrhal conjunctivitis, macular erythematous rash, high fever, and myalgia for 28 days. He had 78% mononuclear cells with 7% atypicals. CMV was recovered from a conjunctival swab on the 12th day of illness. His CF titers rose from less than 1 : 8 on admission to 1 : 128 3 weeks later. No virus was recovered from urine, blood, or saliva. At the onset of the patient's illness, his girl friend had conjunctivitis, but its etiology was not known.

12.4.8. Skin Manifestations

Skin manifestations are relatively common in CMV mononucleosis. Carlström *et al.* (1968) found skin rashes in four out of 11 patients. They were encountered by Klemola (1970) in seven out of 28 patients. Only two out of 23 patients who did not receive ampicillin developed a rash. A macular rash lasted for 5 days in one. In the other case, a maculopapular rash on the upper chest and arms lasted 2 days.

All five patients treated with ampicillin developed a skin rash (Klemola, 1970). It developed between 5 and 10 days after the drug was started, often after it was discontinued. In most cases, it was generalized and maculopapular and lasted for about 1 week. A similar propensity of patients with EB virus mononucleosis who are given ampicillin to develop a rash is well known (Pullen *et al.*, 1967). It has been suggested that the allergen accounting for the rash may result from chemical impurities and not from the ampicillin molecule itself (Shapiro *et al.*, 1969).

Other types of rashes are uncommon in CMV mononucleosis. Müller-Stamou *et al.* (1974) described a 40-year-old male who developed a generalized epidermolysis involving both hands and feet and lasting about a month. The patient also had a rise of CF antibodies, viruria, and cholestatic hepatitis. Skin manifestations in other types of CMV infection are discussed in Section 10.3. Erythema nodosum has been described in a normal adult with clinical and laboratory evidence of CMV mononucleosis (Spear *et al.*, 1988).

12.4.9. Vasculitis

In view of the reports of immunopathological disturbance in neonatal CMV infections in both man (see Section 10.6) and mice (see Section 16.3) and the frequency of vasculitis, particularly with GI involvement (see Section 12.4.6), one might expect to see disseminated vasculitis caused by hCMV. Bamji and Salisbury (1978) described a 57-year-old woman who had fever, subungual splinter hemorrhages, macular rash, congestive failure, and severe myalgia and who was suspected of having bacterial endocarditis. A muscle biopsy showed dense periarteriolar mononuclear infiltrates. She had 39% lymphocytes with atypical cells,

sterile blood cultures, and a diagnostic rise of CMV titers from 1 : 16 to 1 : 256. She responded dramatically to corticosteroids (prednisone).

A type of vasculitis limited to the skin was described in a 7-year-old girl with acquired mononucleosis (Weigand *et al.*, 1980). She presented with papules consistent with "palpable purpura," nodules, and livido on her extremities. Biopsy showed small-vessel vasculitis. To what extent these lesions represented direct viral effects or immunopathology is unknown.

12.4.10. Islets of Langerhans and Diabetes Mellitus

There are numerous suggestions that CMV infection may be associated with insulin-deficient or type 1 diabetes mellitus, but conclusive proof is still lacking. Ward *et al.* (1979) reported a child with congenital CMV infection who became diabetic at the age of 13 months.

Jenson *et al.* (1980) screened 10,000 autopsy cases for viral infections. The pancreases of 250 children with fatal virus infections were examined for lesions in the islets of Langerhans. Viral cytopathology was found in 20 of 45 cases of fatal CMV infections, four of seven cases of Coxsackie B infection, and two cases each of varicella–zoster infection and congenital rubella. In almost all cases with CMV infection, inclusions were seen in islet cells. One case also showed mild insulinitis.

Pak *et al.* (1988) studied the lymphocytes of 59 cases of newly diagnosed type 1 diabetic patients and 38 control subjects. Commercial biotinylated human CMV probes were used to detect infection by *in situ* DNA–DNA hybridization. Cytomegalovirus genomes were found in 22% of the diabetic patients but in only one (2.6%) of the control subjects. Patients who had CMV in their cells had a higher frequency of islet cell antibody (62%) and cytotoxic β-cell surface antibody (69%) than patients who were CMV negative ($P < 0.05$). It is not clear whether the immediate early antigen genes were included in these probes (Sections 5.1 and 9.7.3). These results seem to correlate persistent or latent CMV infection in peripheral leukocytes and the presence of autoantibodies against islet cells. Unfortunately, no routine antibody studies were done to corroborate prior infection by CMV. These studies will clearly need to be confirmed before their important implications are accepted.

13

Human Cytomegalovirus Infections in Immunosuppressed Patients

13.1. CYTOMEGALOVIRUS INFECTION AFTER ORGAN TRANSPLANTATION

Not all virus infections are increased following organ transplantation. The most common are infections by the herpesvirus group, papovavirus (warts and BK virus), hepatitis viruses, and perhaps adenoviruses (Ho, 1977; Rubin and Young, 1988). Symptomatic infections are most commonly caused by herpesviruses and warts (Spencer and Andersen, 1979). Infection with many other common viruses such as rhinoviruses, myxovirus, paramyxoviruses, rubella, enteroviruses, and arboviruses do not appear to be greatly increased, although there are reports of infection with one or another. The exception may be bone marrow transplant recipients, who may develop adenovirus, rotavirus, coxsackievirus, and respiratory synctial virus infections (Townsend *et al.*, 1982; Englund *et al.*, 1988). This may be related to the suppression of humoral as well as cellular immunity in these patients. A virus may be transmitted from the outside and infect *de novo* either a seronegative "nonimmune" subject (primary infection) or a seropositive "immune" subject (reinfection). The virus may also arise from a latent site within a seropositive patient and be activated (reactivation infection). Reinfections and reactivation infections are also called "secondary infections." Primary infections cannot occur if there is no opportunity for exposure to the agent or if preexisting natural or acquired immunity of the patient obviates the risk of exposure. Such immunity may remain operative to some extent despite immunosuppressive measures after transplantation, particularly humoral immunity.

There is increasing evidence, however, that reinfections do occur, not only in

TABLE 13.1
Incidence of Primary and Secondary of CMV Infections after Kidney, Bone Marrow, and Cardiac Transplants

Type of transplant and authors	Total			Primary infection			Secondary infection		
	Inf.	*N*	%	Inf.	*N*	%	Inf.	*N*	%
Kidney transplants									
Craighead *et al.* (1967)	30	41	73%	8	17	47%	22	24	92%
Craighead (1969)	32	53	60%	9	17	53%	23	36	64%
Andersen and Spencer (1969)	33	36	92%	3	5	60%	30	31	97%
Armstrong *et al.* (1971a)	6	6	100%	4	4	100%	2	2	100%
Pien *et al.* (1973)	10	17	59%	2	9	22%	8	8	100%
Spencer (1974)	86	100	86%	19	27	70%	67	73	92%
Fiala *et al.* (1975)	24	26	92%	9	11	82%	15	15	100%
Ho *et al.* (1975)	21	32	66%	13	22	59%	8	10	80%
Betts *et al.* (1975)	31	45	69%	7	21	33%	24	24	100%
Chatterjee *et al.* (1978)	32	35	91%	4	6	67%	28	29	97%
Cappel *et al.* (1978)	22	35	63%	12	22	55%	10	13	77%
May *et al.* (1978)	60	85	71%	15	39	38%	45	46	98%
Whelchel *et al.* (1979)	142	164	87%	21	34	62%	121	130	93%
Ware *et al.* (1979)	106	150	71%	84	102	82%	22	48	46%
Marker *et al.* (1981a)	181	320	57%	59	169	35%	122	151	81%
Ho (1987)	79	131	60%	11	37	30%	68	94	72%
Total kidney transplants	895	1276	70%	280	542	52%	615	734	84%
Cardiac Transplants									
Pollard *et al.* (1978)	32	40	80%	12	20	60%	20	20	100%
Dummer *et al.* (1985)	49	55	89%	16	22	73%	33	33	100%
Total heart transplants	81	95	85%	28	44	64%	53	53	100%
Liver transplants									
Singh *et al.* (1988)	55	93	53%	17	37	46%	38	56	67%
Marrow transplants									
Neiman *et al.* (1977)	32	80	46%	10	44	23%	27	36	75%
Meyers *et al.* (1986)	280	543	52%	139	326	43%	141	217	65%
Wingard *et al.* (1988a)	112	348	32%	15	115	13%	97	232	42%
Total marrow transplants	424	971	44%	164	485	34%	265	485	55%

immunocompetent individuals (see Section 9.8) and in HIV-infected subjects but also after transplantation (Chou, 1986, 1987b; Grundy *et al.*, 1988c). Reactivation infection obviously cannot occur if a virus cannot establish latency or persistence. This is the basic reason why many of the viruses mentioned above do not cause problems after transplantation. Most viruses are eliminated or cured after injection. Alternatively, they may not be readily available in the human or physical environment to infect from the outside. The herpesviruses, and particularly CMV, are in many ways unique. Under conditions of organ transplantation, practically all patients develop CMV infection. Table 13.1 summarizes information on CMV infection after renal, cardiac, liver, and marrow transplantation from sources that dis-

tinguish between primary and secondary types of infection. Operationally, these two types of infections may be recognized if a pretransplant CMV antibody test is available. Primary infection is infection occurring in a subject who had no antibodies before transplantation; secondary infection may be defined as infection in patients previously exposed to CMV as demonstrated by seropositivity before transplantation.

Secondary infections consist of reactivation infections and superinfections in an immune recipient. In the usual series one cannot distinguish between these two possibilities. A number of workers have approached this problem by looking at recipient pairs who have received kidneys from the same seropositive donor. In a number of situations the strains were found to be identical by restriction fragment polymorphism, and hence the virus was assumed to come from the same donor (Chou, 1986; Grundy *et al.*, 1987, 1988c). To further complicate the situation, Chou (1989) showed that at times close examination of restriction enzyme profiles of CMV isolates from corecipients of organs from a common donor shows that two different strains may be shed, presumably from the same donor. The separated strains were also shown on propagation in cell culture to give rise to new, recombinant strains of CMV.

Grundy *et al.* (1988c) report that among seropositive recipients, infection was more common (69%, 18/26) when the kidney came from a seropositive donor than when it came from a seronegative donor (infection in 27%, 4/15). Further, the former group had more symptomatic infections. Their conclusion was that reinfections are more symptomatic than reactivation infection. If true, one should evaluate the importance of preventing infection in all organ recipients, not just seronegative ones. However, other workers do not report more symptomatic disease in seropositive recipients when the donor was seropositive (Smiley *et al.*, 1985) or even a significant increase in infection (Peterson *et al.*, 1983).

In a summary of 16 studies totaling 1276 renal transplant patients, a mean 70% of the entire group became infected with CMV (Table 13.1). All studies were from the United States except ones by Andersen and Spencer (1969) and Spencer (1974) from Denmark and one by Cappel *et al.* (1978) from Belgium. Primary infection occurred in 53% of patients who were susceptible, i.e., nonimmune, before transplantation. In contrast, secondary infection occurred in 84% of the susceptible patients. In ten studies, this rate was 90% to 100%, which suggests strongly that, in view of possible false negatives caused by inability of the laboratory to detect an antibody rise or to isolate CMV, essentially all seropositive individuals activate their latent virus infection after transplantation.

The magnitude as well as the characteristics of infection rates of cardiac and liver transplant recipients were similar to those of the renal transplant group, although differences in morbidity are pointed out below. For reasons that are not entirely clear, the frequency of CMV infection in some series of bone marrow recipients, particularly primary infections, seems somewhat lower (see below). Still, from these statistics, certain deductions may be ventured. (1) Probably all

TABLE 13.2
Risk Factors for Primary and Secondary Infection following Organ Transplantation

Potential source of virus for primary infection
1. Environment: physical and biological environment of patient in hospital, home, or community
2. Persons
3. Blood transfused
4. Organ transplanted
Factors suppressing resistance against reactivation or primary infections
1. Underlying condition of patient (renal or other organ failure, leukemia, aplastic anemia, etc.)
2. Cytotoxic immunosuppressive drugs
3. Corticosteroids
4. Host-versus-graft reaction
5. Graft-versus-host reaction

seropositive individuals harbor latent CMV infection, and serology is an excellent indicator of latency. (2) Most latent CMV infections are activated following transplantation, at least latent infections active enough to produce seropositivity.

We recognize that one cannot assume that the three events, initial infection, antibody development, and latent infection, will always occur together. Antibody levels decrease with time, and in rare individuals, previously infected subjects may become falsely seronegative (Stagno *et al.*, 1975b; Waner *et al.*, 1973; see Section 6.2.3). An unknown but probably small number of seronegative individuals may have been previously infected with CMV, or their antibodies were never stimulated. This possibility will undoubtedly be explored with the availability of newer techniques for detecting virus infection and latency, such as the polymerase chain reaction (see Section 5.3.1).

The primary infection rate, infection among those who were seronegative before transplantation, is usually lower than the rate for reactivation infection in all three forms of transplantation. As expected, this rate varies much more from series to series and may vary with the type of transplant. One obvious reason is different in exposure to a source of CMV.

Potential sources of virus transmission and factors that might depress host resistance are listed in Table 13.2. The latter operate in both primary and secondary types of infections. They range from the underlying pathological process that led to transplantation to measures to preserve the graft. Allograft reactions, whether of the host-versus-graft or graft-versus-host types, are unique to the transplantation process. An attempt is made to evaluate each of these factors using data from the literature.

13.1.1. Sources of Virus in Infection Including Blood

The most common cause for transmission of CMV is man himself. Just how it is transmitted in the well community among children or susceptible adults has been

discussed (see Sections 9.2–9.6). Close personal contact seems essential, and there may be more than one mode of transmission. Contaminated milk, saliva, and urine and sexual intercourse may all play a role.

There is no evidence that any of these means of transmission account for primary infection in the transplant patient, although instances of transmission by close personal contact may be expected to occur from time to time. Two unique potential sources for the virus stand out in transplantation: blood used for transfusions and the donated organ or tissue itself.

The requirement for blood transfusions and their potential risks in practically all transplant operations have already been described. Henle *et al.* (1970) estimated that the risk was proportional to the number of units transfused, which was computed to be 5% to 12% per unit. Prince *et al.* (1971) followed 156 patients who received 1269 units of blood and found 31 who seroconverted. This represents 2.4 infections per 100 units of 2.4% per unit. Our own study of 93 seronegative children undergoing open heart surgery produced a risk of 2.48% per unit (Armstrong *et al.*, 1976). A relatively low risk of infection from blood transfusion (less than 3% per unit) was generally found by six groups of investigators that formed an NIH contract group to study the risk of CMV in transfused blood from 1971 to 1975 (Evans, 1976). More recent data suggest even lower risk of transmission of CMV by blood (see Section 9.7).

In renal transplantation, when the amount of blood transfused is low (3.7 units per patient in our study), one cannot usually demonstrate a correlation between blood transfusion and acquisition of CMV infection (Ho *et al.*, 1976). However, if the number of units increases, the situation may be quite different. Lang *et al.* (1977) studied patients who received an average of 19 units of blood during extracorporeal perfusion. Out of six who were initially seronegative, four seroconverted (67%), and one of four seropositives showed a significant rise in titer. They went on to show that if the blood were depleted of white cells by 60% in a similar series of ten patients, only one showed a significant serologic rise.

That viable white cells may be an essential vehicle of transmission of CMV from blood is also shown by studies by Tolkoff-Rubin *et al.* (1978). None of 21 seronegative patients seroconverted after receiving a total of 157 units of frozen blood (see Section 9.7.3).

Recently we have been unable to document any relationship between CMV infection and blood transfusions in heart, heart–lung, or liver transplantation, where significantly more blood is used (Breinig *et al.*, 1987; Dummer *et al.*, 1985; Singh *et al.*, 1988).

It is possible that the impact of transfused blood on primary infection may be lost if the preponderant source of virus is the seropositive organ donor. The most discriminating study would be one in which primary infections in seronegative recipients were measured in groups that received only seronegative transplants and either seronegative or seropositive blood products. Very few studies of this type have been done. One example was reported by Bowden *et al.* (1986) in seronegative recipients who received only marrow transplants from seronegative donors. Only

one out of 22 (4.6%) recipients who received seronegative blood products became infected, whereas eight out of 25 (32%) who received seropositive blood products became infected ($P < 0.007$). On the other hand, CMV infection in seronegative recipients who received marrow from seropositive donors was not significantly influenced by whether or not they received seronegative or seropositive blood products (25% versus 31%). This study shows clearly that seropositive blood contributed to primary CMV infections at least in marrow recipients. Since each recipient received a mean of 20 units of red blood cells, the risk per unit was 2.3%. This is consistent with the risk calculated from other studies (see Section 9.7).

There is strong epidemiologic evidence that the donated kidney is the source of primary CMV infection. Although suspected for a long time (Hedley-Whyte and Craighead, 1965; Lopez *et al.*, 1974a), the graft was not incriminated earlier because sporadic attempts to isolate CMV from the grafted kidney were unrewarding (Lopez *et al.*, 1974a). Even today, after wide acceptance of the epidemiologic data, search for the virus in the grafted organ has largely been futile. Naraqi *et al.* (1978) studied 130 kidney biopsy samples from live and cadaver donors and from kidney allografts in recipients by cocultivation of intact or homogenized tissue on susceptible human fibroblasts. No virus was isolated from any of the explanted tissues. Cytomegalovirus was isolated from homogenized samples of six out of 57 allograft biopsies, but all positive samples came from viruric recipients. We have cocultivated in larger bottles more than half of the renal cortex in minced fragments. No virus was found in kidneys obtained at autopsy from seropositive individuals (M. P. Meyer, J. A. Armstrong, and M. Ho, unpublished data).

However, another form of independent, albeit still indirect, evidence suggest strongly that donated organs are frequently the source of CMV infection. There are reports of seronegative recipients of hearts or kidneys from the same donor who became infected by identical strains of CMV as determined by restriction fragment polymorphism (Chou, 1986). Transmission of herpes simplex virus by donor organs has also been documented by this method (Dummer *et al.*, 1987), as has transmission of other viruses (HIV, HBV, NANB) and *Toxoplasma* by other methods (Ho and Dummer, 1990), but none of these agents are transmitted by organs with the high frequency of CMV.

The epidemiologic evidence that the kidney is the source of infection first came from prospective studies by Ho *et al.* (1975) and Betts *et al.* (1975) (Table 13.3). In the study by Ho *et al.* (1975), the seronegative recipient group that received kidneys from seropositive donors had an infection rate of 83%, whereas those who received kidneys from seronegative donors had a rate of only 30%. Betts *et al.* (1975) noted a 78% primary infection rate and no CMV infection when seronegative individuals received kidneys from seronegative donors. Tobin *et al.* (1979) provided similar data from the United Kingdom. Confirming data were also provided by Marker *et al.* (1981a) from Minnesota. Of note is the extremely low rate of infection in seronegative recipients of kidneys from seronegative donors in the four studies (8%). This is indirect evidence for the relative infrequency of transmission of CMV by other routes, including transfusions. That the kidney may occasionally transmit

TABLE 13.3
The Kidney Transplant as a Source of Primary CMV Infection
(Infection in Seronegative Recipients)

Donor serology	Number and percent infected in series of				
	Ho *et al.* (1975)	Betts *et al.* (1975)	Tobin *et al.* (1979)	Marker *et al.* (1981a)	Total
Positive	10/12 (83%)	7/9 (78%)	22/33 (67%)	11/33 (33%)	50/87 (57%)
Negative	3/10 (30%)	0/12 (0%)	0/36 (0%)	3/20 (15%)	6/78 (8%)

the virus is not surprising, but the relatively high rate of primary infection in a susceptible recipient who receives the kidneys of a normal seropositive individual also suggests that he is latently infected with CMV and the organs are infected.

The situation is similar in other solid organ transplantation. Pollard *et al.* (1982) showed that six out of seven seronegative patients who received hearts from seropositive donors developed primary CMV infection. More recently the situation was dramatically illustrated in a study of 121 adult liver transplant recipients in Pittsburgh. In donor–recipient pairs whose serologic data were complete, 13 (92%) of 14 seronegative recipients who received livers from CMV-seropositive donors had evidence of CMV infection after transplantation, whereas only one (8%) of 12 seronegative recipients who received livers from seronegative donors developed CMV infection (Singh *et al.*, 1988). Similar data were reported from other centers (Fox *et al.*, 1988).

The situation is not as clear-cut in the case of marrow transplantation. Meyers *et al.* (1980c) earlier provided an interesting breakdown of CMV infections in 158 marrow transplant recipients. The rate of primary infection in seronegative recipients who received marrow from seropositive (57%) or seronegative donors (53%) was similar. However, in a more recent review of 543 marrow recipients, Meyers *et al.* (1986) presented statistics more in line with other types of organ transplantation (Table 13.4). Among seronegative recipients, there was significantly more CMV infection when the donor was seropositive (44/77, 57%) than when the donor was seronegative (58/208, 28%). A similar result was reported from the United King-

TABLE 13.4
The Relationship of Transplanted Marrow to the Development of Primary CMV Infection
in Seronegative Recipients

Donor serology	Number and percentage infected in series of				
	Meyers *et al.* (1980c)	Meyers *et al.* (1986)	Paulin *et al.* (1986)	Miller *et al.* (1986)	Apperely and Goldman (1988)
Positive	12/21 (57%)	44/77 (57%)	1/3 (25%)	6/30 (20%)	4/11 (36%)
Negative	31/58 (53%)	58/208 (28%)	3/14 (21%)	20/107 (19%)	0/17 (0%)

dom (Appereley and Goldman, 1988). Still, many other groups continue to report an absence of an enhancing effect of the donor serostatus on CMV infections or interstitial pneumonia (Paulin *et al.*, 1986; Wingard *et al.*, 1988b; Winston *et al.*, 1990).

Almost all studies point to the importance of seropositivity of the recipient, i.e., the endogenous latency of CMV, as a significant risk factor (Table 13.1). The reason for the apparent lack of more transmission of virus from marrow donor to recipient is obscure, particularly in view of the overwhelming evidence that CMV latently infects leukocytes (see Section 9.7.3). One possibility is that the latently infected donor cell is prevented from reactivating in the presence of an newly engrafted donor immune system, which is essentially the donor's. Indeed, there is some evidence that the seropositive donor imparts protection against CMV infection. Grob *et al.* (1987) report that the incidence, severity, and outcome of CMV pneumonia were significantly worse in seropositive recipients who received marrows from seronegative as opposed to seropositive donors. This acquired immunity against CMV may be transferred to the recipient, and this immunity may be particularly effective against the donor's own infected cells.

There is also evidence that some seropositive donors are better able to transmit the virus that others. Chou and Norman (1988) conducted a 3-year prospective study involving 140 seronegative kidney and heart recipients. Forty of 68 (58%) recipients of a CMV-positive organ developed primary infection, and 42% did not. Four pairs of recipients, each pair of which received organs from the same donor, developed CMV infection, whereas five other pairs of recipients who also received organs from the same donor developed no infection. These observations suggest that seropositive donors may carry latent or abortively infected cells, which may or may not be activable. Toorkey and Carrigan (1989) have shown that normal tissue, such as kidneys, may carry abortively infected cells in which IEA could be demonstrated. It is unknown whether the number of such cells or the nature of the infection determines whether or not CMV will be transmitted.

13.1.2. Morbidity of Cytomegalovirus Infection after Transplantation

There is a remarkably constant incubation period after transplantation when most CMV infection occurs. This is best demonstrated in the renal transplant group but is about the same for other types of transplantation. The evidence suggests that the virus is transmitted or reactivated during or shortly after the transplant operation.

Primary and secondary infections occur about 2 months after transplantation with a range of 1–4 months (Ho *et al.*, 1975; Rubin *et al.*, 1977). The incubation period for primary infections is somewhat shorter (Andersen and Spencer, 1969; Ho *et al.*, 1975). In our series, the mean incubation period for primary infection was 2.0 months, and it was 3.5 months for secondary infection. It should be pointed out that this was based on conversion of CF tests. As already stated, CF antibody response is

TABLE 13.5
Severity of Primary and Reactivation CMV Infections after Organ Transplantations

Type transplant, authors	Primary infections			Reactivation infections		
	No.	Symp.[a]	%	No.	Symp.	%
Kidney transplants						
Suwansirikul *et al.* (1977)	18	14	78	10	2	20
Betts *et al.* (1977)	16	14	88	32	6	19
Whelchel *et al.* (1979)	21	17	81	121	37	31
Marker *et al.* (1981a)	21	18	86	105	72	69
Total kidney transplants	76	63	83	268	117	44
Heart transplants						
Pollard *et al.* (1978)	10	9	90	17	5	29

[a]Symp., symptomatic (at least fever was recorded in association with CMV infection).

slower than FA or ELISA antibody response (see Section 6.2.4). Perhaps primary infection occurs sooner because virus is transmitted at surgery (see below), whereas in reactivation infection, a period of immunosuppression following surgery must precede reactivation of endogenous virus. Based on the development of symptoms, Peterson *et al.* (1980) found that median day of onset of CMV disease in 59 patients was 46 days after transplantation. Six patients became sick 2–3 weeks after transplantation, and 90% had their onsets within 4 months.

Evidence is strong that primary and reactivation CMV infection differ in their clinical implications. Primary infections are more often clinically apparent and cause more disease, an observation made by our group (Ho *et al.*, 1975; Suwansirikul *et al.*, 1977) and Betts *et al.* (1977) and confirmed by others in renal and cardiac transplant groups.

Table 13.5 summarizes the relevant data from four studies on the morbidity of CMV infection after transplantation. The summary shows that after renal transplantation, most (83%) primary CMV infections were symptomatic. The minimum manifestation was fever. On the other hand, the majority (56%) of secondary infections were asymptomatic. A similar pattern emerged when patients who had received cardiac transplant were followed (Pollard *et al.*, 1978).

Marker *et al.* (1981a), however, do not show any significant differences in the morbidity of these two groups of patients, although there was more fever in the primarily infected. The lack of greater difference probably resulted from the unusually high frequency of disease manifestations in patients undergoing a secondary infection (69%). Their patients received antilymphocyte globulin in addition to azathioprine and steroids, and it is possible they were more immunosuppressed than the comparable patients in the other studies.

Weir *et al.* (1987) reported from Baltimore on a series of 167 renal transplant patients who received cyclosporine and prednisone immunosuppression. There were 15 cases of symptomatic CMV disease, and 12 of these or 80% were primary infections that occurred among the 20 seronegative recipients who received organs

from seropositive donors. Only three cases of CMV disease caused by secondary infections occurred among 142 seropositive recipients.

Smiley *et al.* (1985) studied a series of 153 patients from Philadelphia who were on azathioprine–prednisone immunosuppression. Here too, 61% of primary infections developed CMV disease. This group had the highest mean point score of disease severity and also the greatest number of major complications (26%). Only one patient developed CMV disease among 49 seronegative recipients who received kidneys from seronegative donors.

Despite the use of similar cyclosporine–prednisone immunosuppressive regimens in organ transplantation (see below), the frequency of CMV disease may vary greatly depending on the type of transplant (Dummer *et al.*, 1983). Patients undergoing cardiac transplantation had more CMV disease than those who had kidney transplants. In this early mode, antilymphocyte serum was not used in routine immunosuppression after any of our organ transplantations, although it was occasionally used for rejection.

A summary of our experience with CMV infections and morbidity is presented in Table 13.6. It shows that the frequency of infection varied between 59% and 92% in the different transplant groups. Whether this is a true difference or a reflection of differences in the serologic status of donors and recipients and other variables is not known.

What the table does show is the striking incidence of CMV pneumonia in heart–lung recipients (32%). In contrast, out of 131 renal transplant patients, only 10 (8%) had symptomatic CMV infection. The other three transplant groups had significantly more symptomatic CMV disease. Whether there is a significant difference in CMV morbidity among liver, heart, and heart–lung recipients is not clear. Thirty-two percent of the heart–lung recipients had CMV pneumonia. This frequency approximates the symptomatic infection rate and suggests that the lung is particularly vulnerable after heart–lung transplantation. The question arises as to

TABLE 13.6
Infection and Morbidity of CMV Infection in Different Transplant Groups in Pittsburgh after 1981[a]

Type of transplant	Total no. of patients	Number of patients with infections (% total)		Number of symptomatic patients		Number of patients with CMV pneumonia	
				Infected	Total	Infected	Total
Kidney	131	79/131	(60%)	13%	8%	4%	2%
Liver	93	55/93	(59%)	49%	29%	5%	3%
Heart	48	44/48	(92%)	27%	25%	9%	8%
Heart-Lung	31	22/31	(71%)	55%	39%	45%	32%

[a]Data for this table were published by Ho (1987) and Dummer *et al.* (1985, 1986).

whether an allograft undergoing various degrees of allograft rejection is particularly vulnerable to CMV infection.

The situation is comparable to marrow transplantation, where CMV pneumonia has been an unusually difficult problem. Meyers *et al.* (1986) report an incidence of 16.7% in 545 marrow transplant recipients. In bone marrow transplantation the lung is a target organ for graft-versus-host reaction. Graft versus host reactions have consistently been found to be a significant risk factor (see Section 13.3).

Similar results were reported by Wreghitt *et al.* (1988) from Cambridge, England. They investigated 166 heart and 15 heart and lung recipients by serologic methods. Altogether only 45% developed CMV infection, of which 18.5% were primary infections. There were six deaths caused by CMV, all of whom were primarily infected. Cytomegalovirus disease was more severe in heart–lung recipients. Three out of four patients with primary infections in the heart–lung group died, whereas only three out of 16 primary infections in heart recipients died. All deaths in the heart–lung group were from CMV pneumonia.

13.1.3. Role of Immunosuppressive Drugs

Before 1966, when corticosteroids were routinely used after renal transplantation but cytotoxic drugs were not, CMV infections were not observed. It is generally assumed that these became evident only after cytotoxic agents such as azathioprine, cyclophosphamide, methotrexate, and antilymphocyte and antithymocyte serum became available (Kanich and Craighead, 1966; Ho, 1977). However, how the single or combined effects of many other factors might contribute to the risk of infection in the complex transplant patient was unclear (Ho, 1977).

To help clarify the problem, our group made use of patients with connective tissue diseases who were being treated with corticosteroids or cyclophosphamide (Dowling *et al.*, 1976). Studying them permitted us to evaluate the effect of steroids and cytotoxic drugs alone, apart from the complicating factors of a transplant operation, blood transfusions, or allograft reactions. No virus was isolated from the buffy coat or urine of 86 patients who were on cortocosteroids alone. This confirms the impression that steroids alone, at least in the doses given to such patients, do not play a large role in increasing susceptibility to CMV infection. On the other hand, a random urine sample from three out of 15 patients on cyclophosphamide yielded positive cultures for CMV. In a prospective study, 14 patients were followed from the time of initiation of cyclophosphamide therapy. Five of nine who were seropositive developed viruria (56%) compared with only one of five seronegative patients (20%). Interestingly, as in the case of renal transplantation, infection occurred between 6 and 12 weeks after drugs were begun. The rate of infection was expectedly lower than in the renal transplant group, since the rheumatology patients were not exposed to a number of other risk factors peculiar to the transplant recipient.

Between 1966 and 1981 azathoprine and prednisone were the standard immunosuppressive agents used after transplantation. The frequency of infection by CMV during that period is shown in Table 13.1. In most series, the rate of secondary or reactivation infection was close to 100%. As mentioned, the degree of immunosuppression produced by this combination was sufficient to activate almost all latent infection.

The amount of morbidity associated with CMV infection was, however, quite variable, and the type of immunosuppression is an important determinant. For example, in 1978, the Minnesota group reported that in a group of 141 consecutive transplant patients, the incidence of overt CMV disease was 59 (31%) (Peterson *et al.*, 1980). Twenty-five of the 59 symptomatic patients (42%) or 13% of the total had evidence of pulmonary infiltrates. In 1983, Peterson *et al.* (1983) reported from the same institution that from 1980 to 1982, out of 273 renal transplant patients, only 24 (8.8%) developed CMV pneumonia. One difference between the two periods was that during the later period, antilymphocyte serum (ALS) was not used.

That antilymphocyte or antithymocyte globulin in transplant recipients adds an extra dimension of immunosuppression that is conducive to infections has been suggested by a number of studies. Cheeseman *et al.* (1979a) reported that patients who were receiving antithymocyte globulin in addition to prednisone and azathioprine after renal transplantation became viremic more frequently (11/13) than those who were not (3/8). Treatment with interferon reduced viremia in patients who were not receiving antithymocyte globulin, but it did not affect viremia in patients who received it.

The advent of cyclosporine in 1981 (Starzl *et al.*, 1981) has spawned numerous studies on its effect on CMV infection, which is described below. In 1989, large-scale testing began with a new macrolide antirejection drug, FK-506, similar in action to cyclosporine (Starzl *et al.*, 1989; Thomason, 1990). Although results are as yet incomplete, it promises to be an excellent immunosuppressant. Its use may entail fewer infections including CMV infections of clinical significance (Allessiani *et al.*, 1990).

Bia *et al.* (1985) compared 24 renal patients on cyclosporine and 40 on azathioprine. The numbers of symptomatic CMV infections were comparable (58% and 48%, respectively). Both groups also received steroids, and some received antithymocyte globulin (ATG). Excluding patients on azathioprine who received ATG for acute rejections reduced the frequency of symptomatic CMV infection to 25%.

At the University of Pittsburgh, we undertook a comparison of the infections occurring in renal patients on the traditional azathioprine–prednisone combination or on a cyclosporine–prednisone combination (Dummer *et al.*, 1983). No antithymocyte globulin was used. The amount of symptomatic disease may also be illustrated by the amount of CMV pneumonia in the two groups. There were three cases in each group of 138 and 131 transplant recipients (2.2% and 2.3%). The results of these two studies suggest that cyclosporine does not increase morbidity from CMV.

Najarian *et al.* (1985) compared 230 renal recipients on cyclosporine–prednisone with 109 on azathioprine–prednisone–ATG at the University of Minnesota. Symptomatic CMV infection during the first year after transplantation was only 9% in the cyclosporine group but 28% in the azathioprine group. However, the cyclosporine group did not routinely get 14 days of ALS or ATG. Hence, the two groups were not strictly comparable because of the possible overriding role of ATG.

The effect of antilymphocyte preparation on CMV infection was dramatically demonstrated by Rubin *et al.* (1985) in a survey of 1245 renal transplant recipients from 46 transplant centers. They found that cadaveric transplant recipients who received such serum had more than twice as many deaths as those who did not receive (19% versus 5%). Interestingly, the mortality of patients with no evidence of CMV was not affected by the use of antilymphocyte preparation (4%). The excess mortality is assumed to be related to CMV infection that was exacerbated by the antilymphocyte preparation. Unfortunately, there was no description of the actual cause of death in these patients.

Andreone *et al.* (1986) from Minnesota reported a reduction of infections in heart transplant recipients after institution of low-dose "triple"-drug immunotherapy. During the first period (1978–1982), when antilymphocyte serums (ALS), prednisone, and azathioprine were used, and during the second period (1982–1983), when high-dose cyclosporine (blood level: 250–300 ng/ml by high-pressure liquid chromatography) and steroids were used, there were many infections (1.3 or 1.6 episodes of infection per patient), particularly CMV and fungal infections. In the third period, when low-dose "triple" therapy consisting of cyclosporine (blood level: 150–100 ng/ml), azathioprine, and prednisone, but no ALS was used, there were only 0.6 infections per patient. Death from infections during these three periods were 2/11, 3/16, and 2/35, or 18%, 19%, and 6%, respectively. The most striking reduction was in symptomatic CMV infections. During the first two periods the frequency of symptomatic CMV infection was 8/27 or 30%, with three deaths from CMV. During the third period only one out of 35 (3%) patients had symptomatic CMV, and there were no deaths from CMV. This low morbidity is quite comparable to the morbidity from CMV infection in renal transplant recipients who did not receive ALS immunosuppression in other institutions (see above). The last immunosuppressive regimen was also associated with higher actuarial survival rate and lower rejection rates. Two additional measures may have helped reduce the rate of CMV morbidity, particularly from primary infection. One was the use of CMV-negative blood for seronegative recipients, and the second was the administration of CMV hyperimmune globulin. These measures may also have contributed most to the reduction of morbidity from CMV (see Sections 13.1.1 and 14.1).

New monoclonal antilymphocyte serums are also important factors in contributing to infection. They are particularly difficult to evaluate in this regard because they are frequently used only in selected patients, such as those with rejection. Chou and Norman (1985) reported a higher frequency of reactivation of CMV in renal transplant patients on OKT-3, although disease was not increased. Singh *et al.*

(1988) reported on the effect of using OKT3 serum for rejections in 121 liver transplant recipients in Pittsburgh. Patients who received OKT3 had significantly more episodes of disseminated CMV infection (five out of six) and more episodes or oral and genital herpes.

In summary, the type of immunosuppression has a profound effect on infectious complications, but particularly morbidity from CMV infection. Excess use of antilymphocyte and antithymocyte sera is a prime culprit. Newer monoclonal antilymphocyte sera are also suspect, although they have not been studied as carefully. The frequency of infection is not much affected by either antilymphocyte serum or cyclosporine because it is already close to the maximum under all types of modern immunosuppression. Cyclosporine, in controlled doses, does not increase morbidity from CMV infections compared to azathioprine.

13.1.4. Cytomegalovirus Infection, Graft Rejection, and Allograft Reaction

The relationship between CMV infection and the rejection reaction is a complex one. Which is cause and which is effect is difficult to sort out in patients because both occur so frequently in the first few months after transplantation. Although there is no evidence that CMV infection suppresses rejection, it is difficult to determine whether CMV infection is a cause of rejection or if rejection facilitates infection. There is ample evidence from animal work that both reactions occur. We showed that allograft reactions i.e., both host-versus-graft (HvG) and graft-versus-host (GvH) reactions may lead to enhancement of CMV infection (Wu *et al.*, 1975; J. Dowling *et al.*, 1977) (see Section 16.5.2). More recently, it was found that in the mouse model, a GvH reaction can facilitate the production of interstitial pneumonia (Grundy *et al.*, 1985), and conversely, the presence of CMV infection in the host can accentuate a GvH reaction produced by allogenic spleen cells (Grundy *et al.*, 1985; Via *et al.*, 1988) (see Section 16.3). The fact that human cadaveric transplant recipients, who have more rejection than recipients of organs from living related donors, have more CMV infections is presumptive evidence that allograft reactions may also increase frequency of CMV infections in man (Pass *et al.*, 1978; Ho, 1977).

Several effects of CMV infection on the expression of HLA antigens may have some bearing on how infection might affect rejection. Van Es *et al.* (1984) found evidence of activation of CD8 lymphocytes in patients with CMV infection or rejection. In 16 patients who suffered rejection episodes, HLA-DR (class II HLA antigen) expression by CD8 T lymphocytes was significantly increased. Patients with CMV infection also had increased HLA-DR expression on CD8 cells, which coincided with onset of clinical symptoms of CMV infection.

Cytomegalovirus infection has also been found to up-regulate the expression of class II HLA antigens in kidney cells, presumably as a result of γ-interferon production (Von Willebrand *et al.*, 1986).

After successful transplantation, MHC antigens on kidney parenchymal cells are lost, presumably because of administration of glucocorticosteroids. These antigens reappear during rejection. They also appear after CMV infection. It was postulated that increased antigen expression induced by infection led to rejection. Twelve out of 14 patients (86%) with CMV disease rejected, whereas only 17% of the remaining 223 patients rejected ($P < 0.001$).

That such enhancement actually occurs in renal transplant patients has been suspected since 1970 (Simmons *et al.*, 1970), even if evidence for it has been limited. Lopez *et al.* (1974a) found that of 29 infected patients, 21 rejected, whereas only one out of six uninfected patients rejected. Fiala *et al.* (1975) had similar results. The difficulty with these data is that the rate of CMV infection was high in the entire transplant population, irrespective of whether they rejected. Infection and rejection may be unrelated events, or, if related, they may be related to a third unknown event. Other authors have been unable to prove that CMV infection causes rejection. Chatterjee *et al.* (1978) found that the average number of rejection episodes was 1.05 among 20 seronegative patients and 1.06 among 15 seropositive ones. Most patients became infected, particularly the seropositive ones, but it is not clear from their data how many seronegative patients became infected. Flechner *et al.* (1982) followed 100 consecutive cadaveric kidney recipients. The development of either primary or secondary CMV infections with or without overt symptoms had no effect on graft survival. Ho *et al.* (1975) and Pass *et al.* (1978) point out that the reverse may be true; that is, a host-versus-graft reaction may increase CMV infection. As mentioned above, Pass *et al.* (1978) reported that 60 days after transplant, more patients who received cadaver kidneys were infected with CMV than patients who received kidneys from living related donors. This difference was thought to be related to greater host-versus-graft reaction in those who received cadaver kidneys.

Marker *et al.* (1981a) followed 320 transplant patients for 1 to 5 years for CMV infection and its possible complications (see Section 13.1.2). Multivariate analysis was used to examine the importance of several risk factors including the effect of CMV infection on graft survival. Rejection was defined by nephrectomy. Cytomegalovirus infection occurred in 181 patients after transplant and accounted for 20% of graft failures. Fifty-one percent of the infected patients rejected, whereas 41% of patients without infection rejected. Only in the interval 5 to 15 days before rejection was the number of CMV infections somewhat greater than expected (13 expected, 20 occurred). These are not significant differences. The presence of pretransplant antibody offered no protection against rejection. This study confirms the impression that it is difficult to demonstrate a relationship between CMV infection and graft rejection in a general transplant population.

Because of the relatively small number of primary CMV infections, their effect on rejection may be diluted out in a series of renal transplantations unless specifically looked for. Betts *et al.* (1977) studied a series of younger recipients, a significant proportion of whom were seronegative. Rejection occurred in four of 16 patients with primary CMV infection, whereas 24 seronegative patients who did not develop primary infection had no rejection ($P < 0.05$).

There is evidence that CMV infection affects the utility of multiple transfusions before transplantation, which have been found to increase graft survival (Opelz *et al.*, 1973). Andrus *et al.* (1979) studied the effect of CMV infection and the effects of multiple transfusions of frozen red cells on graft survival in a group of 55 recipients who received well-matched (at least two antigens) cadaveric kidneys. In this study, all seropositive recipients reactivated. No infection occurred in seronegative recipients who received kidneys from seronegative donors. Patients who received more than five transfusions before transplantation and who did not develop infection had a 91% graft survival after 24 months. The grafts of those who were infected had only a 41% survival, which suggests that the beneficial effect of transfusions was at least partially offset by CMV. Selection of a seronegative donor and multiple transfusions significantly improved survival of the cadaveric graft in seronegative donors.

Flechner *et al.* (1982) in a similar study of 100 cadaver renal transplants found that those who received more than 5 units of blood had 70% graft survival, whereas those who received no transfusions had only 36% graft survival. Transfusions helped graft survival in patients who became infected as well as in those who remained uninfected, although 1-year graft survival in the uninfected and infected groups (58% versus 7%) was significantly different. These authors found no significant difference in graft survival of patients, transfused and untransfused, who remained seronegative and uninfected after transplantation (71%) and who became infected (61%).

Grattan *et al.* (1989) reported more convincingly on the relationship between CMV rejection and allograft rejection in 301 cardiac transplant recipients at Stanford in the postcyclosporine era. Only 91 patients were infected with CMV (30%), a remarkably low figure (see Section 13.1). It was clearly shown that the frequency of graft rejection was significantly higher in the infected, although most patients ($>$ 84%) had one or more episodes of rejection. What was perhaps more impressive were results of an actuarial analysis that found that graft loss from atherosclerosis that led either to death or to retransplantation was greater for the CMV-infected group. Five years after transplantation, the rate was 68.8% for the CMV-infected and 36.8% ($P < 0.005$) for the noninfected group. Patient survival was also impressively lower in the infected group. Graft atherosclerosis was much more common after transplantation in the infected group as judged by angiographic criteria and by pathological study at autopsy. These data provide additional support for the hypothesis that CMV plays a role in the pathogenesis of atherosclerosis (Melnick *et al.*, 1983; Yamashiroya *et al.*, 1988) (see Section 5.8).

These later data are impressive because, as pointed out previously, it is very difficult to sort out cause–effect relationships between CMV infection and early rejection episodes.

This study found no effect of the severity of CMV infection, i.e., whether the patient was symptomatic or asymptomatic, whether the infection was primary or secondary, on the results. This is surprising and may be related to the low infection

rate and relatively low numbers in the different categories. The main method of detecting infection was serologic, even though cultural methods were said to be used. It is possible that only the more severe and symptomatically infected subjects were being described.

Waltzer *et al.* (1987) analyzed the effect of CMV infection and HLA matching on rejection episodes. Among seronegative recipients who remained free of CMV infection after transplantation, there was a clear benefit of highly matched (three or four antigens) versus poorly matched (one or two antigens) transplants in terms of rejection episodes. The mean number of episodes was, respectively, 0.25 and 1.07 ($P < 0.05$). Similarly, the advantage of high matching was evident in the case of seropositive recipients, most of whom had evidence of secondary CMV infection. However, patients with primary infection had significantly more rejection, and there was no difference in rejection between those who received highly matched and poorly matched kidneys (mean rejection episodes 2.0 versus 1.5). These results suggest that patients who had the more severe type of CMV infection can override the beneficial effect of HLA matching.

Assuming that the morbidity, graft loss, and mortality resulting from primary CMV infection are not acceptable, Ackermann *et al.* (1988) report the results of a policy adopted in Tampa of grafting only kidneys from seronegative donors to seronegative recipients. Compared with historical controls, morbidity caused by CMV infection was reduced from 10.6% to 1.7% ($P < 0.0001$), mortality from 3.7% to 0% ($P = 0.002$), and graft loss from 2.5% to 0% ($P = 0.016$).

There are several other phenomena illustrating the bidirectional relationships between CMV infection and allograft reaction.

There is a strong association between GvH reaction and the frequency of CMV interstitial pneumonia in marrow recipients. The frequency of CMV pneumonia among those who received autologous marrow transplant was strikingly low (Pecego *et al.*, 1986; Wingard *et al.*, 1988a,b). The pathogenesis of this type of pneumonia is assumed to have a strong immunopathological component even though so far the role of the GvH reaction has not been explained (see Section 13.2.2).

Cytomegalovirus infection has been shown to be a risk factor for the development of the pathogenesis of vanishing bile duct after liver transplantation (O'Grady *et al.*, 1988). This syndrome is a variant of chronic graft rejection after liver transplantation and is characterized by the destruction of interlobular bile ducts (Vierling and Fennell, 1985). It is also seen in patients with a complete mismatch for HLA-A/B antigens and paradoxically with one or two matches for HLA-DR antigens (Donaldson *et al.*, 1987).

Burke *et al.* (1984) described in five out of 14 long-term survivors of heart–lung transplantation the development of a progressive obstructive and restrictive ventilatory defect. Biopsy showed an obliterative bronchiolitis (OB).

Obliterative bronchiolitis is known in other disease, but its etiology is unknown. It has been linked to viral infections (Becroft, 1971) and autoimmune diseases such as rheumatoid arthritis (Geddes *et al.*, 1977), eosinophilic faciitis

(Epler *et al.*, 1979), and Sjögren's syndrome (Newball and Brahim, 1977). Chronic graft-versus-host (GvH) reaction has been reported to produce OB within 2 years after bone marrow transplantation (Ralph *et al.*, 1984). Quite possibly OB in the setting of lung transplantation is related to some type of allograft reaction, and CMV infection has been suspected to be involved.

The possible role of CMV infection in OB was studied by Burke *et al.* (1986), who followed 19 survivors of lung–heart transplantation. A total of 12 patients developed CMV infection, of whom six developed OB. Those patients who had CMV pneumonitis also had OB. There were also seven seronegative recipients who received organs from seronegative donors and who did not develop any evidence of CMV infection but who developed progressive OB. This paper suggests that OB may occur in the absence of CMV infection, but CMV infection may still be a cofactor in the development of OB. More patients must be followed before definite conclusions may be drawn.

There are a number of isolated observations that suggest that the grafted organ may be particularly prone to CMV and other viral infections. One may assume that the graft is the site of active allograft reactions. Richardson *et al.* (1981) described seven patients whose allograft dysfunction was attributed to a unique glomerular lesion that was thought to be caused by CMV and was associated with CMV viremia (Section 10.3). Cytomegalovirus hepatitis is a fairly common complication after liver transplantation (Singh *et al.*, 1988; Bronsther *et al.*, 1988; see Section 13.3.3). The grafted liver is also prone to herpes simplex hepatitis (S. Kusne, unpublished data). Patients with heart–lung transplantation were more prone to develop CMV pneumonitis than patients with kidney, liver, or heart transplantation.

In summary, although it is difficult to show that CMV infection affects graft survival, accumulating evidence suggests that at least primary infection may be deleterious. It may also offset the beneficial effect of multiple transfusions.

Several types of actual and potential interactions between CMV and the immune system have been reported that may have a bearing on the subject of immune injury precipitated by CMV on grafts.

The MHC class I antigens consist of three highly pleomorphic α chains associated covalently with β_2-microglobulin (β_2-m). Human CMV has been shown to bind β_2-microglobulin, and viruses to which β_2-microglobulin have been bound are not neutralizable by antiserum (Grundy *et al.*, 1987a, 1988a). Beck and Barrell (1988) predicted on the basis of DNA sequence analysis that CMV encodes a glycoprotein, perhaps of 65 kDa, that is homologous to the α chain of MHC class I antigens of eukaryotes. This protein may be the mediator of binding to β_2-microglobulin. It may play a role in viral adsorption and possibly interact with reactions requiring class I antigens including immunosuppressive ones (see also Section 3.5.1). Evidence exists that class I molecules can serve as receptors for Semliki Forest virus (Helenius *et al.*, 1978) and adenovirus 2 (Signas *et al.*, 1982).

There is also evidence that CMV may have homology with class II MHC antigens. Fujinami *et al.* (1988) presented evidence that IE-2 protein of CMV and HLA β chain share a common epitope. It was shown by computer analysis that one

of the five immediate early (IE) antigens, IE-2 of human CMV, and HLA-DR β chain had common sequences. They synthesized a peptide predicted to be residues 82 to 96 of a 208-amino-acid protein from the IE-2 region. The first five amino acids of this peptide showed sequence homology with the β chain of HLA-DR. The synthesized peptide induced antibodies that recognized the β chain of HLA-DR. Possibly cross reaction of virus-directed antibodies with HLA molecules on the graft may contribute to rejection. This is consistent with the evidence that patients who suffer rejection have increased expression of HLA-DR antigens on their T lymphocytes (Van Es *et al.*, 1984) or on the parenchymal cells of the graft (Von Willebrand *et al.*, 1986).

Such shared antigens may also be involved in enhancement or suppression of cell-mediated reactions. Immune injury may be triggered by CMV by reactions against cells that bear homologous portions of class I or II HLA antigens.

Baldwin *et al.* (1983) described renal dysfunction in cadaver kidney recipients associated with CMV infection, the production of an IgM lymphotoxin, and certain HLA phenotypes. Active CMV infection was found among 23 of 121 transplant recipients. Twelve of 16 patients had broadly reactive IgM lymphotoxins. Ten out of 11 tested were positive for HLA-DR3 or -DR7.

There is relatively little information on the association of CMV infection and HLA haplotypes (see also Section 9.1.4). Roenhorst *et al.* (1985) found that cadaveric renal transplant recipients positive for HLA-DRw6, compared to those negative for this antigen, showed a significantly earlier and higher frequency of CMV infection detected serologically. No relationship to any other HLA antigens were found, including A and B antigens. Recipients positive for HLA-DRw6 more often were virus shedders and showed more clinical symptoms related to CMV ($P < 0.01$).

13.1.5. Immunosuppressive Effect of Cytomegalovirus Infection

CMV is one of many viruses which produce immunosuppressive effects (Rouse and Horohov, 1986).

In addition to its direct clinical effects, CMV infection may have indirect effects that have become apparent. One such effect is its immunosuppressive action. Hence, CMV infection, even an asymptomatic one, should no longer be regarded as an "innocent bystander" (Rand and Merigan, 1978). It may also act as a cofactor in AIDS (see Section 13.2.1).

Not only are primary infections more symptomatic, but they are probably also more immunosuppressive and thus increase susceptibility to other opportunistic infections to which transplant patients are already prone.

It is well known that acute primary murine CMV infection in the mouse depresses many parameters of immunity (see Section 16.6.3). These include development of antibodies against a virus such as NDV or sheep red cells (Osborn *et al.* 1968), production of interferon (Osborn and Medearis, 1967), rejection of skin

grafts across H-2 and H-Y histocompatibility barriers, stimulation of lymphocytes of PHA or in mixed lymphocyte culture (Howard *et al.*, 1974; Booss and Wheelock, 1975), and development of cytotoxic T lymphocytes (Hamilton *et al.*, 1978; Ho, 1980). Grundy and Shearer (1984), however, point out that in the response to alloantigens by CMV-infected mice, there is, following a period of immunosuppression, a period of enhanced cytotoxic reaction against cells exhibiting alloantigens (see also Section 13.1.4).

Similar evidence has recently accumulated in man. Mothers of infants with congenital CMV infection have depressed specific lymphocytotoxicity against CMV-infected targets compared to normal seropositive subjects (Rola-Pleszczynski *et al.*, 1977). A healthy adult with cytomegalovirus mononucleosis had fewer T cells in his blood than normal, and he showed loss of skin reactivity to mumps antigen (Oill *et al.*, 1977). Such patients have decreased ratios of helper to suppressor T lymphocytes that may last as long as 10 months (Carney *et al.* 1981) (Section 7.3.1). Both community-acquired and transfusion-acquired CMV mononucleosis in normal subjects were associated with depression of specific as well as nonspecific parameters of cellular immunity including lymphocyte subset changes (see Section 7.3).

During the immediate posttransplantation period, when many patients are actively infected with CMV, tests show that cell-mediated immunity is suppressed. In cardiac and renal transplant patients, the specific lymphocyte proliferative test against CMV became negative even when it was positive prior to operation (Pollard *et al.*, 1978; Linnemann *et al.*, 1978). Rytel *et al.* (1978) showed that although humoral immunity is unimpaired, cellular immunity against both specific and nonspecific antigens is defective in the renal transplant patient. Eighty percent of patients were unreactive by skin testing with *Candida,* streptokinase–streptodornase, mumps, and PPD antigens. T-lymphocyte ratios as measured by sheep red cell rosetting were suppressed. Six of nine (67%) CMV transplant recipients had no evidence of [^{3}H]thymidine uptake when their lymphocytes were exposed to CMV antigen, although they were infected. In such reports, it is difficult to sort out the immunosuppressive role of CMV infection from the effect of the cytotoxic drugs usually given these patients.

Whereas patients with mild CMV disease mount a brisk antibody response, those with progressive fatal infections may not respond at all (Craighead, 1971; Neiman *et al.*, 1977). Simmons *et al.* (1977) described a form of lethal CMV infection in eight cases out of 377 recipients within 6 months of renal transplantation. It ran a 4-week course beginning with spiking fever, malaise, mild hypoxemia progressing to hypoxia, interstitial pneumonitis, hepatic dysfunction, and renal failure without evidence of graft rejection. There was no antibody response, although CMV was cultured. Chatterjee *et al.* (1978) noted one patient with primary CMV infection and superimposed fungal infections who had CMV inclusions and viral isolation at autopsy; but before death, he had negative blood and urine culture and no serologic response to CMV infection.

Neiman *et al.* (1977) noted that all marrow transplant recipient patients with virologically proven CMV interstitial pneumonia who did not have a fourfold or

greater serologic response died (9/9), whereas only 20% (1/5) of those who had a serologic response died. Thus, suppression of antibody response was correlated with severity of infection.

Suppressed immunity not only is evident by laboratory tests but may have important clinical implications. Rand *et al.* (1978) reported that out of 12 cardiac transplant patients who had serologic evidence of primary CMV infection within 90 days after transplantation, six developed bacterial pneumonias or abscesses. In contrast, only one out of 20 developed such infections if they were seropositive before operation. Mortality in this 90-day period was 32% (9/28) for the seronegative patients and only 10% (2/21) for the seropositive ones.

Chatterjee *et al.* (1978) made similar observations, but different pathogens were incriminated. Of six seronegative recipients, four developed primary CMV infection and died of *Candida* or *Aspergillus* infections within 6 months after transplantation. Among 22 seropositive patients, most of whom had reactivation CMV infection, only one became infected with a fungus that was not lethal. In contrast to fungus infections, bacterial infections occurred with about equal frequency in both seronegative and seropositive groups, but patients who were seronegative had more severe gram-negative infections. Grattan *et al.* (1989) studied, in 301 cardiac transplant recipients, the relationship between CMV infection and rejection (Section 13.1.4). Fungus infections in the 91 CMV-infected patients were significantly more frequent than in the uninfected group. Bacterial infections were not different, probably because all patients were on trimethoprim–sulfamethoxazole prophylaxis.

The basis of increased susceptibility may be a suppression of cell-mediated immunity and altered T-lymphocyte function. There is no evidence of altered leukocyte function, even though CMV may be carried by polymorphonuclear or monocytic leukocytes (Rinaldo *et al.*, 1979b; Fiala *et al.*, 1975).

There are several leads that might explain the immunosuppressive effect of CMV infection, although understanding is incomplete. Several investigators have implicated infected monocytes in *in vitro* studies. Carney and Hirsch (1981) showed that CMV-infected monocytes inhibited the lymphocyte proliferative response induced by concanavalin A. Cytomegalovirus infection of lipopolysaccharide-stimulated monocytes inhibited IL-1 production (Dudding and Garnett, 1987). Rodgers *et al.* (1985) observed that CMV infection inhibited production of IL-1 by monocytes and postulated that an inhibitor was induced. Loh and Hudson (1982), working in the murine system, also postulated that a suppressor substance is elaborated by infected macrophages, although no direct proof was offered (see also Section 16.6.3). Schrier *et al.* (1986) reported that fresh, low-passage clinical isolates of CMV suppressed the NK activity of peripheral blood mononuclear cells (PBMC) against K-562 targets. When monocytes were removed from the reaction mixture, the suppression was abrogated. Kaposi and Rice (1988) studied the inhibitory effect of the Towne strain of CMV on the proliferative response of PBMC induced by phytohemagglutinin. Addition of CMV-infected monocytes or infected lymphocytes abrogated the proliferative response. Infection of either cell type also reduced the production of IL-1 and IL-2 as well as the proliferative response induced by IL-1 or

IL-2. However, exogenous additions of neither IL-1 nor IL-2 could restore the PHA response in populations of CMV-infected lymphocytes and monocytes.

The authors suggest that CMV infection produces a "generalized metabolic depression" of PBMC activity rather than a specific defect. How CMV can do this despite the fact that there is little lytic infection and only a small proportion of lymphocytes or monocytes can be shown to be infected as determined by the expression of immediate early antigen (Rice *et al.*, 1984; Schrier *et al.*, 1985) is unclear. The manner by which CMV can affect general function has also not been fully elucidated.

13.1.6. Late Onset of Cytomegalovirus Infection

As already pointed out, primary and reactivation CMV infections usually occur shortly following transplantation. Late infection is less common, although important complications, especially retinitis, have been identified.

Spencer and Andersen (1979) followed 100 renal transplant patients for 6 to 13 years, of whom 39 completed the follow-up. All were receiving maintenance azathioprine and prednisone immunosuppression. The only two persistent viral problems were warts and shingles. Warts appeared in 25 patients, usually 1 year after transplant. Once they appeared, they never disappeared. In three patients, this presented a cosmetic problem, and in one, a skin tumor (keratoacanthoma) appeared. Six patients had shingles, three within the first 3 months after transplantation and three over 3 years later. This bimodal pattern of occurrence of shingles confirms earlier observations (Spencer and Andersen, 1970).

There were no late cases of symptomatic disease caused by CMV. Those who remained seronegative after 3 months (three) did not convert. In 35, there was an antibody rise 3 months after transplantation, which remained high. Cytomegalovirus was only detected in the urine of two of these patients in late follow-up in low titers. Throat swabs and blood specimens were negative. None had jaundice, and there was no change in the frequency of nonspecific febrile illnesses in these patients. It would appear from this study that transplant patients usually recover from their CMV infection, and as long as they are not overly immunosuppressed, overt infection is cured, and there is no recurrence of morbidity.

On the other hand, every one of the cardiac transplant patients continued to excrete CMV in the urine or through the respiratory tract during a 2-year follow-up once they began excreting virus (Pollard *et al.*, 1982).

Cheeseman *et al.* (1979b) studied 67 patients 2 to 14 years after transplantation. They found that 56% of seropositive patients were excreting the virus in the urine, throat, or blood 2–5 years after transplantation. This declined to 17–25% thereafter. Those excreting virus had higher CF antibody titers. This represents a significantly higher rate of virus excretion than normals and also higher than for herpes simplex virus (14% of seropositive individuals). In agreement with Spencer and Andersen (1970), who found no late cases of CMV disease, only one symptomatic patient was found. He had persistent viruria, viremia, chorioretinitis, and hepatitis.

There are also case reports of late onset of CMV infection and of recurrent infections after transplantation. We have already referred to the late onset of CMV retinitis in renal and cardiac transplant patients (Section 13.3.4). Linnemann *et al.* (1978) described two patients who died of CMV infection more than 12 months after receiving renal transplants from living related donors. One was a 19-year-old college boy with good function of the transplanted kidney who suddenly developed fever, photophobia, headache, myalgias, elevated SGOT levels, nuchal rigidity, and, later, pneumonitis. He died on the 24th hospital day. Disseminated intranuclear inclusions were found in many organs at postmortem. The virus was isolated from the throat, blood, lung, and urine during life and also from many organs including the liver, kidney, and brain at postmortem. This patient was seronegative before transplant and remained negative until the terminal illness, when he developed specific IgG and IgM immunofluorescent CF antibodies. How this patient got his primary infection is unclear.

The second patient was a 53-year-old male who, 16 months after transplant, developed fever, elevated SGOT, leukopenia, and pneumonitis. He died on the 44th day of illness. He had furunculosis and secondary *Enterobacter* and *Candida* infection of the lungs, liver, and brain at autopsy. This patient was also seronegative before transplant but developed transient CF antibodies 6 weeks after surgery. During his terminal infection, CF, immunofluorescent IgG, and IgM antibodies rose. The authors believe that this was a case of late reactivation infection causing death. Reinfection in a patient who may not have developed adequate resistance after the first posttransplant infection is also possible.

Friedman *et al.* (1979) described two cases of relapses of CMV pneumonia after renal transplant. Primary CMV pneumonia was proven by serologic conversion and viral isolations from pleural fluid and inclusions in lung tissue. After a remission of 1 week, a relapse occurred following an increase in immunosuppression. The second episode was associated with a rise in CF titer.

A second patient also seroconverted during the first episode. A buffy coat culture was positive. After a 9-week remission, a second episode occurred while the patient's immunosuppression was increased. He died before a second serum could be obtained.

These two cases show that immunity following CMV disease is probably not complete as long as the patient is immunosuppressed. Vital organs, such as the lung or the eye, may be involved, although it was not clear whether the second episode represented reactivation or reinfection, relapse of a smoldering infection, or systemic spread from a latent site.

13.2. CYTOMEGALOVIRUS INFECTION IN THE AIDS PATIENT AND THE HIV-INFECTED

As an epidemic disease of immunodeficiency, AIDS has served to emphasize the protean disease syndromes that CMV may produce. A major difference between transplant recipients and HIV-infected subjects is that most of the latter are already

infected with CMV prior to HIV infection. Hence, morbidity from CMV in AIDS patients is usually a result of secondary infection, i.e., reactivation or reinfection. Since symptoms of CMV infection in AIDS may be severe and correspond only to what one sees in severely immunosuppressed transplant patients, immunodeficiency must be more severe in AIDS. In this section we first discuss the relationship of CMV infection to AIDS. Then we highlight some of clinical manifestations of CMV in AIDS, leaving the more general aspects of this subject to Section 13.3.

Before the discovery of HIV in 1983 (Barre-Sinoussi *et al.*, 1983; Papovic *et al.*, 1984), CMV was considered as a possible etiologic factor in AIDS. It was known that CMV infection was present in practically all cases of AIDS (Gottlieb *et al.*, 1981), and CMV infection was known to be immunosuppressive and to invert the T-helper to T-suppressor lymphocyte ratio (Rinaldo *et al.*, 1980; Carney *et al.*, 1981); and CMV infection had been shown to contribute to the development of superinfection by bacterial or fungal agents in transplant recipients (Chatterjee *et al.*, 1978; see Section 13.1.5). In addition, CMV was suspected to play a separate direct role in the development of Kaposi's sarcoma.

However, even without the discovery of HIV, it was difficult to explain AIDS on the basis of CMV alone because most people do not develop AIDS when infected with CMV. Cytomegalovirus infection may also be absent in patients with AIDS, particularly when AIDS occurs among nonhomosexual groups. It is still possible that of many cofactors that may contribute to the development of AIDS, CMV may still have a role.

13.2.1. Cytomegalovirus Infection as a Cofactor in AIDS

The possibility of CMV infection as a cofactor for the development of AIDS may be considered from the point of view of theoretical justification and practical evidence.

AIDS is a disease with a very long incubation period. There is still some controversy whether the deterioration of the immune system whose hallmark is the steady decline of the CD4 lymphocyte count can be accounted for by HIV infection alone (Fauci, 1988). Cytomegalovirus infection is immunosuppressive (see Section 13.1.5). Theoretically, it could facilitate the inception of HIV infection or accelerate its progression to AIDS. The reverse is not only possible but recognized, i.e., the facilitation of CMV infection as a result of immunosuppression produced by HIV.

A number of possible mechanisms by which CMV could enhance HIV replication are listed below. These may operate in the microenvironment in which CMV- and HIV-infected cells are in close proximity, or cells may be doubly infected. Other mechanisms result more indirectly from interactions between CMV and HIV infection.

HIV replication is facilitated in activated T lymphocytes. Cytomegalovirus antigen-presenting monocytes or macrophages may initiate such activation.

Cytokines produced by immune stimulation as a result of CMV infection could activate HIV-infected cells.

Cytomegalovirus may act by enhancing HIV viral DNA replication by transactivation. For example, CMV-immune specific stimulation of CD4 lymphocytes could result in enhanced production of cell transcription regulatory proteins such as Spl and NF-kB, which bind to specific sites of the HIV long terminal repeat (LTR) sequences (Sen and Baltimore, 1986; Jones *et al.*, 1986; Nabel and Baltimore, 1987). This could lead to the production of HIV proteins functionally essential for further viral transcription, such as *tat* (Sodroski *et al.*, 1985).

Synergistic infection of CMV and HIV may occur in doubly infected cells. Abortive infection of mononuclear blood cells by CMV accompanied by transcription of immediate early genes is a fairly general phenomenon of CMV infection (see Section 5.5). These gene products are thought to transactivate HIV LTR and thereby enhance expression of HIV (M. G. Davis *et al.*, 1987). Other herpesviruses such as HSV and EBV can also transactivate HIV LTR (Rando *et al.*, 1987; M. G. Davis *et al.*, 1987).

There are a number of more indirect interactions of CMV and HIV infection, the net result of which may accelerate the pathogenesis of AIDS. Raised levels of β_2-microglobulin, assumed to result from increased turnover of HLA molecules as a result of immunologic activation, are associated with a poor prognosis of HIV infection (Moss *et al.*, 1988). This protein binds to CMV and may enhance the infectivity of CMV by inhibiting its neutralization (Grundy *et al.*, 1988a). Cytomegalovirus can also elevate β_2-microglobulin levels (Hutt-Fletcher *et al.*, 1983).

At this time there is no direct evidence that any of these mechanisms are operational in patients. It is quite possible that some interaction between the two viruses exists, and this may be quite complex (Skolnik *et al.*, 1988). Reports showing that brain cells can be coinfected by both viruses (Nelson *et al.*, 1988) lend credence to some of these postulated mechanisms. We next examine epidemiologic data concerning an interaction between CMV and HIV.

We have described CMV infection in the male homosexual in the presence or absence of AIDS (Section 9.5). Compared to persons of similar socioeconomic background, the healthy homosexual without HIV infection has a higher frequency of seropositivity and a greater likelihood of active virus infection as evidenced by positive virus cultures in semen or urine or by the presence of specific IgM antibodies against CMV.

There is evidence of even more CMV infection in the homosexual with HIV infection. Quinnan *et al.* (1984) found evidence of CMV infection proven by virus isolation or by presence of antibody in all patients with AIDS (34/34) and most patients with chronic lymphadenopathy (8/9). There is also evidence of more CMV activity in the HIV-infected nonhomosexual, but this is quantitively less apparent.

Blaser and Cohn (1986) reviewed the frequency of diagnosis of various reactivation and opportunistic infections in 446 patients with AIDS, representing a variety of risk groups in North America, Europe, and Africa. The basis of diagnosis was reports of isolation of the virus or description of typical pathological features. Cytomegalovirus infection was diagnosed in 80% of 165 homosexual or bisexual men, 46% (6/13) of children, 4% (1/25) of intravenous drug abusers, 22% (7/32) of

hemophiliac men, and 12% (3/25) of transfusion recipients. It is clear that homosexual and bisexual men with AIDS seem to have significantly more CMV pathology than any of the other groups. The numbers in the other groups are not large enough to reveal differences among them. HIV infection and AIDS may also be a significant factor enhancing the pathogenecity of congenital or perinatal CMV infection (Pahwa *et al.*, 1986), as it does in adult CMV infection.

Several studies have now compared groups of HIV-infected patients who have more or less CMV infection for differences in mortality or progression to AIDS. Assuming that there was less CMV disease in intravenous drug users or hemophiliacs with AIDS compared to the homosexual with AIDS, it is striking that a study of 4805 patients who received AZT by compassionate release before the drug was licensed showed that there was no significant difference between the probability of survival of drug abusers or homosexuals with AIDS. Actually, patients with transfusion-acquired AIDS survived less well than homosexuals, drug abusers, or hemophiliacs (Creagh-Kirk *et al.*, 1988). Such patients may be older or may also have serious underlying illnesses. According to this report, immunodeficiency does not seem to be more accelerated in homosexuals, who would be expected to have more CMV disease.

Jason *et al.* (1989) compared a cohort of HIV-infected hemophiliacs with a similar one of HIV-infected homosexuals and reported a similar latent period before development of AIDS and similar frequency of development of AIDS. This suggested that the basic disease process, i.e., progression of immunodeficiency, is similar in both groups irrespective of differences in CMV infection. However, it is quite possible that these studies were not discriminating enough because the factor of CMV infection was not directly analyzed. In a study of 164 autopsied cases of AIDS from Los Angeles, the total clinical course defined as the duration from onset to death was the same in those who had CMV infection and in those who did not (Klatt and Shibata, 1988). However, Webster *et al.* (1989) studied the effect of CMV and HSV infection in 108 hemophiliacs. In 1.3 to 9 years from the time of seroconversion, the age-adjusted risk to development of AIDS in CMV-seropositive subjects was consistently 2.5-fold higher than in CMV-seronegative subjects. The results were more striking without age adjustment but remained significant even with age adjustment. In contrast, seropositivity to HSV had no effect on progression to AIDS. This report seems to conclude unambiguously that CMV infection is associated with a more rapid progression to HIV disease. It is still possible that CMV infection is a marker for a third factor. More studies of this type as well as studies on pathogenic mechanisms of CMV as a cofactor are needed before the role of CMV infection in AIDS is fully clarified.

13.2.2. Clinical Aspects of CMV Infection in AIDS

Although active CMV infection is common during HIV infection and AIDS, its clinical significance may be much less clear-cut. Earlier autopsy reports of AIDS cases suggested an alarming amount of significant CMV infection. Macher *et al.*

(1983) reported from the N.I.H. that 14 out of 15 autopsied patients (93%) had anatomic evidence of CMV infection.

More recent data on 164 autopsied AIDS cases from Los Angeles showed that 59% had some anatomic evidence of CMV infection in some organs (Klatt and Shibata, 1988). However, evidence of organ failure caused by CMV leading to the patient's demise was only present in 17 (10%). There was no case in which CMV infection was the only diagnosis of the patient that fulfilled the CDC definition of AIDS. There was always some other AIDS-defining infection or neoplasm.

The sites of anatomic involvement by CMV may be extensive. The major organs involved were the adrenals, lung, gastrointestinal tract, central nervous system, and eye. Other autopsy series also cite the same sites for frequent involvement with CMV (Morgello *et al.*, 1987). Many of the anatomically involved sites may be clinically silent or undiagnosable (Jacobson and Mills, 1988). For example, in the Klatt and Shibata (1988) series cited above, the adrenals of 61 patients (37%) were involved, but such involvement resulted in adrenal failure in only ten cases.

Despite frequent evidence of CMV infection in the *lung,* the clinical importance of such infection can be questioned. In a review of 441 AIDS patients with symptomatic pneumonitis, CMV was diagnosed by culture or characteristic cytopathic effects in bronchial specimens from 17% of patients (Murray *et al.*, 1984). However, two-thirds of these patients had coexistent *Pneumocystis carinii* pneumonia, which was the primary disease because it often responded to specific treatment. Cytomegalovirus was the only pathogen in only 4% of the cases. It is possible that the severe CMV interstitial pneumonitis seen in bone marrow transplantation is caused by a type of immunopathology (see Section 13.3.2) that is not commonly seen in AIDS patients.

Perhaps the most striking clinical manifestation of CMV infection in the patient with AIDS is *CMV retinitis.* Although well recognized in immunosuppressed adults prior to the discovery of AIDS in 1981 (see Section 13.3.4), its classic clinical and ophthalmolgic presentation makes it the only disease caused by CMV that can be diagnosed without laboratory aids. The classical fluffy white exudate often associated with hemorrhages following a vascular pattern is associated with relentless loss of vision. *In situ* hybridization has demonstrated CMV nucleic acid in tissue sections of the eye but not HIV nucleic acid (Kennedy *et al.*, 1986).

Two other organ systems are frequently involved by CMV in AIDS, but specific clinical diagnosis may be much more difficult: the *central nervous system* and the *gastrointestinal tract.* These are described in the next sections.

13.3. CLINICAL MANIFESTATIONS OF CYTOMEGALOVIRUS INFECTIONS IN THE IMMUNOSUPPRESSED

As is the case with most known infections, infection with CMV is frequently asymptomatic, even in the immunosuppressed transplant or HIV-infected patient. The severity of clinical manifestations is a reflection of the degree of immunosuppression. A febrile mononucleosis similar to the disease in the normal adult, already

described, appears to be the most common and least severe presentation in the immunosuppressed patient.

More serious clinical manifestations of CMV infection involve one or more visceral organs or their function. The important ones are described below. The most severe forms will destroy function of a vital organ or threaten life. Usually they are manifestations of a disseminated systemic infection. As mentioned in Section 13.2, many organs may be found to be involved at postmortem without being clinically abnormal. The most severe form of CMV disease in adults is now seen frequently in AIDS, in the allogeneic marrow transplant recipient, and occasionally in the severely immunosuppressed solid organ recipient.

13.3.1. Febrile Syndrome

This occurs usually during a primary infection after transplantation. It has also been described in HIV-infected subjects prior to the development of AIDS (Leport *et al.*, 1987). In such cases it is not clear whether a primary or secondary infection is involved. The cardinal characteristic is prolonged fever. There may also be atypical lymphocytosis and mild hepatitis. Large numbers of atypical lymphocytes are, however, uncommon (Rubin *et al.*, 1977). We found in 13 of 18 primary infections in a series of renal transplant patients at least two of the following: fever, leukopenia, atypical lymphocytes, lymphocytosis, hepatosplenomegaly, myalgia, arthralgia, and pneumonia (Suwansirikul *et al.*, 1977). Hepatic enzyme (SGOT) elevation, usually mild in nature, is common (Betts *et al.*, 1977).

Other febrile syndromes are common after HIV infection and in the course of AIDS. They are frequently undiagnosed etiologically and may be caused by HIV itself. It is particularly difficult to ascribe them specifically to CMV since CMV infections, including positive blood cultures, are so common during HIV infection (see Section 13.2).

The following case from the Presbyterian-University Hospital of Pittsburgh depicts primary CMV mononucleosis in a renal transplant patient:

> T.M.V. was a 17-year-old white boy who had chronic glomerulonephritis, renal failure, secondary hyperparathyroidism, and renal rickets. He was put on chronic hemodialysis. A year later he had bilateral nephrectomies and a kidney transplant from his father, who had a CF titer of 1 : 32 against CMV. Postoperatively, he had a mild rejection episode with a rise in serum creatinine, which responded to a course of solumedrol. He was discharged after 12 days. Thirty-five days after transplantation, the patient developed a dry hacking cough with daily temperature elevations. He was readmitted and was observed to have daily fever spikes of 38 to 40°C for 3 weeks. The white cell count was 5300/mm^3, with 71% polymorphoneuclear leucocytes, 9% band forms, 14% lymphocytes, and two to five atypical lymphocytes. Cryoglobulins and rheumatoid factor were transiently positive. His preoperative CMV CF titer was $< 1:4$, and on January 16, it was $> 1:256$. His urine culture was positive for CMV. Liver enzymes were normal.

This case represents a young seronegative recipient who, 1 month after receiving a transplant from his seropositive father, developed a febrile illness charac-

terized by the presence of only a few atypical lymphocytes and no hepatitis. The seroconversion and isolation of CMV from urine showed this to be a typical case of mild primary CMV mononucleosis.

13.3.2. Interstitial Pneumonitis

After febrile mononucleosis, the next most common symptomatic manifestation of CMV infection is pneumonitis. It is clinically more important because, unlike the febrile syndrome, it is associated with significant morbidity and mortality. Prompt and precise diagnosis is important because therapy is now possible. Cytomegalovirus pneumonitis in the immunosuppressed may be difficult to distinguish from pneumonitis caused by gram-negative bacteria, *Aspergillus, Nocardia, Legionellaceae,* and particularly *Pneumocystis* or a combination of any of these.

The pneumonitis is more likely to show an interstitial rather than lobar or alveolar pattern. A symmetrical, bilateral infiltrate may begin in the periphery or lower lobes and extend centrally and superiorly (Rubin *et al.*, 1977). The infiltrate may also be unilateral, and rarely, lobar consolidation, solitary nodules, or even cavities have been described (Abdallah *et al.*, 1976; Ravin *et al.*, 1977). Atypical lymphocytosis is not a conspicuous part of the clinical picture.

Rubin *et al.* (1977) described nine renal transplant patients with CMV pneumonia, but in only four was CMV the sole organism. The other organisms were gram-negative bacteria. Cytomegalovirus frequently exists together with *Pneumocystis* as a copathogen in both transplant patients (Hardy *et al.*, 1984) and AIDS patients. In AIDS patients the etiologic role of CMV in the pneumonitis is difficult to ascertain, particularly since it may resolve with treatment of the *Pneumocystis* alone (see Section 13.2).

The course of CMV pneumonia varies according to the degree of underlying immunodeficiency and probably other factors as yet not fully elucidated. In the transplant population, there is a wide spectrum of different outcomes. Peterson *et al.* (1980) described CMV pneumonia in a series of renal transplant patients who received antilymphocyte serum as part of routine immunosuppression. They noted pulmonary infiltrates in 25 (42%) of their patients with symptomatic CMV disease. Twenty-four (96%) had bilateral infiltrates, and one had a wedge-shaped left lower lobe infiltrate. Six had late nodular infiltrates. Small unilateral or bilateral effusions were seen in 11 (44%). Mortality was 48% (12/25), which is probably significantly lower than the 84% mortality reported in Meyers' series of interstitial CMV pneumonia in bone marrow recipients (Meyers *et al.*, 1986). Hypoxemia occurred in 65% of all patients with CMV disease and in all with severe or lethal disease. It frequently antedated the development of x-ray abnormalities. Only one of 13 patients who required respiratory assistance survived, also pointing to the poor prognosis of patients with respiratory failure. Abdallah *et al.* (1976) also noted the

importance of functional disturbance. Eleven out of 12 patients who died had hypoxia demonstrated by low Po_2.

Dummer *et al.* (1985) noted eight cases of CMV pneumonia in 55 cases of heart and heart–lung recipients. The frequency of mortality of 75% (6/8) was comparable to that of CMV pneumonia in bone marrow recipients. It is possible that since the lung is the site of an allograft reaction, it is particularly susceptible to CMV pneumonia.

It is not possible to diagnose CMV pneumonia on the basis of clinical signs and symptoms alone. A febrile patient with a pulmonary infiltrate and positive CMV culture at other sites, such as buffy coat or urine, may have CMV pneumonia, particularly if no other cause for the pneumonia is found. But for a definitive diagnosis, evidence of CMV infection must be found in lung tissue.

The preferred method is to obtain lung tissue at open or closed biopsy and demonstrate CMV by culture and by histological evidence. An alternative method is the bronchial alveolar lavage. In Section 5.2, we discuss the procedure that should be undertaken with cells obtained by BAL. The most sensitive method is to culture the cells and detect CMV by the rapid early antigen method. However, there will be some false positives if there is contamination by CMV from the upper respiratory tract. The most specific test is to find tissue evidence of CMV by histological, immunologic, or hybridization methods, but all of these lack required sensitivity.

The simultaneous presence in the lung of other pathogens presents difficult problems of differential diagnosis, which may only be resolved after consideration of all available laboratory and clinical data.

The following is a case of CMV pneumonitis following primary infection after renal transplantation who eventually succumbed:

> M.M. was a 23-year-old white female born in 1952. In 1970 she was discovered to have small kidneys. In 1973, she went into renal failure, was diagnosed to have chronic glomerulonephritis, and was placed on hemodialysis. On May 4, 1974, she received a cadaver kidney whose donor had a positive CF titer. Her own CF titer before transplantation was negative.
>
> One month after transplantation, she had an episode of acute rejection that responded to increased steroids. Six weeks after transplantation, she developed fever, rales, and diminished breath sounds in the lower lobe with corresponding diffuse infiltrates by chest x ray. She was treated with oxacillin and ampicillin, which were without effect on her fever. The infiltrates extended to the right lung. An open lung biopsy revealed intranuclear inclusions consistent with CMV. She received adenine arabinoside, 15 mg/kg per day for 4 days. This was discontinued because her white cell count dropped from 4300 to 1500 per mm^3. Azathioprine was discontinued, and cephalothin and gentamicin were started empirically. The patient gradually improved with clearing of infiltrates.
>
> Eleven weeks after surgery, she developed an infected lymphocoel, which displaced the transplanted kidney and grew *Proteus mirabilis*.
>
> The cyst was drained, and she responded temporarily to ampicillin. However, she continued to drain from the abdominal wound, developed an infected pleural effusion (*Pseudomonas*), and had increasing renal failure. Fifteen weeks after transplantation, chest films revealed a new infiltrate in the right lower lobe. She required an arterial–venous shunt for hemodialysis; respiratory distress increased, and the patient expired on August 23, 1974.

> At autopsy the patient's kidneys were extremely small with changes consistent with chronic sclerosing glomerulonephritis. The transplanted kidney showed changes consistent with allograft rejection, focal acute inflammation, and CMV infection. The lungs showed acute and/or severe organizing bronchopneumonia with intranuclear inclusions.

Inclusions were also found in the salivary glands and spleen. This renal transplant recipient died from almost pure CMV infection. This is not common in our experience after kidney transplantation but is consistent with the lethal group described by Peterson *et al.* (1980) and is seen more often in other types of transplantation (see above).

Interstitial pneumonia is a special problem after bone marrow transplantation (Meyers *et al.*, 1982a; Meyers, 1989). It is particularly common in association with graft-versus-host (GvH) reactions characteristic of that type of transplantation.

There is a growing body of evidence that CMV pneumonia in the bone marrow recipient is at least partly an immunopathological disease (Grundy *et al.*, 1987b). (1) Cytomegalovirus pneumonia is strongly associated with graft-versus-host disease in allogeneic marrow transplantation. In contrast, in syngeneic marrow transplantation none of 100 patients developed CMV pneumonia despite a high incidence of infection (Appelbaum *et al.*, 1982). (2) Suppressing the virus by an antiviral agent such as ganciclovir, which has been shown to be effective in other types of CMV disease such as retinitis, does not cure CMV pneumonia in the marrow recipient (Section 14.1.1). Nine out of ten patients went on to die (Shepp *et al.*, 1985).

Grundy *et al.* (1987b) support the pathogenetic role of immunopathology by pointing to three models of murine CMV pneumonia (see also Section 16.3). In the first model, a fatal diffuse pneumonitis in Fl hybrid mice developed only when a GvH reaction in addition to murine CMV (mCMV) infection was produced (Grundy *et al.*, 1985). The GvH reaction was produced by injection of parental spleen cells. In the absence of a GvH reaction, the virus replicated in the lung without producing a pneumonitis. Further ganciclovir could cure the virus infection in the lung without curing the pneumonitis (Shanley *et al.*, 1987). In the second model, immunosuppression by one dose of cyclosphosphamide facilitates the development of mCMV pneumonitis, but additional doses, which presumably prevented development of immunopathology, prevented its development (Shanley *et al.*, 1982). The third model utilized the observation that mCMC replicates in the lungs of T-lymphocyte-deficient athymic nude mice without producing pneumonitis (Shanley *et al.*, 1987). After these mice were reconstituted with syngeneic T cells, mCMV and cyclosphosphamide produced a pneumonitis.

Together these models and the data from humans argue well for the idea that some aspect of cellular immunity is needed to produce a pathological reaction in the lung. The precise pathogenesis is still unclear. Grundy *et al.* (1987b) postulate that T-cell recognition of a virus-coded protein may be involved. The relationship between CMV interstitial pneumonia and GvH reaction first reported by Neiman *et al.* (1977) from Seattle, Washington, is still illustrative and has not been superseded (see Section 13.1). Among 28 recipients with GvH disease, 79% developed in-

terstitial pneumonia with a mortality of 77%. Of 52 patients without GvH disease, 40% developed interstitial pneumonia, and 52% died. Not all cases of interstitial pneumonia were proved to have been caused by CMV, although it was the most common pathogen. In 43 cases, 20 were caused by CMV. Other cases were caused by other opportunistic agents such as *Pneumocystis carinii,* and 40% of cases were idiopathic. Cytomegalovirus was recovered from 57% of recipients with GvH disease, and 43% developed CMV-associated pneumonia. It was recovered from 34% of patients without GvH disease, and 15% developed CMV associated with pneumonia. Diagnosis was usually made by virological studies of an open lung biopsy.

The following case, reported by Dr. P. Neiman of the University of Washington, illustrates some cardinal features of fatal CMV interstitial pneumonia following marrow transplantation:

> V.T. was a 36-year-old male who was found to have acute myelogenous leukemia in January, 1975. A complete remission of his disease was achieved with chemotherapy for a period of 6 months, when malignant myeloblasts reappeared in the marrow and peripheral blood. In June, he was admitted for marrow transplantation from his brother with whom he matched at the major histocompatibility loci. He was prepared for engraftment with 400 mg/m^2 of BCNU, 120 mg/kg of cyclosphosphamide, and 1000 rad of total-body irradiation. He tolerated the intravenous infusion of allogeneic marrow well, and both normal and leukemic cells disappeared from his marrow and peripheral blood over a period of 10 days. Six days after irradiation he developed a perirectal infection and bacteremia. This was successfully treated with gentamicin and carbenicillin and leukocyte transfusions from his marrow donor. Fifteen days following transplantation, recovery of hemopoietic function became apparent. Thirty days after transplantation, the patient began to complain of a pruritic, generalized erythematous rash, which, on punch biopsy, was found to represent grade 11 graft-versus-host disease (GvHD) of the skin. This was followed over the next 10 days by a deterioration in his performance status and a rising serum bilirubin level to 2.5 mg/dl. A percutaneous liver biopsy 40 days after transplantation also showed changes indicating GvHD. Histological examination for CMV inclusions and viral cultures was negative. In addition to the standard weekly dose of 10 mg/m^2 of methotrexate, treatment with rabbit antihuman thymocyte globulin (ATG) was instituted in an intramuscular dose of 8 mg/kg every other day. On this treatment his skin rash improved, but the bilirubin continued to rise to the level of 5 to 6 mg/dl, and he continued to do poorly with respect to nutrition and general performance.
>
> The patient had serial serum samples examined for CF antibody to CMV weekly, which remained negative throughout his course. His marrow donor had a low CF titer to CMV at 1 : 16. Both the patient and donor had negative urine, throat, and buffy coat cultures for CMV at the time of transplantation. Fifty-six days thereafter, CMV was isolated from a buffy coat culture. The antibody titer remained less than 1 : 8. One week later, the patient developed a fever to 103°F and dyspnea. His chest x ray revealed diffuse bilateral interstitial and alveolar infiltrates (see Fig. 13.1). Arterial blood gas examination showed a Po_2 of 42.1, a Pco_2 of 30, and pH of 7.52. The patient had no cough or sputum. An open lung biopsy of the right lung revealed histological findings of advanced interstitial pneumonia with intranuclear inclusions typical of CMV infection. No *P. carinii* was seen. Culture of the biopsy was positive for CMV and negative for other viruses, bacteria, or fungi. Over the next 9 days, the patient's condition progressively worsened with deteriorating respiratory function. Over the last 5 days, adenine arabinoside in a dose of 10 mg/kg per day was administered without apparent clinical benefit. The patient died of inadequate oxygenation and cardiac arrest 74 days following marrow transplantation.

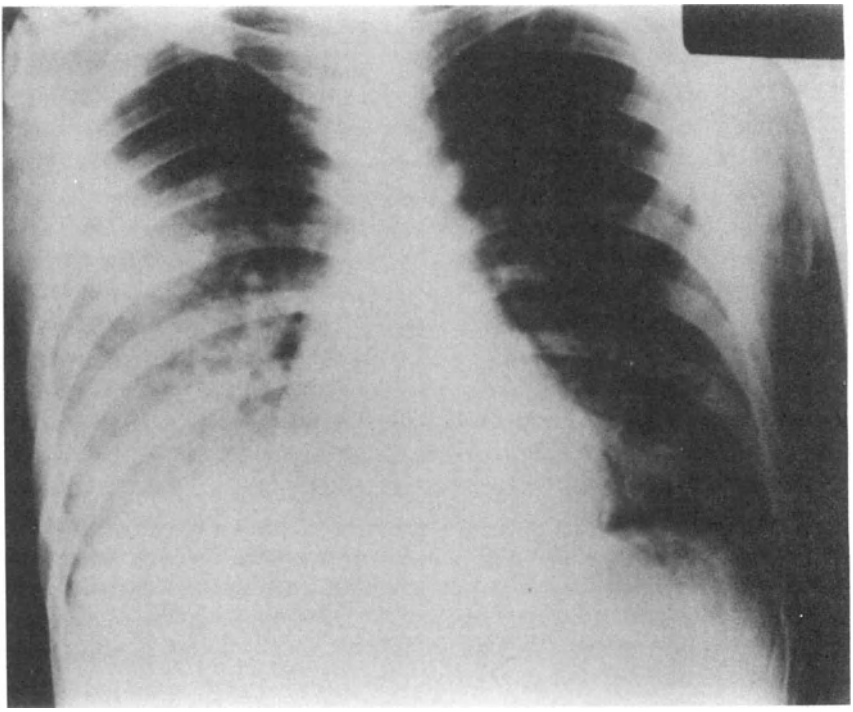

Figure 13.1. Chest x ray from case V.T. on the day of open lung biopsy. Bilateral interstitial infiltrates ar seen, more pronounced on right side. (Courtesy of Dr. P. Neiman.)

This case illustrates a number of features. (1) The patient had a refractory hematological malignancy and intensive exposure to irradiation, cytotoxic drugs and immunosuppressive drugs before and after transplantation. (2) He had heavy exposures to fresh blood products, platelets, and granulocyte transfusions immediately after transplantation, a risk to which other transplant groups are not exposed. (3) Severe GvH disease developed in the skin and liver and may also have involved the lung. (4) Cytomegalovirus was isolated from buffy coat cells before the onset of what appears to be pure CMV pneumonia. (5) The patient failed to respond serologically to the CMV infection, a hallmark of severe adult disease (see Section 13.1.5). (6) Adenine arabinoside was not effective, and ganciclovir would also not have been effective in treatment (Section 14.1.1). This is consistent with more recent data that ganciclovir, an antiviral that is significantly more effective against CMV, is also ineffective (Shepp *et al.*, 1985). The pathological progression of the lung disease characteristic of such patients is seen in Fig. 13.2.

13.3.3. Hepatitis

Hepatitis after CMV infections has already been discussed in terms of pathology, congenital infection, and its occurrence after acquired CMV infection in

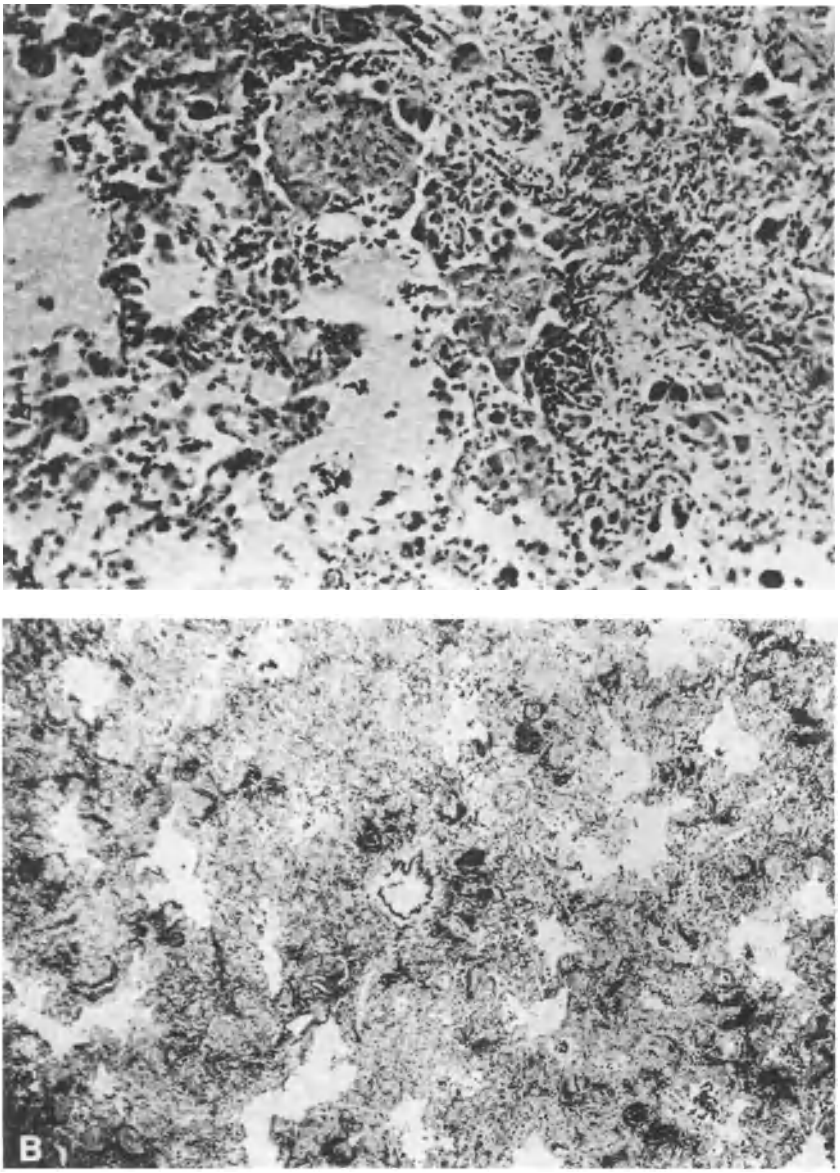

Figure 13.2. (A) An open lung biopsy from a bone marrow transplant recipient with interstitial pneumonia (×160). It shows moderate pulmonary edema (left side) and severe confluent involvement (right side). Many inclusion-body-bearing cells are seen. (B) A lung section (×100) from the autopsy of a marrow transplant recipient with end-stage interstitial CMV pneumonia. There are markedly thickened septa and residual dilated air spaces and characteristic "honeycombing." Graft-versus-host disease probably contributes to this picture. (Courtesy of Dr. P. Neiman.)

the normal patient (see Sections 10.2 and 12.4.2). Although chemical hepatitis is common in febrile mononucleosis caused by CMV, clinically significant hepatitis is not usually a common or serious problem even after transplantation. There are, however, reports to the contrary after renal transplantation, and it is a definite problem after liver transplantation.

Luby *et al.* (1974) noted that in Dallas, 20 of 44 renal transplant patients developed hepatitis, of whom nine (20%) had serologic evidence of CMV infection. Five developed chronic hepatitis, i.e., abnormal liver function tests continued beyond 120 days. Ware *et al.* (1979) reported that 24 out of 150 (16%) transplant patients with CMV infection developed acute liver disease, and 36 (24%) developed chronic liver disease. However, these cases did not have biopsy evidence of CMV hepatitis. It is difficult without such evidence to prove CMV to be the cause of either acute or chronic hepatitis after transplantation, since CMV infection is so common.

Aldrete *et al.* (1975) also reported that 22% of 126 renal transplant patients developed hepatitis, of whom seven had severe disease, and CMV was isolated from blood, urine, sputum, or feces. Autopsies on five of these patients revealed CMV in the liver. This means that 7% of this transplant group developed fatal CMV hepatitis, a finding that should be confirmed at other centers.

The incidence of liver disease in the renal transplant group in Iowa was 9% (Anuras *et al.*, 1977). Nine out of 14 patients developed chronic active hepatitis, most of which was of unknown etiology. Five went on to develop cirrhosis. Of the nine, two patients had acute cytomegalovirus hepatitis. However, the etiologic role of CMV was not clearly proven. That CMV may be the cause of chronic lupoid hepatitis, a form of chronic active hepatitis with immunologic disturbance, has also been suspected (Robson and MacKay, 1969). Cytomegalovirus is not recognized as a significant cause of chronic hepatitis in any age group irrespective of immunosuppression. LaQuaglia *et al.* (1981) reported on a 10-year experience of 405 consecutive renal transplant recipients in Boston. Sixty-two percent of the cases (or 6.5% of the total) were classified by exclusion as non-A and non-B hepatitis. Chronic hepatitis developed frequently with the development of significant morbidity and mortality, but CMV was not implicated

It has been noted in Pittsburgh that acute symptomatic CMV hepatitis may be a peculiar complication of liver transplantation (Bronsther *et al.*, 1988; Singh *et al.*, 1988). Bronsther *et al.* (1988) reported 17 adults and children proven by biopsy evidence. Eight represented primary, and six represented secondary CMV infection. They occurred 15–132 days after transplantation. Nine (53%) patients survived. The most prominent manifestations were prolonged fever and elevation in total bilirubin and liver enzymes. Clinically the most important diagnosis to exclude was rejection, since the method of therapy for CMV hepatitis may be to reduce immunosuppressants, whereas for rejection they are often increased. Another infectious hepatitis we have seen in the posttransplant period is caused by disseminated herpes simplex hepatitis (Taylor *et al.*, 1981). It may be distinguished from CMV hepatitis by occurring sooner after transplantation, by leukocytosis with a shift to the left, and by a higher mortality (S. Kusne, unpublished data). The only way to discriminate

between these different causes of hepatitis is by biopsy. For these and other reasons, periodic "protocol" liver biopsies are commonly taken after liver transplantation.

Paya *et al.* (1989) found 13 (17%) cases of CMV hepatitis in 78 patients after liver transplantation. This contrasts sharply with the experience elsewhere, where CMV hepatitis was found in only 1–4% of patients (Bronsther *et al.*, 1988; Snover *et al.*, 1987; Kusne *et al.*, 1988). One reason may be the prospective nature of this study, in which periodic protocol biopsies were undertaken five times during the first year after transplantation.

Cytomegalovirus hepatitis was more common after primary as opposed to secondary CMV infection. It was significantly more common in seronegative recipients who received a seropositive organ (64%) than in seropositive recipients who received a transplant from either a seronegative (3%) or a seropositive (6%) donor ($P < 0.001$). Cytomegalovirus hepatitis was not fatal or fulminant in any of the cases, and only six were treated with ganciclovir.

The diagnosis of CMV hepatitis was established histologically in 12 of 14 episodes in 13 patients. In two cases, no histological abnormality was seen, but biopsy culture was positive. From 17 histologically specimens from 13 patients, nine were negative when cultured for CMV. This is evidence that culture was not as sensitive as histological examination, and more sensitive virological detection methods may be needed.

13.3.4. Retinitis

Cytomegalovirus retinitis is quite rare in the well adult (Foerster, 1959), although it is well recognized as part of congenital cytomegalic inclusion disease (Christensen *et al.*, 1957). Since 1970, it has been frequently described in the immunosuppressed patient. By 1978, there were 26 case reports, of whom 19 were renal transplant recipients on immunosuppressive drugs (Murray, 1978). By 1980 (Pollard *et al.*, 1980; Egbert *et al.*, 1980), there were 44 patients reported, all of whom except one (Chawla *et al.*, 1976) were immunosuppressed. Most were organ transplant recipients.

However, it is with the emergence of AIDS since 1981 that CMV retinitis, not unlike *Pneumocystis* pneumonia, has achieved epidemic proportions. Cytomegalovirus retinitis has been observed in 15–46% of AIDS patients in the United States (Freeman *et al.*, 1984; Palestine *et al.*, 1984), but it has not been seen in AIDS patients in Africa (Kestelyn *et al.*, 1985). Cytomegalovirus retinitis is usually a complication of disseminated CMV infection and severe immunosuppression. In 20 patients with AIDS, the mean time to death from the time of diagnosis of retinitis was 3.1 months (Buhles *et al.*, 1988).

Cytomegalovirus retinitis is so characteristic that it is one of the few CMV diseases that can be diagnosed clinically without direct viral isolation from the eye. The patient is usually severely immunosuppressed and has evidence of systemic

CMV infection. The virus has been cultured from the vitreous humor (Friedman *et al.*, 1981) and from the retina (Palestine *et al.*, 1984). The patient may be asymptomatic or complain of blurred vision, decreased visual acuity, and scotomata. The course is frequently progressive and irreversible. By funduscopic examination, there are white granular necrotic patches, which may be superimposed with flame-shaped intraretinal hemorrhages. Wyhinny *et al.* (1973) pointed out that adult CMV retinitis is restricted by Bruch's membrane and does not extend to the choroid as in neonates. Viral inclusions may be seen in the retina and retinal pigment epithelium but not in the pars plana.

Egbert *et al.* (1980) described the natural history of CMV retinitis in 11 posttransplantation patients and three patients with lymphoma (Fig. 13.3). Retinitis was actually asymptomatic in seven patients and was discovered by routine funduscopic examination. Both eyes of half of the patients were eventually involved. Their description applies equally well to patients with AIDS. The earliest sign was an opaque white granular patch with ragged edges. Progression usually occurred by a brushfirelike expansion of an original focus. The advancing edge was preceded by faint white dots, which coalesced and formed a new edge. At times, progressive retinitis was accompanied by vascular, usually venous, sheathing, which is a poor prognostic sign. One-third of the patients showed no progression of an initial lesion, which may heal spontaneously. In others, the inflammation may recur after healing of an original retinitis. The infection tended to be in the peripheral retina and to avoid the macula. Nevertheless, central visual acuity was impaired because of cytomegalovirus in half of the eyes in this series. The patients at risk for significant visual loss were those with advancing lesions near the macula. Marked uveitis with secondary cataracts and band keratopathy occurred in only one patient and is also rare in patients with AIDS.

Before 1984, CMV retinitis was not treatable except for modification of immunosuppression in transplant recipients. The use of ganciclovir since then has been extremely important in altering its course (Felsenstein *et al.*, 1985a; Palestine *et al.*, 1986) (see also Section 14.1.1). About 80–90% of treated patients improved as measured by resolution or arrest of progression of retinitis. However, all treated patients regress within 2–10 weeks of therapy. Since oral medication is not available, indefinite intravenous maintenance therapy is advocated. Relapses may occur despite such maintenance, and very often therapy cannot be continued because of leukopenia or anemia resulting from the therapy, the basic AIDS disease, or other therapy such as zudovidine.

Retinitis did not always occur at the time of initial infection a few months after transplantation. It may occur months or years later, and after resolution, relapses were frequent. In this respect, unlike most other manifestations of CMV infection, retinitis is one that remains a risk throughout the period of immunosuppression. It is unknown whether reactivation infection or primary infection is the greater risk for the development of retinitis. In AIDS patients, retinitis is associated with evidence of active CMV infection elsewhere. It is almost always a result of secondary infection, as AIDS patients are rarely seronegative for CMV.

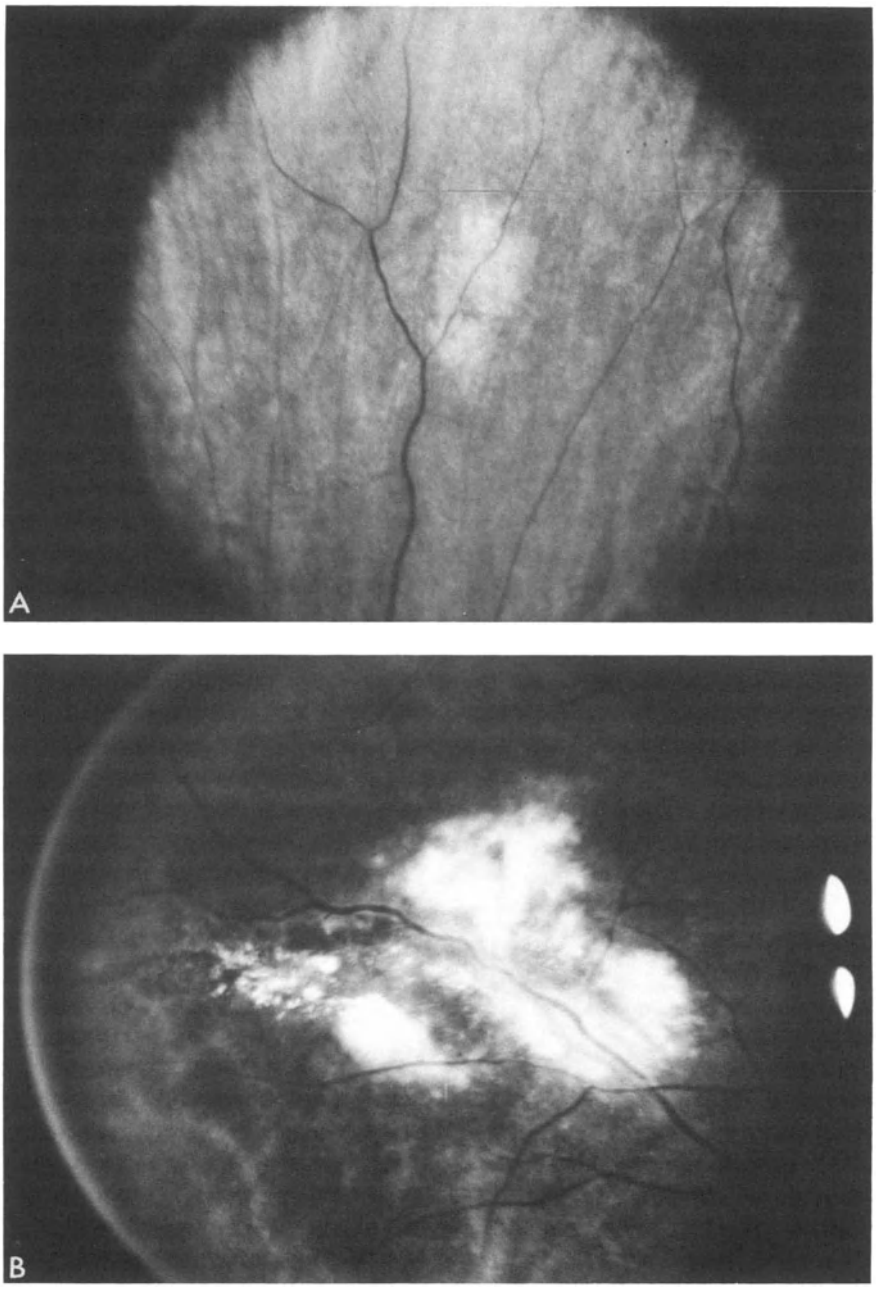

Figure 13.3. (A) An early lesion of CMV retinitis. It expanded from 1 × 2 mm to 6 mm over 3 months before resolving. (B) Brushfirelike lesion of advanced retinitis, with central scar and advancing edges. (Egbert *et al.*, 1980. Courtesy of Dr. P. R. Egbert and by permission from the editors of *The Annals of Internal Medicine*.)

13.3.5. Gastrointestinal Disease

The identification of many disease syndromes in AIDS and immunosuppressed patients caused by CMV in the GI tract strengthens the belief that such syndromes may occur in immunocompetent subjects (see Section 12.4.6). In the immunosuppressed patient, bowel disease caused by CMV ranks among its most frequent manifestations. Peterson *et al.* (1980) described renal transplant patients who developed gastrointestinal hemorrhage or perforation traced to submucosal ulcerations anywhere in the gastrointestinal tract from the esophagus to the rectum. Gastric ulcerations were described in marrow recipients (Strayer *et al.*, 1981). In addition to CMV hepatitis, involvement of any region of the bowel has been noted in other types of transplanted patients, such as liver recipients on cyclosporine and OKT3. Ulcerations are particularly common and may occur as part of disseminated CMV disease (Singh *et al.*, 1988; Kusne *et al.*, 1988).

Reference has already been made to the possibility of developing CMV colitis in immunocompetent individuals following transmission of the virus after passive anal intercourse (see Section 12.4.6; Surawicz and Myerson, 1988). With the onset of the HIV and AIDS epidemic, the gastrointestinal tract from the oral cavity to the anus has also become a common site of clinical abnormalities. This is even more striking in the case of gay males. Large-scale studies indicate that 30% to 90% of AIDS patients develop chronic diarrhea and wasting (Bartlett *et al.*, 1988). In Africa this syndrome is called in some places, such as rural Uganda, "slim disease," characterized by diarrhea and weight loss (Serwadda *et al.*, 1985). In many of these cases it is not possible to determine which virus (e.g., HIV, CMV, HSV), bacterium (e.g., *Campylobacter*, enteric bacteria), parasite (e.g., cryptosporidium, amoeba), or fungus (e.g., *Candida*) or combination of these agents may be responsible. What is clear is that CMV may play an important role, although it is rarely unmistakenly diagnosed in individual patients.

Esophagitis with severe dysphagia and/or odynophagia, with or without other signs of systemic infection, may be caused by CMV (St. Onge and Bezahler, 1982; Pitchenick *et al.*, 1983). Typical radiographic findings based on a study of 16 proven cases include (1) segmented or focal involvement; (2) mucosal granularity, erosions, ulcerations, and irregular thickening of mucosal folds; (3) isolated deep ulcerations; and (4) ulcers that project intraluminally (Balthazar *et al.*, 1987). The most important differential is *Candida* esophagitis and herpes simplex esophagitis. Segmental ulcers and large ulcers are less common in moniliasis. However, it may be impossible to distinguish these three conditions without a biopsy, and one may be required for clear distinction.

In a study of radiologic findings with pathological correlations in 12 AIDS patients with CMV infection of the gastrointestinal tract, Teixidor *et al.* (1987) reported three instances of the involvement of the esophagus, two of the stomach, six of the small intestines, six of the colon, and one of the bile ducts. Others have reported that the colon is most frequent site involved (Knapp *et al.*, 1983; Balthazar *et al.*, 1985; Frager *et al.*, 1986; Meiselman *et al.*, 1985). Of 11 patients with

enteritis and/or colitis, seven had significant lower gastrointestinal bleeding, and five died as a result of it. Radiologic and CT findings included superficial and deep mucosal ulcerations, perforation or fistula formation, luminal narrowing, rigidity and thickening of the intestinal wall, and inflammatory infiltration of the mesentery. Cytomegalovirus inclusions were found in seven out of 12 patients in whom autopsy was performed. Only one patient had clinical pancreatitis. In some cases, flexible endoscopy of the sigmoid colon may suggest pseudomembranous colitis (Gertler *et al.*, 1983). Other presentations show diffusely erythematous and ulcerated mucosa throughout the colon, consistent with Crohn's colitis but also including the stomach and small bowel (Knapp *et al.*, 1983). Multiple submucosal erythematous lesions may also be misinterpreted as Kaposi's sarcoma. Colitis may progress to gangrenous bowl (Meiselman *et al.*, 1985).

As pointed out above, the exocrine portion of the pancreas may be involved with CMV, and occasionally clinical pancreatitis caused by CMV may occur. Acute hemorrhagic pancreatitis is not uncommon after renal transplantation, and a small number of cases have been ascribed to CMV based on histological findings in the pancreas (Tilney *et al.*, 1966; Parham, 1981). Cytomegalovirus pancreatitis has also been documented in AIDS (Teixidor *et al.*, 1987). More often its etiology is undocumented. One of the our patients was a 30-year-old gay, nonalcoholic male who presented with an acute self-limited episode of pancreatitis. He was proven to be HIV seropositive.

Acalculous biliary disease is another manifestation of AIDS whose etiology is frequently unclear, but CMV may be highly suspect.

Blumberg *et al.* (1984) and Kavin *et al.* (1986) described three cases of acalculous cholecystitis in AIDS associated with but not proven to be caused by CMV infection. There were complex multiply infected patients. Saraux *et al.* (1987) described a case of acalculous cholecystitis as the initial manifestation of AIDS:

> A 33-year-old black woman from Zaire presented with a 1-month history of fever, weight loss, and pain in the right upper quadrant. Murphy's sign was positive. HIV western blot and cultures for CMV in blood and urine were positive. Examination of the gallbladder after cholecystectomy showed thickening of the wall but no calculi. Microscopically, CMV inclusions were seen in endothelial and epithelial cells associated with inflammatory cells. Her abdominal symptoms were relieved, but the patient later developed herpes simplex virus and *Mycobacterium avium-intracellulare* infections, which established the diagnosis of AIDS.

This case suggests that any patient with unexplained abdominal complaints suggestive of pancreatitis or cholecystitis should be considered for HIV and CMV infections, and these complications are not limited to gay men.

A related but pathologically different biliary disease in AIDS that may be caused by CMV is papillary stenosis and sclerosing cholangitis (Schneiderman *et al.*, 1987). Patients with right upper quadrant pain, nausea, vomiting, and occasionally fever. Increased hepatic enzyme levels, particularly serum alkaline phosphatase, suggested biliary obstruction. Endoscopic retrograde cholangiopancreato-

graphy showed strictures and dilation of intra- and extrahepatic biliary ducts. Therapy consisted of endoscopic papillotomy. In this series of eight, seven showed evidence of cryptosporidiosis, six were infected by cytomegalovirus, and five showed infection with both. Ampullary biopsy specimens were diagnostic for cryptosporidiosis or cytomegalovirus infection in three. At this time, it is unclear which of these two agents is more important in causing this syndrome. It is possible that both may be involved (Margulis *et al.*, 1986).

13.3.6. Neurological Disease

We have already described the occasional nervous system manifestations of CMV infection in the immunocompetent subject (Section 12.4.5) and in congenital disease (Section 11.4.1). Involvement of CNS by CMV is not common after organ transplantation. Antemortem diagnosis has not usually been possible, and most studies have been postmortem. Schneck (1965) and Bailey *et al.* (1968) described glial nodule encephalitis in renal transplant recipients who had histological evidence of CMV infection in other organs. Dorfman (1973) presented four additional cases of diffuse glial nodule encephalitis, three of whom were in renal transplant recipients. Rare inclusion bodies were found. The clinical findings of cerebral involvement were frequently nonspecific. In retrospect, it is now clear from studies in AIDS patients that CMV glial nodule encephalitis is a distinct pathological entity even if it is still not easy to diagnose clinically (see Section 10.6 and below). It represents one point on the spectrum of CMV neurological diseases than can be found in AIDS.

Cytomegalovirus meningoencephalitis can occur in immunocompetent subjects (see Section 12.4.5), and it can occasionally occur in AIDS. Edwards *et al.* (1985) described a 36-year-old gay male with HIV infection and Kaposi's sarcoma. He had meningoencephalitis, and CMV was isolated from CSF. This was not thought to be the result of "overwhelming" CMV infection and resembled much more what might happen in immunocompetent subjects.

The most common and most devastating neurological syndrome earlier considered related to CMV is the "AIDS dementia complex," also variously referred to as "subacute encephalitis" or "subacute encephalopathy" (Snider *et al.*, 1983; Levy *et al.*, 1985; Navia *et al.*, 1986a,b). Subcortial dementia denotes a diffuse dementia with psychomotor slowing characteristic of this type of patients, and aphasia and apraxia, findings typical of "cortical" dementias, are uncommon early in the course. Of 70 autopsied AIDS patients, 46 suffered from this syndrome (Navia *et al.*, 1986a,b; see Section 10.6). The classical course and picture are quite easily identified. The patients present early with signs of "subcortical dementia," i.e., impaired memory and concentration with psychomotor slowing. In about one-half of the cases, motor deficits or behavioral changes predominate. These include ataxia, weakness, tremor, apathy, withdrawal, and occasionally organic psychosis. Cortical atrophy, frequently accompanied by ventricular dilation, is the most typical

finding by CT scan. The course is usually relentless. Terminally, one observes severe dementia, mutism, incontinence, paraplegia, and occasionally myoclonus.

As pointed out in Section 10.6 on the neuropathology of this condition, this syndrome was earlier thought to be caused by CMV. It is more likely that the classical syndrome is caused largely by HIV, although the possible participation of CMV at least in some cases has not been ruled out (Wiley and Nelson, 1988). Both HIV and CMV frequently infect the brain together. The fact that they can coinfect a single brain cell suggests a possible interaction.

The frequency of finding evidence of CMV infection in the CNS is very similar to that of HIV. Morgello *et al.* (1987), in a study of 107 autopsies of cases of AIDS, found 30 (28%) on the basis of the presence of inclusion bodies. The actual frequency may be considerably higher, as DNA as well as antigen probing have shown that many infected cells show no evidence of inclusion bodies (Wiley *et al.*, 1986; Wiley and Nelson, 1988). The neuropathological processes of CMV infection have already been described (Morgello *et al.*, 1987) (see Section 10.6).

Cytomegalovirus encephalitis is a poorly described clinical entity, and most of the reports are pathological in nature. Two types may be distinguished. (1) There is a group of encephalitis characterized by the presence of glial nodules described above (Dorfman *et al.*, 1973; Section 10.6). No diagnostic clinical picture has emerged. In the series of 30 patients by Morgello *et al.* (1987), microglial nodule encephalitis was asymptomatic in two patients and associated with dementia in five. Dorfman's (1973) cases were characterized by nonspecific neurological signs such as disorientation, psychosis, confusion, diffuse motor weakness with muscle wasting, spasticity, diffuse loss of sensory perception, paresthesias, and absent or hyperactive reflexes. (2) There is a type of periventricular encephalitis characterized by severe involvement of periventicular, ependymal, and subependymal cells similar to encephalitis seen in cytomegalic inclusion disease (see Section 10.6). It may be associated with hemorrhagic and necrotizing foci. This pathological type has been described in AIDS (Morgello *et al.*, 1987; Hawley *et al.*, 1983) ad other immune deficiency states (Kauffman *et al.*, 1979).

> A 61-year-old woman had thymoma and immunoglobulin deficiency. The thymoma was removed 10 years before. The patient's CMV illness began with headaches, went on to right-sided weakness, aphasia, and fifth and 12th nerve lesions. She deteriorated invariably and developed quadriparesis, global aphasia, and coma before death.

The neurological picture of a similar pathologically diagnosed case described by Hawley *et al.* (1983) in an AIDS patient was overshadowed by fever, diarrhea, pneumonia, and severe gastrointestinal hemorrhage. Autopsy revealed a periventricular encephalitis, vasculitis, and cytomegalic inclusions.

Polyradiculopathy associated with Guillain–Barré syndrome has already been described as a complication of CMV infection even in immunocompetent subjects (see Section 12.4), although direct virological proof of etiology has not usually been available. Cytomegalovirus polyradiculoencephalopathy and myelopathy are possi-

bly the best-defined clinical CMV neuropathies in AIDS (Moskowitz *et al.*, 1984; Behar *et al.*, 1987). The following case was described by Behar *et al.* (1987):

> A 33-year-old gay male with AIDS developed difficulty walking, became paraplegic, and had urinary retention. The legs were flaccid and atrophic with diffuse fasciculations, and Babinski's were present bilaterally. Sensation was normal. The CSF showed 99 leucocytes per mm^3; 57% were granulocytes. The patient lost movement of all his legs. He died of pneumonia 5 days after a trial of high-dose steroids. At postmortem the ventral roots of the spinal cord showed numerous cytomegalic cells and dense periventicular infiltrate. In addition, there was CMV infection in the subependymal region, retina, optic nerve, and exit zones of motor nerve roots.

This represents a case of profound polyradiculopathy and CNS involvement in AIDS that is clearly caused by CMV infection. This type of evidence has not so far been available in cases of CMV-associated polyradiculopathy without AIDS (see Section 12.4.5).

Other cases (also called myeloradioculitis) have remarkably similar clinical pictures (Mahieux *et al.*, 1989). Patients often complain of lumbar or radicular pain and progress rapidly to flaccid paraplegia, severe loss of sphincter control, and a rapid death in weeks. In some cases demyelination may be a more prominent picture. Moskowitz *et al.* (1984) described three such patients, one of whom was as follows:

> A 34-year-old Haitian with AIDS and tuberculosis complained of inability to walk and general debility. He had diffuse loss of motor strength and died 3 days after admission. At post, there was severe degeneration of the posterior columns and sparing of the central gray matter. Cytomegalic inclusion bodies were seen.

This case had severe loss of myelin, suggesting a primary demyelinating process.

Virus cultures are of limited help in the diagnosis of CMV disease in the nervous system. Cultures of throat, urine, and even blood are not of diagnostic value because CMV is so often found in these body fluids in AIDS. Fiala *et al.* (1986) found CMV viremia in all AIDS patients tested. Cultures of the spinal fluid may be more significant. It was positive in two out of nine patients tested in a series of 30 cases of CMV encephalitis (Morgello *et al.*, 1987). Both had clinical ascending myelopathy and necrotizing radiculomyelitis at postmortem. However, other such cases may be culture negative (Behar *et al.*, 1987; Wiley *et al.*, 1986). Interestingly, the CSF in such cases may show a preponderance of polymorphonuclear leukocytes. Cytomegalovirus has also been isolated from the CSF in a case of meningoencephalitis (Edwards *et al.*, 1985).

Even virus cultures of brain biopsies or sections may be negative in CMV encephalitis (Nielsen *et al.*, 1984). Morgello *et al.* (1987) cultured five of their 30 cases post-mortem, and only one with foci of parenchymal necrosis was positive. However, attempts at culturing CSF and nerve tissues are to be encouraged because results may be significant. In time, application of newer methods of diagnosis such as hybridization and monoclonal antibody tests to cells and tissues should be considered (see Chapter 5).

13.3.7. Dermatitis

Dermatitis as an accompliment of CMV disease in neonates (Section 11.4), in immunocompetent subjects (Section 12.4.8), and in immunosuppressed patients (Section 10.3) have been described. Please consult these sections.

13.3.8. Endocrine Systems

The involvement of various endocrine organs as part of disseminated CMV disease is well known, but clinical endocrinopathies were rare until the advent of AIDS. The involvement of the ovaries, endometrium, placenta, and thymus are described in Chapter 10. The possible role of CMV in the pathogenesis of type I diabetes has already been described (see Sections 10.3, 11.4, and 12.4.10).

One of the striking pathological findings in autopsies of AIDS patients is the frequent involvement of the adrenal glands. Klatt and Shibata (1988) found that 81 out of 164 patients who died of AIDS had CMV infection, even though in only 10% of all patients was CMV infection the cause of organ failure. The most common site of involvement was the adrenal gland (75%), but clinical evidence of Addison's disease was uncommon. Findings included cortical and medullary necrosis, inflammatory infiltrates, and fibrosis. In only two cases was necrosis extensive, and in each case death resulted from adrenal failure. Glasgow *et al.* (1985) also noted adrenal necrosis in nearly all of 41 autopsy cases without clinical evidence of Addison's disease.

We have seen a case of both anterior and posterior pituitary involvement, although an autopsy was not available (data unpublished). This 32-year-old male AIDS patient developed CMV retinitis, had CMV in his CSF, and developed adrenal insufficiency associated with lack of adrenal corticotropic hormone and diabetes insipidus controlled by vasopressin. It should be pointed out, however, that many AIDS patients develop salt and water disturbances, even severe hyponatremia, without evidence of adrenal insufficiency.

Cytomegalovirus may cause epididymitis (Randazzo *et al.*, 1986). One 33-year-old homosexual AIDS patient had repeated episodes of unilateral epididymitis with fever that were resistant to antibiotics and finally treated with epididymectomy. Inclusions were seen in the ductal epithelium.

Other involvement by CMV in immunosuppressed adults includes a number of pathological findings without necessarily any clear clinical involvement. Oophoritis (Subietas *et al.*, 1977; LiVolsi and Merino, 1979) and thyroditis (Frank *et al.*, 1987) have been described. In an AIDS patient, we have also seen extensive involvement of the thyroid by *Pneumocystis*, which may confuse matters (unpublished data). A syndrome has also been described in AIDS patients consisting of hypercalcemia, suppressed parathyroid activity, and disseminated CMV infection (Zaloga *et al.*, 1985).

13.4. CYTOMEGALOVIRUS INFECTION IN PATIENTS WITH MALIGNANCIES*

Historically, the occurrence of CMV infections in patients with advanced malignancies was noted before such occurrence in transplant patients. Like many opportunistic agents, such as gram-negative bacteria, *Pneumocystis, Candida,* and *Toxoplasma,* CMV afflicts patients with leukemias and lymphomas. Despite a number of studies in such patients, even simple estimates of the prevalence of CMV infection are difficult to make. Variations in the available data result from different methods of measuring CMV infection and the fact that different patient groups are not comparable. They may differ demographically or in terms of the type and stage of cancer they have and the extent and duration of treatment.

Unlike transplantation studies, which have a natural point of beginning at the time of surgery, CMV infection is not usually a problem of early cancer and is studied at various periods of the disease. In general, the longer patients with malignant diseases have been examined for evidence for CMV, the higher was the frequency of infection found.

Another problem not easily overcome in studies on cancer patients is ignorance of the serologic state of the patient before onset of cancer. Hence, it is often not possible to arrive at an estimate of primary versus secondary disease. Without such knowledge, some of the risk factors discussed above, such as the role of possible sources of exogenous infection, cannot be intelligently discussed.

13.4.1. Autopsy Studies

The first association between cancer and CMV was made from autopsy observations. In 1962, Wong and Warner (1962) reviewed 40 cases of C.I.D. in adults reported in the literature and added 14 cases observed over a 5-year period. At least 20 of this collection of 54 cases had neoplastic disease, primarily lymphomas and leukemias. Many patients had received chemotherapy, corticosteroids, or both. The localized form of infection was usually limited to the lungs or gastrointestinal tract. Five of their own 14 patients were living, and CMV infection was diagnosed by biopsies or surgical resections. Others had disseminated disease found at autopsy. Gottman and Beatty (1962) described three children with acute lymphocytic leukemia and one with lymphosarcoma who had C.I.D. at autopsy. All had been treated with cytotoxic drugs or radiation. Cytomegalovirus pneumonia was felt to be the principal cause of one child's death.

During the period from 1953 to 1963, 13 of the 394 (3%) patients at the N.I.H. with acute leukemia who came to autopsy were found to have had C.I.D., most of whom (11) were discovered in the last year of the study (Bodey *et al.,* 1965). Patients with C.I.D. had had more corticosteroid therapy during the last 90 days of

*I (M. Ho) am indebted to Dr. J. N. Dowling for much of this section (Ho and Dowling, 1979).

life than controls, and this excess amount of immunosuppression was thought to underlie the infections.

In a similar study, Rosen and Hajdu (1971) reviewed autopsies on patients with all types of cancer at the Memorial Hospital, New York, during the 12-year period 1957 to 1968. Cytomegalic inclusions were identified in only 0.3% of 5788 cases. Even so, six of the 19 patients identified had Hodgkin's disease, and the others had sarcomas (four), chronic leukemias (four), solid tumors (four), and acute leukemia (one). Again, the risk of hematologic malignancies was clear. The lungs and adrenal glands and the margins of gastric or duodenal ulcers most frequently revealed CMV inclusions.

However, at the same institution in the 2 years of 1971 and 1972, 13 cases of disseminated CMV infection were recognized at necropsy in patients with malignancies (Armstrong and Rosen, 1975). This represented a fourfold increase from 1.6 cases per year in the previous series to 6.5 cases per year. Twelve of the 13 patients had a lymphoma, and ten of these had Hodgkin's disease. The authors believed that the increased prevalence of CMV infection was caused by increased immunosuppressive therapy employed in the latter years.

13.4.2. Cytomegalovirus Infection in Children with Malignancies

Hanshaw and Weller (1961) attempted to measure the prevalence of CMV infection in 50 children with leukemia or lymphoma by examining 98 specimens for viruria. Cytomegalovirus was isolated two or more times from the urine of four of the 50 (8%) patients, three of the 43 children with acute leukemia and one of three with Hodgkin's disease. One of the viruric patients had recurrent fever and respiratory symptoms, and CMV inclusions were found in the lung at autopsy. Two other excreters had no necropsy evidence of C.I.D. In retrospect, it is impossible to say whether these frequencies were abnormal, although the case with CMV disease was.

Benyesh-Melnick *et al.* (1964) examined 515 urines from 101 children and found no difference in the frequency of viruria between children with a malignancy and those with other serious diseases or normal children. Cytomegalovirus was isolated from the urine of seven of 42 (17%) children with acute leukemia, one of nine (11%) with other malignancies, four of 21 (19%) with other diseases, and six of 29 (21%) normal controls. Similarly, Dyment *et al.* (1968) isolated CMV from single urine specimens of two of 97 children with acute leukemias and from none of 50 controls, a difference without significance. In addition, review of autopsy material from 82 leukemic children revealed only two cases (2.4%) with disseminated cytomegalic inclusions. The problem with monitoring for virus excretion is that one may be detecting persistent infection, which occurs even in normal children, and acute episodes of infection may not be revealed. Viruria detects prevalence of infection, and this may not be increased even if incidences of new infections and autopsy evidence of infection are.

Armstrong *et al.* (1971b) studied CF antibody levels as well as virus excretion

in 42 children being treated for acute leukemia, primarily lymphoblastic. Five children (12%) excreted CMV in urine, saliva, or both; CMV could not be isolated from the blood obtained from four of the excreters. Two of these five had one or two negative specimens within a year of diagnosis, followed by virus isolations. One had a 32-fold increase in titer, and the other seroconverted in relation to isolation. In the remaining three excreters, antibody levels were stable. Postmortem examination of two excreters revealed no inclusions in various organs. No clinical events associated with CMV were found. They could find no evidence for a higher rate of CMV infection in leukemias.

Sutton *et al.* (1971) tried to correlate serologic evidence of CMV infection with clinical illness. Leukemic children were followed for a mean of 18.5 months. First, most episodes of fever, pneumonitis, and hepatitis compatible with CMV infection were not caused by CMV based on serologic evidence. Second, there were six out of 27 children (22%) who seroconverted, but only three had illnesses related to the conversion, none of which were severe. Severe illnesses caused by CMV are not to be expected in small prospective studies.

A higher prevalence of CMV infections, including clinically apparent infections, was detected in a more extensive virological study on leukemias by Henson *et al.* (1972a). Eighty-eight children receiving therapy for acute leukemia were followed for 2–16 (average, 8) months. An average of 9.5 urine and throat cultures from each child were examined. Twenty-four (27%) of the 88 children excreted virus, six only in the urine, 11 only in the throat, and seven in both. Of the 24 ultimate virus shedders, six were positive on the first specimens collected. Based on each patient's highest recorded titer, 87% of CMV excreters had indirect hemagglutination antibody to CMV, compared to 39% of nonexcreters, which shows that the antibody-forming capacity is preserved in leukemic children. Children who excreted virus had significantly more episodes of pneumonitis and of rash with fever than nonexcreters. Virus excreters also spent more time in hematologic relapse than did nonexcreters. Episodes of hepatitis or upper respiratory infection and fever were not increased.

If patients are examined early in their course and then followed serologically, an increase in the incidence of CMV infections may also be detected. Sullivan *et al.* (1968) did serial determinations of CMV CF antibody in 41 leukemic children. Eight of the 14 (57%) initially seronegative children underwent seroconversion 1 to 6 (median, 2) months after the initiation of chemotherapy. Four of these eight seroconverters had clinical symptoms suggestive of a viral pneumonia before the rise in antibody, compared to one of six who did not seroconvert. Two of 16 (12.5%) sera obtained before or within 1 month of the initiation of chemotherapy contained CF antibody. Twelve of the 25 (48%) patients whose initial sera were tested more than a month after initiation of chemotherapy were seropositive. These data suggest a significant incidence of primary infection 1–6 months after beginning chemotherapy. The rate is somewhat higher than that found by Cox and Hughes (1975) (see below). The source of virus for these infections is unknown.

In a similar study, Cox and Hughes (1975) studied by serology and virus

isolation 92 children with acute lymphoblastic leukemia. They had CMV CF titers performed at the time of the first clinic or hospital admission and subsequently at variable intervals until seroconversion occurred or the study terminated. Ten percent were seropositive when first tested. Of the remaining 83 patients, 17 (20%) underwent seroconversion while under observation, about 1½–11 months later. The temporal relationship of the initial specimens to the date of diagnosis or beginning of therapy is not clear. Seroconversion appeared to be related to the number of cytotoxic drugs employed for maintenance chemotherapy. Three of 35 (8.6%) children maintained on one or two drugs seroconverted, compared with 14 of 45 (31%) treated with three or four agents. Application of the χ^2 test to these data indicates a significant difference. All seroconverters and initially seropositive individuals and a randomly selected group of seronegative patients had urine cultures for CMV on one or more occasions. Urine was positive for virus in 11 of 25 (44%) seroconverters and four of nine (44%) children who were seropositive when first tested. Only three of the 55 (5.5%) seronegative patients whose urines were cultured were excreting CMV.

Although the rate is not precisely defined, the foregoing studies indicate that children with leukemia are at increased risk for acquiring CMV infection. A relatively small proportion, 10–12%, of such children have antibody to CMV at the time of diagnosis. This figure would vary somewhat depending on the incidence of primary infection in the general population and the age of the patients studied. Of those children without serologic evidence of prior infection, 20–60% undergo seroconversion within a year following diagnosis and institution of therapy. Presumably, this rate of seroconversion far exceeds that which would be found if healthy children of the same age were followed for a similar period of time. However, it is not clear why the incidence of *de novo* CMV infections had varied so widely among studies. A significant factor is the nature and intensity of the therapy employed in the individual case. Another factor, unexplored in this patient group, is the source of the virus. Blood transfusions, interpersonal contact, and even environmental factors should be considered. It should be noted that Cox and Hughes (1975) found that isolation of leukemic children did not prevent CMV infection.

13.4.3. Cytomegalovirus Infection in Adults with Malignancies

There are no data on the incidence of primary and reactivation infections in adults with malignancies. The only systematic study of the prevalence of CMV infection in adults with neoplastic disease is by Duvall *et al.* (1966). These authors collected urine and sputum or saliva specimens from 64 patients over a 2-month period. Cytomegalovirus was isolated from the urine, sputum, or both of 11 of the 32 (34%): five of 15 (33%) with chronic myelogenous leukemia, two of six (33%) with Hodgkin's disease, and four of 11 (36%) selected patients with other malignancies. As would be expected in an adult population, none of 17 family members and ward personnel, who served as controls, yielded virus. In the 11 positive patients, a

total of 54 specimens were examined, and 34 (63%) contained CMV. More culture-positive patients were receiving corticosteroids within 1 month of their initial culture (7/11, 64%) than culture-negative patients (5/21, 24%), a significant difference. Autopsies were performed on five patients with positive cultures, and CMV was isolated from, or inclusions were seen in, four of these. An equal percentage of excreters, nonexcreters, and controls had CF antibody to CMV, although excreters tended to have somewhat higher titers than nonexcreters.

Langenhuysen *et al.* (1974) reported that CMV antibody titers were significantly elevated in adults with Hodgkin's disease compared with healthy individuals matched for age and sex. However, in a number of other studies, no difference has been found in the incidence or magnitude of CMV antibody titers between patients with Hodgkin's disease (Levine *et al.*, 1971; Andersen *et al.*, 1976; Hilgers and Hilgers, 1976), non-Hodgkin's lymphomas (Andersen *et al.*, 1976; Dumont *et al.*, 1976), or leukemias (Andersen *et al.*, 1976) and normal controls. These results, taken together with those of Duvall *et al.* (1966), may indicate that in adults with neoplasia, most of whom are seropositive, little difference in antibody status is appreciated compared to the population at large.

13.4.4. Risk Factors for Cytomegalovirus Infection

At present, the most identifiable risk factor for CMV in children with lymphoma or leukemia is the chemotherapy employed for therapy. In two studies (Sullivan *et al.*, 1968; Sutton *et al.*, 1971) in which children were examined specifically before and after beginning chemotherapy, the rate of seroconversion was high for several months following initiation of the drugs. The prevalence of CMV antibody was increased in children who were not sampled until after chemotherapy had been given, indicating that many of these had already undergone seroconversion. And the rate of seroconversion appeared related to the number of drugs employed. It is interesting that in these circumstances, chemotherapeutic agents are apparently a predisposing factor for primary CMV infection, since the children are initially seronegative. As previously noted, in adults with connective tissue diseases, cytotoxic drug therapy was found to be a risk factor primarily for reactivation of clinically latent CMV infection (Dowling *et al.*, 1976).

In the two series (Bodey *et al.*, 1965; Duvall *et al.*, 1966) retrospective analysis indicated a relationship between corticosteroid therapy and CMV infection. It is difficult to separate the effect of corticosteroids from other therapies or the activity of the underlying disease. In children without malignancies who were on corticosteroids, Chiba *et al.* (1972) reported five out of ten had a fourfold or greater rise in CMV antibody titer, compared with only one of 21 not so treated. Further analysis of this and other putative risk factors will only be possible when primary and secondary infections in patients with malignancies are meticulously distinguished in large prospective studies using serologic and virological methods.

13.4.5. Clinical Manifestations of Cytomegalovirus Infection

At this time, we do not understand what factors make CMV infections symptomatic in cancer patients, including whether primary infections are more severe than secondary ones. In general, clinical manifestations may be more severe than in the normal subject and resemble what has been described for the transplant and other immunosuppressed patients with the exception that AIDS patients have significantly more CMV disease (see Section 13.3). Certain clinical syndromes related to CMV have been identified in autopsy series, studies concerned with the prevalence of infection in patients with neoplasia, and in case reports. These include pneumonia (Gottman and Beatty, 1962; Hanshaw and Weller, 1961; Henson *et al.*, 1972a; Sullivan *et al.*, 1968), rash and fever (Henson *et al.*, 1972a), myocarditis (Bodey *et al.*, 1965), and gastrointestinal involvement (Bodey *et al.*, 1965). Some studies revealed no significant morbidity because of CMV (Armstrong *et al.*, 1971b). Cangir and Sullivan (1966) reported on three children with acute leukemia in whom C.I.D. was found at autopsy and was believed to be the cause of death. The clinical findings in these patients included persistent high temperatures, cough, vomiting, diarrhea, hepatosplenomegaly, rales, and terminal icterus. Evidence of pneumonia appeared 3 weeks after the onset of clinical symptoms, which is also a characteristic of CMV pneumonia in transplant patients (see Section 13.3.2). Abnormal liver function tests were also late findings. In another similar study (Cangir *et al.*, 1967), the same group then identified five children with acute leukemia and CMV disease associated with fever, cough, dyspnea, and roentgenographic evidence of pneumonia. The diagnosis was documented by the finding of intranuclear inclusion-bearing cells in the urine or sputum of only two of the five patients. These patients were treated with fluorodeoxyuridine (see Section 14.1).

Cox and Hughes (1975) attempted to isolate CMV from the blood of 36 children with acute lymphocytic leukemia who were excreting CMV in their urine or saliva. Eleven (31%) had viremia. Three of the 11 viremic patients had clinical disease ascribed to CMV. Two had episodes of chorioretinitis, and one had a CMV mononucleosis syndrome. Of the 21 patients with CMV retinitis reported in the literature through 1977 (not including the preceding two children), one had Hodgkin's disease, and another breast carcinoma; one patient had no predisposing condition, and the remainder were renal transplant recipients (Murray, 1978). Both patients with preexisting neoplastic disease had received cytotoxic drugs, corticosteroids, and radiation therapy. In addition, CMV was shown to be the cause of optic nerve head swelling without retinitis in a patient under treatment for lymphoma (Marmor *et al.*, 1978). A series of 14 patients with retinitis included 11 transplant patients, two with non-Hodgkin's lymphoma, and one with leukemia (Egbert, 1980). If these numbers are any indication, acquired CMV retinitis is almost exclusively a disease resulting from the immunosuppression of AIDS, transplantation, or lymphoreticular malignancies, and the latter is, by comparison, a less frequent cause.

Abdallah *et al.* (1976) described 16 patients in whom CMV pulmonary infection was diagnosed over a 3⅓-year period at Stanford. Seven of the 16 patients had

underlying malignancies including lymphoma (four), leukemia (two), and solid tumor; the remainder of the series were transplant recipients. Thirteen of the 16 patients had other pathogens found in addition to CMV. Two of the three patients whose pneumonitis was caused by CMV alone were diagnosed ante mortem by lung biopsy. One had an underlying lymphoma, and the other leukemia. Both died of progressive respiratory failure within 2 to 3 weeks of biopsy, and CMV was isolated from lung at autopsy without any other organisms being found. Thus, CMV alone can cause clinically significant pneumonia in patients with underlying malignancies, although, as in the case of retinitis, it seems to be less common than in the transplant group.

Whether CMV infection can affect the outcome of the underlying malignancy also requires further clarification. Two studies suggest that children with acute leukemia infected with CMV spend a greater amount of time in hematologic relapse than those not infected (Bodey *et al.*, 1965; Henson *et al.*, 1972a), but another study denies this (Armstrong *et al.*, 1971b). An alternate explanation is that relapse, or the therapy employed to reverse it, increases the patient's susceptibility to CMV. This problem is similar to the relationship between CMV and graft rejection, except that few studies have looked at it rigorously.

13.5. CYTOMEGALOVIRUS INFECTION ASSOCIATED WITH MISCELLANEOUS CONDITIONS

Cytomegalovirus infection seems to be a fellow traveler and opportunist *par excellence*. The discovery of the agent was intimately associated with congenital syphilis (see Section 1.1). The congenital disease may occur with obvious immunodeficiency (see Section 9.4.2). This chapter has dealt with the association of CMV infection with immunologically compromised patients, particularly those who have received an organ transplant or who have a malignancy or AIDS.

Many other conditions predispose to infection, and in most cases it is suspected that immunosuppression is involved. McCordock and Smith (1934) reported cytomegalic inclusions in 18 out of 40 (45%) children who died of pertussis. They even suspected that the virus might be etiologically associated with pertussis. When Medearis (1957) reviewed 132 cases of C.I.D., he noted that 31 were associated with pertussis. The association was particularly strong in those cases with primary pulmonary manifestations. Olson *et al.* (1970) found that 33% of the children with pertussis had CMV in their respiratory secretions (see Section 6.2.3). The reason for this association was never clarified. Seifert and Oehme (1957) also report the association of C.I.D. and cystic fibrosis.

Cytomegalovirus may be associated with pathology peculiar to a locality. In South Africa, inapparent infection in the salivary glands and disseminated disease may be found in infants with congenital biliary cirrhosis or with kwashiorkor (Wainwright, 1972). The important role of malnutrition as an immunosuppressant that may predispose to primary and reactivation infection and dissemination is an

important possibility in man. A low-protein diet and mCMV infection in neonatal mice acted synergistically to produce immunosuppression, morbidity, and death (Cruz and Waner, 1978).

We have already discussed the possible etiologic association of CMV infection with diabetes mellitus (Sections 11.4 and 12.4.10), Alzheimer's disease (see Section 12.4.5), Kaposi's sarcoma (see 5.7), carcinoma of the prostate, carcinoma of the colon (see Section 5.7), ulcerative colitis (see Section 12.4.6), gastrointestinal hemorrhage (see Section 12.4.6), lupoid hepatitis (Section 13.3.3), and Hodgkin's disease (Section 13.4.3).

Ranchod and Bissell (1979) report a 5-year-old boy with acute lymphoblastic leukemia who died with pulmonary alveolar proteinosis and CMV infection. The CMV was suspected to have caused the proteinosis.

Tindall *et al.* (1978) found that of 13 patients with myasthenia gravis, 48% were seropositive for CMV, whereas only 24% of 19 controls were positive. The thought was that persistent viral antigenic stimulation in the thymus was instrumental in producing autoimmune antibodies against acetylcholine-receptor-bearing thymic cells. Further epidemiologic as well as theoretical evidence is needed to prove this hypothesis.

Infantile spasm lasting 10–15 days appeared in three infants who were routinely immunized. Two had serologic evidence of CMV infection (Midulla *et al.*, 1976). Ménétrier disease, a hypertrophic gastritis, and protein-loss malnutrition occurred in a 3½-year-old boy. Cytomegalovirus inclusions were found in the gastric glands. (Leonidas *et al.*, 1973).

Cytomegalovirus infection has frequently been associated with a number of other agents. In postwar Europe, *Pneumocystis* pneumonia was observed in 23 out of 25 consecutive autopsy cases of C.I.D. in children under 1 year of age (Seifert, 1954). The association was stronger in the more generalized cases. The two agents have also been frequently associated in immunosuppressed adults, particularly in patients who have an underlying malignancy, hematologic disorder, connective tissue disease (Symmers, 1960), or AIDS (see Section 13.2.2). *Pneumocystis,* like CMV, is an opportunist in all three conditions, particularly if patients have been treated with immunosuppressive agents (Walzer *et al.*, 1974). Wang *et al.* (1970) even suggest that CMV may grow in *Pneumocystis.* Whether the two agents are coincidentally present or whether parasitism with one synergistically facilitates parasitism with the other is not known.

Toxoplasma, also an opportunistic intracellular parasite (Ruskin and Remington, 1976), has likewise been observed in infections together with CMV. Gelderman *et al.* (1968) produced doubly infected fibroblasts *in vitro.* Vietzke *et al.* (1968) described two cases of double infection, both of whom suffered from cancer. Vinh *et al.* (1970) studied nine cases of congenital toxoplasmosis and 44 cases of congenital C.I.D. Two cases of combined infection were found.

Mueller *et al.* (1988) found an association of testicular carcinoma with high antibody titers against varicella–zoster virus, herpes simplex virus, and CMV. The strongest association was with CMV. The reason for this association is unknown.

14

Treatment and Prevention of Cytomegalovirus Infections

14.1. TREATMENT OF CYTOMEGALOVIRUS INFECTIONS

Treatment of systemic virus diseases is still largely experimental, although rapid progress is being made. The basic problem has been that putative antiviral agents may not be effective enough or are too toxic. Evaluation of the effect of these drugs may also be difficult. Frequently, the disease that is treated has a variable course, and the demonstration of efficacy of an antiviral may not be possible except by a blind, controlled study in which the drug-treated group is compared to a placebo-treated group. Such studies are expensive to undertake, and although the knowledge gained is usually solid, it is often limited. On the other hand, when a drug seems to be effective, controlled studies are also difficult to undertake.

The treatment of virus infections in the immunosuppressed patient also presents special problems. In bacterial infections, the efficacy of certain antibiotics may be depressed because ancillary host defenses are essential for optimum effect of the drug. In similar situations, antiviral agents may also have diminished effectiveness because of inadequate host defenses. Concurrent infections from other causes may complicate the picture. Compromised renal or other organ function may alter the pharmacodynamics of drugs tested. Frequently, the immunosuppressive properties of antiviral agents themselves may accentuate their considerable toxicity.

In addition, CMV seems to present special problems. As a DNA herpesvirus, it would be expected to respond to the same antivirals as herpes simplex. However, in almost every case, the susceptibility of CMV seems to be less, whether tested in cell culture or in the host. This is partly because of the lack of virus-specified thymidine kinase (see Section 4.4.2), which can phosphorylate and activate nucleoside antivirals (see below). Even under the best circumstances, as in the case of ganciclovir,

the antiviral may suppress CMV during treatment but fail to cure it, such that virus infection reappears after the drug is stopped.

14.1.1. Nucleoside Antivirals

Treatment of systemic virus diseases, especially those caused by the herpesviridae, has made rapid progress in the last 10 years. Nucleoside antivirals, the most important and approved one being acyclovir, have been shown in extensive clinical trials to be effective against herpes simplex types 1 and 2 and varicella–zoster viruses (Shaeffer, 1982). Nucleosides discovered so far are less effective against CMV, but a compound closely related to acyclovir, ganciclovir, has been shown to be effective in suppressing CMV replication in patients and ameliorating retinitis (see below). However, the ideal nucleoside antiviral against CMV is still not at hand.

Different nucleoside antiviral agents have been used in CMV infections in normal and in immunosuppressed subjects for over 20 years. Cangir *et al.* (1967) treated five children with acute leukemia with fluorodeoxyuridine. Two had CMV pneumonia demonstrated by intranuclear inclusions in cells of sputum, and three were suspected of having the disease. The dosage used was the same as for the treatment of acute leukemia. The patients showed "prompt" clinical response with eventual complete resolution of their pneumonitis. Nausea, vomiting, diarrhea, and bone marrow suppression were noted. It is difficult to know whether these patients had CMV disease and whether the response to the drug was based on its antileukemic or antiviral effect.

The experience with cytosine arabinoside (ara-C), collected mostly by measurement of viruria in congenital CMV disease, is that it will suppress virus concentration but rarely cure the infection. Clinically the patient usually showed no response. Doses that could consistently suppress viruria or even cure it (15 mg/kg) were too toxic for routine administration (McCracken and Luby, 1972).

Idoxuridine also decreased viruria in two of three patients with congenital CMV disease, but the clinical response was disappointing (Barton and Tobin, 1970).

Adenine arabinoside (ara-A), the antiherpes agent licensed for treatment of herpesvirus encephalitis (Whitley *et al.*, 1977), is also, unfortunately, not very effective against human CMV. Ch'ien *et al.* (1974) treated five patients with congenital CMV disease, two with mononucleosis, and five immunosuppressed renal transplant recipients with 5–20 mg/kg intravenously for 6 to 18 days. In the nonimmunosuppressed infants and patients with mononucleosis, viruria was temporarily suppressed by the treatment but returned 1 to 3 weeks after it was stopped. In immunosuppressed patients, viruria was only reduced in titer, and viremia was not affected. They did not improve clinically. One patient cultured after death still had CMV in many tissues. On the other hand, two infants and two patients with mononucleosis improved. No significant toxicity was observed. Thus, ara-A is

ineffective in eliminating CMV in the immunosuppressed, and its transient virostatic effect appears to be without clinical benefit. Rytel and Kauffman (1976) saw no benefit from ara-A given to two renal transplant patients with CMV pneumonitis and one with retinitis. At 5–10 mg/kg, they observed hematologic toxicity. Fourteen cases of clinically evident CMV infection were treated with 10 mg/kg ara-A per day for 5–7 days by Marker *et al.* (1980). No therapeutic effect was noted, and four developed dramatic neurological signs, including tremors and mycoclonus, which may be side reactions more common in the immunosuppressed.

Pollard *et al.* (1980) reported beneficial effects of up to 20 mg/kg ara-A for 5–14 days for several courses. In five out of seven patients, lesions and inflammatory activity in the retina improved along with quantitative decreases in urinary virus excretion. Two patients failed to respond at all. Additionally, ara-A in elevated doses produced significant nausea, vomiting, diarrhea, suppression of the bone marrow, and neuralgia. Granulocytopenia and thrombocytopenia led to sepsis, hemorrhage, and death in one patient.

Nucleoside analogues obtained in the 1970s had more selective antiherpes activity because they relied on virus-coded thymidine kinase present in infected cells to be transformed to the active 5'-monophosphate and from there to the active di- and triphosphate forms.

The development of 1-(2-deoxy-2-fluoro-β-*d*-arabinofluranosyl)-5-methyluridine (FAMAU), 1-(2-deoxy-2-fluoro-β-*d*arabinofuranosyl)-5-iodocytosine (FIAC), 1-(2-deoxy-2-fluoro-β-*d*-arabinofuranosyl)-5-iodouridine (FIAU), 1-(2-deoxy-2-fluoro-β-*d*-arabinofluraosyl)-5-ethyluridine (FEAU), (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), and 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) generated much interest (Verheyden, 1988).

The most effective licensed antiherpes agent to emerge from this group is the nucleoside acyclovir (acycloguanosine), an acyclic analogue of 2′-deoxyguanosine that is selectively phosphorylated by the thymidine kinase of herpes simplex virus (HSV), and its triphosphate is a potent inhibitor of herpesvirus DNA polymerase (Elion *et al.*, 1977; Schaeffer *et al.*, 1978; Furman *et al.*, 1979), including CMV DNA polymerase (St. Clair *et al.*, 1980; Mar *et al.*, 1981). It is effective against herpes simplex types 1 and 2 topically, orally, and parenterally. However, since CMV does not code for specific thymidine kinase (Estes and Huang, 1977) (see Section 4.4.2), acyclovir triphosphates are not readily formed in CMV-infected cells, and acyclovir is not effective against CMV in cell cultures. Only at acyclovir concentrations greater than 100 μM was there a greater than 50% reduction in plaque formation by clinical isolates (Lang and Cheung, 1982).

As a therapeutic agent for CMV infections, acyclovir has also been a failure. It was found ineffective against CMV pneumonia in bone marrow recipients (Meyers *et al.*, 1982b; Wade *et al.*, 1983).

Of this group of compounds, FIAC was the only nucleoside that inhibited CMV replication at a nontoxic concentration for cells (0.6 mM), and FMAU was effective at 1.0 μM (Colacino and Lopez, 1983). FIAC was postulated to act by better utilization of its triphosphate by viral rather than by host DNA polymerase

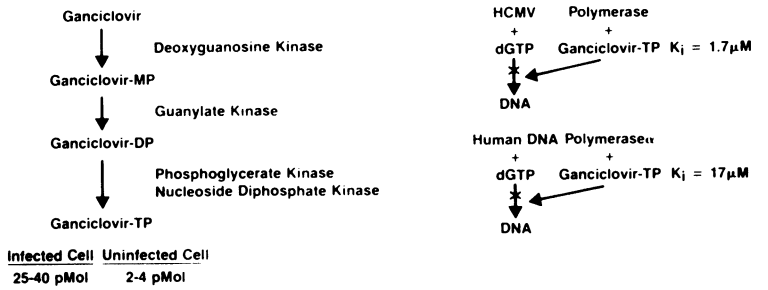

Figure 14.1. Cellular metabolism and mechanism of action of ganciclovir. The intracellular metabolism of ganciclovir is shown at left. The enzymes catalyzing the successive phosphorylating steps are indicated, as well as the concentrations of ganciclovir-5'-triphosphate (ganciclovir-TP) generally achieved in uninfected and cytomegalovirus (hCMV)-infected cells. The effects of ganciclovir-TP on DNA polymerase are shown at right. Ganciclovir-TP competes with deoxyguanosine triphosphate (dGTP) for binding to DNA polymerases, producing the indicated K_i values for human DNA polymerase α and hCMV polymerase. MP and DP, monophosphate and diphosphate, respectively. (From Matthews and Boehme, 1988; by permission of Dr. Thomas Mathews and the University of Chicago Press.)

(Colacino and Lopez, 1985). However, both FMAU and FIAC were virostatic. Viral DNA replication resumed on removal of the drug.

Ganciclovir {[9-(1,3-dihydroxy-2-propoxy)methyl]- guanine; DHPG} is an acyclovir nucleoside synthesized in 1982 and is a potent inhibitor of the herpesviridae (Ashton *et al.*, 1982; Cheng *et al.*, 1983; Field *et al.*, 1983). Human CMV is inhibited at concentrations of ganciclovir of 10 μM or less (Cheng *et al.*, 1983; Field *et al.*, 1983). Other viruses inhibited in decreasing order of effectiveness are HSV, EBV, varicella–zoster virus, murine CMV, and guinea pig CMV (1 to 70 μM; Matthews and Boehme, 1988). Ganciclovir is unique among the nucleosides in being unusually effective against human CMV.

The action of these nucleoside analogues depends on the formation of the triphosphates, which inhibit viral DNA synthesis by inhibiting the viral polymerase. The rate-limiting step in the formation of triphosphates (TP) is the formation of the monophosphates (Fig. 14.1). Despite the fact that CMV does not code for a viral thymidine kinase (Estes and Huang, 1977) as does HSV, which facilitates the formation of ganciclovir monophosphates intracellularly (Smee *et al.*, 1985), ganciclovir-TP levels in CMV infected cells are significantly higher than in uninfected cells (Freitas *et all.*, 1985). They are also higher than acyclovir triphosphate levels in CMV-infected cells (Biron *et al.*, 1985), which partly explains why ganciclovir is more effective against CMV than acyclovir.

The phosphorylation of ganciclovir in CMV-infected cells is thought to be mediated by host enzymes that are induced during infection. A deoxyguanosine kinase is thought to be one of these host enzymes induced by CMV infection. It catalyzes the formation of ganciclovir monophosphate (Lewis *et al.*, 1984; Meijer *et al.*, 1984; Smee, 1985; Smee *et al.*, 1985). Ganciclovir diphosphates and triphosphates are formed by other cellular kinases.

Ganciclovir-TP is a potent inhibitor of CMV DNA polymerase. It acts by competitively inhibiting the incorporation of deoxyguanosine triphosphate (dGTP) into DNA, hence terminating DNA chain elongation (Biron *et al.*, 1985; Freitas *et al.*, 1985; Mar *et al.*, 1985a,b). The mean 50% inhibitory dose of ganciclovir for different isolates from patients ranged from 0.54 to 5.9 μM (Shepp *et al.*, 1985; Felsenstein *et al.*, 1985a,b; Mar *et al.*, 1983; Tymus *et al.*, 1984; Plotkin *et al.*, 1985a, Cole and Balfour, 1987).

One of the most gratifying findings in antiviral therapy was the efficacy of ganciclovir against CMV retinitis (Felsenstein *et al.*, 1985a,b; Bach *et al.*, 1985). Masur *et al.* (1986) used 7.5–15 mg/kg per day intravenously in three divided doses. Improvement or stabilization of lesions occurred in six of seven patients while on therapy. Urine or blood cultures become negative. Holland *et al.* (1986, 1987) treated CMV retinitis of 40 patients with AIDS with 7.5–10 mg/kg per day for 14–20 days. Thirty-one (97% of evaluable patients) had objective evidence of improvement. However, reactivation of disease occurred even in 50% of 20 patients who had more than 3 weeks of therapy. All ten who responded initially but stopped reactivated. Neutropenia developed in nearly 40% of patients. To prevent relapses, most authors administer single doses of 5–6 mg/kg 5–7 days per week (Mills *et al.*, 1988). Intravitreal administration of ganciclovir has also been reported to be effective (Henry *et al.*, 1987).

Although these anecdotal reports are unanimous in reporting suppression of CMV retinitis by ganciclovir, efficacy has not yet been proven in controlled studies. No studies of interaction between ganciclovir and zidovudine (AZT) are available, although very likely leukopenia is a greater hazard when both marrow-toxic agents are combined.

Ganciclovir has also been found to be effective in suppressing CMV disease in the gastrointestinal tract, which as already pointed out is frequently a serious problem in AIDS patients (see Section 13.3.5). For example, Dietrich *et al.* (1988) treated 69 AIDS patients with CMV disease of the gastrointestinal tract. The sites of involvement were colon (67%), esophagus and stomach (22%), rectum (7%), liver 3%), and small bowel (1.4%). Ganciclovir was given intravenously at 20 mg/kg in two divided daily doses for 14 days. Maintenance therapy consisted of 6 mg/kg daily. Improvement in clinical response was seen in 75%, stable response in 13%, and no response in 12%. Urine cultures became negative in 69%. Moderate leukopenia was observed in seven patients, and severe leukopenia in three. The clinical response lasted a minimum of 4 weeks from start of therapy. These results are comparable to the effect of ganciclovir on CMV retinitis but quite different from the effect of ganciclovir on pneumonia (see below).

Early treatment of CMV interstitial pneumonia with ganciclovir was disappointing. For example, Shepp *et al.* (1985) treated ten such patients, from whom CMV was eliminated from the respiratory secretions after 8 days. However, only one patient survived.

Some recent data on the treatment of CMV pneumonia and other systemic disease caused by CMV are somewhat more encouraging. Erice *et al.* (1987) report

that 17 (55%) of 31 immunosuppressed patients with various types of CMV disease who received 2.5 mg/kg i.v. every 8 hr improved clinically during ganciclovir therapy. Viremia ceased in 93% (14/15) of patients after 4.7 days of therapy. Unfortunately, in these anecdotal trials it is difficult to document efficacy because a control group is not available for comparison. Let us consider only the case of CMV pneumonia in marrow recipients, for which there are a great deal of historical data. In this study, 11 such patients were treated, and five (45%) were said to improve with therapy, while six did not. However, three of the five eventually died, giving a mortality rate for the total group of 82% (9/11), which is the same as the mortality for untreated CMV pneumonia in marrow recipients (84%). As for the favorable responses in CMV disease in other types of transplant recipients, such as CMV pneumonitis in renal transplant recipients or gastrointestinal disease or hepatitis, there are insufficient historical data to be sure that untreated patients would not have fared as well.

The potentially important observation has been made in anecdotal studies that the combination of ganciclovir and immune globulin may be effective in treating CMV interstitial pneumonia in marrow recipients (Reed *et al.*, 1988; Emanuel *et al.*, 1988). Reed *et al.* (1988) reported from Seattle on 25 consecutive patients proven by biopsy or BAL to have CMV interstitial pneumonia and treated with 7.5 mg/kg ganciclovir per day for 14 days and CMV immunoglobulin, 400 mg/kg on days 1, 2, and 7 and 200 mg/kg on day 14. Thirteen (52%) of these patients survived, which was significantly better than the survival of 89 historical controls (15%, $P < 0.001$). Emanuel *et al.* (1988) reported similar results on ten patients and 11 controls from the Sloan Kettering Institute in New York. The treated group was given ganciclovir, 7.5 mg/kg per day for 20 days, and immunoglobulin twice a week for four or more weeks. The controls received either ganciclovir or immune globulin alone. None of the controls survived, whereas seven (70%) of the treated patients survived ($P < 0.001$). The inadequacy of antiviral treatment alone in interstitial pneumonia in marrow recipients suggests that viral cytopathology is not the sole pathogenetic factor in this disease and that there is an immunopathological element (see Sections 13.3.2 and 16.3). How immunoglobulin functions in this setting is unclear. One theory is that it neutralizes a viral antigen on lung cells recognized by cytotoxic T cells, which produce immune pathology (Grundy *et al.*, 1987b; Frank and Friedman, 1988).

These results should be confirmed by better-controlled studies. In addition, the conclusions should not be generalized to CMV pneumonitis in other types of patients, in whom the pathogenesis may well be different.

With the extensive use of ganciclovir, problems of resistance have already arisen. Erice *et al.* (1989) report recovery of three strains of CMV resistant to ganciclovir from the blood of three patients on prolonged ganciclovir therapy. All were immunosuppressed patients, one a woman with leukemia and two men with AIDS who eventually died with virological evidence of CMV disease. In each of the three patients, resistance evolved by a different mechanism. In the first patient, the initially isolated strain was already relatively resistant to ganciclovir. In the second

patient, an initially sensitive strain became resistant. The genetic identity of the two strains was proven by restriction enzyme profile. In the third patient, a genetically different resistant strain emerged.

Development of resistance to ganciclovir is paralleled by development of resistance of herpes simplex virus to acyclovir. Such resistance may result from single base changes in virus-coded thymidine kinase or DNA polymerase genes (Larder and Darby, 1984; Larder *et al.*, 1987; Kit *et al.*, 1987). The mechanism of resistance of CMV to ganciclovir has not yet been elucidated.

14.1.2. Phosphonoformic Acid

Phosphonoformic acid (PFA, Foscarnet) appears to be an equally effective but less toxic analogue of phosphonoacetic acid (PAA). Both drugs selectively inhibit viral DNA synthesis and are noncompetitive inhibitors of herpesvirus-induced DNA polymerase (Huang *et al.*, 1976; Leinbach *et al.*, 1976; Mao *et al.*, 1975; Helgestrand *et al.*, 1978; Cheng *et al.*, 1981; Sabourin *et al.*, 1978). When clinical studies showed PAA to be retained in bone, further trials were stopped. Phosphonoformic acid also accumulates in bone, but no toxic effects have been observed, and PFA is not found in bone marrow (Helgestrand *et al.*, 1980).

Phosphonoformic acid has been found to be promising against CMV infection in anectodal reports. It must be given intravenously at 3–7 mg/kg per hour for 4–21 days in order to maintain a plasma level of 30–50 μg/ml (100–165 μmol/liter). Klintmalm *et al.* (1985) treated six immunosuppressed patients with life-threatening CMV infection in this manner and observed favorable responses in five. Ringden *et al.* (1986) treated 57 episodes of CMV infection in 13 bone marrow and 33 renal transplant recipients. The patients received courses ranging from 2 to 399 g. Improvement, indicated by eradication of CMV, was seen in 57% (8/14), resolution of fever in 50% (11/22), and laboratory improvement was noted in 70%. However, 48% (12/25) of the patients died. Adverse effects such as reduced hemoglobin, decreased renal function, and increased serum calcium were observed in a few patients. Anecdotal reports also indicate response of CMV retinitis in AIDS to PFA. No cross resistance occurs with ganciclovir. This drug clearly awaits controlled evaluation and may well be an alternative to ganciclovir, although like it, it is a suppressive and not a curative drug.

14.1.3. Other Antivirals

Interferon, a natural antiviral protein made from human leukocytes or other virus-infected cells, is not very effective in suppressing CMV infection either in cell cultures or in laboratory animals. Cytomegalovirus is more resistant to interferon than other viruses, such as Sindbis, vaccinia, and vesicular stomatitis virus (Glasgow *et al.*, 1967). It has also been shown that if infected cells rather than free

virus are used as the challenge in an interferon assay, they are up to 300-fold less sensitive (Holmes *et al.*, 1978). The reason may be that such cells continuously release virus, which can overcome the interferon-protected cells. This suggests that virus transmitted by infected cells in the body may be particularly resistant to interferon. However, Postic and Dowling (1977) showed that the prototype Davis strain and six clinical isolates were all sensitive to interferon, although less so than vesicular stomatitis virus.

Arvin *et al.* (1976) treated five patients with neonatal CMV infection with 1.7–3.5 $\times$ 10^5 units/kg intramuscularly for 7–14 days. One infant had transient suppression of viruria. Emodi *et al.* (1976) suppressed viruria in three of four infants with congenital CMV infection and in two of five patients with acquired infections by administering 500,000 to 1 million units of interferon per day. Viremia was not suppressed. In two patients, the lymphocyte response to phytohemmagglutinin was suppressed, apparently as a result of the immunosuppressive effect of interferon. Arvin *et al.* (1976) noted slower weight gain, transient elevation of SGOT, and fever in the treated babies. Interferon, at least in the doses given, was inadequate in controlling CMV infection.

Meyers *et al.* (1980a) treated CMV pneumonia in six bone marrow recipients with 6 to 50 million units of recombinant α-interferon per day. Fever and gastrointestinal side effects were seen in two patients each. Hepatic function abnormalities were observed in all. However all six patients succumbed, and four were proven to have CMV pneumonia at autopsy. Interferon is not effective for treating CMV infection, at least not as single therapy.

Wade *et al.* (1983) treated 13 bone marrow recipients with 500 and 1000 mg/m^2 and doses of leukocyte interferon between 2 $\times$ 10 and 4 $\times$ 10^5 units/kg per day. Ten of the 13 patients died of pneumonia, and CMV was isolated from all patients. Hence, the combination of interferon and acyclovir was also not effective against CMV pneumonia.

Transfer factor can transfer delayed hypersensitivity of the donor to a recipient. When one uses it as a therapeutic agent, one hopes that specific cellular immunity is also transferred. It is prepared from peripheral white cells, and the active component is a dialyzable substance with a molecular weight less than 10,000 (Lawrence, 1976). The precise mechanism by which transfer factor works is unclear. It may work by inhibiting suppressor cells, by increasing helper activity, or by providing specific information to lymphocytes.

Rytel *et al.* (1975) treated one renal transplant recipient suffering from CMV retinitis with transfer factor (TF) that was prepared from lymphocytes of a donor who recovered from CMV mononucleosis and who had cell-mediated immunity to CMV as assayed by lymphocyte transformation and migration-inhibition factor. After 20 doses of TF, the patient's retinal inflammation became inactive, and the patient's viruria was reduced but not eliminated.

Thomas *et al.* (1977) injected two doses of TF from CMV-seropositive donors into three infants with symptomatic congenital CMV infection. The efficacy of the injection was monitored by conversion of the tuberculin and/or *Candida* skin tests.

After injection, there was a short period of time when occasional negative urines were noted, whereas previously all urines contained CMV. No clinical change was noted.

Another interesting anecdotal report comes from Jones *et al.* (1981). Based on the facts that transfer factor is not adversely affected by gut enzymes and that transfer of specific immunity is thought to occur by oral absorption of lymphoid cells during breast feeding, these authors gave transfer factor orally to a 4-year-old boy suffering from chronic CMV disease. The transfer factor was prepared from blood leukocytes of a cow that had skin sensitivity to bovine rhinotracheitis virus, a virus homologous to CMV. Prior to treatment, the patient had persistent viruria and an absent lymphocyte proliferative response to CMV antigen. After treatment, his lymphocytes became responsive, viruria stopped, and his recurrent fevers, lethargy, abdominal pain, and vomiting disappeared.

Transfer factor is a poorly standardized biological, and neither its efficacy nor its safety has been established in the treatment of any disease.

14.2. PREVENTION OF CYTOMEGALOVIRUS INFECTION

Since CMV infection and morbidity are so common after tissue and organ transplantation, and treatment remains unsatisfactory, various methods of prophylaxis have been explored, and some success has been reported. At this time, the most promising measures are the administration of acyclovir or immune globulin.

14.2.1. Chemical Prophylaxis: Acyclovir

As pointed out above, as a therapeutic agent for CMV infections, either in cell cultures or in patients, acyclovir has been a failure (see Section 14.1.1) (Meyers *et al.*, 1982b; Wade *et al.*, 1983). Despite these discouraging prospects, acyclovir has been reported to be effective in preventing CMV infection or disease in transplant recipients.

Earlier attempts to prevent CMV infection along with HSV infections with oral acyclovir were either unconfirmed or unsuccessful (Gluckman *et al.*, 1983; Prentice, 1983; Wade *et al.*, 1984). Meyers *et al.* (1988) administered acyclovir at 500 mg per square meter of body surface every 8 hr to 86 CMV-seropositive bone marrow recipients from 5 days before transplantation to 30 days afterward. These patients were also seropositive for HSV. Sixty-five patients who were seropositive for CMV but not for HSV served as controls. Cytomegalic disease, mostly interstitial pneumonia, developed in 19 (22%) acyclovir recipients and 25 (38%) controls ($P = 0.008$). Survival in the first 100 days after transplantation was significantly increased in acyclovir recipients. Cytomegalovirus infection was also significantly decreased in acyclovir recipients, from 87% to 70%.

The above study could be criticized because it was not randomized or blind.

Balfour *et al.* (1989) conducted a randomized double-blind controlled study of oral acyclovir, 800 to 3200 mg per day for 12 weeks depending on the patient's renal function. During the first year after cadaveric kidney transplantation, four of 53 patients (7.5%) in the acyclovir groups had symptomatic disease compared to 15 of 51 (29%) in the placebo groups ($P = 0.002$). The greatest prophylactic benefit was in the seronegative recipients who received a kidney from a seropositive donor. Only one out of six such patients in the treated group had CMV disease, compared to seven out of seven in the placebo group. Acyclovir, unlike most immune globulins (see Section 14.2) also reduced the CMV infection rate from 61% to 36% ($P = 0.01$).

It is unclear why acyclovir was effective. The mean inhibitory dose (ID_{50}) for local isolates was 63.1 mM, whereas peak acyclovir levels in patients who received the drug 1 week after transplantation was only 25 mM. It is possible that these measurements do not truly reflect the biologically important intracellular triphosphate levels, which may have been higher.

Acyclovir is therefore a viable candidate to use for CMV prophylaxis. It is in some ways more attractive than immune globulin. It can be administered orally and it is less expensive. It may be more effective because, unlike immune globulin, it seems to reduce infection as well as morbidity. However, these results need to be confirmed by other investigators and in other transplant groups.

14.2.2. Immune Globulin

Immune globulin or hyperimmune globulin is useful in the prevention of a number of viral disease (Benenson, 1980). Hyperimmune globulin against CMV might be effective in preventing primary infection in patients at risk such as seronegative recipients of organ transplants. Conceivably, even if it does not prevent infection, it might reduce the morbidity of infection. It would not be expected to be effective in preventing the reactivation of latent infection in seropositive subjects, as they already have circulating antibodies. But it may affect morbidity.

Condie *et al.* (1979) showed that large doses of human γ-globulin (200 mg/kg) could be given intravenously over 10 days without side effects. The material used contained 10 mg/ml largely monomeric undenatured IgG. In an uncontrolled study, 71% of patients benefited from the injections in terms of life-threatening infections. In those who benefited, there was a significant increase in antibodies against CMV following administration of IgG.

Zaia *et al.* (1979) collected 200 units of plasma with a titer of at least 1 : 64 against CMV after screening 3080 units. This was fractionated by Cohn's cold ethanol method. The resultant fraction, called CMV IG, represented a 20-fold concentration. It has a CF and neutralizing titer of about 1 : 1024. Meyers *et al.* (1980b) gave 6 ml/m^2 of this material to 14 seronegative marrow recipients three times before the procedure and weekly to day 77 after the procedure. A CF titer of

1 : 16 was maintained in the patient. The treated group and the 12 controls had about the same number of CMV infections (50% and 42%), and one patient from each group had CMV pneumonia. The authors postulated that the antibody was not effective because the virus was cell associated at the time of first exposure.

Six more recent studies of hyperimmune plasma or globulin in bone marrow transplant recipients (Winston *et al.*, 1982, 1987; Meyers *et al.*, 1983; O'Reilly *et al.*, 1983; Kubanek *et al.*, 1985; Bowden *et al.*, 1986); and one study in kidney recipients (Snydman *et al.*, 1987) have demonstrated the efficacy of high-titered immune globulin in preventing disease, although not in preventing infection, in seronegative transplant recipients.

Winston *et al.* (1987), for example, studied 38 recipients who were given an equivalent of 1.0 gm/kg immune globulin once weekly until day 120 after marrow transplantation. There were 37 controls. The CMV infection rates in the two groups (47% and 57%) were not significantly different, although symptomatic infection rates differed (21% versus 46%). Interstitial pneumonia caused by CMV (16% versus 32%) and deaths from interstitial pneumonia (6% versus 14%) were reduced. Most of the subjects in this study (63/75 or 84%) were negative for CMV antibodies prior to transplantation. If only primary infections were counted, these were also significantly reduced by immune globulin (25% versus 52%).

The antibody titer of the preparation used in this study was 1 : 6400 by ELISA, and 1 : 64 by neutralization. It should be pointed out that the best antibody test to titer immune serum is still unknown.

In the one negative study in the series of six studies (Bowden *et al.*, 1986), immune globulin alone did not reduce primary infection in seronegative subjects (4/14 versus 4/11) or affect the number of symptomatic infections (2/14 versus 2/11). On the other hand, seronegative blood products significantly reduced CMV infection (1/32 versus 8/25). Perhaps the main problem with this study was that the number of symptomatic infections (four) in the entire study was too small to detect any differences.

These results suggest that immune globulin may be effective in reducing but not eliminating CMV disease in seronegative transplant marrow recipients. Many centers are using immune globulins in the study of patients. Whether or not other types of transplant recipients should also be treated is not clear, particularly in view of the low morbidity from CMV in some patient groups, the large amount of immune globulin required, and its high cost. Snydman *et al.* (1987) showed in an important study of 59 seronegative renal transplant recipients that 150 mg/kg of a hyperimmune globulin given within 72 hr of transplantation and 50–100 mg/kg every 2 weeks up to 16 weeks after transplantation did not reduce the rate of CMV infection (71% versus 77%) but did reduce the rate of symptomatic disease (21% versus 60%). Rates of death ascribed to CMV were also reduced (1/17 versus 5/27). These results are provocative and suggest that immune prophylaxis should be considered for seronegative kidney allograft recipients. However, it should be noted that this group of renal transplant patients seemed to have unusually high rates of

symptomatic CMV disease and deaths from CMV, possibly because of the use of ATG. As pointed out, the risk of developing CMV disease in kidney recipients varies with the type of immunosuppression (see Section 13.1.3). This risk must also be assessed for other types of transplant recipients. Even in the case of marrow transplantation, the risk of CMV interstitial pneumonia has declined significantly in some institutions (Wingard *et al.*, 1988b), so that the cost efectiveness of expensive prophylaxis needs to be evaluated from time to time.

14.2.3. Other Antiviral Prophylaxis

Interferon has received some attention as an antiviral agent that might function in the prophylaxis against CMV infection, although this line of research is no longer being pursued because of the pleiotropic effect of interferon, including its role in enhancing host-versus-graft reactions such as rejection (see Section 13.1.4).

Cheeseman *et al.* (1979a) conducted a controlled double-blind study on the effect of leukocyte interferon on CMV infection in renal transplant patients. Interferon in 3×10^6 units was given intramuscularly twice weekly for 15 doses (6 weeks) after surgery (total 36 million units per patient). There was evidence of some efficacy. Virus excretion was not prevented, but it was delayed in onset from 4.2 weeks after surgery in controls to 7.2 weeks in the treated group. Cytomegalovirus viremia correlated with clinical disease, which was more apparent in patients who had received antithymocyte globulin (see Section 13.1.3). Among patients who did not receive the globulin, no viremia occurred in four patients in the interferon-treated group, whereas three out of four who received placebo treatment had viremia. In the same study, interferon had no effect on the development of herpes simplex infection in patients. However, this infection occurred earlier than CMV in the posttransplant period. Thus, infections with this virus were preceded by fewer treatments with interferon. These results, although suggestive, were not enough to establish the prophylactic role of interferon.

Weimar *et al.* (1979), in a similar study but using fibroblast interferon, found no change in disease or serologic response to CMV and other viruses. Virus cultures were not monitored.

Hirsch *et al.* (1983) extended the previously reported interferon treatment regimen by Cheeseman *et al.* (1979a) from 6 to 8 weeks (total 102 million units per patient). Only CMV-seropositive subjects were enrolled. Out of 22 placebo recipients, seven developed symptomatic CMV disease, and only one developed such disease out of 20 interferon recipients ($P < 0.03$). Opportunistic superinfection from *Aspergillus fumigatus* and *Pneumocystis carinii* occurred only in placebo patients. Thus, this study showed a significant clinical effect, perhaps because only preinfected patients and longer treatments were used. However, interferon is no longer considered a candidate prophylactic agent, particularly since both chemoprophylaxis and immune globulin may be more effective and less toxic (see Sections 14.2.1 and 14.2.2).

14.2.4. Immunization

The purpose of immunization is to produce or improve acquired specific immunity against CMV infection. It is probably impossible to prevent establishment of latent infections, since natural acquired immunity is unable to do this. What one hopes to accomplish is to prevent the type of infection that produces the most serious disease syndromes. The type of infection one thinks of is a primary infection of the neonate and the immunosuppressed. Thus, immunization probably has little role in HIV-infected individuals because symptomatic CMV infections in these individuals are usually secondary (Section 13.2).

Congenital infections are a significant cause of mental retardation and neurological disabilities. The precise extent of this problem is not known, but in view of the late sequelae of infection, there may be at least 5000 brain-damaged children produced per year in the United States (Hanshaw, 1971; Stagno *et al.*, 1984). Theoretically, immunization, particularly with a live vaccine, might prevent these.

The main conceptual objections to a live CMV vaccine are the following: (1) Primary infection may not be the only cause of morbidity caused by CMV. Reactivation of a latent infection or reinfection in an immunosuppressed patient may still be important (Section 13.3). (2) Like the natural virus, a live vaccine strain may persist in the recipient, and it may reactivate at unpredictable times and/or be associated with or cause cancer (Section 5.6). (3) An objection to developed vaccine strains is that they have no clear markers of attenuation, either in cell cultures (see below) or in experimental animals. Unfortunately, there is no suitable animal model available for CMV. However, Quinnan *et al.* (1984) have reported that in experimental infection of volunteer subjects, none of four who received the Towne vaccine strain (see below) became systemically symptomatic, although some who received a recent clinical isolate developed mononucleosis.

Another argument frequently heard is that if there are many strains of CMV, why develop a vaccine from an arbitrary strain before we know if there is cross immunity among the various stains (see Chapter 8)? There is no simple way to sort out the problem, although there is less concern about it than previously. Strains of CMV do differ, and antigenic differences may be found. Different strains isolated from various parts of the world or from different parts of the body seem much the same. Strains also differ in nucleotide sequences, but there is also a great deal of nucleotide homology (see Section 8.6). Superinfection by different strains has been found in both immunocompetent and immunosuppressed subjects (Sections 9.4, 9.5, and 13.1). Hence, immunity against reinfection and against disease appears to be present.

Probably all herpesviruses have some oncogenic potential. Cytomegalovirus has been observed in the laboratory to transform human cells as well as hamster embryo cells (Albrecht and Rapp, 1973). The association of CMV with Kaposi's sarcoma is unproven (see Section 5.7). In veterinary medicine, effective vaccines have been developed against both oncogenic and nononcogenic herpesviruses. A highly efective vaccine has been developed against Marek's disease, which is a

tumor-causing herpesvirus. Infectious bovine rhinotracheitis virus may also be prevented by a live vaccine. This virus, like CMV, can transform hamster embryo cells (Michalski and Hsiung, 1975). None of these veterinary vaccines causes tumors in the animal recipients.

Another possible consequence of immunization may be persistence of the vaccine strain and subsequent symptomatic reactivation if the patient becomes immunosuppressed. So far, there is little evidence that this will be a problem, but long-term follow-ups are lacking (see below).

One might ask whether the major benefits of a vaccine against CMV might not be achieved by a killed-virus or subunit vaccine. Dead vaccines would not be able to produce latent infection, and it would not be oncogenic if the gene (DNA) responsible for transformation is inactivated or eliminated. Elek and Stern (1974) point out, however, that killed vaccine may, as in the case of measles and respiratory syncytial virus vaccines, render subsequent natural infection more hazardous, although killed vaccine may have a beneficial role in immunosuppressed patients. Whether this happens in the case of CMV again cannot be determined short of human trials.

14.2.4.1. AD169 Vaccine

Elek and Stern (1974) first described a live CMV vaccine and some human trials. The vaccine was derived from strain AD169 and underwent 56 passages in cell cultures: 14 passages in M.A.F. cells, 18 in diploid human embryonic lung fibroblasts, and eight in M.R.C.-5 cells (Jacobs *et al.*, 1970), a well-characterized strain of human fetal lung diploid fibroblasts developed in the United Kingdom.

Administration of up to 10,000 tissue culture doses (TCD_{50}) of the vaccine intradermally did not evoke an antibody response, but 300,000 TCD_{50} raised CF and neutralizing antibodies.

A study was undertaken with 26 susceptible (seronegative) individuals who received 10,000-TCD_{50} subcutaneously. Twelve developed a local reaction. Twenty-five responded serologically against AD169, 11 in 2 weeks and 14 in 3–8 weeks. There was no evidence of virus excretion in the vaccines. Two subjects followed for more than a year did not show a drop in antibody titer. Challenge of two volunteers who were successfully vaccinated failed to produce a skin lesion, which was also the case in the naturally immune. Ten successfully vaccinated subjects were tested for antibodies to other strains of CMV. Eight weeks after vaccination, nine had antibodies against the Kerr strain, and five against the Davis strain. At this early stage, there was some degree of strain specificity in response to vaccination. Three other successfully vaccinated subjects examined 1 year later had neutralizing antibodies to all three strains, suggesting that the antibody spectrum broadened with time.

A similar CMV vaccine was described and studied by the Merck group (Neff *et al.*, 1979). The 53rd passage of the Elek and Stern vaccine strain (Elek and Stern, 1974) was passed another five times in WI-38 cell cultures. It titered $10^{-3.4}$ per 0.1 ml, and 0.5 ml, or about $10^{4.0}$ tissue culture doses, which is what Elek and Stern

(1974) used, were injected in 24 male priests and seminarians. Twenty subjects who were seronegative before injection developed CF, immune adherence (IA), and neutralizing antibodies. No booster effect was observed in seropositive individuals. The CF and IA tests were all positive by 1 month, and neutralization titers developed by 2 months (19/20). Antibody levels declined over 1 year, particularly CF antibodies. One individual who developed neutralizing antibodies eventually lost all antibodies. Most subjects developed local soreness and induration. Five of the 24 subjects developed fever. Virus was not isolated from blood, urine, or throat. Contacts of vaccinees did not develop antibodies or virus infection.

14.2.4.2. Towne 125 Vaccine

The Towne 125 strain was propagated in WI-38 human diploid cell strain for 129 passages (Plotkin *et al.*, 1975). Three possible markers of attenuation were identified. The first is an increase in production of virus with continued passage. The second, less well described, is greater thermostability of the Towne and AD169 strains for 24 hr at 40°C, whereas C-87, Davis, and Kerr strains are paradoxically less stable at 4°C (see Section 4.5.1; Vonka and Benyesh-Melnick, 1966). Third, the virus increased in trypsin sensitivity on continued passage. It is not known whether any of these "markers" is related to attenuation for the human host. Prichett (1980) compared by reciprocal DNA–DNA reassociation kinetics the DNAs of the Towne and AD169 strains. Approximately 10% heterogeneity was demonstrated, and each strain contained unique nucleotide sequences.

The Plotkin group (1976) administered the vaccine intranasally and subcutaneously in two early human trials. No serologic response resulted from administration of nose drops containing $10^{4.7}$ PFU. But seronegative patients responded to the subcutaneous injection of $10^{4.7}$ PFU by producing CF, IgG immunofluorescence, and IgM immunofluorescence antibodies. Two seropositive subjects developed a booster response in CF and IgG antibodies. They also developed an IgM response, albeit earlier (1 week) than the seronegative subjects, who responded during the second and third weeks. No complications resulted from the vaccinations except for local reactions consisting of induration, erythema, and tenderness 7–12 days after inoculation and lasting for about 1 week.

Gehrz *et al.* (1980) inoculated four seronegative normal adults with the Towne strain vaccine.. Cytomegalovirus-specific CF and IFA antibodies developed within 3 weeks as described. After 41 weeks, the CF titers had declined to 1 : 4 or less, and the IF titers declined to 1 : 20 to 1 : 80. Measured by lymphocyte proliferation, cellular immunity induced by the vaccine also rose in 3 weeks but declined in three of the four subjects by the 12th week. The authors report an enigmatic secondary rise in this response at around 300 days after immunization. This "biphasic" response cannot be considered to be proven because of the small number of subjects studied and the inherent variability of the proliferation test. It should be noted that, unlike true infection, the vaccine virus was never isolated from the subjects, and there was no suppression of other cellular immune reactions as measured by the

proliferative response to three nonspecific mitogens and five common antigens.

Twelve seronegative prospective renal transplant candidates received the Towne 125 strain of live mCMV vaccine (Glazer *et al.*, 1979). As in the case of normal vaccinees (Plotkin *et al.*, 1976), there were no significant side reactions except erythema and induration at the site of inoculation. All seroconverted by 6 weeks after inoculation, which is slower than in normal subjects. Three patients tested developed CMV-specific blastogenic responses.

Subsequently, four vaccinees received kidneys from seronegative donors, and only one became infected. Five received kidneys from seropositive donors; all of these developed viruria, which was accompanied by a serologic rise in four. One died 30 weeks after transplantation after having experienced fever, leukopenia, thrombocytopenia, abnormal liver function, and polyneuropathy. Typical CMV intranuclear inclusions were seen in various organs at autopsy. Restriction endonuclease analysis of four of the six isolates showed that they were not the vaccine strain. This limited trial showed that prospective transplant patients could be successfully immunized without developing serious side effects or apparent infection.

The Towne attenuated vaccines produced an asymptomatic, apparently self-limited infection that resulted in the production of detectable antibodies and the development of CMV-specific lymphocyte proliferative response in almost all healthy volunteers (Starr *et al.*, 1981). However, in a controlled clinical trial of 91 renal transplant recipients by Plotkin *et al.* (1984), nine out of 37 (24%) seronegative patients who received the vaccine failed to produce antibodies. Five out of six also failed to develop a specific positive lymphocyte response, whereas all eight normal controls responded. Primary infection developed in both vaccinated and unvaccinated seronegative recipients who received kidneys from seropositive donors. The frequency of such infection was similar in vaccinees and the placebo-treated subjects (15/16 or 94% versus 11/14 or 78%). Also, the proportion of those infected who were symptomatic was not significantly different (60% versus 91%). However, the degree of morbidity in the vaccines measured according to a quantitative scoring system was significantly lower (2.7 versus 5.7).

Vaccinated seronegative patients who received kidneys from seronegative donors and were not infected extraneously did not shed vaccine virus in the urine, throat, or blood, suggesting that the vaccine strain neither spread nor persisted in the host. Furthermore, CMV strains isolated from primary infections in vaccinees have been shown by restriction endonuclease fragment polymorphism to be unrelated to the vaccine strain (Glazer *et al.*, 1979).

The modest reduction in morbidity of CMV by the vaccine was not supported in a study employing the same vaccine in Minnesota (Balfour *et al.*, 1985). A total of 117 received the vaccine (6.6×10^3 viral infectious plaques), and 119 received placebo. Seventy-six percent of 63 seronegative subjects converted their antibody status after being immunized. Seropositive subjects also had a boost in their antibody titers.

Forty-seven (20%) of the 236 study subjects developed CMV disease. There was no difference in incidence or severity of CMV disease between vaccinees and

the placebo group among seropositive subjects or seronegative subjects who received a kidney from a seronegative donor. There was a suggestion of protection by the vaccine among seronegative subjects who were given a seropositive kidney. Two of eight vaccinees with CMV disease had severe or lethal disease, whereas five of six placebo subjects with CMV disease experienced severe or lethal illness. This suggests that CMV disease was ameliorated, although the frequency of CMV disease in the vaccinated and placebo groups (38% and 43%) was not changed.

More recently, the Plotkin group (Brayman *et al.*, 1988) provided data that seemed to support the type of protection suggested by Balfour *et al.* (1985). As previously reported, the vaccine did not decrease infection rates or symptomatic CMV infection. However, morbidity in seronegative recipients who received kidneys from seropositive donors as measured by disease scores was reduced (ten versus two "serious diseases"). More interestingly, the 5-year graft survival rate for this group of patients was significantly higher in the vaccinated group (62% versus 25%). The mechanism of this improvement was not clear.

In summary, the Towne vaccine does not prevent primary infection but may have a marginal effect in alleviating CMV disease resulting from primary infection or its consequences. One problem may be that the transplant patient does not produce an optimum immunologic response. The vaccine seems well tolerated, and none of the feared complications have arisen. More trials are needed. Future developments should aim at improving the immunogenicity of the vaccine in potential transplant recipients. Other classes of vaccines, such as subunits and recombinant vaccines, remain to be developed.

15

Nonhuman Cytomegaloviruses

15.1. INTRODUCTION

As discussed in Chapter 4, the criteria for assigning the name "cytomegalovirus" to a herpesvirus discovered in animals other than man are not entirely clear-cut and appear to be in the process of evaluation. I have decided not to review comprehensively all the so-called cytomegaloviruses of other animals; such a review properly belongs to a separate treatise or to one on herpesviruses.

However, development of knowledge of some of the these agents has in the past and up to the present influenced our understanding of human CMV. It would be a mistake to ignore the nonhuman cytomegaloviruses altogether, and this view is reflected in my own approach to this group of agents. I have found that maintaining an interest in the murine as well as the human agent improves understanding of the virology and pathogenesis of the human agent.

What follows then is a selective review of some aspects of the nonhuman viruses. The point of focus is how knowledge of these agents has complemented and furthered our understanding of human CMV.

We begin by presenting a history of the development of knowledge of nonhumn CMVs. Much of the knowledge common to this group of viruses has been developed in the animal agents. Then, we concentrate on selected topics of guinea pig and murine CMV. Particularly in the case of murine CMV, important contributions have been made in its virology, experimental infection, pathogenesis, genetics of infection, and immunology.

By and large, there is very little interspecies crossing between the different CMVs. One-way cross-neutralizations have been described between certain primate CMVs and human CMV. That is, antiserum against the primate agent will neutralize human CMV, but antiserum against the human agent will not neutralize the primate agent (see Section 5.3). This has been described for the agents of the chimpanzee (Vogel and Pinkerton, 1955), African green monkey (Black *et al.*, 1963), and rhesus

monkey (Asher *et al.*, 1969; Swack *et al.*, 1971), but much less for the baboon (Graham *et al.*, 1971). The cross-reactive titers are low.

15.2. HISTORY OF NONHUMAN CYTOMEGALOVIRUSES

As knowledge about human CMV in man was developed, parallel discoveries were made in animals. As in the case of hCMV, two phases may also be identified, a morphological–pathological phase and the virological phase initiated by the discovery of murine CMV by Margaret G. Smith (1954) (Table 15.1). In the beginning, studies in animals trailed behind studies in man, but knowledge of nonhuman CMV, particularly murine CMV, developed more rapidly later, greatly influencing the concepts and understanding of the human agent.

Early discoveries of animal CMVs were made by intent or accident after histopathological examination of the salivary glands of various animals. Jackson (1920), for example, discovered guinea pig CMV when she examined the salivary glands of guinea pigs. In other cases, the animal in question was used for experimental infection of hCMV (Kuttner and Wang, 1934). More recently, a number of agents were discovered by examining animal tissue used for cell cultures (Rabson *et al.*, 1969).

Leila Jackson (1920) first described what she called an intracellular protozoan parasite in the ducts of the salivary glands of 26 out of 48 guinea pigs. Similar structures were seen in the kidneys of 12 out of 44 guinea pigs. The illustrations that accompany her paper show a close resemblance between these structures and cytomegalic inclusions in human materials described by previous writers. Jackson

TABLE 15.1
Cytomegalovirus in Animals Other Than Man

Animal	Author
Discovery by histopathology	
Guinea pig	Jackson (1920)
Mice	Findley (1932)
White mice	Kuttner and Wang (1934)
Rats	Kuttner and Wang (1934)
Hamsters	Kuttner and Wang (1934)
Monkeys	Covell (1932)
Moles	Rector and Rector (1934)
Chimpanzees	Vogel and Pinkerton (1955)
Rodent	Raynaud and Raynaud (1947)
Discovery by isolation of agent	
Mice and voles	Smith (1954), Raynaud *et al.* (1969), Diosi *et al.* (1972)
Dogs	Habermann *et al.* (1960)
Cercopethicus monkeys	Black *et al.* (1963)
Rhesus monkeys	Asher *et al.* (1969)
Horses	Hsiung *et al.* (1969)
Wild rats	Rabson *et al.* (1969)

thought they were "coccidial" (protozoan) in nature. She also described the "earliest form of the parasite" embedded in the cytoplasm of a duct epithelial cell, which is the first description of the cytoplasmic inclusion of a CMV. She was also the first to note inclusion bodies in the kidney, the salivary glands and kidney being the primary sites of persistent infections by CMV of various species.

Pearson (1930) described in detail cytoplasmic inclusions in the duct cells of the submaxillary gland of guinea pigs. They were more limited in distribution than the intranuclear inclusions, and he concluded that they were not a manifestation that was *sine qua non* in his virus infection.

Cole and Kuttner (1926) found that 84% of submaxillary glands from 75 guinea pigs over 6 months of age showed intranuclear inclusions. They undertook the first experimental transmission studies. Suspensions of salivary glands positive for inclusion bodies were injected into the maxillary glands of very young guinea pigs free of inclusion body infection. Inclusion-containing cells and mononuclear infiltration were produced. Injections were also made in the testes, brain, lung, and tongue. Most striking results were obtained after injection into the brain. The inoculated animals developed a temperature of 105–106°F 48 hr after injection. Later they became jerky, irritable, and paralyzed and usually died in 5–7 days. The most striking pathological feature was a meningeal exudate with large cells containing intranuclear inclusions. Attempts to infect rabbits, rats, and kittens by injecting their submaxillary glands were unsuccessful. This was the first indication of the species specificity of a cytomegalovirus.

Cole and Kuttner were unable to find a consistent method for serial transfer of the infectious material from guinea pig to guinea pig. The best they could do was to transfer infected submaxillary gland material into the testes and from thence to the brain and then the testes of a third and final recipient. Probably the animals were largely immune because of prior infection.

It was determined by transfer experiments that the infective material was destroyed by heating at 54°C for 1 hr and preserved in 50% glycerol for as long as 11 days, presumably at room temperature. After 28 days, infectivity was lost. The infective agent was not held back by a Berkefield N filter, which held back *E. coli.* This was the first conclusive evidence that the infective agent was a filterable virus.

Subsequently, Kuttner (1927) found that the virus localized in the maxillary glands of young guinea pigs 12–15 days following subcutaneous, intraperitoneal, or intravenous injection of infective maxillary gland material. Virus could be transmitted continuously from guinea pig to guinea pig by inoculating infective material by direct inoculation in the submaxillary gland or by subcutaneous injection.

She also discovered that after the development of an infection in the submaxillary gland, the animal became refractory to an ordinarily lethal intracerebral inoculation. Immunity also developed in young guinea pigs that received injections of heat-killed virus, a finding that anticipated studies on vaccines.

Andrews (1930) grew guinea pig testes in Maitland cultures and showed for the first time that inoculation of salivary gland virus resulted in development of characteristic inclusions in cells of such cultures.

Over the years, it became evident that agents producing cytomegaly could be found in many other mammals besides man and guinea pigs.

Cowdry and Scott (1930) discovered that two out of 58 dogs without clinical disease showed intranuclear inclusions in the liver. Whether this represented a type of CMV is not known.

Kuttner and Wang (1934), working in Peking, attempted to transmit an infectious agent from the salivary glands of four Chinese infants with cytomegaly to guinea pigs, rabbits, hamsters, white mice, wild rats, and two monkeys. The animals were inoculated intracerebrally or directly into the submaxillary gland. Although the transmission experiments were predictably negative, in the course of examining the submaxillary glands of these species, inclusions were found in hamsters, white mice, and wild rats, but not in rabbits, *Macaca* monkeys, or squirrels. The inclusions were similar to those found in man and in guinea pigs. Intracerebral injection of the homologous salivary gland material into hamsters, white mice, and wild rats produced meningeal irritation and death. However, infectivity could not be serially demonstrated with meningeal material. They also showed that if immune serum was first added to such materials, inclusion bodies did not develop. Further, virus mixed with immune serum and inoculated subcutaneously into young guinea pigs was partly or wholly neutralized as demonstrated by the scant number or absence of inclusion bodies when the salivary glands were subsequently examined.

Rector and Rector (1934) discovered inclusions in moles. Cowdry and Scott (1935a) published excellent plates showing cytomegalic cells in the renal tubules but not the salivary glands of about 10% of apparently normal *Macaca* monkeys or in those used for other experiments. In contrast, inclusions were more rarely seen in the cebus monkey and then only in the salivary gland (Cowdry and Scott, 1935b).

Vogel and Pinkerton (1955), during an epidemic of illness and deaths in their chimpanzee colony, discovered disseminated C.I.D. in three out of 12 animals. In each case, the adrenals had cytomegalic inclusions, and in one case, the myocardium was affected. Five other animals were not clinically ill but had cytomegalic cells in their salivary glands. This was thought to be the first observation of the natural disseminated disease in animals.

Smith (1954) observed large intranuclear inclusions in mouse embryonic explant tissue cultures after they were inoculated with salivary gland material from mice infected with salivary gland virus. They were reproduced in three serial subcultures. That an infection was involved was verified when intranuclear inclusions were produced in mouse salivary gland after intraperitoneal inoculation of supernatant fluid from cultures. Calculation of dilution factors showed that a significant increase in virus replication had occurred in tissue culture. Smith's work heralded the virological period of development in the study of all cytomegaloviruses.

With increased use of cell cultures from many tissues of many animals, adventitious agents including CMV were isolated from tissues cultured. Kidney cell cultures were a source of CMV isolated from cercopethicus (African green) monkeys (Black *et al.*, 1963), rhesus monkeys (Asher *et al.*, 1969), and horses (Hsiung *et al.*, 1969). In these cases, the agents would appear spontaneously when the cultures were observed for long periods of time.

As mentioned above, Smith (1954) initially isolated mouse (*Mus musculus*) CMV. Later, Raynaud *et al.* (1969) isolated an agent from another species, the mole *Apodemus sylvaticus*, with a number of interesting properties including replication in human cells. But this could not be confirmed (Kim *et al.*, 1974). Plummer *et al.* (1969) found that these two strains have different base ratios, suggesting that they were distinct. Diosi *et al.* (1972) isolated a third agent from moles (field mice) (*Microtus arvalis*) in mouse embryonic fibroblasts. Kim *et al.* (1975) found that the Smith, Raynaud, and Diosi strains were identical in terms of growth in cell culture, buoyant density, antigenicity, and electron microscopic appearance. This is curious because mice and moles are unrelated animals.

Direct isolation from the salivary glands was also made in the case of the coercopethicus monkey (Black *et al.*, 1963). Isolation was usually carried out in homologous cell cultures, and in each case, the agent was thought to be species specific. However, in retrospect, in almost all cases, the agent could be grown in cells from other species, and a general characteristic of CMVs is that they are not strictly species specific. For example, the coercopethicus agent was grown in human fibroblasts (Black *et al.*, 1963), the horse agent was grown in primary rabbit kidney cells (Hsiung *et al.*, 1969), and the rat agent was grown in hamster kidney cells (Rabson *et al.*, 1969). Often the cells were from a closely related, heterologous species, but this was not the case with the horse agent growing on rabbit cells. The latter are also permissive for guinea pig CMV (Hsiung *et al.*, 1969).

15.3. EXPERIMENTAL INFECTION WITH GUINEA PIG CYTOMEGALOVIRUS

Although CMV infection in mice has been the most extensively studied model and is discussed in Chapter 16, the guinea pig model has also received attention in the last 20 years (Bia *et al.*, 1983).

In some respects guinea pig CMV (gpCMV) resembles human CMV more than murine CMV. Electron microscopic examination reveals that following gpCMV infection of cells the nucleocapsid assembly, envelopment of nucleocapsids at the inner nuclear membrane, and the development of both intranuclear and intracytoplasmic inclusions resemble those of human CMV infection. The presence of virus in salivary duct cells rather than acinar cells is a characteristic of human, simian, and gpCMV (Henson and Strano, 1972). Guinea pig CMV, like human CMV, also produces cytoplasmic and extracellular dense bodies that possess viral antigenicity (Fong *et al.*, 1980).

Biologically, infection with gpCMV also has many similarities with human infection. In at least two respects, the guinea pig model has advantages over the mouse model. First, a model of congenital transplacental infection can be established with gpCMV but not readily with murine CMV. Secondly, there has been more work done on inner ear infection by gpCMV.

In the last analysis animal CMV models are limited because each animal CMV is distinct from human CMV, even though a name is shared. What one learns from

animal models can not be directly applied to human infection but can only serve as reference points or points for comparison.

15.3.1. Acute and Persistent Infections with Guinea Pig Cytomegalovirus

After either intraperitoneal or subcutaneous inoculations of gpCMV in the guinea pig, an acute infection followed by a chronic persistent one is established, similar to what has been described for murine CMV (see Section 16.2). As in the case of the human or murine virus, the nature of persistent and latent infection remains to fully characterized.

By cocultivation techniques for virus isolation, viruria and viremia can be demonstrated after the first week following infection, with transient virus replication in various organs. Persistent high titers (10^3–10^5 $TCID_{50}$) of full virus in the salivary gland for as long as 20 weeks after inoculation may be found (Connor and Johnson, 1976; Hsiung *et al.*, 1976, 1978). Even though virus was often undectable in the blood leukocyte fraction (buffy coat), gpCMV was transmitted by blood to nonimmune susceptible animals (Hsiung *et al.*, 1978). It did not matter whether the recipient was syngeneic or allogeneic with respect to the donor cells (Bia *et al.*, 1979). Virus when found was present in the granulocyte and mononuclear-cell-enriched phase (Griffith *et al.*, 1981).

As in the case of murine CMV, the gpCMV of salivary gland origin is more virulent than tissue-culture-passaged strains. After subcuteaneous inoculation of the more virulent salivary gland strain (gpCMV-SG) in Hartley guinea pigs, examination of the hematologic and lymphoid systems revealed that the mononucleosis syndrome could be reproduced (Griffith *et al.*, 1981). Atypical lymphocytes were often seen in peripheral blood associated with anemia, neutropenia, lymphocytosis, splenomegaly, and lymphadenopathy.

During the stage of acute mononucleosis 1 week after infection, mobilization of neutrophils to the site of a peritoneal exudate was diminished compared to uninfected controls. Neutrophils from infected animals also released less hydrogen peroxide than controls in response to a bacterial stimulus. These neutrophils were also less efficient in killing bacteria (Youtree *et al.*, 1982). These observations parallel those in man and in mice, where cytomegalovirus infection has been found to be immunosuppressive (see Sections 13.1.5 and 16.6.3).

Inbred strain 2 guinea pigs are more susceptible to gpCMV than the outbred Hartley strain. They are always killed by an acute disseminated infection after being inoculated with a high dose (7.5×10^5 $TCID_{50}$) of gpCMV-SG. Lesions and infectious virus were present in the spleen, liver, lungs, heart, pancreas, brain, salivary gland, and other organs (Fong *et al.*, 1983).

Strain 2 animals may develop severe interstitial pneumonia during such acute infection as part of a more generalized viremic illness (Bia *et al.*, 1982). This may serve as a model for pneumonia, although to study the contribution of complex

immunopathological factors of pathogenesis, work with inbred mice has been more useful (see Section 16.3).

Booss *et al.* (1989) produced a central nervous system infection by intracerebral inoculation of gpCMV. Virus titers in the brain peaked 6 days after infection and was accompanied by leptomeningitis. Monocytes in contrast to lymphocytes were the primary inflammatory cells. They were also found in parenchymal foci, which were also notable for the absence of T lymphocytes.

15.3.2. Congenital Infection with Guinea Pig Cytomegalovirus

The fact that both human and guinea pig placentas have a single layer of trophoblast cells between maternal and fetal circulation makes the successful development of congenital gpCMV infection particularly interesting (Choi and Hsiung, 1978; Kumar and Nankervis, 1978; Johnson and Connor, 1979). The gestation period in the guinea pig is 69 days.

Kumar and Nankervis (1978) inoculated 19 guinea pigs with $10^{5.5}$ $TCID_{50}$ i.p. during the latter part of their pregnancies. Six out of 15 animals that carried their pregnancies to term had at least one CMV-infected newborn as defined by positive explant cultures of spleen, lung, or brain. In all, seven (12%) out of 58 offspring were congenitally infected. The six mothers that had litters containing infected newborns delivered an average of 3.1 offspring per litter, fewer than the normal average of 4.3. No gross congenital abnormalities were noted.

The antibody status of the mother prior to the onset of pregnancy was the most important determinant of fetal infection. All three previously nonimmune mothers delivered infected litters, whereas only three out of 12 immune mothers delivered infected litters, with virus isolation restricted to the lung. Tissue-culture-passaged virus was less lethal for the mothers, but its ability to produce congenital infection was not diminished.

Griffith and Hsiung (1980) also studied intrauterine infection with gpCMV. After subcutaneous inoculation of 10^6 $TCID_{50}$ guinea pig virus in mothers during the first, second, or third trimester of gestation, stillbirths were more frequent when mothers were infected during the third trimester (47%) as compared to mothers infected during the first and second trimesters (11%, 3%). Irrespective of the trimester, viral recovery in the offspring was most frequent 10 to 15 days after inoculation of the mothers (51% in placenta, 60% in fetal tissues). After 2 weeks of maternal infection, recovery of virus declined significantly. Virus could be found in the salivary glands of some offspring 14 weeks after delivery.

Transplacental transmission of virus occurred only when acute primary infection was initiated during pregnancy in either outbred Hartley or inbred strain 2 guinea pigs (Griffith *et al.*, 1986). Offspring of mothers that were inoculated prior to pregnancy were generally immune. However, a small number of offspring from chronically infected mothers showed histological lesions consistent with infection even though no virus was isolated. Johnson and Connor (1979) reported that fetal

CMV infections could occur in seropositive mothers reinfected with gpCMV during pregnancy, analogous to what has been reported to happen in humans (see Section 11.3.3).

The Hsiung group has developed a high-cell-culture-passaged gpCMV that can act as a safe vaccine that produced no viruria, symptomatic infection, or reactivation of virus infection during pregnancy. It was effective in preventing generalized infection, interstitial pneumonia, and protected fetuses from stillbirth, abortion, and transplacental viral transmission (Bia *et al.*, 1982, 1984).

15.3.3. Infection of the Inner Ear with Guinea Pig Cytomegalovirus

Sensorineural deafness is a significant complication of congenital human CMV infection (see Section 11.4.3). Relatively little is known about the pathogenesis and histopathology of such deafness, and knowledge contributed by animal models may be important. Cytomegalic inclusions were identified in the membranous labyrinth, including cochlea, saccule, utricle, and semicircular canals (Davis, 1981). Endolabyrinithitis was considered a fundamental characteristic.

By direct inoculation into the scala tympani (perilabyrinthine compartment) of the inner ear, gpCMV has been used to establish a reproducible model of CMV labyrinthitis and hearing loss in seronegative guinea pigs (Woolf and Harris, 1986; Harris *et al.*, 1984). Impairment of cochlear function was documented by electrophysiological recordings of cochlear microphonic (CM) and eighth nerve N1 compound action potential (AP) thresholds. Striking elevations of both CM and AP thresholds indicating severe functional disturbance were observed 8 days after infection.

In this guinea pig model, the labyrinithitis was primarily in the perilymphatic compartment. The stria vascularis and the organ of Corti of the endolymphatic compartment were also involved. Whether the primary involvement of the perilymphatic compartment was artifactual or a result of inoculation is unclear. Since this compartment was not found to be involved in man, these results also suggest the possibility that such involvement may have been missed so far.

16

Murine Cytomegalovirus

16.1. BASIC VIROLOGY OF MURINE CYTOMEGALOVIRUS

The genome of mCMV appears to be considerably larger than those of most other herpesviruses. Mosmann and Hudson (1973) found by velocity sedimentation in neutral and alkaline sucrose gradients and by equilibrium centrifugation in cesium chloride the molecular weight of mCMV DNA to be 132×10^6. Like human CMV, it is a linear double-stranded DNA molecule consisting of about 235 kilobase pairs. Unlike human CMV, however, the genome is one long unique sequence that lacks large terminal and internal repeat regions (Ebeling *et al.*, 1983; Mercer *et al.*, 1983).

As in the case of human CMV and other herpesviruses, the transcription and translation of CMV are coordinately regulated and take place in an orderly sequence, as in the case of human CMV (see Chapter 2). Transcription of immediate early (IE) genes takes place first without prior protein or DNA synthesis and hence can occur in the presence of the antimetabolite cycloheximide. Protein products can be translated 2 hr after infection. Transcription of early-phase genes requires prior synthesis of at least one of these IE proteins and cannot be expressed in the presence of cycloheximide. The late gene transcription requires viral DNA synthesis and occurs about 16 hr after infection (Keil *et al.*, 1984).

The IE genes originate from 0.769 to 0.815 map units of the genome (Keil *et al.*, 1987). This region encodes the major IE protein, pp89, a phosphoprotein. The 84- and 76-kDa phosphoproteins share with pp89 antigenic components recognized by monoclonal antibodies and represent posttranslational modification products of pp89 (Keil *et al.*, 1985).

The predominant transcript of the major IE gene, ieI, a 2.75-kilobase mRNA, is, as in the case of the ieI of human CMV, generated by splicing of transcripts of four exons. However, there is no significant homology between the nucleotide sequence of the human and mouse CMV genes (Keil *et al.*, 1987).

Immediate early proteins are thought to be essential for expression of late gene

function. Direct evidence of the ability of pp89 to transactivate gene expression was obtained (Koszinowski *et al.*, 1986). L cells were transfected with appropriate DNA, and pp89 was constitutively expressed. When such cells were transfected with constructs containing the bacterial chloramphenicol acetyltransferase (CAT) gene under control of viral promotors, CAT was efficiently expressed.

The regulatory function of the product of the IE genes was confirmed by transient expression assays in which mCMV IE genes were cotransfected into L cells together with recombinant constructs of the CAT gene. Hence, the IE region of murine CMV takes its place among similar regions in other DNA viruses, particularly herpes simplex virus and human CMV, as being powerful regulators of viral gene functions.

The pp89 is thought to play a role in recognition by immune lymphocytes. After transfection of the DNA sequences that code for pp89 in L cells, this major IE antigen was expressed on the cell membrane and was recognized by cloned cytotoxic T cells (Koszinowski *et al.*, 1987a,b; also see below). Thus, in this function pp89 resembles the latent membrane protein of EBV and the T antigen of SV-40, both of which are gene products of latently infected cells, are partially expressed on cell membranes, and are recognized by specific cytotoxic T cells (Tevethia *et al.*, 1980; Keil *et al.*, 1987).

16.2. MURINE CYTOMEGALOVIRUS IN CELL CULTURE

Like hCMV, mCMV grows well in fibroblast cultures of the homologous species. Mouse fibroblast cultures may be readily prepared from trypsinized embryos. Cytopathic effects develop faster in mCMV- than in hCMV-infected cultures. It is observable in 3–5 days, and plaques form under tragacanth (Mirchamsy and Rapp, 1968) or other types of soft overlay in less than 1 week. As in the case of hCMV, and quite unlike herpes simplex virus, replication of mCMV depends on the cell cycle (see Section 4.4.1). Cells infected during G_0 phase can only support nonproductive infection, and only five nonstructural viral polypeptides are synthesized. Viral DNA replication does not occur. During the productive S phase, viral DNA synthesis occurs, and progeny virus is subsequently produced. It is possible that this dependence on cell cycle may be related to the persistence and latency of mCMV (Muller *et al.*, 1978).

Centrifugal enhancement is a phenomenon peculiar to mCMV discovered by Osborn and Walker (1968). When mCMV was centrifuged at low speed (1900 $\times$ *g*) onto a monolayer culture, a 10- to 100-fold increase in the number of infectious particles resulted. Other herpesviruses such as HSV-1 and HSV-2 preparations also showed enhancement of infectivity after centrifugation, but less (four- to sixfold) (Hudson *et al.*, 1976). Hodgkins *et al.* (1988) postulate that this procedure enhanced the phase of reversible virus binding while not affecting irreversible binding. This phenomenon is of practical importance in the "shell vial" method of virological diagnosis (see Section 5.1.3).

Another interesting property of mCMV is its attenuation in cell culture after only one passage through mouse embryo cell cultures (Osborn and Walker, 1970). The passaged mCMV became much less lethal for suckling mice, and its ability to multiply in liver, spleen, and pancreas but not in the salivary gland after parenteral injection was also diminished. The immunosuppressive capacity of mCMV, such as its ability to inhibit antibody formation and interferon formation for 4–6 days, was also eliminated. Virulence and ability to multiply in the liver were restored when cell-culture-attenuated virus was passed only once in mice or virus from the salivary gland was used.

Dutko and Oldstone (1981) described an interesting cell culture model in which mCMV did not productively infect an undifferentiated murine teratocarcinoma cell line. But when such cells were differentiated in the presence of dimethylacetamide, viral RNA transcription and replication occurred. A similar model has been developed in the human system (Gonczol *et al.*, 1984; LaFemina and Hayward, 1986; Section 5.5.6). Permissive replication of CMV in the host may require differentiated cells.

16.3. EXPERIMENTAL MURINE CYTOMEGALOVIRUS INFECTIONS

Infection with mCMV in its definitive host has served as the major experimental model of CMV infection. Despite the fact that mCMV and human CMV are separate herpesviruses, they are biologically similar. The mouse models of infection have provided insight concerning the virulence, persistence, latency, chronicity, and specific immunity of these viruses. Recent works elucidating the genetics of the infection and the nature of mCMV interstitial pneumonia are particularly interesting. Contributions from mCMV continue to be needed for understanding of the nature of latency. We first review the historical development of the mouse model and then some of the more recent developments.

The early work showed that mCMV produces a wide range of morbidity in mice depending on strain, age of the host, and route of administration. Pathological changes were noted in many tissues, but the salivary gland emerged as the organ of viral persistence. McCordock and Smith (1936) produced extensive visceral lesions after injecting infected salivary gland material intraperitoneally and intracerebrally in C57 black mice and in "Buffalo strain" mice. About 50% of the latter died or were moribund when killed between the fourth and seventh day after inoculation. The most extensive lesions were in the liver, spleen, adrenals, lymph nodes, and subperitoneal connective tissue and fat. Focal areas of necrosis, inflammation, and cells with intranuclear inclusions were observed in these tissues. Less extensive changes were found in the lungs, kidneys, intestines, and pancreas. Changes were found in the lungs and pancreas of about half of the animals. No lesions were found in the salivary gland before the eighth day after infection, but inclusion bodies were found in the acinar cells when they were examined thereafter. Brodsky and Rowe

(1958) found lesions in the salivary gland 60 days but not 120 days after infection. In animals that survived, the pancreas and salivary gland were the only sites of pathology.

Similar lesions were produced by intracerebral inoculation of newborn Swiss–Webster CD-1 mice (Lussier, 1975). In addition, calcium deposits were found in the head, neck, lumbar, and paravertebral muscles and interscapular brown fat. Necrotic lesions in the brown fat persisted 56 days after infection.

Mannini and Medearis (1961) determined that the susceptibility of Swiss–Webster mice, as determined by cumulative mortality after inoculation, varied with the dose of the virus, decreased with age, and increased after intravenous injection. After intravenous injections of $10^{5.6}$ TCD_{50} virus, 30% of the mice died by the fifth day, but deaths (20%) were postponed to the eighth day after intraperitoneal injection of the same dose.

After sublethal infection weanling mice were followed up to 181 days. At that time, more than 10^5 TDC_{50} viruses were recovered from a gram of salivary gland, and more than 10^4 TDC_{50} were recovered from throat swabs. Smaller amounts of virus were also recovered from eye swabs, urine, and kidney, but no virus was recovered from stools. Virus excretion in the eye paralleled excretion in the salivary gland. One assumes that the lacrimal gland is the site of infection, although I know of no study of this problem.

By immunofluorescence, Mims and Gould (1979) confirmed that the salivary gland was the primary site of infection of CD-1 mice. Infection initially involved the perivascular interstitial cells and later spread to the acinar cells. Virus was recoverable from saliva up to 58 days after infection. The corpora lutea of the ovaries (but not ova) also became infected after intravenous infection of pregnant mice. The medullas of the adrenals were infected in suckling mice, and the adrenal cortex in older ones. The dermis was involved only locally after intradermal injection of the virus.

Among mice surviving neonatal infection, about half carried virus in the kidney for 12–26 months, and a smaller number carried it in the salivary gland. Nearly all showed chronic glomerulonephritis and acellular deposits of unknown nature in the adrenals and salivary glands.

In addition to the parenteral routes of administration, Mannini and Medearis (1961) demonstrated infection by the oral and intranasal route but not by conjunctival swabbing or by placing uninfected animals with infected ones. Pregnant mice examined 2 days after an intravenous inoculation of CMV showed signs of incipient abortion or macerated embryos. Virus, however, could not be recovered from the embryos, newborns, or stillborn offspring despite successful infection of maternal tissues. However, cell cultures from embryos of latently infected pregnant mice developed evidence of mCMV infection proven by immunofluorescence and *in situ* nucleic acid hybridization (see below; Chantler *et al.*, 1979).

The striking effect of the route of administration was also shown by Jordan (1982). No evidence of virus latency was demonstrable by cocultivation of spleen cells after mice were infected subcutaneously. On the other hand, latent infection was established after intraperitoneal inoculation (see Section 16.5).

After intraocular injection of $10^{4.3}$ PFU of mCMV in ICR/HA mice less than 24 hr of age, lymphoid cell necrosis and thymic atrophy were observed (Schwartz *et al.*, 1975). The authors were originally interested in development of chorioretinitis. These mice, 94% of which died in 14 days, developed severe runting and loss of weight. There was marked depletion of lymphocytes in the subcapsular and cortical areas of the thymus, spleen, lymph nodes, and Peyer's patches. The spleen showed fewer lymphocytes than normal. Inclusions were found in the bone marrow, along with a decrease in the cell populations and necrosis. These reactions may be similar to the acute splenic necrosis seen after mCMV infection of certain strains of white mice, such as Balb/c (see Section 16.3; Chalmer, 1979). Relatively few reports about the cytopathology in the thymus have been described. Other viruses that affect the thymus are lymphocytic choriomeningitis, rubella, rubeola, canine distemper, and feline enteritis viruses. This study suggests that when infants present at birth with both immune deficiency and hCMV infection, the immune deficiency may result from infection of the lymphoid tissues (see case in Section 11.4.2). This intraocular route of infection resulted in a picture seen in some cases of human congenital C.I.D.

Chronic infection with mCMV produces some evidence of autoimmunity. Olding *et al.* (1976) reported that latently as well as chronically infected mice that survived after being inoculated *in utero* showed moderate amounts of IgG and C3 deposits in the glomeruli by 6–8 weeks of age. A discontinuous granular pattern was seen predominantly in the mesangial region and along the walls of the capillary basement membrane. Deposits of mCMV antigen were also found. A significant proportion (26%) of the IgG was shown to be specific against mCMV. There was only minimal evidence of glomerulonephritis by light microscopy, and no report of functional disease.

Other evidence of autoimmunity was the detection of elevated antinuclear antibody titers in *in-utero*-infected C57Bl/cdj mice but not C3H/St, C57Bl/t, BALB/c/St, or other strains. There was also evidence of antibody to DNA and soluble nuclear protein. Cytomegalovirus infection in man has also been associated with elevated antinuclear antibodies (Kantor *et al.*, 1970) (see Sections 10.5 and 12.3).

Cytomegalovirus disease was first discovered in human neonates, and it should not be surprising that age plays an important role in determining pathogenicity of this group of viruses. Booss and Wheelock (1975) found that 90% of weanling 22-day-old mice inoculated with 3×10^4 PFU died, whereas only 30% of the 25-day-old mice and none of the 28-day-old mice inoculated with this dose died. The fourth week of life represents a period of rapid change in pathogenicity. Chalmer (1979) found that aging after this period had little further effect. Little is known about the reason for the susceptibility of younger animals. One possible explanation may be the absence of NK cells in young mice (Moller, 1979).

Attempts have been made to produce a model of congenital infection with mCMV. Earlier attempts were unsuccessful (Medearis, 1964a,b), but later reports have been more positive. Chantler *et al.* (1979) observed a decrease in litter size of a female mouse infected with mCMV. One of three fetuses yielded mCMV on serial

tissue culture passages, which suggests that transplacental transmission had occurred. No gross abnormality in fetal development was noted. Baskar *et al.* (1985) produced latent mCMV infection by injecting newborn female mice. Sixteen abnormal offspring were found among 189 (8.5%), including craniofacial and ocular malformations. Virus was demonstrated by cocultivation in the tissues and placenta of two, and four were positive by *in situ* cRNA hybridization. Despite these reports, the mouse is still not a popular model for congenital transmission, probably because of the low yield of successful infection and the basically different placental structure of mice.

Interstitial pneumonia associated with CMV is one of the best-recognized and most severe diseases caused by CMV (see also Section 13.3.2). Depending on the pathogenetic mechanism it is quite possible that there is more than one form of CMV pneumonia. This formulation is supported by the many types of interstitial pneumonias in the mouse model.

As pointed out in Section 16.1, the parenteral administration of mCMV results in viral replication in a number of organs, including the lung. If immunosuppression is added, viral titers can be significantly enhanced, and a frank pneumonitis may result. Brody and Craighead (1974) observed that when C3H mice were inoculated with mCMV, they developed interstitial pneumonia during prolonged administration of antilymphocyte serum.

Interstitial pneumonia associated with viral replication in the lung was observed after mCMV infection of the footpad of Balb/c mice that had received total-body irradiation in a single dose of 6 Gy (600 rads) (Reddehase *et al.*, 1985).

Given the right strain of mice, the simplest model of mCMV interstitial pneumonia may be produced by an intranasal inoculation of mCMV (Jordan, 1978). A minimum of 100 PFU of virus inoculated in BRUS mice produced a pulmonary inflammatory response similar to what is seen in man. However, this strain of mice is not readily available. The same type of inoculation did not consistently produce interstitial pneumonia in other susceptible strains such as Balb/c (Shanley *et al.*, 1982).

Shanley *et al.* (1982) found that injection of a single dose of cyclophosphamide 24 hr after intranasal virus inoculation produced a severe interstitial pneumonitis 10–14 days after inoculation. If the mice received additional doses of cyclophosphamide every 5 days after the initial dose, the virus titers in the lungs increased, but interstitial pneumonia was no longer observable. This suggests that repeated doses of cyclophosphamide had suppressed an immunopathological process essential for the development of interstitial pneumonia.

Grundy *et al.* (1985) noted that murine CMV did not produce interstitial pneumonia in F_1 hybrid mice of B10 genetic background unless a graft-versus-host (GvH) reaction was produced by an intravenous injection of parental cells. Virus titers had no predictive value, as they were about the same in infected mice with or without pneumonia. A graft-versus-host (GvH) reaction was produced in F_1 mice of C57BL and BIO.BR parentage by infusing 20×10^6 parental BIO.BR spleen cells. The donor cells ($H\text{-}2^k$) reacted against cells expressing the C57BL ($H\text{-}2^b$) antigens

in the recipients. Murine CMV was given intraperitoneally at the same time as donor cells. A consistent interstitial pneumonia was developed. Compared to sham-infected controls or controls without a GvH reaction, these mice showed by bronchoalveolar lavage an increased number of inflammatory cells from the lung, 85% of which were of donor origin. The majority were T lymphocytes of the helper ($T3T4^+$) and $Lyt2^+$ varieties (Shanley *et al.*, 1987).

Another indication that viral replication alone did not account for the pneumonia in this model was the effect of ganciclovir therapy. Daily subcutaneous injections of ganciclovir beginning 1 day after infection reduced the titers of mCMV in lung tissue to undectable levels, but not the inflammatory response (Shanley *et al.*, 1988).

Interstitial pneumonia may also be produced in nude mice, but only under certain conditions. Murine CMV given to T-cell-deficient athymic nude mice replicated extensively in the lung, but diffuse pneumonitis did not develop even when one dose of cyclosphosphamide was given. However, if T-cell function was restored by reconstitution with syngeneic cells, the combination of mCMV and cyclophosphamide did produce an interstitial pneumonitis.

In summary, interstitial pneumonia caused by mCMV may be produced in numerous ways depending on the route of inoculation, immunosuppression, GvH response, and the integrity of the immune system. Several models point to the importance of immunosuppression as well as an immunopathological component. Immunosuppression allows the virus to replicate, but the immunopathological component allows inflammation to occur. Excessive immunosuppression will eliminate the latter response (see also Section 16.6.3).

16.4. GENETIC DETERMINANTS OF MURINE INFECTION

The genetics of infection cannot easily be studied in man. It is best studied in mice because many inbred mouse strains are available and because the pathology and immunology are more easily explored in this animal. McCordock and Smith (1936) observed that outbred Swiss mice were more susceptible to mCMV than the Buffalo strain and that inbred C57Bl were least susceptible. Selgrade and Osborn (1974) found that DBA mice were more resistant than C57Bl by the intraperitoneal route and that F_1 hybrids of the two strains were resistant, which suggests that resistance was a dominant trait.

Diosi *et al.* (1974) recovered a strain of mCMV from wild mice that was not infectious for Swiss mice by the intracerebral route. F_1 hybrids of the wild mice and Swiss mice were susceptible to this virus. Backcrosses of the F_1 hybrids with Swiss mice produced progeny one-third of which were susceptible. In contrast to the findings of Selgrade and Osborn (1974), susceptibility rather than resistance was thought to be controlled by a unifactorial autosomal dominant gene. The routes of inoculation in the two studies were, however, not comparable, and the number of animals Diosi *et al.* used for the crosses was very small (three or five).

The outcome of infection with mCMV in terms of both (1) resistance to the lethal effects of the infection and (2) characteristics of the virus-induced morbidity have been found to be associated with H-2- (mouse major histocompatibility complex) and non-H-2-associated genes (Chalmer *et al.*, 1977; Chalmer, 1979; Bancroft *et al.*, 1981; Quinnan and Manischewitz, 1987). Chalmer (1979) and Chalmer *et al.* (1977) found that the strains C3H (H-2^k) and CBA (H-2^k) were relatively resistant to the lethal effect of mCMV by intraperitoneal injection, whereas BALB/c (H-2^d), C57Bl (H-2^b), DK-black, Simpson, and PHI mice were relatively susceptible. The F_1 hybrid between C3H and BALB/c mice had a susceptibility between those of the parents. The backcross of the F_1 hybrid to the resistant C3H mice produced a segregation of resistant and susceptible progeny in the ratio of 1 : 1. This result suggests that there is a single gene controlling resistance (or susceptibility) and that it is partially dominant.

To examine the effect of H-2 haplotypes on resistance and susceptibility, congenic strains of BALB/c mice that have an identical genetic makeup except for their H-2 haplotypes were tested for their response to infection. The LD_{50} for BALB/c mice carrying H-2^b, H-2^d, H-2^g, and H-2^k alleles were determined. H-2^g is a recombinant haplotype between b and d. Of these four congenic strains, only the H-2^k haplotype was resistant. The b and d haplotypes were associated with susceptibility.

Recombinant strains with a B10 background are available to examine the relative importance of various regions of the H-2 major histocompatibility complex. It was found that the presence of the k alleles at either the K or IA loci or the D locus was associated with resistance. However, the possibility that other haplotypes might be associated with either resistance or susceptibility was not ruled out. Susceptibility determined at an H-2 locus was inherited as a dominant trait. It was postulated that there were two loci in the H-2 region that determine resistance. One mapped at the K end of the H-2 complex at either the K or IA locus; the other mapped at the D locus or close to it. Differences among various stains were only apparent in adult mice. They did not exist in very young mice, as all of them were equally susceptible.

Quinnan and Manischewitz (1987) also found that the susceptibility trait of Balb/c mice segregated in backcross mice as if carried by a single dominant gene.

Chalmer (1979) found that certain types of pathology were associated with certain H-2 haplotypes. Whereas varying degrees of hepatitis and thrombocytopenia were associated with mCMV infection of all strains, splenic necrosis was only seen in Balb/c mice and H-2^d haplotypes.

Mims and Gould (1978) described splenic necrosis in Balb/c, CDI, LACA, and NIH Swiss mice but not in C57Bl and CBA mice. By specific immunofluorescence, the cells infected were identified as perifollicular round cells that did not have any B- or T-cell marker. They were also not macrophages. It was assumed they are poorly defined dendritic cells.

Massive liver necrosis was seen in C57Bl mice, ovarian involvement was seen in H-2^b haplotypes (C57Bl and Balb/B). Interestingly, the more resistant H-2^k

haplotypes (C3H, Balb/k) developed a unique myocarditis with myocardial calcifications, and C3H mice developed a unique hemolytic anemia (Chalmer, 1979). These are examples of associations. It was not proven that the relationship was exclusive; that is, it was not ruled out that other haplotypes or recombinants may manifest one or more of these pathological conditions.

Until fairly recently it was not clear that the susceptibility of cultured cells from different inbred strains corresponded to that of the intact host. Harnet and Shellam (1982) first correlated mCMV replication in fibroblasts from different mouse strains *in vitro* with *in vivo* resistance, although the differences observed *in vitro* were small. Quinnan and Manischewitz (1987) reported similar findings with spleen cells (see below). More striking findings were found recently with peritoneal macrophages by the Australian group (Price *et al.*, 1987). Fewer than 10% of macrophages from the resistant H-2^k mice infected *in vitro* produced viral antigen, DNA, or cytopathic effect, whereas 80–90% of macrophages similarly infected from more sensitive H-2^d, H-2^b, and H-2^a strains became affected. In H-2^a cells (B10.A), the D^d gene conferred sensitivity despite the resistant K^k and class II k phenotype. These data are consistent with the conclusion that susceptibility of infection of macrophages by mCMV is determined by H-2 phenotype and that there may be a critical association between class I antigens and an early stage in the infectious process.

The mechanism by which H-2 genes mediate susceptibility and resistance is not clear. Such a mechanism must be effective early in the infection, since susceptible mice may die within 3 days of virus administration. Several putative mechanisms have been studied. By and large they point to the operation of multiple factors, some of which are not entirely controlled by the H-2 gene complex.

This point may be illustrated by the effect of the Nu/Nu gene on genetically determined resistance to mCMV. Mice homozygous for this gene are nude and athymic and are unable to mount a specific T-cell response. Genetically susceptible Balb/c (H-2^d) mice showed a greater than 316-fold increase in susceptibility as determined by the LD_{50} in homozygous nude mice as opposed to heterozygotes. On the other hand, athymic mice of the resistant CBA (H-2^k) strain showed only a 16-fold increase in susceptibility compared to their heterozygous littermates. They were also no more susceptible than T-cell-competent Balb/c mice. These results emphasize the importance of H-2-associated genetic resistance and suggest that this resistance cannot be fully explained by T-lymphocyte responses.

The NK-cell response is a preformed defense mechanism that reacts early during infection. Bukowski *et al.* (1984) studied the effect of NK depletion by administering to mice antibody to asialo-G_{M1}, a neutral glycosphingolipid present at high concentration on NK cells. The LD_{50} of mCMV for C57Bl/6 mice was decreased, and virus titers in infected animals were increased up to 1000-fold in their lungs, spleens, and livers. These acute effects of NK depletion were produced when NK-cell depletion was produced 2 to 3 days after infection. Ballooning hepatocytes and splenic necrosis, typically seen in the more susceptible Balb/c strain after mCMV infection, was noted. The NK-cell depletion did not activate latent infec-

tion, but enhanced virus titers in the persistently infected salivary gland were seen 30 days after infection. Thus, NK-cell depletion increased the severity, extent, and duration of acute infection.

Another approach to the elucidation of the role of the NK cell is to study the *beige* (bg) mutation in various inbred strains of mice (Roder, 1978). The ability of NK cells from beige mice to lyse cells is depressed. Homozygous beige (bg/bg) mice were more susceptible to the lethal effects of mCMV, and virus titers were higher in the organs of these mice than their bg/+ littermates. The greatest effect of this mutation was seen in CBA mice, which are genetically most resistant to mCMV and also have the highest endogenous levels of NK cells. These results are consistent with NK cells being an important mediator of genetically determined resistance to mCMV (Allan and Shellam, 1985).

Another indication that NK activity may be related to resistance is the clear association between augmentation of NK-cell response during an infection and resistance to the lethal effects of mCMV (Bancroft *et al.*, 1981). This correlation existed in ten out of 11 strains examined. For example, the more resistant CBA and C3H strains showed significantly greater augmentation of NK activity during infection than the more susceptible Balb/K or Balb/c strains. This augmentation, probably largely as a result of induction of interferon during infection, was evident in resistant strains 10 hr after infection, which is consistent with the role of NK cells in early defense mechanisms before the development of specific immunity. However, the most resistant strain, B10.BR, showed no augmentation at all, demonstrating that the NK-cell response is not always an indispensable component of genetic resistance.

Comparison of the induction of plasma interferon showed that higher plasma interferon levels were induced by large doses of mCMV in resistant CBA than in susceptible Balb/c mice (Allan and Shellam, 1985).

Quinnan and Manischewitz (1987) attempted to sort out which genetic factor of resistance was interferon dependent by studies of infection in animals and in cell culture. Harnett and Shellam (1982) had originally shown that replication of mCMV in fibroblasts correlated with *in vivo* resistance (see above). This was confirmed for BIO.BR, C3H, C57Bl, and Balb/c strains of mice and their adherent spleen cells, which, as listed, had decreasing order of resistance to mCMV *in vivo* and in cell cultures. Neutralization of interferon in cultures of adherent spleen cells from these strains enhanced the susceptibility of C3H ($H\text{-}2^k$) cells but not of the other strains. Hence, resistance in $H\text{-}2^k$ mice (CBA, C3H) seemed to be interferon dependent. However, the mechanism of resistance of BIO.BR was still largely unexplained. Not only was its resistance independent of interferon formation and NK cytotoxicity but specific antibody and cytotoxic T-cell responses in BIO.BR mice were similar or lower than in other more susceptible strains.

Another putative resistance factor studied is the development of *specific delayed-type skin sensitivity* (DTH) to mCMV. Resistant $H\text{-}2^k$ (C3H, CBA) strains developed more DTH of the skin to inactivated mCMV than susceptible strains such as Balb/c ($H\text{-}2^d$) and C57Bl ($H\text{-}2^b$). Among genetically resistant strains, BIO.BR ($H\text{-}2^k$) and Balb.K ($H\text{-}2^k$) produced a significantly greater DTH response than the less resistant congeneic strains $C_{57}Bl/10$ ($H\text{-}2^b$), Balb/c ($H\text{-}2^d$), and BALB.B

(H-2^b). The greater DTH response and resistance of C_{57}Bl compared to Balb.B mice, both of the H-2^b type, shows the importance of non-H-2-linked genes in determining the extent of the DTH response and resistance. Although DTH response and resistance seem to be correlated, the role of DTH in resistance is unclear (Lawson *et al.*, 1987).

16.5. LATENCY OF MURINE CYTOMEGALOVIRUS AND ITS ACTIVATION

Murine CMV persists in mice for varying periods after acute infection. Free virus is demonstrable in the salivary gland, throat, kidney, or urine of outbred Swiss mice for months to a year after infection (Brodsky and Rowe, 1958; Medearis, 1964b). Such animals are said to be persistently infected.

Latent infection defined as viral activity in the absence of "lytic" or complete active viruses was first demonstrated by Henson *et al* (1972b). At a time when free virus could not be demonstrated, spleen cells from C3H and BALB/c mice infected 14–55 days earlier were cocultivated on mouse embryo cell culture, and viral CPE was observed 9–21 days later. In one case, CPE took as long as 55 days to develop. Similar observations were made with cells from abdominal lymph nodes. These findings suggest that in the absence of free virus, virus in latently infected cells may be activated by cocultivation. The nature of latency is unknown in the case of both human and murine CMV. The definition of latency in our present context in essentially operational: it implies the absence of free virus that is demonstrable by usual virological methods. The key problems associated with latency in mCMV as in hCMV (see Section 5.5) are how it is brought about, how reactivation occurs, and where cellular sites of latency are.

Wise *et al.* (1979) described a method of reactivating virus in minced spleen tissue from Balb/c mice infected for 4 weeks. Explants were planted on collagen-coated bottles and became positive in 1 week. This method was more sensitive than cocultivation and worked with either allogeneic or syngeneic fibroblasts.

Olding *et al.* (1975, 1976) described a model of latent infection and some interesting properties of latently infected cells. They injected inbred strains of mice *in utero* or neonatally with tissue-culture-passaged virus. Adult survivors (10% to 50%) had no evidence of free virus in any organ. However, in a small number of these mice, when their spleen cells were cocultivated on allogeneic but not syngeneic mouse embryo fibroblasts, virus was recovered. The latently infected cells were all in the B-lymphocyte fraction of spleen cells. By DNA–DNA hybridization, three to four virus genomes equivalent per 100 cells were found in the spleen and less often in the salivary gland but not in other tissues. The conclusion was that B lymphocytes were carriers of latent mCMV infection and that cell division stimulated either by mitogens or by allogeneic reaction was needed for activation. This model for allogeneic reactivation has not been confirmed.

The La Jolla group also described a type of latent infection in macrophages. By cocultivation on either syngeneic or allogeneic fibroblasts, thioglycollate-stimulated

macrophages from mice latently infected at birth produced mCMV in 12 out of 18 and 15 out of 18 cases, respectively (Brautigam *et. al.*, 1979). The method of infection was presumably the same method of producing latency as previously described (Olding *et al.*, 1975). Virus could be recovered as long as 30 months after initial infection. This suggests that thioglycollate stimulation is able to reactivate viral genomes that are in macrophages, although the possibility exists that they became permissive and were infected by virus from another source.

Various immunosuppressive regimes that reactivate latent mCMV infections have been developed. Jordan (1980) "immunized" C3H mice with cell-culture-passed live "vaccine" virus. Although immunized animals were protected from exogenous infection, 19 out of 20 developed disseminated infection when immunosuppressed with antilymphocyte serum and cortisone.

Mayo *et al.* (1977) infected DBA/2 mice with mCMV and found that by 16 weeks, all tested organs, including the spleen and salivary glands, were free of detectable virus. The following methods were used to demonstrate that spleen cells from these mice were latently infected:

1. Cyclophosphamide and azathioprine (Mayo *et al.*, 1978) administered to these mice activated their latent infection so that free virus became detectable in the salivary gland.
2. Spleen cells (10^8) from latently infected mice transferred to syngeneic recipients or allogeneic C57Bl recipients treated with cyclophosphamide infected the salivary glands of the recipients. Presumably, in order for virus to activate, continued survival of injected cells was necessary. Immunosuppression of the allogeneic host may have facilitated this.
3. Spleen cells from these mice could be activated by cocultivation on either syngeneic or allogeneic fibroblasts (Mayo *et al.*, 1978).

Jordan *et al.* (1977) obtained similar results with C3H/St mice, which received 1000 PFU salivary-gland-passed mCMV. Between 4 and 8 months after infection, 90% of salivary glands became negative for virus. The gland was surgically biopsied before it was studied to ascertain that it was free of lytic virus. Virus, however, was reactivated by a combination of antilymphocyte serum (ALS) and cortisone. Viremia as well as virus was consistently found in the salivary gland, kidney, liver, and spleen.

Reactivation of latent infections in donated organs is described in the section below. One variant of that is reactivation by transfusion. Cheung and Lang (1977) transferred blood cells from latently infected C3H and Balb/c mice to syngeneic and allogeneic Balb/c or C3H recipients. Virus was activated and detected by its presence in the salivary gland in allogeneic recipients but not in syngeneic recipients about 4 weeks after cell transfer. In addition, they "activated" latency in allogeneic recipients by injection of uninfected blood but there were many exceptions. These experiments were intended to test the interesting but unconfirmed hypothesis that human CMV infection is not transmitted but is activated by blood transfusions (Foster, 1966; Lang, 1972).

It is quite clear that a variety of cells and tissues can become infected during an acute infection. The question of which types of cells carry latent infection is still unresolved. Results vary depending on the experimental model and virus detection method used.

As pointed out above, work from the La Jolla group suggested that the B lymphocyte and macrophages may be the site for latency.

Wu and Ho (1979) suggested that there may be more than one kind of latently infected cell. Balb/c mice were infected with mCMV, and white-cell fractions were followed for free and latent virus infection for 4 weeks. By 8–10 days after infection, no free virus was demonstrable in serum or blood cells. However, when either B or T cells from blood were cocultivated on syngeneic or allogeneic fibroblasts, virus was activated from a small number of cells. Further, when latently infected lymphocytes were placed in a 450-nm Millipore® chamber and cocultivated on fibroblasts, the T lymphocytes produced free virus, but the B lymphocytes did not. This suggests that both B and T lymphocytes may be latently infected but that infection in B lymphocytes was more restrictive and direct contact of B cells with the feeder cell layer was needed for activation.

Jordan and Mar (1982) studied the site of latency in spleens of mCMV-infected C3H mice. They made suspensions of spleen cells by forcing spleen fragments through a screen. Virus could only be detected by cocultivation in the nonadherent fraction, which was Thy-1-negative and adhered to nylon wool and IgG affinity bead columns. They also concluded from these data that B lymphocytes were latently infected.

Different conclusions were arrived at by Mercer *et al.* (1988), who studied the site of infection in the spleens of acute and latently infected C3H and Balb/c mice. Acute infection, as defined by combined application of immunocytochemical staining, *in situ* hybridization, and electron microscopy, was present predominantly in sinusoidal lining cells, which were positive for factor-VIII-related antigen and negative for Ia, Thy-1, and F4/80 macrophage marker antigen. Lymphocytes and macrophages were not infected. Spleens of latently infected mice could not be studied by these methods because there were too few infected cells. However, by cocultivation on mouse embryonic fibroblasts, virus was recovered only from fragments of spleen suspensions sedimentated 10 to 20 min at $1 \times g$. Removal of immunoglobulin-bearing cells and depletion of Thy-1- and Ia-bearing cells did not affect reactivation. Although the precise cell type was not identified, B and T lymphocytes, macrophages, and dendritic cells were all ruled out. Sinusoidal or endothelial cells were considered possible sites for latent infection. These findings are potentially important to explain why organs are so much more efficient in transmitting CMV than blood cells (Section 13.1.1).

16.5.1. Latency and Reactivation in Transplantation

A number of organs and tissues of infected animals such as kidney, heart, and skin have been shown to transmit mCMV to uninfected recipients (Hamilton and

Seaworth, 1985; Rubin *et al.*, 1984; Shelby and Shanley, 1987). Infection may be transmitted either by the lytic or "complete" virus or by latently infected cells. In some reports transmission was only possible if complete virus was demonstrable in tissues, or only if tissues from actively infected animals were transplanted. This was the case of Shelby and Shanley (1987), who used skin, and Billingsley *et al.* (1983), who used kidneys of C3H mice. However, Rubin *et al.* (1984) and Hamilton and Seaworth (1985) showed that heart or kidney transplants from latently infected Balb/c mice could also transmit mCMV.

In the system of Hamilton and Seaworth (1985), virus was undetectable in any tissue or body fluid 8 to 12 months after primary infection. Treatment of the mice with antithymocyte serum reactivated the infection in 82% of the mice, suggesting that the original infection had resulted in latent infection in most of the animals. Direct experiments to test for infection by cocultivation of kidney minces with mouse embryonic fibroblasts showed that 53% (8/15) were positive (Porter *et al.*, 1985). Of 22 such latently infected donors, 13 (59%) were shown to transmit the virus after transplantation of kidney tissue. Blood transfusions transmitted latent virus to 28% of recipients. Transmission from either organ transplantation or blood transfusion was more apparent in syngeneic than in allogeneic recipients.

Klotman *et al.* (1985, 1987) showed that when previously infected recipients were transplanted with an organ from a donor infected with another mCMV strain distinguishable by restriction endonuclease pattern, the latent recipient strain was activated in preference to the donor strain. This interesting finding may have relevance to the human situation, where seropositive recipients frequently receive organs from seropositive donors (see Section 13.1).

The cells responsible for transmitting mCMV from the donor to recipients, as in the case of human transplantation, have not been identified (Section 13.1.1). Many organs in latently infected animals including the kidney have now been found to contain mCMV DNA. This was shown when DNA extracted from tissue was subjected to polymerase chain reaction (PCR) amplification using synthetic oligonucleotide primers flanking a Bam HI B fragment or a 116-bp region of the major immediate early antigen gene (Henry *et al.*, 1988).

16.5.2. Cytomegalovirus Infection and Alloreactivity

Allograft reactions may be a stimulus for activation of CMV infection. Reactivation of latent CMV infection is almost universal after most types of major organ or tissue transplantations (see Section 13.1). Whether a host-versus-graft (HvG) or a graft-versus-host (GvH) reaction, which almost always follows these allografts, can activate or enhance a latent infection has been studied in the mouse model. Mouse leukemia virus was activated by a HvG reaction produced by a combination of skin graft and antilymphocyte serum (Hirsch *et al.*, 1972) as well as by a GvH reaction (Hirsch *et al.*, 1970a).

Wu *et al.* (1975) found that when a skin graft from C3H/He was placed on a chronically infected Balb/c recipient, there was significant elevation of virus titers at all points of observation within 10 days after grafting.

J. Dowling *et al.* (1977) produced a GvH reaction in F_1 hybrids (C3H/He × DBA/2) that had been infected 5 weeks previously by multiple injections of parenteral DBA/2 spleen cells. Compared to controls, the organs of those animals undergoing a GvH reaction were more frequently positive for mCMV than controls.

As pointed out in Section 16.5.1, transmission from organ transplantation or second transfusion was more evident in syngeneic than in allogeneic donor–recipient pairs (Porter *et al.*, 1985). Presumably survival of latently infected grafted cells may facilitate successful infection (see also Section 13.1.1).

Murine CMV infection can affect the host response to foreign histocompatibility antigens in two phases (Grundy and Shearer, 1984). The response of spleen cells to allogeneic H-2 antigens was measured by an *in vitro* cell-mediated lympholysis assay. As has been shown for other immunologic responses (see Section 16.5), the response to allogeneic antigens was suppressed 2–4 days after infection. However, by 7–13 days after infection, spleen cells of certain strains, such as C57Bl but not Balb/c mice, showed *enhanced* alloreactivity. A similar biphasic pattern of suppression and enhancement of cytotoxic response to hapten (trinitrophenyl)-modified self antigens was also observed. It is possible that this type of enhanced allogeneic response may explain the enhancing effect of CMV infection on allograft rejection (see Section 13.1.4). It also suggests that CMV infection may augment the GvH reaction.

The latter point was directly tested in the F_1 hybrid model of GvH reaction, in which parental spleen cells are given intravenously to F_1 hybrids (Grundy and Shearer, 1984; Grundy *et al.*, 1985). The GvH reaction, as described above, markedly inhibited *in vitro* cell-mediated lympholysis response to allogeneic H-2 antigens. Murine CMV infection plus GvH markedly augmented the immunosuppression. It also greatly augmented the GvH reaction as measured by spleen weight. A GvH reaction was produced in infected mice with as few as 1×10^6 spleen cells, which was 20 times less than the amount needed to produce a GvH reaction in reinfected mice. This may be related to the enhanced alloresponse seen 7–13 days after mCMV infection in mice of B10 background (see above paragraph). Moreover, murine CMV combined with a GvH reaction produced an interstitial pneumonitis that resulted in deaths 16–21 days after infection. Viral titers in the lung had no predictive value for pneumonia, since they were about the same in infected mice that did not have a GvH reaction or interstitial pneumonia. Intranuclear inclusions and antibody titers were also absent.

Via *et al.* (1988) studied in greater detail the kinetics of the effect of CMV infection on GvH reaction and immunodeficiency. When donor mice were infected with murine CMV 3 days prior to spleen cell transfer, the ability of these cells to induce GvH was *reduced.* In contrast, infection of the recipient 3 days prior to cell transfer *increased* their susceptibility to GvH reaction and immunodepression. This

suggests that CMV infection in the recipient may enhance the GvH reaction. Infection of either donor or recipient mice 10 to 17 days before spleen cell transfer had no effect. Thus, acute mCMV infection early after transplantation can alter the course and severity of the GvH reaction, a finding of relevance to human bone marrow transplantation (see Section 13.1.4).

16.6. THE IMMUNE RESPONSES TO MURINE CYTOMEGALOVIRUS

16.6.1. Responses of B Cells

One characteristic of virus infection, contrary to many infections by bacteria, fungi, and parasites, is the stimulation of potent neutralizing antibodies. Such antibodies are important in terminating infection and accounting for acquired immunity. The immunologic responses to mCMV are the same as to other classical viral pathogens. However, one difference shared by CMV and other herpesviruses is that the host often fails to clear up the infection, so that persistent or latent infection ensues. Whether this failure can be traced to some deficiency in the humoral or cellular immune response is a question of abiding interest and one that has not yet been solved.

Early evidence seemed to indicate that antibodies develop late. Two weeks after infection by mCMV, neutralizing antibodies were found in serum, and complement-fixing antibodies were not detected until 1 month after infection (Medearis, 1964b). The relatively late development of these antibodies has been ascribed to the immunosuppressive effect of the virus infection (Osborn *et al.*, 1968). Antibody was measured by antibody-dependent cell-mediated cytotoxicity (see Sections 7.1 and 7.2.2) 8–10 days after infection of Balb/c mice, and titers were tenfold higher than neutralization titers (Manischewitz and Quinnan, 1980).

Cruz-Araullo *et al.* (1978) showed that in CD-1 mice, serum from animals infected for 3 days had specific 7 S γ-globulin and could be used passively to protect animals. Similar results were reported by Shanley *et al.* (1981), who showed, in addition, that immune mice immunosuppressed by antilymphocyte serum were protected from the acute infection by mCMV. Establishment of the latent infection, however, was not prevented. These experiments suggest that humoral immunity accounts to a large extent for acquired immunity to an outside challenge.

One method of assessing the importance of an immune response is to observe infections in animals that cannot mount the response. Brody and Craighead (1974) reported that the majority of mice treated with antilymphocyte serum and inoculated with a small dose of virus developed fatal infections with extensive pulmonary pathology. Selgrade *et al.* (1976) observed that the LD_{50} for homozygous athymic nude (Nu/Nu) mice deficient in T cells was $10^{3.9}$ PFU, whereas it was $10^{5.3}$ for heterozygotes (Nu/+). Balb/c mice whose B (Ig+) lymphocytes were reduced to

5% by injection of goat antimouse IgM (μ chain) also had a lower $LD/_{50}$ ($10^{4.1}$ versus $10^{5.1}$ PFU in controls). It is interesting that mice in both deficient strains took longer to die than controls. Starr and Allison (1977) also found that nude mice had fewer inflammatory changes in the liver than controls. In addition to being protective, both humoral and cellular immunity may participate in immunopathological reactions that hasten death or account for specific pathological reactions in certain organs.

Another type of experiment suggests that at least one B-lymphocyte function is unimportant in resistance to an acute infection in a nonimmune host. CBA/Hn mice possess a sex-linked B-cell defect expressed in inability to form antibody against T-cell-independent antigens such as DNP-Ficoll. They all respond poorly to B-cell mitogens (Scher *et al.*, 1975). When CBA/Hn female mice are mated with normal DNA/2 male mice, the F_1 male carries the defect, and the congenic F_1 female does not. Males and females infected with mCMV did not differ in LD_{50}.

16.6.2. Cell-Mediated Immunity

Several developments have focused on the role of cellular immunity against CMV infections. First, despite the presence of neutralizing antibodies, CMV infection persists in man and animals, and, under proper circumstances, latent infection may be activated. Second, cytotoxic immunosuppressants commonly used in human medicine, which primarily affect cell-mediated immunity, activate or enhance CMV infection in both man and animals. A great deal of knowledge concerning cellular immunity against CMV comes from studies in mice.

As in the case of human CMV infection (Section 7.3.1), during an acute infection, helper (Lyt 1.2) T lymphocytes are decreased, and suppressor (Lyt 2.2) cells are increased. The helper-to-suppressor cell ratio is lowest 5 days after infection (Sell *et al.*, 1985).

Antithymocyte serum given to wild mice can convert an asymptomatic salivary gland infection into a fatal disseminated infection that produces inclusion bodies and mCMV in various viscera such as liver and spleen (Gardner *et al.*, 1974). As pointed out in Section 16.3, a favorite method to reactivate a latent infection or enhance a persistent one is to administer some form of immunosuppression, most types of which affect T-cell function in one way or another.

Starr and Allison (1977) found that 10^8 immune spleen cells obtained 6 days after infection protected Balb/c mice from death when they were given 24 hr after 1.5 LD_{50}. The protective effect of the spleen cells was eliminated by pretreatment with anti-θ serum and complement. Serum from mice infected for 6 days failed to protect. These findings suggest that the protective cells were T lymphocytes.

Kelsey *et al.* (1978) and Howard *et al.* (1978) studied the specific proliferative response of T lymphocytes against mCMV. The antigen used by Kelsey *et al.* (1978) was UV-irradiated mCMV-infected mouse embryo fibroblasts, whereas Howard *et al.* (1978) used semipurified heat-inactivated virus. Kelsey *et al.* reported a signifi-

cant proliferative response 2 days after infection, whereas Howard *et al.* noticed maximum proliferation 15 days after infection. The capacity of spleen cells to respond was demonstrable for at least 60 days. Kelsey *et al.* (1978) point out that during the period of nonspecific immunosuppression (4–10 days after infection), a specific proliferative response could still be demonstrated.

16.6.2.1. NK and Cytotoxic T Cells

NK cells are preformed nonspecific cytotoxic cells that are active before the development of acquired immunity and play an important role in determining the course of infection (see Section 16.4.1). NK-cell activity is also enhanced by interferon induced within days of a virus infection (Welsh, 1978).

Quinnan and Manischewitz (1979) found that before the development of specific cytotoxic T cells, NK-cell activity was elevated in the spleen between the second and sixth day of infection; NK activity was measured by lysis of RBL5 target cells. Balb/c mice were infected with 10^4 PFU of *in-vivo*-passed mCMV. Interferon was first detected 2 days after infection, reaching maximum levels of $10^{3.1}$ units/ml. It then declined over the next 4 days to undetectable levels. These NK cells were thought to be stimulated by interferon induced by mCMV infection.

It is difficult to prove unequivocally the role of cytotoxic T lymphocytes in defense against CMV infection. One cannot separate clearly their function from other T-cell functions. However, as in the case of human infections, there is now a body of evidence to suggest that they are important (see Section 7.3).

Quinnan *et al.* (1978) reported that 3 days to about 3 weeks after intraperitoneal infection, various inbred strains of mice developed specific cytotoxic T cells that lysed mCMV-infected mouse embryo fibroblast targets. The same response was observed in cells of the spleen, buffy coat, and anterior cervical lymph nodes after Balb/c mice were infected intransally (Quinnan *et al.*, 1980). The maximal response was seen about 10 days after infection. This is consistent with the time interval during which cytotoxic cells may be found in the circulation after other viral infections, which is usually about 1–2 weeks after infection (Blanden, 1974).

Ho (1980) found that spleen cells from 6-day-infected animals given 24 hr after a virus challenge reduced spleen virus titers. Cells from animals 3 days before or 30 days after infection were not protective. The time interval corresponds to the time needed to generate cytotoxic T cells *in vivo* according to Quinnan *et al.* (1978). Further, Ho and Ashman (1979) and Ho (1980) generated cytotoxic T cells by *in vitro* incubation of spleen cells from immune animals with UV-irradiated mCMV-infected fibroblasts. Such cells also reduced spleen virus titers when given 24 hr after a virus challenge. Protection was eliminated by anti-θ serum and complement. These cells were protective only in animals that shared with the donor cells identical H-2K and/or H-2D antigens. This H-2-restricted recognition and passive protection was typical of specific cytotoxic T cells (Zinkernagel and Doherty, 1974). These data taken together suggested that cytotoxic T cells were active in passive cell-mediated protection.

More recently, the dynamics of the generation of cytotoxic T lymphocytes (CTL) in the murine CMV (mCMV) system and their specificities and function have been studied in some detail by the Tübingen group (Reddehase *et al.*, 1984a,b). Lymphocytes were collected from lymph nodes draining the footpad acutely inoculated with mCMV. They could not be used to demonstrate cytotoxicity directly, but functional cytotoxic T cells (CTL) were produced *in vitro* in the presence of IL-2 or in the presence of viral antigen. Precursors that responded to IL-2 stimulation alone were the minority (15%) of responsive cells and represented more mature differentiated CTL. Those that responded to viral antigen represented more immature precursors.

A major contribution of the Tübingen group was to show that these CTL were primarily directed against the immediate early (IE) antigen of mCMV. Fibroblast targets expressing IE antigen were produced by sequential addition of cycloheximide and then actinomycin D to suspensions of freshly infected cells. Cycloheximide prevented protein synthesis and allowed early messenger RNA to accumulate. Actinomycin added after cycloheximide was removed prevented transcription of late messages and synthesis of late proteins. To prepare fibroblast targets expressing "late" antigens, infection was carried out in the absence of these two antimetabolites, and viral replication was allowed to proceed for 20 hr. About one out of 6900 lymphocytes collected from a draining popliteal lymph node during an acute mCMV infection was a precursor CTL that recognized targets expressing IE antigens. Only half as many precursors recognized targets expressing late antigens (Reddehase *et al.*, 1984a,b; Koszinowski *et al.*, 1987a).

This recognition pattern was also confirmed by first constructing target cells by transfection of the different parts of the cloned IE gene region into L cells that were histocompatible with the effector CTL derived from Balb/c (H-2^d) mice (Koszinowski *et al.*, 1987b). As described in Section 16.1, the IE region consists of at least three transcription units that code for regulatory IE proteins. The main transcript is ie1, which codes for pp89, an 89-kDa phosphoprotein that is the major regulatory protein (Keil *et al.*, 1987). Mice were also vaccinated with a recombinant vaccinia virus, mCMV-ie1-VAC, which contains the ie1 gene and expressed only pp89. They produced CTL precursor, which lysed mCMV-infected fibroblasts (Volkmer *et al.*, 1987).

The Tübingen group have also provided evidence that specific CTL are protective in an experimental mCMV model of infection. Mice of the Balb/c strain were immunosuppressed by total body γ irradiation before intraplantar infection by mCMV. Pneumonitis (Reddehase *et al.*, 1985), splenitis, and adrenalitis (Reddehase *et al.*, 1987a,b) were produced. T lymphocytes obtained from a draining lymph node or memory cells from latently infected mice were protective when given prophylactically before infection or therapeutically after mCMV had colonized host tissues. Prior *in vitro* expansion of cell numbers was not required. The lymphocyte subset responsible was the cytotoxic $CD8^+$ phenotype. $CD4^+$ helper cells were not protective and did not add to the protective effect of CD8 cells (Reddehase *et al.*, 1987a, 1988).

In contrast, Shanley studied adoptive immunity in another infection model and obtained different results. Adrenalitis was produced by mCMV infection in homozygous nude mice, which are selectively defective in mature T lymphocytes. Adoptive transfer of immune T lymphocytes from the spleen of heterozygous mice infected 21 days before suppressed viral replication in the adrenal gland. Spleen cells from naive mice were ineffective. Transfer of sorted cells showed that T cells of the CD4 (L3 T4 helper) phenotype were responsible for the protective effect. CD4 cells are class II MHC antigen restricted and may help in antibody formation as mediators of delayed hypersensitivity or even cytotoxicity. Cells of the cytotoxic CD8 (Lyt-2) phenotype were not protective (Shanley *et al.*, 1987).

The possible explanations for the differences between the two models are many. Very likely the functions of the many components of T-cell immunity may be brought out by different models of infection.

16.6.2.2. Murine Cytomegalovirus and Macrophages

Macrophages are a double-edged sword. They can inhibit virus replication (Morahan *et al.*, 1980), but they may also be carriers of virus infection. Johnson (1964) found that herpes simplex virus is spread from the peritoneum to the central nervous system by macrophages in the suckling mouse. Macrophages from adult mice are not permissive for herpes simplex virus, and such spread does not occur (Hirsch *et al.*, 1970b).

Numerous attempts have been made to implicate macrophages in the pathogenesis of mCMV. There is no simple parallel to the situation with herpes simplex virus. No difference in permissiveness of macrophages from mice of different ages has been found.

Tegtmeyer and Craighead (1968) used virus passaged four times in cell culture and were able to infect mouse macrophages. Selgrade and Osborn (1974) showed, however, with stock salivary gland virus, that peritoneal macrophages were not well infected. They may be considered to be essentially nonpermissive.

Selgrade and Osborn (1974) studied macrophages of a resistant (CBA/J) and a susceptible strain (C57Bl/6J) of mice. Injection of 60 mg silica 2 hr prior to infection decreased mortality in both strains but did not eliminate the innate resistance of CBA mice. Titers in liver were increased by silica administration, but not those in the spleen. In general, C57Bl mice (even without silica) showed more hepatitis than CBA mice.

Suckling or younger mice are more susceptible to mCMV. They were injected with adult syngeneic macrophages or lymphocytes prior to infection to see if resistance was increased. Mortality was reduced by macrophages more effectively than by lymphocytes, but the recipients were still more susceptible than the donors. Macrophages from susceptible and resistant mice did not replicate mCMV virus *in vitro,* although cells were infected. An increase in infectious centers to a maximum of 50% 9 days after infection was noted. This contrasts with the findings of Tegtmeyer and Craighead (1968), who found good replication of mCMV.

This study shows that although macrophages are important, they are not the only defense factor, and their function differs from the situation in HSV.

Mims and Gould (1978) restudied the problem. Tissue-culture-passaged virus was ten times more ineffective than salivary gland virus. But even with this virus, about 100 PFU was needed to infect one macrophage or to produce one fluorescent focus. Macrophages activated by broth were even more resistant.

Brautigam *et al.* (1979) made the observation that macrophages stimulated by thioglycollate were more permissive. After such macrophages were infected with cell-culture-passed virus, 5–7 log PFU units per ml were produced in 3–9 days. They were able to show that 20–50% of macrophages infected at high multiplicity were infected by infectious center assay, whereas only 11–18% of unstimulated macrophages were infected. By *in-situ* nucleic acid hybridization, 82% of macrophages, whether or not stimulated by thioglycollate, contained mCMV DNA. Macrophages from both highly susceptible ($H\text{-}2^d$) Balb/cSt or resistant ($H\text{-}2^k$) C3H mice replicated equally well. Activation of macrophages, perhaps accompanied by DNA stimulation, may be important in increasing permissiveness for viral replications.

It is difficult to understand the biological meaning of this phenomenon, as tissue-culture-passed virus is less virulent and does not infect the liver, spleen, or central nervous system after an intraperitoneal infection. Also, in view of the finding of Mims and Gould (1978) that broth-stimulated macrophages are more resistant, there must be subtle determinants of permissiveness following different methods of "elicitation" and "stimulation" of macrophages (Morahan, 1980).

16.6.3. Immunosuppressive Activity of Murine Cytomegalovirus

16.6.3.1. Effect on Humoral Immunity, Interferon, and Nonspecific Cell-Mediated Immunity

Many viruses are immunosuppressive (Fenner *et al.*, 1974; Rouse and Horohov, 1986), and we are familiar with the foremost one of all, HIV (Section 13.2). In the case of mCMV infection, the study of its immunosuppressive effect may have relevance to human infection and disease by hCMV, which is also immunosuppressive (Section 13.1.5). The suppressive effect of acute mCMV infection on various parameters of immunity is very well documented.

Osborn and Medearis (1967) first showed that during acute mCMV infection, the antibody response is profoundly suppressed. Sheep red cells injected on the fourth to the eighth day of mCMV infection stimulated neligible amounts of hemagglutinin 7 days later. Jerne-type plaque-forming centers (PFC) (Jerne and Nordin, 1963) in the spleen were also measured (Osborn *et al.*, 1968). Red cells were injected at various times after mCMV infection, and PFCs were assayed 4–6 days later. Maximum suppression occurred 4 days after infection, at which time, compared to controls, there was more than a 100-fold reduction of 19 S and 7 S PFCs in

response to sheep red blood cells. Recovery was, however, rapid. All measurements of 19 S and 7 S PFCs were normal by day 8 of virus infection.

Howard *et al.* (1974) showed that suppression of cellular immunity also occurs following mCMV infection. A skin graft from C57Bl/6 male to female represents grafting across a weak H-Y histocompatibility barrier. Its survival was prolonged to over 180 days compared to 20 days in controls if the recipient was infected on the day of grafting. Infection 5 and 7 days before grafting but not 13 days before grafting also prolonged graft survival.

Similar results were obtained with skin grafting across a strong (H-2) histocompatibility barrier. Grafts from A/J mice survived longer in C3H recipients if they were infected on the day of grafting or 3, 5, or 7 days before but not 21 days before grafting. The survival of the graft was 7.0 days in controls compared to a maximum of 11.7 days in mice infected 3 days before grafting.

Phytohemagglutinin stimulation was studied in 4-week-old C57Bl/6 females infected with mCMV. Background incorporation of tritiated thymidine was elevated in spleen cells from infected mice for 5 days after infection, but response to PHA was depressed for the entire 26 days of study.

Response to nonspecific B-cell antigens such as pokeweed mitogen (PWM) and lipopolysaccharide (LPS) is also suppressed (Selgrade *et al.*, 1976).

Mixed lymphocyte culture (MLC) reaction involved irradiated A/J spleen cells as stimulating cells (Howard *et al.*, 1974). Responding cells were obtained from infected and control C57Bl/6 mice. As in the PHA studies, the background uptake of tritiated thymidine was higher in the culture containing cells from infected mice. The degree of stimulation by allogeneic cells was depressed in cells obtained from mice 5 or 12 but not 19 or 26 days after infection.

Booss and Wheelock (1975) tried to correlate the mortality of DBA/2 female mice infected with mCMV infection with depression of response to nonspecific mitogens. Spleen cells from both adults and weanling mice given a lethal dose of mCMV were essentially unresponsive to concanavalin A 4 days after infection. Cells from adult, nonlethally infected mice were significantly stimulated, although less so than controls.

They were maximally suppressed between days 3 and 11 after infection, and the stimulation index was normal by day 15. The cells from lethally infected animals had less than 22% of normal activity from day 3 to day 8 of mCMV infection and less than 10% between day 6 and day 8. Cells from nonlethally infected animals always had more than 10% of the activity of controls (Booss and Wheelock, 1977).

On the other hand, the number of focus-forming anti-sheep-red-cell antibodies was equally suppressed in spleens from both lethally and nonlethally infected animals. Suppression of this parameter did not predict the lethal outcome of an infection.

Acute mCMV infection also inhibits interferon formation (Osborn and Medearis, 1967). From 68 hr to 12 days after mCMV infection, weanling CD-1 outbred

mice failed to produce detectable circulating interferon after an intravenous dose of 10^5 PFU of Newcastle disease virus (NDV). After 4 days of mCMV infection, adult mice in response to $10^{8.2}$ PFU produced at most 20 units of interferon, whereas controls produced 80 to 160 units. Interestingly, endotoxin-induced interferon was not suppressed. It is thought that lymphocytes are the cells responsible for interferon production after an injection of NDV, whereas macrophages are largely responsible for endotoxin-induced interferon (Ho and Armstrong, 1975). There is no accepted explanation for suppression of interferon formation. The most plausible one is probably that mCMV infection induces interferon formation in lymphocytes during the first week of infection (Tarr *et al.*, 1978) and that this produces the state of "hyporeactivity." This is a well-recognized condition that follows systemic induction of interferon, during which the animal is refractory to repeat induction by the same inducer or another type of inducer (Ho and Armstrong, 1975). Ordinarily, this condition lasts about 1 week. In what way this condition may be related to one or another phenomenon of immunosuppression is not known.

Another parameter of nonspecific defense inhibited by mCMV is the clearance of microbial agents by the reticuloendothelial system, of which the liver and the spleen are the major components. The clearance curve from the bloodstream of $10^{8.2}$ PFU NDV injected intravenously lagged about 1 log behind controls in 4-day infected mice. The respective titers in serum 5 hr after injection of NDV were about 10^3 and 10^2 PFU NDV/ml (Osborn and Medearis, 1967). Hamilton *et al.* (1976) and Hamilton and Overall (1978) reported that 3 days after infection with mCMV, an ordinarily nonlethal dose of *Pseudomonas aeruginosa* (2×10^6) failed to be cleared from blood 4 hr after injection and remained in high titers in organs. Smaller doses were adequately cleared. On the other hand, Howard *et al.* (1974) reported that carbon clearance was not affected in CMV-infected mice. It would be interesting to know whether serum virucidal factors (particularly against myxoviruses such as NDV) and bactericidal factors and complement are affected by mCMV infection.

16.6.3.2. Effect on Cytotoxic Immunity

Ho (1980) studied the effect of acute mCMV infection on the development of primary cytotoxic T-cell response against ectromelia virus. Spleen cells were collected from Balb/c mice, and the assay measured lysis of ^{51}Cr-labeled ectromelia-infected P815 (a mouse lymphoma cell line) targets.

Three types of experiments were done. In the first, mice were infected with mCMV 3, 7, and 14 days before infection with attenuated ectromelia virus. Spleen cells were collected 5 days after ectromelia infection at a time when the specific cytotoxic response was at its maximum. Cells from mice that received mCMV 3 or 7 days previously showed only 10% cytotoxicity against ectromelia-infected targets. However, mCMV given 14 days before ectromelia no longer had a demonstrable suppressive effect.

In the second type of experiment, mCMV injected 3 days before spleen cells

were collected from mice previously infected with ectromelia suppressed the ability of these cells to develop into cytotoxic T cells on stimulation *in vitro* with ectromelia-infected lymphocytes.

In the third type of experiment, mCMV was injected 3 days before ectromelia virus. Five weeks later, spleen cells were collected from these mice and from controls that did not receive mCMV. The two sets of cells were stimulated *in vitro* to generate secondary cytotoxic cells against ectromelia virus. Cells from mice that received mCMV generated less cytotoxic activity.

These three types of experiments show that an acute mCMV infection of between 3 and 7 days inhibited (1) the development of a primary cytotoxic response, (2) the ability of lymphocytes from mice with established memory to develop cytotoxic cells, and (3) the ability of mice to develop memory cells for the cytotoxic response to ectromelia.

Hamilton *et al.* (1978) found that prior infection of mice with mCMV reduced the ability of the animals to respond to EL4, a mouse ascites tumor, by developing cytotoxic lymphocytes against the tumor. As in other cases of immunosuppression by mCMV, the greatest depression was noted in animals infected 7 days before grafting.

In all studies of the immunosuppressive effect of mCMV, maximum inhibition was observed within the first week of infection, typically between 3 and 5 days after infection. None of the observed effects lasted more than 3 weeks after infection despite the well-known fact that acutely infected mice frequently remain persistently infected indefinitely. More recent data on GVH reactions are consistent with this conclusion (see Section 16.5.2). There have been no reports showing that persistently infected mice are immunosuppressed.

The mechanism of immunosuppression by acute infection has not been completely elucidated. Suppression of both B and T lymphocyte functions of the infected spleen are correlated with peak infection. Loh and Hudson (1981) report that infection is largely in the adherent cell (macrophage) fraction and that immunosuppression is correlated with the peak in the number of infected cells. However, the number of infected macrophages or lymphocytes (usually $< 1\%$) in the spleen is too small to explain immunosuppression by productive infection or cell killing alone. So far, there is no report on the possible role of abortive infection or infection by defective particles. A peculiar characteristic of the problem is that generally it is difficult to produce either infection or immunosuppression by directly inoculating spleen cells with virus *in vitro*.

The prevailing theory is that immunosuppression is produced by indirect effects of virus infection. A suppressor cell or suppressor factor is being sought. Bixler and Booss (1981) used an *in-vitro* antibody stimulation system (Mishell–Dutton cultures) to study the antibody production by spleen cells stimulated with sheep red blood cells. The diminished response of spleen cells given infected mice was only observed when adherent antithymocyte serum resistant cells were present. Hence, suppressor T cells do not appear responsible. The possible role of activated

or altered macrophages, either directly or through the medium of a poorly diffusible factor, has received further attention (see Section 16.6.3.4).

16.6.3.3. Murine Cytomegalovirus Accentuates Infection by Other Agents

In view of the prolonged immunosuppressive effect of mCMV infection, one would expect an additional infection to be more severe, at least when this occurs during the acute phase of mCMV infection. This is so.

Osborn and Medearis (1967) reported that when NDV, which is ordinarily noninfective for mice, is injected 4 days after mCMV infection, it is able to replicate up to 10^6 PFU per gram of spleen tissue 48–72 hr later. Viremia was also occasionally present. It should be noted that NDV may replicate to low titers in mouse embryo cell cultures (Kono, 1962), so mouse cells are not entirely restricted for NDV. Despite the fact that antibody as well as interferon formation induced by NDV was inhibited in these mice (see Section 16.6.3.1), replication of NDV ceased spontaneously 72 hr after its injection.

Hamilton *et al.* (1976) and Hamilton and Overall (1978) studied the effect of mCMV on bacterial and fungal infections in Swiss–Webster mice. Three days after mCMV infection, a 10% lethal dose of *Staphylococcus aureus* (8×10^6) given intravenously produced 90% mortality. Similarly, a 20% lethal dose of *Pseudomonas aeruginosa* (1.8×10^6) and a nonlethal dose of *Candida albicans* became 90% and 100% fatal. Interestingly, the mCMV infection, measured by virus titers in various organs, was not enhanced in combined infections with *Pseudomonas*.

It was not clear why these infections could not be handled in mCMV-infected mice. Leukopenia and decreased ability of the reticuloendothelial system to clear the organisms were factors considered.

16.6.3.4. Cellular Basis of Immunosuppressive Effect of Murine CMV

The cellular basis of the immunosuppressive effect of murine CMV, as in the case of human CMV (see Section 13.1.5), is presumed to result from the direct or indirect effect of the virus on immunologically active cells, the lymphocytes or macrophages. Possible effects of the virus on other types of cells have not been explored.

Loh and Hudson (1981, 1982) postulated that infected macrophages played a key role in the explanation of the immunosuppressive of murine CMV. They first showed that immunosuppression, as measured by the response of spleen cell suspensions from infected mice to mitogens (concanavalin A or lipopolysaccharide), was correlated with the number of infected macrophages in the suspensions measured by infectious centers (Loh and Hudson, 1981). Secondly, removal of macrophages from such infected spleen cell suspensions largely abrogated the immunosuppressive effect (Loh and Hudson, 1982). Thirdly, these authors postulated that

infected macrophages acted by production of an immunosuppressive factor. There is only indirect evidence for this hypothesis, as such a factor has not actually been isolated. Even though immunosuppression was correlated with the number of infected macrophages determined by infectious center titration, this number was still very low: as few as 100 infected macrophages abrogated immunosuppression. In addition, it was found that lymphocytes from infected spleens were poorly responsive to mitogens even in the presence of uninfected macrophages. It was assumed that these lymphocytes had been affected by the putative macrophage-inhibiting factor *in vivo*. In view of the experiments of Kaposi and Rice (1988) in the human system (see Section 13.1.5), it is just as likely that this phenomenon may be explained by the direct effect of CMV on T lymphocytes.

To summarize: there is a great deal of evidence in mice and in man (Section 13.1.5) to implicate the macrophages in mediating immunosuppression. However, no suppressive factor has been found in either system. It is very likely that both murine and human CMV may directly affect lymphocytes and that the role of macrophages may be necessary but not sufficient.

16.7. SUMMARY COMPARISON OF MURINE AND HUMAN INFECTIONS

Murine CMV resembles hCMV morphologically and in its behavior in cell cultures and in the host, but there are also significant differences. The two viral genomes have little homology. In the salivary gland, mCMV replicates to high titer without the production of multicapsid virions, which are similar to dense bodies of hCMV and may be related to the lower efficiency of mCMV replication in other tissues. Cell-culture-attenuated mCMV continues to replicate in the salivary gland but not other organs. Congenital infection with mCMV has been described in mice but is not a popular model, because the mouse placenta presents a greater barrier to infection than the human or guinea pig placenta.

The genetic determinants of infection with mCMV should provide insight for the human infection. The major histocompatibility complex of the mouse, the H-2 complex, is an important genetic determinant. H-2^d and H-2^b genotypes are more susceptible, and H-2^k is more resistant. How this complex works is not clear. But other determinants are NK-cell activity, interferon induction, T-cell immunity, and delayed hypersensitivity. Both human and mouse newborns are more susceptible to their respective CMV.

The nature of latency seems similar in mCMV and hCMV, although neither has been fully characterized. Murine CMV appears to be latent in a number of different cells, i.e., B and T lymphocytes, macrophages, and sinusoidal cells of the spleen. The molecular events leading to reactivation by cocultivation have not been elucidated.

Cytotoxic T-cell immunity has been characterized in mCMV. An important target antigen seems to be the pp89 of the immediate early antigen (IE) group. Since

the structure of the IE region is similar in both viruses, this finding has been a strong stimulus for parallel studies in hCMV, which have yielded similar results.

Like hCMV, mCMV is broadly immunosuppressive. As in the case of the human, mouse lymphocytes are poorly permissive for the virus, but mCMV exerts extensive pathological and functional effects on lymphoid tissues.

References

Abdallah, P. S., Mark, J. B. D., Merigan, T. C. Diagnosis of cytomegalovirus pneumonia in compromised hosts. *Am. J. Med.* **61**:326–332, 1976.

Ackermann, J. R., LeFor, W. M., Weinstein, S., *et al.* Four-year experience with exclusive use of cytomegalovirus antibody (CMV-Ab)-negative donors for CMV-Ab-negative kidney recipients. *Transplant. Proc.* **20**:469–471, 1988.

Ada, G. L., Jackson, D. C., Blanden, R. V., *et al.* Changes in the surface of virus-infected cells recognized by cytotoxic T cells. I. Minimal requirements for lysis of ectromelia-infected P815 cells. *Scand. J. Immunol.* **5**:23–30, 1976.

Adler, R., Glorioso, J. C., Cossman, J., Levine, M. Possible role of Fc receptors on cells infected and transformed by herpesvirus: Escape from immune cytolysis. *Infect. Immun.* **21**:442–447, 1978.

Adler, S. P. Cytomegalovirus infection in parents of children at day-care centers. *N. Engl. J. Med.* **315**:1164, 1986a.

Adler, S. P. Molecular epidemiology of cytomegalovirus: Evidence for viral transmission to parents from children infected at a day care center. *Pediatr. Infect. Dis.* **5**:315–318, 1986b.

Adler, S. P. The molecular epidemiology of cytomegalovirus transmission among children attending a day care center. *J. Infect. Dis.* **152**:760–767, 1988.

Adler, S. P. Cytomegalovirus and child day care. Evidence for an increased infection rate among day-care workers. *N. Engl. J. Med.* **321**:1290–1296, 1989.

Adler, S. P., Baggett, J., McVoy, M. Transfusion-associated cytomegalovirus infections in seropositive cardiac surgery patients. *Lancet* **2**:743–746, 1985.

Ahlfors, K., Ivarsson, S. A., Johnsson, T., Svensson, I. Congenital and acquired cytomegalovirus infections. Virological and clinical studies on a Swedish infant population. *Acta Paediatr. Scand.* **67**:321–328, 1978.

Ahlfors, K., Harris, S., Ivarsson, S., Svanberg, L. Secondary maternal cytomegalovirus infection causing symptomatic congenital infection. *N. Engl. J. Med.* **305**: 284, 1981a.

Ahlfors, K., Ivarsson, S. A., Johnsson, T., Renmarker, K. Risk of cytomegalovirus infection in nurses and congenital infection in their offspring. *Acta Paediatr. Scand.* **70**:819–823, 1981b.

Ahlfors, K., Ivarsson, S. A., Harris, S., *et al.* Congenital cytomegalovirus infection and disease in Sweden and the relative importance of primary and secondary maternal infections. *Scand. J. Infect. Dis.* **16**:129–137, 1984.

Ahlfors, K., Ivarsson, S. A., Bjerre, I. Microcephaly and congenital cytomegalovirus infection: A combined prospective and retrospective study of a Swedish infant population. *Pediatrics* **78**:1058–1063, 1986.

Ahlfors, K., Forsgren, M., Griffiths, P., Nielsen, C. M. Comparison of four serological tests for the

detection of specific immunoglobulin M in cord sera of infants congenitally infected with cytomegalovirus. *Scand. J. Infect. Dis.* **19:**303–308, 1987.

Akrigg, A., Wilkinson, G. W. G., Oram, J. D. The structure of the major immediate early gene of human cytomegalovirus strain AD169. *Virus Res.* **2:**107–121, 1985.

Albrecht, T., Rapp, F. Malignant transformation of hamster embryo fibroblasts following exposure to UV-irradiated human cytomegalovirus. *Virology* **55:**53–61, 1973.

Albrecht, T., Nachtigal, M., St. Jeor, S. C., Rapp, F. Induction of cellular DNA synthesis and increased mitotic activity in Syrian hamster embryo cells abortively infected with human cytomegalovirus. *J. Gen. Virol.* **30:**167–177, 1976.

Albrecht, T., Cavallo, T., Cole, N. L., Graves, K. Cytomegalovirus. Development and progression of cytopathic effects in human cell culture. *Lab. Invest.* **42:**1–7, 1980.

Aldrete, J. S., Sterling, W. A., Hathaway, B. M., *et al.* Gastrointestinal and hepatic complications affecting patients with renal allografts. *Am. J. Surg.* **129:**115–24, 1975.

Alexander, E. R., Maternal and neonatal infection with cytomegalovirus in Taiwan. *Pediatr. Res.* **1:**210, 1967.

Alford, C., Hayes, K., Britt, W. Primary cytomegalovirus infection in pregnancy: Comparison of antibody responses to virus-encoded proteins between women with and without uterine infection. *J. Infect. Dis.* **158:**917–924, 1988.

Allan, J. E., Shellam, G. R. characterization of interferon induction in mice of resistant and susceptible strains during murine cytomegalovirus infection. *J. Gen. Virol.* **66:**1105–1112, 1985.

Allessiani, M., Kusne, S., Martin, F. M., *et al.* Infections under FK506 immunosuppression: Preliminary results with primary therapy. *Transplant. Proc.* **22:**44–46, 1990.

Alter, H. J., Purcell, R. H., Feinstone, S. M., Tegtmeier, G. E. Non-A, non-B hepatitis: Its relationship to cytomegalovirus, to chronic hepatitis, and to direct and indirect test methods. In W. Szmuness, H. J. Alter, and J. E. Maynard, (eds.), *Viral Hepatitis: 1981 International Symposium.* Franklin Institute Press, Philadelphia, 1982, p. 279.

Ambinder, R. F., Newman, C., Hayward, G. S., *et al.* Lack of association of cytomegalovirus with endemic African Kaposi's sarcoma. *J. Infect. Dis.* **156:**193–197, 1987.

Anders, D., Gibson, W. Location, transcript analysis and partial nucleotide sequence of the cytomegalovirus gene encoding for an early DNA-binding protein with similarities to ICP8 of herpes simplex virus type 1. *J. Virol.* **62:**1364–1372, 1988.

Anders, D. G., Irmiere, A., Gibson, W. Identification and characterization of a major early cytomegalovirus DNA-binding protein. *J. Virol.* **58:**253–262, 1986.

Anders, D., Kidd, J., Gibson, W. Immunological characterization of an early cytomegalovirus single-strand DNA-binding protein with similarities to the HSV major DNA-binding protein. *Virology* **161:**579–588, 1987.

Andersen, H. K. Complement-fixing and virus neutralizing antibodies in cytomegalovirus infection as measured against homologous and heterologous antigen. *Acta Pathol. Microbiol. Scand. [B]* **78:**504–508, 1970.

Andersen, H. K. Serologic differentiation of human cytomegalovirus strains using rabbit hyperimmune sera. *Arch. Ges. Virusforsch.* **33:**189–191, 1971.

Andersen, H. K. Studies of human cytomegalovirus strain variations by kinetic neutralization tests. *Arch. Ges. Virusforsch.* **38:**297–305, 1972.

Andersen, H. K., Spencer, E. S. Cytomegalovirus infection among renal allograft recipients. *Acta Med. Scand.* **186:**7–19, 1969.

Andersen, P., Godtfredsen, A., Spencer, E. J. Studies on the specificity of the complement fixation test on cytomegalovirus infection. *Scand. J. Infect. Dis.* **3:**183–187, 1971.

Andersen, P., Molgaard, J., Andersen, I., Andersen, H. K. Smooth-muscle antibodies and antibodies to cytomegalovirus and Epstein–Barr virus in leukemias and lymphomata. *Acta Pathol. Microbiol. Scand. [C]* **84:**86–92, 1976.

Andreone, P. A., Olivari, M. T., Elick, B., Arentzen, C. E., Sibley, R. K., Bolman, R. M., Simmons, R. L., Ring, W. S. Reduction of infectious complications following heart transplantation with triple-drug immunotherapy. *J. Heart Transplant.* **5:**13–19, 1986.

Andrews, C. H. Immunity to the salivary virus of guinea-pigs, studies in the living animal, and in tissue culture. *Br. J. Exp. Pathol.* **11**:23–34, 1930.

Andrus, C. H., Betts, R. F., May, A. G., Freeman, R. B. CMV infection blocks the beneficial effect of pre-transplant blood transfusion on renal allograft survival. *Transplantation* **28**:451–456, 1979.

Anuras, S., Piros, J., Bonney, W. W., *et al.* Liver disease in renal transplant recipients. *Arch. Intern. Med.* **137**:42–48, 1977.

Appereley, J. F., Goldman, J. M. Cytomegalovirus: Biology, clinical features and methods for diagnosis. *Bone Marrow Transplant* **3**:253–264, 1988.

Applebaum, F. R., Meyers, J. D., Fefer, A., *et al.* Nonbacterial nonfungal pneumonia following marrow transplantation in 100 identical twins. *Transplantation* **33**:265–268, 1982.

Aquila, R., Hayward, G., Summers, W. Physical mapping of the human cytomegalovirus (HCMV) (Towne) DNA polymerase gene: DNA-mediated transfer of a genetic marker for an HCMV gene. *Virology* **171**:312–316, 1989.

Armstrong, D., Rosen, P. Cytomegalovirus and herpes simplex pneumonia complicating neoplastic disease. In A. vonGraevenitz and T. Sall (eds.), *Pathogenic Microorganisms from Atypical Clinical Sources*. Marcel Dekker, New York, 1975, pp. 89–99.

Armstrong, D., Balakrishnan, S. L., Steger, L., *et al.* Cytomegalovirus infections with viremia following renal transplantation. *Arch. Intern. Med.* **127**:111–115, 1971a.

Armstrong, D., Haghbin, M., Balakrishnan, S. L., Murphy, M. L. Asymptomatic cytomegalovirus infection in children with leukemia. *Am. J. Dis. Child.* **122**:404–407, 1971b.

Armstrong, J. A., Tarr, G. C., Ho, M., *et al.* Cytomegalovirus infection in children undergoing open-heart surgery. *Yale J. Biol. Med.* **49**:83–91, 1976.

Arthur, R. R., Shah, K. V., Charache, P., Saral, R. BK and JC virus infections in recipients of bone marrow transplants. *J. Infect. Dis.* **158**:563–569, 1988.

Arvin, A. M., Yeager, A. S., Merigan, T. C. Effect of leukocyte interferon on urinary excretion of cytomegalovirus by infants. *J. Infect. Dis.* **133S**:A205–210, 1976.

Asher, D. M., Gibbs, C. J., Jr., Lang, D. J. Rhesus monkey cytomegalovirus; Persistent asymptomatic viruria. *Bacteriol. Proc.* **191**:, 1969.

Ashton, W. T., Karkas, J. D., Field, A. K., Tolman, R. L. Activation by thymidine kinase and potent antiherpetic activity of 2′-nor-2′-deoxyguanosine (2′NDG). *Biochem. Biophys. Res. Commun.* **108**:1716–1721, 1982.

Bach, M. C., Bagwell, S. P., Knapp, N. P., *et al.* 9-(1,3-Dihydroxy-2-propoxymethyl)guanine for cytomegalovirus infections in patients with the acquired immunodeficiency syndrome. *Ann. Intern. Med.* **103**:381–384, 1985.

Bailey, O. T., Wolf, A., Schneck, S. A. Iatrogenic modification of tissue responses to infectious agents in the central nervous system. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **44**:445, 1968.

Baldwin, W. M. III, Claas, F. H. J., Van Es, A., *et al.* Renal graft dysfunction during infection with cytomegalovirus: Association with IgM lymphocytotoxins and HLA-DR3 and DR7. *Br. Med. J.* **287**:1332–1334, 1983.

Bale, J. F., Jr., O'Neil, M. E., Hart, M. N., *et al.* Human cytomegalovirus nucleic acids in tissues from congenitally infected infants. *Pediatr. Neurol.* **5**:216–220, 1989.

Balfour, C. L., Balfour, H. H. Cytomegalovirus is not an occupational risk for nurses in renal transplant and neonatal units. *J.A.M.A.* **256**:1909–1914, 1986.

Balfour, H. H., Jr., Welo, P. K., Sach, G. W. Cytomegalovirus vaccine trial in 400 renal transplant candidates. *Transplant. Proc.* **17**:81–83, 1985.

Balfour, H. H., Chace, B. A., Stapleton, J. T., *et al.* A randomized, placebo-controlled trial of oral acyclovir for the prevention of cytomegalovirus disease in recipients of renal allografts. *N. Engl. J. Med.* **320**:1381–1387, 1989.

Ballard, R. B., Drew, W. L., Hufnagle, K. G., Riedel, P. A. Acquired cytomegalovirus infection in preterm infants. *Am. J. Dis. Child.* **133**:482–485, 1979.

Balthazar, E. J., Megibow, A. J., Fazzini, E., *et al.* Cytomegalovirus coltis in AIDS: Radiologic findings in 11 patients. *Radiology* **155**:585–589, 1985.

Balthazar, E. J., Megibow, A. J., Hulnick, D., *et al.* Cytomegalovirus esophagitis in AIDS: Radiographic features in 16 patients. *Am. J. Radiol.* **149:**919–923, 1987.

Bamji, A., Salisbury, R. Cytomegalovirus and vasculitis. *Br. Med. J.* **1:**623–624, 1978.

Bancroft, G. J., Shellam, G. R., Chalmer, J. E. Genetic influences on the augmentation of natural killer (NK) cells during murine cytomegalovirus infection: Correlation with patterns of resistance. *J. Immunol.* **126:**998–994, 1981.

Banks, T., Huo, B., Kousoulas, K., *et al.* A major neutralizing domain maps within the carboxyl-terminal half of the cleaved cytomegalovirus B glycoprotein. *J. Gen. Virol.* **70:**979–985, 1989.

Baron, J., Youngblood, L., Siewers, C. M. F., Medearis, D. N., Jr. The incidence of cytomegalovirus, herpes simplex, rubella, and toxoplasma antibodies in microcephalic, mentally retarded and normocephalic children. *Pediatrics* **44:**932–939, 1969.

Barre-Sinoussi, F., Cherman, J. C., Rey, F., *et al.* Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **223:**1086–1087, 1983.

Bartlett, J. G., Laughon, B., Quinn, T. C. Gastrointestinal complications of AIDS. In V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), *AIDS, Etiology, Diagnosis, Treatment and Prevention* 2nd ed. Lippincott, Philadelphia, 1988, pp. 227–244.

Barton, B. W., Tobin, J. O'H. The effect of idoxuridine on the excretion of cytomegalovirus in congenital infection. *Ann. N.Y. Acad. Sci.* **173:**90–95, 1970.

Baskar, J. F., Stanat, S. C., Huang, E.-S. Congenital defects due to reactivation of latent murine cytomegaloviral infection during pregnancy. *J. Infect. Dis.* **152:**621–624, 1985.

Bayer, W. L. The effect of frozen blood on the relationship of cytomegalovirus and hepatitis virus to infection and disease. In R. B. Dawson and A. Barnes, Jr. (eds.), *Clinical and Practical Aspects of the Use of Frozen Blood.* American Association of Blood Banks, Washington, 1977, p. 133.

Bayer, W. L., Tegtmeier, G. E. The blood donor: Detection and magnitude of cytomegalovirus carrier states and the prevalence of cytomegalovirus antibody. *Yale J. Biol. Med.* **49:**5–12, 1976.

Beck, S., Barrell, B. G. Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens. *Nature* **331:**269–272, 1988.

Becroft, D. M. O. Bronchiolitis obliterans bronchiectasis and other sequelae of adenovirus type 21 infection in young children. *J. Clin. Pathol.* **24:**72–82, 1971.

Becroft, D. M. O. Prenatal cytomegalovirus infection and muscular deficiency (eventration) of the diaphragm. *J. Pediatr.* **94:**74–75, 1979.

Behar, R., Wiley, C., McCutchan, A. Cytomegalovirus polyradiculoneuropathy in acquired immune deficiency syndrome. *Neurology (N.Y.)* **37:**557–561, 1987.

Bell, W. E., McCormick, W. F. Guillain–Barre syndrome. In A. J. Schaffer (ed.), *Neurologic Infections in Children.* Saunders, Philadelphia, 1975, pp. 423–432.

Beneke, J. S., Tegtmeier, G. E., Alter, H. J., *et al.* Relation of titers of antibodies to CMV in blood donors to the transmission of cytomegalovirus infection. *J. Infect. Dis.* **150:**883–888, 1984.

Benenson, A. S. *Control of Communicable Diseases in Man,* 13th ed. American Public Health Association, Washington, 1980, p. 443.

Benko, D., Gibson, W. Primate cytomegalovirus glycoproteins: Lectin-binding properties and sensitivities to glycosidases. *J. Virol.* **59:**703–713, 1986.

Benko, D. M., Haltiwanger, R. S., Hart, G. W., Gibson, W. Virion basic phosphoprotein from human cytomegalovirus contains O-linked N-acetylglucosamine. *Proc. Natl. Acad. Sci. U.S.A.* **85:**2573–2577, 1988.

Benyesh-Melnick, M., Dessy, S. I., Fernback, D. J. Cytomegaloviruria in children with acute leukemia and in other children. *Proc. Soc. Exp. Biol. Med.* **117:**624–630, 1964.

Benyesh-Melnick, M., Vonka, V., Probstmeyer, F., Wimberly, I. Human cytomegalovirus: Properties of the complement-fixing antigen. *J. Immunol.* **90:**261–267, 1966b.

Berenberg, W., Nankervis, G. Long-term follow-up of cytomegalic inclusion disease of infancy. *Pediatrics* **46:**403–410, 1970.

Bernischke, K., Mendoza, G. R., Bazeley, P. L. Placental and fetal manifestations of cytomegalovirus infection. *Virchows Arch. (Cell Pathol.)* **16:**121–139, 1974.

Bernstein, M. T., Stewart, J. A. Indirect hemagglutination test for detection of antibodies to cytomegalovirus. *Appl. Microbiol.* **21**:84–89, 1971.

Betts, R. F., Schmidt, S. G. Cytolytic IgM antibody to cytomegalovirus to primary cytomegalovirus infection in humans. *J. Infect. Dis.* **143**:821–826, 1981.

Betts, R. F., Freeman, R. B., Douglas, R. G., Jr., *et al.* Transmission of cytomegalovirus infection with renal allograft. *Kidney Int.* **8**:387–394, 1975.

Betts, R. F., George, S. D., Rundell, B. R., *et al.* Comparative activity of immunofluorescent antibody and complement-fixing antibody in cytomegalovirus infection. *J. Clin. Microbiol.* **4**:151–156, 1976.

Betts, R. F., Freeman, R. B., Douglas, R. G., Jr., Talley, T. E. Clinical manifestations of renal allograft derived primary cytomegalovirus infection. *Am. J. Dis. Child.* **131**:759–763, 1977.

Betts, R. F., Cestero, R. V. M., Freeman, R. B., Douglas, R. G., Jr. Epidemiology of CMV infection in end stage renal disease. *J. Med. Virol.* **4**:89–96, 1979.

Bia, F. J., Hastings, K., Hsiung, G. D. Cytomegalovirus infection in guinea pigs. III. Persistent viruria, blood transmission and viral interference. *J. Infect. Dis.* **140**:914–920, 1979.

Bia, F. J., Lucia, H. L., Fong, C. K. Y., *et al.* Effects of vaccination on cytomegalovirus-associated interstitial pneumonia in strain 2 guinea pigs. *J. Infect. Dis.* **145**:742–747, 1982.

Bia, F. J., Griffith, B. P., Fong, C. K. Y., Hsiung, G. D. Cytomegaloviral infections in the guinea pig: Experimental models for human disease. *Rev. Infect. Dis.* **5**:177–195, 1983.

Bia, F. J., Miller, S. A., Lucia, H. L., Griffith, B. P., Tarsio, M., Hsiung, G. D. Vaccination against transplacental cytomegalovirus transmission: Vaccine reactivation and efficacy in guinea pigs. *J. Infect. Dis.* **149**:355–362, 1984.

Bia, M. J., Andiman, W., Gaudio, K., Kliger, A., Siegel, N., Smith, D., Flye, W. Effect of treatment with cyclosporine versus azathioprine on incidence and severity of cytomegalovirus infection post-transplantation. *Transplantation* **40**:610–614, 1985.

Biggar, R. J., Anderson, H. K., Ebbesen, P., *et al.* Seminal fluid excretion of cytomegalovirus related to immunosuppression in homosexual men. *Br. Med. J.* **286**:2010–2012, 1983.

Billingsley, A. M., Shelby, J., Corry, R. J. Transfer of murine cytomegalovirus infection by heart transplantation. *Transplantation* **36**:584–586, 1983.

Birnbaum, G., Lynch, J. I., Margileth, A. M., Lonergan, W. M., Sever, J. L. Cytomegalovirus infections in newborn infants. *J. Pediatr.* **75**:789–795, 1969.

Biron, K. K., Stanat, S. C., Sorrell, J. B., *et al.* Metabolic activation of the nucleoside analog 9-([2-hydroxy-1-(hydroxymethyl)ethoxy]methyl)guanine in human diploid fibroblasts infected with human cytomegalovirus. *Proc. Natl. Acad. Sci. U.S.A.* **82**:2473–2477, 1985.

Bixler, G. S., Jr., Booss, J. Adherent spleen cells from mice acutely infected with cytomegalovirus suppress the primary antibody response *in vitro*. *J. Immunol.* **127**:1294–1299, 1981.

Black, P. H., Hartley, J. W., Rowe, W. P. Isolation of a cytomegalovirus from African green monkey. *Proc. Soc. Exp. Biol. Med.* **112**:601–605, 1963.

Blanar, M. A., Burkly, L. C., Flavell, R. A. NF-kappa-B binds within a region required for B-cell-specific expression of the major histocompatibility complex class II gene E d. *Mol. Cell. Biol.* **9**:844–846, 1989.

Blanden, R. V. T cell response to viral and bacterial infections. *Transplant. Rev.* **19**:56–88, 1974.

Blanden, R. V. Cell-mediated immune response to acute viral infection. *Prog. Immunol.* **3**:463–471, 1977.

Blanton, R., Tevethia, M. Immunoprecipitation of virus-specific immediate-early and early polypeptides from cells lytically infected with human cytomegalovirus strain AD 169. *Virology* **112**:262–273, 1981.

Blaser, M. J., Cohn, D. L. Opportunistic infections in patients with AIDS: Clues to the epidemiology of AIDS and the relative virulence of pathogens. *Rev. Infect. Dis.* **8**:21–30, 1986.

Blatt, J., Kastner, P., Hodes, D. S. Cutaneous vesicles in congenital cytomegalovirus infection. *J. Pediatr.* **92**:509, 1978.

Blumberg, R. S., Kelsey, P., Perrone, T. R., Dickersin, R., Laauaglia, M., Ferruci, J. Cytomegalovirus-

and Cryptospordium-associated acalculous gangrenous cholecystitis. *Am. J. Med.* **76:**1118–1123, 1984.

Bodey, G. P., Wertlake, P. T., Douglas, G., Levin, R. H. Cytomegalovirus inclusion disease in patients with acute leukemia. *Ann. Intern. Med.* **62:**899–906, 1965.

Boezi, J. A., Lee, L. F., Blakesley, R. W., *et al.* Marek's disease herpesvirus-induced DNA polymerase, *J. Virol.* **14:**1209–1219, 1974.

Boldogh, I., Bonczol, E., Gartner, L., Vaczi, G. Expressions of the human cytomegalovirus genome in mouse cells and in human–mouse heterokaryons. *Arch. Virol.* **53:**101–108, 1977.

Bonkowsky, H. L., Lee, R. L., Klatskin, G. Acute granulomatous hepatitis. Occurrence in cytomegalovirus mononucleosis. *J.A.M.A.* **233:**1284–1288, 1975.

Booss, J., Wheelock, E. F. Correlation of survival from murine cytomegalovirus infection with spleen cell responsiveness to concanavalin A. *Proc. Soc. Exp. Biol. Med.* **149:**443–446, 1975.

Booss, J., Wheelock, E. F. Progressive inhibition of T-cell function preceding clinical signs of cytomegalovirus infection in mice. *J. Infect. Dis.* **135:**478–481, 1977.

Booss, J., Dann, P. R., Griffith, B. P., Kim, J. H. Host defense response to cytomegalovirus in the central nervous system. *Am. J. Pathol.* **134:**71–78, 1989.

Booth, J. C., Hannington, D., Tryhorn, Y., Stern, H. Cytomegalovirus complement-fixing IgM antibody. *J. Med. Virol.* **5:**183–193, 1980.

Borgemeyer, U., Nowock, J., Sippel, A. E. The TGGCA-binding protein: A eukaryotic nuclear protein recognizing a symmetrical sequence on double-stranded linear DNA. *Nucleic Acids Res.* **12:**4295–4311, 1984.

Borysiewicz, L. K., Morris, S., Page, J. D., Sissons, J. G. P. Human cytomegalovirus-specific cytotoxic T lymphocytes: Requirements for *in vitro* generation and specificity. *Eur. J. Immunol.* **13:**804–809, 1983.

Borysiewicz, L., Graham, S., Hickling, J., *et al.* Human cytomegalovirus-specific cytotoxic T cells: Their precursor frequency and stage specificity. *Eur. J. Immunol.* **18:**269–275, 1988a.

Borysiewicz, L., Hickling, J., Graham, S., *et al.* Human cytomegalovirus specific cytotoxic T cells—relative frequency of stage specific CTL recognizing the 72kDa immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses. *J. Exp. Med.* **168:**919–931, 1988b.

Boshart, M., Weber, F., Jahn, G., *et al.* A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* **41:**521–530, 1985.

Bowden, R. A., Sayers, M., Flournoy, N., *et al.* Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. *N. Engl. J. Med.* **314:**1006–1010, 1986.

Bowden, R. A., Day, L. M., Amos, D. E., Meyers, J. D. Natural cytotoxic activity against cytomegalovirus-infected target cells following marrow transplantation. *Transplantation* **44:**504–508, 1987.

Brady, M. T., Milam, J. D., Anderson, D. C., *et al.* Use of deglycerolized red blood cells to prevent posttransfusion infection with cytomegalovirus in neonates. *J. Infect. Dis.* **150:**334–339, 1984.

Brandt, J. A., Kettering, J. D., Lewis, J. E. Immunity to human cytomegalovirus measured and compared by complement fixation, indirect fluorescent-antibody, indirect hemagglutination, and enzyme-linked immunosorbent assays. *J. Clin. Med.* **19:**147–152, 1984.

Brasfield, D. M., Stagno, S., Whitley, R. J., Cloud, G., Cassell, G., Tiller, R. E. Infant pneumonitis associated with cytomegalovirus, *Chlamydia, Pneumocystis,* and ureaplasma: Follow-up. *Pediatrics* **79:**76–83, 1987.

Brautigam, A. R., Dutko, F. J., Olding, L. B., Oldstone, M. B. A. Pathogenesis of murine cytomegalovirus infection: The macrophage as a permissive cell for cytomegalovirus infection, replication and latency. *J. Gen. Virol.* **44:**349–359, 1979.

Brayman, K. L., Dafoe, D. C., Smythe, W. R., Barker, C. F., Perloff, L. J., Naji, A., Fox, I. J., *et al.* Prophylaxis of serious cytomegalovirus infection in renal transplant candidates using live human cytomegalovirus vaccine. *Arch. Surg.* **123:**1502–1508, 1988.

Breinig, M. K., Zitelli, B., Starzl, T. E., Ho, M. Epstein–Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. *J. Infect. Dis.* **156:**273–279, 1987.

Britt, W. J. Neutralizing antibodies react with a complex of cytomegalovirus envelope glycoproteins. In F. Rapp (ed.), *Herpesvirus.* Alan R. Liss, New York, 1984a, pp. 217–226.

Britt, W. J. Neutralizing antibodies detect a disulfide-linked glycoprotein complex within the envelope of human cytomegalovirus. *Virology* **135:**369–378, 1984b.

Britt, W. J., Auger, D. Synthesis and processing of the envelope gp55–116 complex of human cytomegalovirus. *J. Virol.* **58:**185–191, 1986a.

Britt, W. J., Auger, D. Human cytomegalovirus virion-associated protein with kinase activity. *J. Virol.* **59:**185–188, 1986b.

Britt, W. J., Vugler, L. Structural and immunological characterization of the intracellular forms of an abundant 68,000 M_r human cytomegalovirus protein. *J. Gen. Virol.* **68:**1897–1907, 1987.

Britt, W. J., Vugler, L. Antiviral antibody responses in mothers and their newborn infants with clinical and subclinical congenital cytomegalovirus infections. *J. Infect. Dis.* **161:**214–219, 1989.

Britt, W. J., Vugler, L., Stephens, E. Induction of complement-dependent and -independent neutralizing antibodies by recombinant-deviced human cytomegalovirus gp55–116 (gB). *J. Virol.* **62:**3309–3318, 1988.

Brodsky, I., Rowe, W. P. Chronic subclinical infection with mouse salivary gland virus. *Proc. Soc. Exp. Biol. Med.* **99:**654–655, 1958.

Brody, A. R., Craighead, J. E. Pathogenesis of pulmonary cytomegalovirus infection in immunosuppressed mice. *J. Infect. Dis.* **129:**677–689, 1974.

Bronsther, O., Makowka, L., Jaffe, R., Demetris, A. J., Breinig, M. K., Ho, M., Esquivel, C. O., Gordon, R. D., Iwatsuki, S., Tzakis, A., Marsh, J. W., Jr., Mazzaferro, V., Van Thiel, D., Starzl, T. E. Occurrence of cytomegalovirus hepatitis in liver transplant patients. *J. Med. Virol.* **24:**423–434, 1988.

Brough, A. J., Jones, D., Page, R. H., Mizukami, I. Dermal erythropoiesis in neonatal infants. A manifestation of intra-uterine viral disease. *Pediatrics* **40:**627–635, 1967.

Buhles, W. C., Jr., Mastre, B. J., Tinker, A. J., *et al.* The Syntex Collaborative Ganciclovir Treatment Study Group. Ganciclovir treatment of life- or sight-threatening cytomegalovirus infection: Experience in 314 immunocompromised patients. *Rev. Infect. Dis.* **10:**S495–S506, 1988.

Bukowski, J. F., Woda, B. A., Welsh, R. M. Pathogenesis of murine cytomegalovirus infection in natural killer cell-depleted mice. *J. Virol.* **52:**119–128, 1984.

Burke, C. M., Theodore, J., Dawkins, K. D., *et al.* Post-transplant obliterative bronchiolitis and other late lung sequelae in human heart–lung transplantation. *Chest* **86:**824–829, 1984.

Burke, C. M., Glanville, M. B., Macoviak, J. A., *et al.* The spectrum of cytomegalovirus infection following human heart–lung transplantation. *Heart Transplant* **5:**267–272, 1986.

Byrne, P. J., Silver, M. M., Gilbert, J. M., *et al.* Cyclopia and congenital cytomegalovirus infection. *Am. J. Med. Genet.* **28:**61–65, 1987.

Cabau, N., Labadie, M. D., Vesin, C., *et al.* Seroepidemiology of CMV infections during the first years of life in urban communities. *Arch. Dis. Child.* **54:**286–290, 1979.

Cabau, N., Labadie, M. D., Duros, C., *et al.* Freeze-dried erythrocytes for an indirect hemagglutination test for detection of cytomegalovirus antibodies. *J. Clin. Microbiol.* **13:**1026–1030, 1981.

Cai, W., Gu, B., Person, S. Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion. *J. Virol.* **62:**2596–2604, 1988.

Cameron, J., Rigby, R. J., van Deth, A. G., Petrie, J. J. B. Severe tubulo-interstitial disease in a renal allograft due to cytomegalovirus infection. *Clin. Nephrol.* **18:**321–325, 1982.

Campbell, D. A., Piercey, J. R. A., Shnitka, T. K., *et al.* Cytomegalovirus-associated gastric ulcer. *Gastroenterology* **72:**533–535, 1977.

Campbell, M. E. M., Preston, C. M. DNA sequences which regulate the expression of the pseudorabies virus major immediate early gene. *Virology* **157:**307–316, 1987.

Cangir, A., Sullivan, M. P. The occurrence of cytomegalovirus infections in childhood leukemia. *J.A.M.A.* **195:**616–622, 1966.

Cangir, A., Sullivan, M. P., Sutow, W. W., Taylor, G. Cytomegalovirus syndrome in children with acute leukemia. *J.A.M.A.* **201:**612–615, 1967.

Cappel, R., Hestermans, O., Toussaint, C., *et al.* Cytomegalovirus infection and graft survival in renal graft recipients. *Arch. Virol.* **56:**149–156, 1978.

Carlström, G. Virologic studies on cytomegalic inclusion disease. *Acta Paediatr. Scand.* **54:**17–23, 1965.

Carlström, G., Jalling, B. Cytomegalovirus infections in different groups of paediatric patients. *Acta Paediatr. Scand.* **59:**303–309, 1970.

Carlström, G., Alden, J., Belfrage, S., *et al.* Acquired cytomegalovirus infection. *Br. Med. J.* **2:**521–525, 1968.

Carney, W. P., Hirsch, M. S. Mechanisms of immunosuppression in cytomegalovirus mononucleosis. II. Virus–monocyte interactions. *J. Infect. Dis.* **144:**47–54, 1981.

Carney, W. P., Rubin, R. H., Hoffman, R. A., *et al.* Analysis of T lymphocyte subsets in cytomegalovirus mononucleosis. *J. Immunol.* **126:**2114–2116, 1981.

Carney, W. P., Iacoviello, V., Hirsch, M. S. Functional properties of T lymphocytes and their subsets in cytomegalovirus mononucleosis. *J. Immunol.* **130:**390–393, 1983.

Castellano, G. A., Hazzard, G. T., Madden, D. L., Sever, J. L. Comparison of the enzyme-linked immunosorbent assay and the indirect hemagglutination test for detection of antibody to CMV. *J. Infect. Dis.* **136S:**S337–S340, 1977.

Caul, E. O., Clarke, S. K. R., Mott, M. G., Perham, T. G. M., Wilson, R. S. E. Cytomegalovirus infections after open heart surgery. *Lancet* **1:**777–779, 1971.

Centers for Disease Control: *Pneumocystis* pneumonia—Los Angeles. *Morbid. Mortal. Week. Rep.* **30:**250–252, 1981.

Chalmer, J. E. *Murine Cytomegalovirus Infection: A Study of Genes Controlling Resistance and Their Effect on Pathogenesis.* Ph.D. Thesis, University of Western Australia, Perth, 1979, p. 200.

Chalmer, J. E., MacKenzie, J. S., Stanley, N. F. Resistance to murine cytomegalovirus linked to the major histocompatibility complex of the mouse. *J. Gen. Virol.* **37:**107–114, 1977.

Chanarin, I., Walford, D. M. Thrombocytopenic purpura in cytomegalovirus mononucleosis. *Lancet* **1:**238–239, 1973.

Chandler, S. H., Holmes, K. K., Wentworth, B. B., *et al.* The epidemiology of cytomegaloviral infection in women attending a sexually transmitted disease clinic. *J. Infect. Dis.* **152:**597–605, 1985a.

Chandler, S. H., Alexander, E. R., Holmes, K. K. Epidemiology of cytomegaloviral infection in a heterogeneous population of pregnant women. *J. Infect. Dis.* **152:**249–255, 1985b.

Chandler, S. H., Handsfield, H. H., McDougall, J. K. Isolation of multiple strains of cytomegalovirus from women attending a clinic for sexually transmitted diseases. *J. Infect. Dis.* **155:**655–660, 1987.

Chang, C., Malone, C., Stinski, M. A human cytomegalovirus gene that has three inducible promoters that are regulated differently at various times after infection. *J. Virol.* **63:**281–290, 1989a.

Chang, C., Vesole, D., Nelson, J., Oldstone, M., Stinski, M. Identification and expression of a human cytomegalovirus early glycoprotein. *J. Virol.* **63:**330–337, 1989b.

Chantler, J. K., Misra, V., Hudson, J. B. Vertical transmission of murine cytomegalovirus. *J. Gen. Virol.* **42:**621–625, 1979.

Charpentier, B., Michelson, S., Martin, B. Definition of human cytomegalovirus-specific target antigens recognized by cytotoxic T cells generated *in vitro* using an autologous lymphocyte system. *J. Immunol.* **137:**330–336, 1986.

Chatterjee, S. N., Fiala, M., Wiener, J., Stewart, J. A., Stacey, B., Warner, N. Primary cytomegalovirus and opportunistic infections. Incidence in renal transplant recipients. *J.A.M.A.* **240:**2446–2449, 1978.

Chawla, H. B., Ford, M. J., Munro, J. F., Scorgie, R. E., Watson, A. R. Ocular involvement in cytomegalovirus infection in a previously healthy adult. *Br. Med. J.* **2:**281–282, 1976.

Chee, M., Lawrence, G., Barrell, B. Alpha-, beta-, and gammaherpesviruses encode a putative phosphotransferase. *J. Gen. Virol.* **70:**1151–1160, 1989a.

Chee, M., Rudolf, S., Plachter, B., Barrel, B., Jahn, G. Identification of the major capsid protein gene of human cytomegalovirus. *J. Virol.* **63:**1345–1353, 1989b.

Chee, M. A., Bankier, A. T., Beck, S., *et al.* An analysis of the protein coding content of the sequence of human cytomegalovirus strain AD169. *Curr. Top. Microbiol. Immunol.* 125–169, 1990, 1989c.

Cheeseman, S. H., Rubin, R. H., Stewart, J. A., *et al.* Controlled clinical trial of prophylactic human-leukocyte interferon in renal transplantation. Effect on cytomegalovirus and herpes simplex virus infections. *N. Engl. J. Med.* **300:**1345–1349, 1979a.

Cheeseman, S. H., Stewart, J. A., Winkle, S., *et al.* Cytomegalovirus excretion 2–14 years after renal transplantation. *Transplant. Proc.* **11:**71–74, 1979b.

Cheng, Y.-C., Grill, S., Derse, D., Chen, J.-Y., *et al.* Model of action of phosphonoformate as an antiherpes simplex virus agent. *Biochim. Biophys. Acta* **652:**90–98, 1981.

Cheng, Y.-C., Huang, E.-S., Lin, J.-C., *et al.* Unique spectrum of activity of 9-[(1,3-dihydroxy-2-propropoxy)methyl]guanine against herpes simplex virus type 1. *Proc. Natl. Acad. Sci. U.S.A.* **80:**2767–2770, 1983.

Cherrington, J. M., Mocarski, E. S. Human cytomegalovirus IE1 transactivates the alpha promoter-enhancer via an 18-base-pair repeat element. *J. Virol.* **63:**1435–1440, 1989.

Chess, L., MacDermott, R. P., Schlossman, S. F. Immunologic function of isolated human lymphocyte populations. II. Antigen triggering of B and T cells *in vitro*. *J. Immunol.* **113:**1122–1127, 1974.

Cheung, K. S., Lang, D. J. Transmission and activation of cytomegalovirus with blood transfusion: A mouse model. *J. Infect. Dis.* **135:**841–845, 1977.

Chiang, W. T., Wentworth, B. B., Alexander, E. R. The use of an immunofluorescence technique for the determination of antibodies to cytomegalovirus strains in human sera. *J. Immunol.* **104:**992–999, 1970.

Chiba, S., Ikeda, S., Chiba, Y., *et al.* Epidemic of subclinical infection with cytomegalovirus in an infant home. *Tohoku J. Exp. Med.* **106:**387–389, 1972.

Chiba, S., Motokawa, T., Tamura, T., *et al.* Seroconversion to virus-specific pre-early nuclear antigens in infants with primary CMV infection. *Infect. Immun.* **30:**135–139, 1980.

Chiba, S., Kamada, M., Yoshimura, H., *et al.* Congenital cytomegalovirus infection in Japan. *N. Engl. J. Med.* **310:**350, 1984.

Ch'ien, L. T., Cannon, N. J., Whitley, R. J., *et al.* Effect of adenine-arabinoside on cytomegalovirus infections. *J. Infect. Dis.* **130:**32–39, 1974.

Chin, W., Magoffin, R., Firerson, G., Lennette, E. H. Cytomegalovirus infection. A case with meningoencephalitis. *J.A.M.A.* **225:**740–741, 1973.

Chiou, H. C., Weller, S. K., Coen, D. M. Mutations in the herpes simplex virus major DNA-binding protein gene leading to altered sensitivity to DNA polymerase inhibitors. *Virology* **145:**213–226, 1985.

Choi, Y. C., Hsiung, G. D. Cytomegalovirus infection in guinea pigs. II. Transplacental and horizontal transmission. *J. Infect. Dis.* **138:**197–202, 1978.

Choi, Y. C., Swack, N. S., Hsiung, G. D. Effect of heparin on cytomegalovirus replication. *Proc. Soc. Exp. Biol. Med.* **157:**569–571, 1978.

Chou, S. Acquisition of donor strains of cytomegalovirus by renal-transplant recipients. *N. Engl. J. Med.* **314:**1418–1423, 1986.

Chou, S. Reactivation and recombination of multiple cytomegalovirus strains from individual organ donors. *J. Infect. Dis.* **160:**11–15, 1989.

Chou, S., Merigan, T. C. Rapid detection and quantitation of human cytomegalovirus in urine through DNA hybridization. *N. Engl. J. Med.* **308:**921–925, 1983.

Chou, S., Norman, D. J. Effect of OKT3 antibody therapy on cytomegalovirus reactivation in renal transplant recipients. *Transplant. Proc.* **17:**2755–2756, 1985.

Chou, S., Norman, D. J. The influence of donor factors other than serologic status on transmision of cytomegalovirus to transplant recipients. *Transplantation* **46:**89–93, 1988.

Chou, S., Scott, K. M. Latex agglutination and enzyme-linked immunosorbent assays for

cytomegalovirus serologic screening of transplant donors and recipients. *J. Clin. Microbiol.* **26**:2116–2119, 1988.

Chou, S., Kim, D. Y., Norman, D. J. Transmission of cytomegalovirus by pretransplant leukocyte transfusions in renal transplant candidates. *J. Infect. Dis.* **155**:565–567, 1987a.

Chou, S., Kim, D., Scott, K. M., Sewell, D. L. Immunoglobulin M to cytomegalovirus in primary and reactivation infections in renal transplant recipients. *J. Clin. Microbiol.* **25**:52–55, 1987b.

Chretien, J. H., McGinnis, C. G., Muller, A. Venereal causes of cytomegalovirus mononucleosis. *J.A.M.A.* **238**:1644–1645, 1977.

Christiansen, L., Beeman, H. W., Allen, A. Cytomegalic inclusion disease. *Arch. Ophthalmol.* **57**:90–99, 1957.

Chua, C. C., Carter, T. H., St. Jeor, S. Transcription of the human cytomegalovirus genome in productively infected cells. *J. Gen. Virol.* **56**:1–11, 1981.

Churchill, M. A., Zaia, J. A., Forman, S. J., *et al.* Quantitation of human cytomegalovirus DNA in lungs from bone marrow transplant recipients with interstitial pneumonia. *J. Infect. Dis.* **155**:501–509, 1987.

Clanton, D. J., Jariwalla, R. J., Kress, C., Rosenthal, L. J. Neoplastic transformation by a cloned human cytomegalovirus DNA fragment uniquely homologous to one of the transforming regions of herpes simplex virus type 2. *Proc. Natl. Acad. Sci. U.S.A.* **80**:3826–3830, 1983.

Clark, B., Zaia, J., Balce-Directo, L., Ting, Y. Isolation and partial chemical characterization of a 64,000-dalton glycoprotein of human cytomegalovirus. *J. Virol.* **49**:279–282, 1984.

Cockley, K. D., Rapp, F. Analysis of long-term human cytomegalovirus latency *in vitro*. *Intervirology* **26**:129–139, 1986.

Colacino, J. M., Lopez, C. Efficacy and selectivity of some nucleoside analogs as anti-human cytomegalovirus agents. *Antimicrob. Agents Chemother.* **24**:505–508, 1983.

Colacino, J. M., Lopez, C. Antiviral activity of 2′-deoxy-2′-fluoro-beta-*d*-arabinofuranosyl-5-iodocytosine against human cytomegalovirus in human skin fibroblasts. *Antimicrob. Agents Chemother.* **28**:252–258, 1985.

Cole, N. L., Balfour, H. H., Jr. *In vitro* susceptibility of cytomegalovirus isolates from immunocompromised patients to acyclovir and ganciclovir. *Diag. Microbiol. Infect. Dis.* **6**:255–261, 1987.

Cole, R., Kuttner, A. G. A filterable virus present in the submaxillary glands of guinea pigs. *J. Exp. Med.* **44**:855–873, 1926.

Collaborative Study. Cytomegalovirus infection in the northwest of England. A report on a two year study. *Arch. Dis. Child.* **45**:513–522, 1970.

Collier, A. C., Meyers, J. D., Corey, L., *et al.* Cytomegalovirus infection in homosexual men. *Am. J. Med.* **82**:593–600, 1987.

Condie, R. M., Hall, B. L., Howard, R. J., *et al.* Treatment of life-threatening infections in renal transplant recipients with high-dose intravenous human IgG. *Transplant. Proc.* **11**:66–68, 1979.

Conley, A. J., Knipe, D. M., Jones, P. C., Roizman, B. Molecular genetics of herpes simplex virus VII. Characterization of a temperature-sensitive mutant produced by *in vitro* mutagenesis and defective in DNA synthesis and accumulation of a-polypeptides. *J. Virol.* **37**:191–206, 1981.

Connor, W. S., Johnson, K. P. Cytomegalovirus infection in weaning guinea pigs. *J. Infect. Dis.* **134**:442–449, 1976.

Cook, M. L., Bastone, V. B., Stevens, J. G. Evidence that neurons harbor latent herpes simplex virus. *Infect. Immun.* **9**:946–951, 1974.

Coombs, R. R. H. Cytomegalic inclusion-body disease associated with acquired autoimmune haemolytic anaemia. *Br. Med. J.* **2**:743–744, 1968.

Corey, L., Spear, P. G. Infections with herpes simplex viruses. *N. Engl. J. Med.* **314**:686–691, 749–757, 1986.

Cosimi, A. B., Colvin, R. B., Burton, R. C., *et al.* Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. *N. Engl. J. Med.* **305**:308–314, 1981.

Costa, J. C., Rabson, A. S. Role of Fc receptors in herpes simplex virus infection. *Lancet* **1**:77–78, 1975.

Coulson, A. S., Lucas, Z. J., Condy, M., Cohn, R. Forty-day fever. An epidemic of cytomegalovirus disease in a renal transplant population. *West. J. Med.* **120**:1–7, 1974.

Coutinho, R. A., Van Dillen, P. W., Van Lent, P. A., *et al.* Infection with cytomegalovirus in homosexual men. *Br. J. Vener. Dis.* **60**:249–252, 1984.

Covell, W. P. Occurrence of intranuclear inclusions in monkeys unaccompanied by specific signs of disease. *Am. J. Pathol.* **8**:151–158, 1932.

Cowdry, E. V. The problem of intranuclear inclusions in virus disease. *Arch. Pathol.* **18**:525–542, 1934.

Cowdry, E. V., Scott, G. H. A comparison of certain intranuclear inclusions found in the livers of dogs without history of infection with intranuclear inclusions characteristic of the action of filterable viruses. *Arch. Pathol.* **9**:1184–1196, 1930.

Cowdry, E. V., Scott, G. H. Nuclear inclusions in the kidneys of *Macacus* monkeys. *Am. J. Pathol.* **11**:659–668, 1935a.

Cowdry, E. V., Scott, G. H. Nuclear inclusions suggestive of virus action in salivary glands of the monkey. *Cebus fatuellus* L. *Proc. Soc. Exp. Biol. Med.* **32**:709–711, 1935b.

Cox, F., Hughes, W. T. Fecal excretion of cytomegalovirus in disseminated cytomegalic inclusion disease. *J. Infect. Dis.* **129**:732–736, 1974.

Cox, F., Hughes, W. T. Cytomegaloviremia in children with acute lymphocytic leukemia. *J. Pediatr.* **87**:190–194, 1975.

Cox, F., Meyer, D., Hughes, W. T. Cytomegalovirus in tears from patients with normal eyes and with acute cytomegalovirus chorioretinitis. *Am. J. Ophthalmol.* **80**:817–824, 1975.

Craighead, J. E. Immunological response to cytomegalovirus infection in renal allograft recipients. *Am. J. Epidemiol.* **90**:506–513, 1969.

Craighead, J. E. Pulmonary cytomegalovirus infection in the adult. *Am. J. Pathol.* **63**:487–504, 1971.

Craighead, J. E., Hanshaw, J. B., Carpenter, C. G. Cytomegalovirus infection after renal allotransplantation. *J.A.M.A.* **201**:725–728, 1967.

Craighead, J. E., Kanich, R. E., Almeida, J. D. Non-viral microbodies with viral antigenicity produced in cytomegalovirus-infected cells. *J. Virol.* **10**:766–775, 1972.

Cranage, M., Kouzarides, T., Bankier, A., *et al.* Identification of the human cytomegalovirus glyprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. *EMBO J.* **5**:3057–3063, 1986.

Cranage, M., Smith, G., Bell, S., *et al.* Identification and expression of a human cytomegalovirus glyprotein with homology to the Epstein–Barr virus BXLF2 product, varicella–zoster virus gpIII, and herpes simplex virus type 1 glycoprotein H. *J. Virol.* **62**:1416–1422, 1988.

Creagh-Kirk, T., Doi, P., Andrews, E., *et al.* Survival experience among patients with AIDS receiving zidovudine. *J.A.M.A.* **260**:3009–3015, 1988.

Cremer, N. E., Schmidt, N. J., Jensen, F., *et al.* Complement-fixing antibody in human sera reactive with viral and soluble antigens of cytomegalovirus. *J. Clin. Microbiol.* **1**:262–267, 1975.

Cremer, N. E., Hoffman, M., Lennette, E. H. Analysis of antibody assay methods and classes of viral antibodies in serodiagnosis of cytomegalovirus infection. *J. Clin. Microbiol.* **8**:153–159, 1978.

Cremer, N., Cossen, C., Shell, G., Pereira, L. Antibody response to cytomegalovirus polypeptides captured by monoclonal antibodies on the solid phase in enzyme immunizes. *J. Clin. Microbiol.* **21**:517–521, 1985.

Crome, L. Microgyria. *J. Pathol. Bacteriol.* **64**:479–495, 1952.

Crome, L., France, N. E. Microgyria and cytomegalic inclusion disease in infancy. *J. Clin. Pathol.* **12**:427–434, 1959.

Cruz, J. R., Waner, J. L. Effect of concurrent cytomegaloviral infection and undernutrition on the growth and immune response of mice. *Infect. Immun.* **21**:346–441, 1978.

Cruz, J. R., Mata, L. J., Urrutia, J. J. Citomegalovirua durante el primer ano de vida: Estudia prospecto en una poblacion indigena de Guatemala. *Bol. Of. Sanit. Panam.* **83**:218–222, 1977.

Cruz-Araullo, T. P., Ho, M., Armstrong, J. A. Protective effect of early serum from mice after cytomegalovirus infection. *Infect. Immun.* **21**:840–842, 1978.

Cutforth, R., Mitchell, R. M., Mundy, G. R. Cytomegalovirus mononucleosis following renal hemodialysis. *Med. J. Aust.* **2**:1103–1104, 1968.

D'Antonio, D., Angelini, A., Iacone, A., *et al.* Rapid detection of cytomegalovirus with monoclonal antibody in blood component donors: A simple screening for hemotherapy in a bone marrow transplantation program. *Haematologica* **73**:495–497, 1988.

Davis, G. L. Cytomegalovirus in the inner ear. Case report and electron microscopic study. *Ann. Otol. Rhinol. Laryngol.* **78**:1179–1188, 1969.

Davis, L. E., Tweed, G. V., Stewart, J. A., *et al.* Cytomegalovirus mononucleosis in a first trimester pregnant female with transmission to the fetus. *Pediatrics* **48**:200–206, 1971.

Davis, L. E., James, C. G., Fiber, F., McLaren, L. C. Cytomegalovirus isolation from a human inner ear. *Ann. Otol. Rhinol. Laryngol.* **88**:424–426, 1979.

Davis, L. E., Johnsson, L. G., Kornfeld, M. Cytomegalovirus labyrinthitis in an infant: Morphological, virological and immunofluorescent studies. *J. Pathol. Exp. Neurol.* **40**:9–19, 1981.

Davis, L. E., Stewart, J. A., Rarey, K. E., McLaren, L. C. Recovery and probable persistence of cytomegalovirus in human inner ear fluid without cochlear damage. *Ann. Otol. Rhinol. Laryngol.* **96**:380–383, 1987.

Davis, M., Mar, E., Wu, Y., Huang, E. Mapping and expression of a human cytomegalovirus major viral protein. *J. Virol.* **52**:129–135, 1984.

Davis, M., Huang, E. Nucleotide sequence of a human cytomegalovirus DNA fragment encoding a 67-kilodalton phosphorylated viral protein. *J. Virol.* **56**:7–11, 1985.

Davis, M. G., Kenney, S. C., Kamine, J., *et al.* Immediate-early gene region of human cytomegalovirus trans-activates the promoter of human immunodeficiency virus. *Proc. Natl. Acad. Sci. U.S.A.* **84**:8642–8646, 1987.

Davison, A. J. Structure of the genomic termini of varicella zoster virus. *J. Gen. Virol.* **65**:1969–1977, 1984.

Davison, A. J., Wilkie, N. M. Nucleotide sequences of the joint between the L and S segments of herpes simplex virus type 1 and 2. *J. Gen. Virol.* **55**:315–331, 1981.

Deibel, R., Fairley, R. J., Ducharme, C. *Serological Studies with Cytomegalovirus. Annual Report 56-58 (1965).* Division of Laboratories and Research, New York State Department of Health, Albany, 1965.

De la Monte, S. M., Ho, D. D., Schooley, R. T., *et al.* Subacute encephalomyelitis of AIDS and its relation to HTLV-III infection. *Neurology (N.Y.)* **37**:562–569, 1987.

DeMarchi, J. M. Human cytomegalovirus DNA: Restriction enzyme cleavage maps and map locations for immediate-early, early and late RNAs. *Virology* **114**:23–38, 1981.

DeMarchi, J. M. Post-transcriptional control of human cytomegalovirus gene expression. *Virology* **124**:390–402, 1983a.

DeMarchi, J. M. Correlation between stimulation of host cell DNA synthesis by human cytomegalovirus and lack of expression of a subset of early virus genes. *Virology* **129**:274–286, 1983b.

DeMarchi, J. M. Nature of the block in the expression of some early virus genes in cells abortively infected with human cytomegalovirus. *Virology* **129**:287–297, 1983c.

DeMarchi, J. M., Kaplan, A. S. Replication of human cytomegalovirus DNA: Lack of dependence on cell DNA synthesis. *J. Virol.* **18**:1063–1070, 1976.

DeMarchi, J. M., Kaplan, A. S. Physiological state of human embryonic lung cells affects their response to human cytomegalovirus. *J. Virol.* **23**:126–132, 1977.

DeMarchi, J. M., Blankship, M. L., Brown, G. D., Kaplan, A. S. Size and complexity of human cytomegalovirus DNA. *Virology* **89**:643–646, 1978.

DeMarchi, J. M., Schmidt, C. A., Kaplan, A. A. Patterns of transcription of human cytomegalovirus in permissively infected cells. *J. Virol.* **35**:277–297, 1980.

Demetris, A. J., Lasky, S., Van Thiel, D. H., *et al.* Pathology of hepatic transplantation: A review of 62 adult allograft recipients immunosuppressed with a cyclosporine/steroid regimen. *Am. J. Pathol.* **118**:151–161, 1985.

Demmler, G. J., Brady, M. T., Bijou, H., *et al.* Posttransfusion cytomegalovirus infection in neonates: Role of saline-washed red blood cells. *J. Pediatr.* **108:**762–765, 1986.

Demmler, G. J., Buffone, G. J., Schimbor, C. M., May, R. A. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. *J. Infect. Dis.* **158:**1177–1184, 1988.

Depto, A. S., Stenberg, R. M. Regulated expression of the human cytomegalovirus pp65 gene: Octamer sequence in the promoter is required for activation by viral gene products. *J. Virol.* **63:**1232–1238, 1989.

DeSilva, L. M., Klampfner, G. L., Lister, C. M., Tobin, J. O'H. Identification of pregnancies at risk from cytomegalovirus infection. *J. Hyg. (Camb.)* **79:**347–354, 1977.

Desmonts, G., Couvreur, J. Congenital toxoplasmosis. A prospective study of 378 pregnancies. *N. Engl. J. Med.* **290:**1110–1116, 1974.

Dias, M. J. M., Harmant-van Rijckevorsel, G., Landrieu, P., Lyon, G. Prenatal cytomegalovirus disease and cerebral microgyria: Evidence for perfusion failure, not disturbance of histogenesis as the major cause of fetal cytomegalovirus encephalopathy. *Neuropediatrics* **15:**18–24, 1984.

Dienstag, J. Serologic testing for hepatitis. *Lab. Manage.* **22:**21–28, 1982.

Dienstag, J. L., Cline, W. L., Purcell, R. H. Detection of cytomegalovirus antibody by immune adherence hemagglutination. *Proc. Soc. Exp. Biol. Med.* **153:**543–548, 1976.

Dieterich, D. T., Chachoua, A., Lafleur, F., Worrell, C. Ganciclovir treatment of gastrointestinal infections caused by cytomegalovirus in patients with AIDS. *Rev. Infect. Dis.* **10**(Suppl. 3):S532–S537, 1988.

Diethelm, A. G., Gore, I., Ch'ien, L. T., Sterling, W. A., Morgan, J. M. Gastrointestinal hemorrhage secondary to cytomegalovirus after renal transplantation. A case report and review of the problem. *Am. J. Surg.* **131:**371–374, 1976.

Diezel, P. B. Mikorgyrie infolge cerebraler Speicheldrusenvirus Infektion in Rahmen einer generalisierten Cytomegalie bei einen Saugling. Zugleich ein Beitrag zur Theorie der Windungsbildung. *Virchows Arch. (Pathol. Anat.)* **325:**109–130, 1954.

Diosi, P., Moldovan, E., Tomescu, N. Latent cytomegalovirus infection in blood donors. *Br. Med. J.* **4:**660–662, 1969.

Diosi, P., Arcan, P., Babusceac, L., Moldovan, E. Identification of a cytomegalovirus recovered from field mice (*Microtus arvalis*). *Pathol. Microbiol. (Basel)* **38:**178–183, 1972.

Diosi, P., Arcan, P., Plavosin, L. Genetic control of resistance to mouse cytomegalovirus infection. *Arch. Ges. Virusforsch.* **44:**23–27, 1974.

Doldi, K., Leroux, M., Kirchner, H., Bienzle, U., Ritter, K., Kalden, J. R. 74 Proliferative responses to human cytomegalovirus in lymphocyte cultures of male homosexuals. *J. Clin. Lab. Immunol.* **17:**111–114, 1985.

Donaldson, P. T., O'Grady, J., Portmann, B., *et al.* Evidence for an immune response to HLA class I antigens in the vanishing-bile duct syndrome after liver transplantation. *Lancet* **1:**945–948, 1987.

Donner, L., Sainerova, H., Svoboda, J., Scherneck, S. Potentiation of RSV rescue from RSV-transformed "poorly virogenic" cell lines by 5-bromodeoxyuridine treatment before transfusion with chick embryo fibroblasts. *Int. J. Cancer* **13:**37–42, 1974.

Dorfman, L. Cytomegalovirus encephalitis in adults. *Neurology (Minneap.)* **23:**136–143, 1973.

Dorsch-Hasler, K., Keil, G. M., Weber, F., *et al.* A long and complex enhancer activates transcription of the gene coding for the highly abundant immediate early mRNA in murine cytomegalovirus. *Proc. Natl. Acad. Sci. U.S.A.* **82:**8325–8329, 1985.

Dowling, J. N., Saslow, A. R., Armstrong, J. A., Ho, M. Cytomegalovirus infection in patients receiving immunosuppressive therapy for rheumatologic disorders. *J. Infect. Dis.* **133:**399–408, 1976.

Dowling, J. N., Wu, B. C., Armstrong, J. A., Ho, M. Enhancement of murine cytomegalovirus infection during graft versus host reaction. *J. Infect. Dis.* **135:**990–994, 1977.

Dowling, P., Menonna, J., Cook, S. Cytomegalovirus complement fixation antibody in Guillain–Barre syndrome. *Neurology (Minneap.)* **27:**1153–1156, 1977.

Dreesman, G. R., Benyesh-Melnick, M. Spectrum of human cytomegalovirus complement-fixing antigens. *J. Immunol.* **99:**1106–1114, 1967.

Dresler, S., Linder, D. Non-cirrhotic portal fibrosis following neonatal cytomegalic inclusion disease. *J. Pediatr.* **93:**887–894, 1978.

Drew, W. L., Miner, R. C., Ziegler, J. L., *et al.* Cytomegalovirus and Kaposi's sarcoma in young homosexual men. *Lancet* **II:**125–127, 1982.

Drew, W. L., Mintz, L., Miner, R. C., Sands, M., Ketterer, B. Prevalence of cytomegalovirus infection in homosexual men. *J. Infect. Dis.* **143:**188–192, 1981.

Drew, W. L., Sweet, E. S., Miner, R. C., *et al.* Multiple infections by cytomegalovirus in patients with acquired immune deficiency syndrome: Documentation by Southern blot hybridization. *J. Infect. Dis.* **150:**952–953, 1984.

Duchowny, M., Caplan, L., Siber, G. Cytomegalovirus infection in the adult nervous system. *Ann. Neurol.* **5:**458–461, 1979.

Dudding, L. R., Garnett, H. M. Interaction of strain AD169 and a clinical isolate of cytomegalovirus with peripheral monocytes: The effect of lipopolysaccharide stimulation. *J. Infect. Dis.* **155:**891–896, 1987.

Duff, R., Rapp, F. Properties of hamster embryo fibroblasts transformed in vitro after exposure to ultraviolet irradiated herpes simplex virus type 2. *J. Virol.* **8:**469–477, 1971.

Dummer, J. S., Hardy, A., Poorsattar, A. Early infections in kidney, heart and liver transplant recipients on cyclosporine. *Transplantation* **36:**259–267, 1983.

Dummer, J. S., Ho, M., Rabin, B., Griffith, B. P., Hardesty, R. L., Bahnson, H. T. The effect of cytomegalovirus and Epstein–Barr virus infection of T lymphocyte subsets in cardiac transplant patients on cyclosporine. *Transplantation* **38:**433–435, 1984.

Dummer, J. S., White, L. T., Ho, M., *et al.* The morbidity of cytomegalovirus infection in heart and hear–lung transplant recipients on cyclosporine. *J. Infect. Dis.* **152:**1182–1191, 1985.

Dummer, J. S., Montero, C. G., Griffith, B. P., *et al.* Infections in heart–lung transplant recipients. *Transplantation* **41:**725–729, 1986.

Dummer, J. S., Armstrong, J., Somers, J., *et al.* Transmission of infection with herpes simplex virus by renal transplantation. *J. Infect. Dis.* **155:**202–206, 1987.

Dumont, J., Liabeuf, A., Henle, W., *et al.* Anti-EBV antibody titers in non-Hodgkin's lymphomas. *Int. J. Cancer* **18:**14–23, 1976.

Dutko, F. N., Oldstone, M. B. A. Cytomegalovirus causes a latent infection in undifferentiated cells and is activated by induction of cell differentiation. *J. Exp. Med.* **154:**1636–1651, 1981.

Duvall, C. P., Casazza, A. R., Grimley, P. M., Carbone, P. P., Rowe, W. P. Recovery of cytomegalovirus from adults with neoplastic disease. *Ann. Intern. Med.* **64:**531–541, 1966.

Dworsky, M. E., Welch, K., Cassady, G., Stagno, S. Occupational risk for primary cytomegalovirus infection among pediatric health-care workers. *N. Engl. J. Med.* **360:**950–952, 1983.

Dworsky, M., Lakeman, A., Stagno, S. Cytomegalovirus transmission within a family. *Pediatr. Infect. Dis.* **3:**236–238, 1984.

Dyment, P. G., Orlando, S. J., Isaacs, H., Jr., Wright, H. T., Jr. The incidence of cytomegaloviruria and post-mortem cytomegalic inclusions in children with acute leukemia. *J. Pediatr.* **72:**533–536, 1968.

Ebeling, A., Keil, G. M., Knust, E., Koszinowski, U. H. Molecular cloning and physical mapping of murine cytomegalovirus DNA. *J. Virol.* **47:**421–433, 1983.

Edwards, R. H., Messing, R., McKendall, R. R. Cytomegalovirus meningoencephalitis in a homosexual man with Kaposi's sarcoma: Isolation of CMV from CSF cells. *Neurology (N.Y.)* **135:**560–562, 1985.

Egbert, P. R., Pollard, R. B., Gallagher, J. B., Merigan, T. C. Cytomegalovirus retinitis in immunosuppressed hosts. II. Ocular manifestations. *Ann. Intern. Med.* **93:**664–670, 1980.

Einhorn, L., Ost, A. Cytomegalovirus infection of human blood cells. *J. Infect. Dis.* **149:**207–214, 1984.

El-Beik, T., Razzaque, A., Jariwalla, R., Cihlar, R. L. Multiple transforming regions of human cytomegalovirus DNA. *J. Virol.* **60:**645–652, 1986.

Elek, S. D., Stern, H. Development of a vaccine against mental retardation caused by cytomegalovirus infection *in utero*. *Lancet* **1**:1–5, 1974.

Elion, G. B., Furman, P. A., Fyfe, J. A., *et al.* Selectivity of action of an antiherpes agent, 9-(2-hydroxyethoxymethyl)guanine. *Proc. Natl. Acad. Sci. U.S.A.* **74**:5716–5720, 1977.

Emanuel, D., Peppard, J., Chehimi, J., *et al.* The diagnostic, prophylactic, and therapeutic uses of monoclonal antibodies to human cytomegalovirus. *Trans. Proc.* **19**:132–137, 1987.

Emanuel, D., Cunningham, I., Jules-Elysee, K., *et al.* Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. *Ann. Intern. Med.* **109**:777–782, 1988.

Embil, J. A., Folkins, D. F., Haldane, E. V., VanRooyen, C. E. Cytomegalovirus infection following extra-corporeal circulation in children: A prospective study. *Lancet* **2**:1151–1155, 1968.

Embil, J. A., Ozere, R. L., Haldane, E. V. Congenital cytomegalovirus infection in two siblings from consecutive pregnancies. *J. Pediatr.* **77**:417–421, 1970.

Emini, E., Luka, J., Armstrong, M., *et al.* Identification of an Epstein–Barr virus glycoprotein which is antigenically homologous to the varicella–zoster virus glycoprotein II and the herpes simplex virus glycoprotein B. *Virology* **157**:552–555, 1987.

Emodi, G., O'Reilly, R., Muller, A., *et al.* Effect of human exogenous leukocyte interferon in cytomegalovirus infections. *J. Infect. Dis.* **133**:A199–A204, 1976.

Ende, N., Gelpi, A. P. Pathologic changes noted in bone marrow in a case of Q fever. *Arch. Intern. Med.* **100**:793–796, 1957.

Enders, J. F., Weller, T. H., Robbins, F. C. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* **109**:85–87, 1949.

Englund, J. A., Sullivan, C. J., Jordan, M. C., *et al.* Respiratory syncytial virus infection in immunocompromosed adults. *Ann. Intern. Med.* **109**:203–208, 1988.

Epler, G. R., Snider, G. L., Gaensler, E. A., *et al.* Bronchiolitis and bronchitis in connective tissue diseases. *J.A.M.A.* **242**:528–532, 1979.

Erice, A., Jordan, M. C., Chace, B. A., *et al.* Ganciclovir treatment of cytomegalovirus disease in transplant recipients and other immunocompromised hosts. *J.A.M.A.* **257**:3082–3087, 1987.

Erice, A., Chou, S., Biron, K. K., *et al.* Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. *N. Engl. J. Med.* **320**:289–293, 1989.

Estes, J. E., Huang, E. S. Stimulation of cellular thymidine kinase by human cytomegalovirus. *J. Virol.* **24**:13–21, 1977.

Evans, A. S. Infectious mononucleosis and other mono-like syndromes. *N. Engl. J. Med.* **286**:836–838, 1972.

Evans, A. S. *Viral Infections of Humans, Epidemiology and Control.* Plenum Press, New York, 1976, p. 584.

Evans, A. S., Niederman, J. C. Epstein–Barr virus. In A. J. Evans (ed) *Viral Infections of Humans,* 2nd Edition. Plenum Press, New York, 1982, pp. 253–282.

Evans, D. J., Williams, E. D. Cytomegalic inclusion disease in the adult. *J. Clin. Pathol.* **21**:311–316, 1968.

Fabricant, C. G., Fabricant, J., Litrenta, M. M., Minick, C. R. Virus induced atherosclerosis. *J. Exp. Med.* **148**:355–340, 1978.

Farber, I., Wurtzler, P., Schweizer, H., Sprossig, M. Human CMV induced changes in rabbit cells. *Arch. Virol.* **59**:257–261, 1979.

Farber, S., Wolbach, S. G. Intranuclear and cytoplasmic inclusions ("protozoan-like bodies") in the salivary glands and other organs of infants. *Am. J. Pathol.* **8**:123–135, 1932.

Farrer, G. H., Greenaway, P. J. Characterization of glycoprotein complexes present in human cytomegalovirus envelopes. *J. Gen. Virol.* **67**:1469–1473, 1986.

Farrer, G. H., Oram, J. D. Characterization of the human cytomegalovirus envelope glycoproteins. *J. Gen. Virol.* **65**:1991–2001, 1984.

Fauci, A. S. The human immunodeficiency virus: Infectivity and mechanisms of pathogenesis. *Science* **239**:617–622, 1988.

Feldman, R. A. Cytomegalovirus in stored urine specimens. *J. Pediatr.* **73**:611–614, 1968.

Felsenstein, D., D'Amico, D. J., Hirsch, M. S., *et al.* Treatment of cytomegalovirus retinitis with 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine. *Ann. Intern. Med.* **103**:377–380, 1985a.

Felsenstein, D., D'Amico, D. J., Hirsch, M. S., *et al.* Treatment of cytomegalovirus retinitis with 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine and high-dose corticosteroids. *Ann. Intern. Med.* **105**:214–215, 1985b.

Felsenstein, D., Carney, W. P., Iacoviello, V. R., Hirsch, M. S. Phenotypic properties of atypical lymphocytes in cytomegalovirus-induced mononucleosis. *J. Infect. Dis.* **152**:198–203, 1985c.

Fenner, F., McAuslan, B. R., Mims, C. A., *et al. The Biology of Animal Viruses*, 2nd ed. Academic Press, New York, 1974, p. 834.

Fetterman, G. H. A new laboratory aid in the clinical diagnosis of inclusion disease of infancy. *Am. J. Clin. Pathol.* **22**:424–425, 1952.

Fetterman, G. H., Sherman, F. E., Fabizio, N. S., Studnicki, F. M. Generalized cytomegalic inclusion disease of the newborn: Localization of inclusions in the kidney. *Arch. Pathol.* **86**:86–94, 1968.

Fiala, M., Kattlove, H., Lemkin, T. Cytomegalovirus mononucleosis with severe thrombocytopenia. *Ann. Intern. Med.* **79**:450–451, 1973.

Fiala, M., Payne, J. E., Berne, T. V., *et al.* Epidemiology of cytomegalovirus infection after transplantation and immunosuppression. *J. Infect. Dis.* **132**:421–433, 1975.

Fiala, M., Honess, R. W., Heiner, D. C., *et al.* Cytomegalovirus proteins. I. Polypeptides of virions and dense bodies. *J. Virol.* **19**:243–254, 1976.

Fiala, M., Cone, L. A., Chang, C. M., Mocarski, E. S. Cytomegalovirus viremia increases with progressive immune deficiency in patients infected with HTLV-III. *AIDS Res.* **2**(3):175–181, 1986.

Fiche, M., Soulilou, J. P., Bignon, J. D., *et al.* T lymphocyte monitoring in kidney transplant recipients undergoing cytomegalovirus infection or rejection episodes. *Transplantation* **37**:421–423, 1984.

Field, A. K., Davies, M. E., DeWitt, C., *et al.* 9-([2-Hydroxy-1-(hydroxymethyl)ethoxy]methyl) guanine: A selective inhibitor of herpes group virus replication. *Proc. Natl. Acad. Sci. U.S.A.* **80**:4139–4143, 1983.

Figueroa, M. E., Geder, L., Rapp, F. Infection of human amnion cells with CMV. *J. Med. Virol.* **2**:369–375, 1978.

Findlay, G. M. Intranuclear bodies in the liver cells of mice. *Br. J. Exp. Pathol.* **13**:223–229, 1932.

Fioretti, A., Pollini, C. Use of gel precipitin test in the diagnosis of CMV infections. *Acta Virol. (Praha)* **22**:68–69, 1978.

Fioretti, A., Fuirukawa, T., Santoli, D., Plotkin, S. A. Non-productive infection of guinea pig cells with human cytomegalovirus. *J. Virol.* **11**:998–1003, 1973.

Flechner, S. M., Novick, A. C., Steinmuller, D. Improved cadaver allograft survival in transfused recipients who remain serologically negative for cytomegalovirus. *J. Urol.* **127**:644–647, 1982.

Foerster, H. W. Pathology of granulomatous uveitis. *Surv. Ophthalmol.* **4**:296, 1959.

Fong, C. K. Y., Bia, F., Hsiung, G. D. Ultrastructural development and persistence of guinea pig CMV in duet cells of guinea pig submaxillary gland. *Arch Virol.* **64**:97–108, 1980.

Fong, C. K. Y., Lucia, H., Bia, F. J., Hsiung, G. D. Histopathologic and ultrastructural studies of disseminated cytomegalovirus infection in strain 2 guinea pigs. *Lab. Invest.* **49**:183–194, 1983.

Forghani, B., Schmidt, N. J. Humoral immune response to virions and dense bodies of human cytomegalovirus determined by enzyme immunofluorescence assay. *J. Med. Virol.* **6**:119–127, 1980.

Forman, S., Zaia, J., Clark, B., Wright, C., Mills, B., Pottathil, R., Racklin, B., Gallagher, M., Welte, K., Blume, K. A 64,000 dalton matrix protein of human cytomegalovirus induces *in vitro* immune response similar to those of the whole viral antigen. *J. Immunol.* **134**:3391–3395, 1985.

Fortunato, J., Goldschmidt, B., Menonna, J., Dowling, P., Cook, S. Rapid detection of antibodies to cytomegalovirus by counterimmunoelectrophoresis. *J. Infect. Dis.* **135**:432–437, 1977.

Foster, K. M. Post-transfusion mononucleosis. *Aust. Ann. Med.* **15**:305–310, 1966.

Foster, K. M., Jack, I. Isolation of cytomegalovirus from the blood leucocytes of a patient with post-transfusion mononucleosis. *Aust. Ann. Med.* **17**:135–140, 1968.

Foster, K. M., Jack, I. A prospective study of the role of cytomegalovirus in post-transfusion mononucleosis. *N. Engl. J. Med.* **280**:1311–1316, 1969.

Fox, A. S., Tolpin, M. D., Baker, A. L., *et al.* Seropositivity in liver transplant recipients as a predictor of cytomegalovirus disease. *J. Infect. Dis.* **157**:383–385, 1988.

Frager, D. H., Frager, J. D., Brandt, L. J., *et al.* Gastrointestinal complications of AIDS: Radiologic features. *Radiology* **158**:597–603, 1986.

Frank, I., Friedman, H. M. Progress in the treatment of cytomegalovirus pneumonia. *Ann. Intern. Med.* **109**:769–771, 1988.

Frank, T. S., LiVolsi, V. A., Connor, A. M. Cytomegalovirus infection of the thyroid in immunocompromised adults. *Yale J. Biol. Med.* **60**:1–8, 1987.

Freeman, W. R., Lerner, C. W., Mines, J. A., Lash, R. S., Nadel, A. J., Starr, M. B., Tapper, M. L. A prospective study of the opthalmologic findings in the acquired immune deficiency syndrome. *Am. J. Pathol.* **97**:133–142, 1984.

Freitas, V. R., Smee, D. F., Chernow, M., Boehme, R., Matthews, T. R. Activity of 9-(1,3-dihydroxy-2-propoxymethyl)guanine compared with that of acyclovir against human, monkey, and rodent cytomegaloviruses. *Antimicrob. Agents Chemother.* **28**:240–245, 1985.

Frenkel, N., Deiss, L. P. Spaete, R. R. Studies of HSV DNA propagation using HSV amplicons. In F. Rapp (ed.), *Herpesvirus, UCLA Symposia on Molecular and Cellular Biology, New Series.* Alan R. Liss, New York, 1984, pp. 289–300.

Friedman, A. H., Orellana, J., Freeman, W. R., *et al.* Cytomegalovirus retinitis: A manifestation of the acquired immune deficiency syndrome (AIDS). *Br. J. Ophthalmol.* **67**:372–380, 1981.

Friedman, H. M., Grossman, R. A., Plotkin, S. A., *et al.* Relapse of pneumonia caused by cytomegalovirus in two recipients of renal transplants. *J. Infect. Dis.* **139**:465–473, 1979.

Fuccillo, D. A., Moder, F. L., Traub, R. G., *et al.* Micro indirect hemagglutination test for cytomegalovirus. *Appl. Microbiol.* **21**:104–107, 1971.

Fujinami, R. S., Nelson, J. A., Walker, L., Oldstone, M. B. A. Sequence homology and immunologic cross-reactivity of human cytomegalovirus with HLA-DR beta chain: A means for graft rejection and immunosuppression. *J. Virol.* **62**:100–105, 1988.

Furman, P. A., St. Clair, M. H., Fyfe, J. A., *et al.* Inhibition of herpes simplex virus-induced DNA polymerase activity and viral DNA replication by 9-(2-hydroxyethoxymethyl)guanine and its triphosphate. *J. Virol.* **32**:72–77, 1979.

Furukawa, T., Fioretti, A., Plotkin, S. A. Growth characteristics of cytomegalovirus in human fibroblasts with demonstration of protein synthesis early in viral replication. *J. Virol.* **11**:991–997, 1973.

Furukawa, T., Tanaka, S., Plotkin, S. A. Stimulation of macromolecular synthesis in guinea pig cells by human CMV. *Proc. Soc. Exp. Biol. Med.* **148**:211–214, 1975a.

Furukawa, T., Hornberger, E., Sakuma, S., Plotkin, S. A. Demonstration of immunoglobulin G receptors induced by human cytomegalovirus. *J. Clin. Microbiol.* **2**:332–336, 1975b.

Furukawa, T., Sakuma, S., Plotkin, S. A. Human cytomegalovirus infection of WI-38 cells stimulates mitochondrial DNA synthesis. *Nature* **262**:414–416, 1976.

Furukawa, T., Yoshimura, N., Jean, J. H., Plotkin, S. A. Chronically persistent infection with human cytomegalovirus in human lymphocytes. *J. Infect. Dis.* **139**:211–214, 1979.

Gajl-Peczalska, K. Cytomegalic inclusion diseae. *Arch. Dis. Child.* **42**:14–19, 1967.

Galloway, D. A., Nelson, J. A., McDougall, J. K. Small fragments of herpesvirus DNA with transforming activity contain insertion sequence-like structures. *Proc. Natl. Acad. Sci. U.S.A.* **81**:4736–4740, 1984.

Gao, M., Isom, H. C. Characterization of the guinea pig cytomegalovirus genome by molecular cloning and physical mapping. *J. Virol.* **52**:436–447, 1984.

Garau, J., Kabins, S., DeNosaquo, S., Lee, G., Keller, R. Spontaneous cytomegalovirus mononucleosis with conjunctivitis. *Arch. Intern. Med.* **137:**1631–1632, 1977.

Gardner, M. B., Officer, J. E., Parker, J., Estes, J. D., Rongey, R. W. Induction of disseminated virulent cytomegalovirus infection by immunosuppression of naturally chronically infected wild mice. *Infect. Immun.* **10:**966–969, 1974.

Garnett, H. M. Isolation of human cytomegalovirus from peripheral blood T cells of renal transplant patients. *J. Lab. Clin. Med.* **99:**92–97, 1982.

Geballe, A. P., Mocarski, E. S. Translational control of cytomegalovirus gene expression is mediated by upstream AUG condons. *J. Virol.* **62:**3334–3340, 1988.

Geballe, A. P., Leach, F. S., Mocarski, E. S. Regulation of cytomegalovirus late gene expression: (gamma) genes are controlled by posttranscriptional events. *J. Virol.* **57:**864–874, 1986a.

Geballe, A. P., Spaete, R. R., Mocarski, E. S. A cis-acting element within the 5′ leader of a cytomegalovirus (beta) transcripts determines kinetic class. *Cell* **46:**865–872, 1986b.

Geddes, D. M., Corrin, B., Brewerton, D. A., *et al.* Progressive airway obliteration in adults and its association with rheumatoid disease. *Q. J. Med.* **46:**427–444, 1977.

Geder, L. Evidence for early nuclear antigens in cytomegalovirus infected cells. *J. Gen. Virol.* **32:**315–319, 1976.

Geder, L., Skinner, G. R. B. Differentiation between type 1 and type 2 strains of herpes simplex virus by an indirect immunofluorescent technique. *J. Gen. Virol.* **12:**179–182, 1971.

Geder, L., Lausch, R., O'Neill, F., Rapp, F. Oncogenic transformation of human embryo lung cells of human cytomegalovirus. *Science* **192:**1134–1137, 1976.

Geder, L., Kreider, J., Rapp, F. Human cells transformed *in vitro* by human cytomegalovirus: Tumorigenicity in athymic nude mice. *J. Natl. Cancer Inst.* **58:**1003–1007, 1977.

Geelen, J. L. M. C., Westrate, M. W. Organization of the human cytomegalovirus genome. In Y. Becker (ed.), *Herpesvirus DNA.* Martinus Nijhoff Medical Publishers, The Hague, 1981, pp. 325–343.

Geelen, J. L. M. C., Walig, C., Wertheim, P., Van Der Noordaa, J. Human cytomegalovirus DNA. I. Molecular weight and infectivity. *J. Virol.* **26:**813–816, 1978.

Gehrz, R. C., Rutzick, S. R. Cytomegalovirus (CMV)-specific lysis of CMV-infected target cells can be mediated by both NK-like and virus-specific cytotoxic T lymphocytes. *Clin. Exp. Immunol.* **61:**80–89, 1985.

Gehrz, R. C., Marker, S. C., Knorr, S. O., *et al.* Specific cell-mediated immune deficit in active cytomegalovirus infection of young children and their mothers. *Lancet* **2:**844–847, 1977.

Gehrz, R. C., Christianson, W. R., Linner, K. M., *et al.* Cytomegalovirus vaccine. Specific humoral and cellular immune responses in human volunteers. *Arch. Intern. Med.* **140:**936–939, 1980.

Gehrz, R. C., Christianson, W. R., Linner, K. M., *et al.* Cytomegalovirus-specific humoral and cellular immune responses in human pregnancy. *J. Infect. Dis.* **143:**391–395, 1981.

Gelderman, A. H., Grimley, P. M., Lunde, M. N., Rabson, A. S. *Toxoplasma gondii* and cytomegalovirus: Mixed infections by a parasite and a virus. *Science* **160:**1130–1132, 1968.

Gergely, L., Czedledy, J., Vaczi, L. Human antibody response to human cytomegalovirus-specific DNA-binding proteins. *Acta Virol. (Praha)* **32:**1–5, 1988.

Gerna, G., McCloud, C. J., Chambers, R. W. Immunoperoxidase technique for detection of antibodies to human cytomegalovirus. *J. Clin. Microbiol.* **3:**364–372, 1976a.

Gerna, G., Vasquez, A., McCloud, C. J., Chambers, R. W. The immunoperoxidase technique for rapid human cytomegalovirus identification. *Arch. Virol.* **50:**311–321, 1976b.

Gerna, G., Cereda, P. M., Cattaneo, E., *et al.* Immunoglobulin G to virus-specific early antigens in congenital, primary and reactivated human CMV infections. *Infect. Immun.* **22:**833–841, 1978.

Gershon, A., Cosio, L., Brunnell, P. A. Observations on the growth of varicella–zoster virus in human diploid cells. *J. Gen. Virol.* **18:**21–31, 1973.

Gertler, S. L., Pressman, J., Price, P., *et al.* Gastrointestinal cytomegalovirus infection in a homosexual man with severe acquired immunodeficiency syndrome. *Gastroenterology* **85:**1403–1406, 1983.

Ghazal, P., Lubon, H., Fleckenstein, B., *et al.* Binding of transcription factors and creation of a large nucleoprotein complex on the human cytomegalovirus enhancer. *Proc. Natl. Acad. Sci. U.S.A.* **84:**3658–3662, 1987.

Ghazal, P., Lubon, H., Hennighausen, L. Specific interactions between transcription factors and the promoter-regulatory region of the human cytomegalovirus major immediate early gene. *J. Virol.* **62:**1076–1079, 1988a.

Ghazal, P., Lubon, H., Henninghausen, L. Multiple sequence-specific transcription factors modulate cytomegalovirus enhancer activity *in vitro*. *Mol. Cell. Biol.* **8:**1809–1811, 1988b.

Gibson, W. Immediate-early proteins of human cytomegalovirus strains AD 169, Davis, and Towne differ in electrophoretic mobility. *Virology* **112:**350–354, 1981a.

Gibson, W. Structural and nonstructural proteins of strain Colburn cytomegalovirus. *Virology* **111:**516–537, 1981b.

Gibson, W. Protein counterparts of human and simian cytomegaloviruses. *Virology* **128:**391–406, 1983.

Gibson, W., Irmiere, A. Selection of particles and proteins for use as human cytomegalovirus subunit vaccines. In *Birth Defects* **20:**305–324, 1984.

Gibson, W., Murphy, T., Roby, C. Cytomegalovirus-infected cells contain a DNA-binding protein. *Virology* **111:**251–262, 1981.

Gilbert, G. L., Hudson, I. L., Hayes, K., James, J. Prevention of transfusion-acquired cytomegalovirus infection in infants by blood filtration to remove leucocytes. *Lancet* **I:**1128–1231, 1989.

Giraldo, G., Beth, E., Coeur, P., *et al.* Kaposi's sarcoma: A new model in the search for viruses associated with human malignancies. *J. Natl. Cancer Inst.* **49:**1495–1507, 1972a.

Giraldo, G., Beth, E., Haguenau, F. Herpes-type virus particles in tissue culture of Kaposi's sarcoma from different geographic regions. *J. Natl. Cancer Inst.* **49:**1509–1526, 1972b.

Giraldo, G., Beth, E., Kourilsky, F. M., *et al.* Antibody patterns to herpesviruses in Kaposi's sarcoma: Serological association of European Kaposi's sarcoma with cytomegalovirus. *Int. J. Cancer* **15:**839–848, 1975.

Giraldo, G., Beth, E., Henle, W., *et al.* Antibody patterns to herpesviruses in Kaposi's sarcoma. II. Serological association of American Kaposi's sarcoma with CMV. *Int. J. Cancer* **22:**126–131, 1978.

Giraldo, G., Beth, E., Huang, E. W. Relationship of CMV to Kaposi's sarcoma. In *International Conference on Human Herpesviruses,* Atlanta, 1980.

Glaser, R., Rapp, F. Rescue of Epstein–Barr virus from somatic cell hybrids of Burkitt's lymphoblastoid cells. *J. Virol.* **10:**288–296, 1972.

Glaser, R., Geder, L., St. Jeor, S., *et al.* Partial characterization of a herpes-type virus (K9V) derived from Kaposi's sarcoma. *J. Natl. Cancer Inst.* **59:**55–60, 1977.

Glaser, R., Strain, E. C., Tarr, K. L., *et al.* Changes in Epstein–Barr virus antibody titers associated with aging. *Proc. Soc. Exp. Biol. Med.* **179:**352–355, 1985.

Glasgow, L. A., Hanshaw, J. B., Merigan, T. C., Petralli, J. K. Interferon and cytomegalovirus *in vivo* and *in vitro*. *Proc. Soc. Exp. Biol. Med.* **125:**843–849, 1967.

Glasgow, B. J., Steinsapir, K. D., Anders, K., *et al.* Adrenal pathology in the acquired immune deficiency syndrome. *Am. J. Clin.* **84:**594–597, 1985.

Glazer, J. P., Friedman, H. M., Grossman, R. A., Starr, S. E., Barker, C. F., Perloff, L. J., Huang, E. S., Plotkin, S. A. Live cytomegalovirus vaccination of renal transplant candidates. A preliminary trial. *Ann. Intern. Med.* **91:**676–683, 1979.

Gleaves, C. A., Smith, T. F., Shuster, E. A., Pearson, G. R. Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *J. Clin. Microbiol.* **21:**217–221, 1985.

Gluckman, E., Lotsberg, J., Devergie, A., *et al.* Prophylaxis of herpes infections after bone-marrow transplantation by oral acyclovir. *Lancet* **2:**706–708, 1983.

Godowski, P. J., Knipe, D. M. Transcriptional control of herpesvirus gene expression: Gene functions required for positive and negative regulations. *Proc. Natl. Acad. Sci. U.S.A.* **83:**256–260, 1986.

Goins, W. F. *Post-transcriptional Regulation of a Human Cytomegalovirus Late Gene.* Thesis, University of Iowa, Iowa City, 1986.

Goins, W. F., Stinski, M. F. Expression of a human cytomegalovirus late gene is post-transcriptionally regulated by a 3′-end-processing event occurring exclusively late after infection. *Mol. Cell. Biol.* **6:**4202–4213, 1986.

Gold, D., Ashley, R., Handsfield, H., *et al.* Immunoblot analysis of the humoral immune response in primary cytomegalovirus infection. *J. Infect. Dis.* **157:**319–326, 1988.

Gold, E., Nankervis, G. A. Cytomegalovirus. In A. S. Evans (ed.), *Viral Infections of Humans.* Plenum Press, New York, 1976, pp. 43–161.

Gompels, U., Minson, A. The properties and sequence of glycoprotein H of herpes simplex virus type 1. *Virology* **153:**230–247, 1986.

Gonczol, E., Andersen, H. K. Studies on human cytomegalovirus strain variations by membrane fluorescence. *Arch. Ges. Virusforsch.* **44:**147–149, 1974.

Gonczol, E., Andrews, P. W., Plotkin, S. A. Cytomegalovirus replicates in differentiated but not in undifferentiated human embryonal carcinoma cells. *Science* **224:**159–161, 1984.

Gonczol, E., Andrews, P. W., Plotkin, S. A. Cytomegalovirus infection of human tetracarcinoma cells in culture. *J. Gen. Virol.* **66:**509–515, 1985.

Gonczol, E., Hudecz, F., Ianocone, J., *et al.* Immune response to isolated human cytomegalovirus envelope proteins. *J. Virol.* **58:**661–664, 1986.

Gonczol, E., Ianacone, J., Starr, S., Plotkin, S. Immunization of seronegative and seropositive individuals with a protein complex of the HCMV envelope. In *Twelfth International Herpesvirus Workshop Abstracts,* Philadelphia, 1987, p. 253.

Gonczol, E., Ianacone, J., Furlini, G., *et al.* Humoral immune response to cytomegalovirus Towne vaccine strain and to Toledo low-passage strain. *J. Infect. Dis.* **159:**851–865, 1989.

Goodheart, C. R., McAllister, R. M., Filbert, J. E. Human cytomegalovirus. DNA synthesis and migration in infected cells studied autoradiographically. *Virology* **23:**603–608, 1964.

Goodman, M. D., Porter, D. D. Cytomegalovirus vasculitis with fatal colonic hemorrhage. *Arch. Pathol.* **96:**281–284, 1973.

Goodpasture, E. W. Intranuclear inclusions in experimental herpetic lesions of rabbits. *Am. J. Pathol.* **1:**1–9, 1925.

Goodpasture, E. W., Talbot, F. B. Concerning the nature of "protozoan-like" cells in certain lesions of infancy. *Am. J. Dis. Child.* **21:**415–425, 1921.

Gottlieb, M. S., Schroff, R., Schanker, H. M., *et al. Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men. Evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* **305:**1425–1431, 198i.

Gottman, A. W., Beatty, E. C. Cytomegalic inclusion disease in children with leukemia or lymphosarcoma. *Am. J. Dis. Child.* **104:**180–184, 1962.

Grafe, M. R., Wiley, C. A. Spinal cord and peripheral nerve pathology in AIDS: The roles of cytomegalovirus and human immunodeficiency virus. *Ann. Neurol.* **25:**561–566, 1989.

Graham, B. J., Minammashima, Y., Dreesman, G. R., *et al.* Complement-requiring neutralizing antibodies in hyperimmune sera to human cytomegalovirus. *J. Immunol.* **107:**1618–1630, 1971.

Graham, C. B., Jr., Thal, A., Wassum, C. S. Rubella-like bone changes in congenital cytomegalic inclusion disease. *Radiology* **94:**39–43, 1970.

Granström, M. L., Leinikki, P. Illnesses during the first two years of life and their association with perinatal CMV infection. *Scand. J. Infect. Dis.* **10:**257–264, 1978.

Granström, M. L., Leinikki, P., Santavuori, P., Pettay, O. Perinatal cytomegalovirus infection in man. *Arch. Dis. Child.* **52:**354–359, 1977.

Granström, M. L., Leinikki, P., Santavuori, P., Pettay, O. Perinatal cytomegalovirus in man. In *International Conference on Human Herpesviruses,* Atlanta, 1980.

Grattan, M. T., Moreno-Cabral, C. E., Starnes, V. A., *et al.* Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. *J.A.M.A.* **261:**3561–3566, 1989.

Gray, J. J., Alvey, B., Smith, D. J., Wreghitt, T. G. Evaluation of a commercial latex agglutination test for detecting antibodies to cytomegalovirus in organ donors and transplant recipients. *J. Virol. Methods* **16:**13–19, 1987.

Green, M. Oncogenic viruses. *Annu. Rev. Biochem.* **39:**701–756, 1970.

Greenberg, S. B., Linder, S., Baxter, B., *et al.* Lymphocyte subsets and urinary excretion of

cytomegalovirus among homosexual men attending a clinic for sexually transmitted diseases. *J. Infect. Dis.* **150:**330–333, 1984.

Gregory, W. W., Menegus, M. A. Practical protocol for cytomegalovirus isolation: Use of MRC-5 cell monolayers incubated for 2 weeks. *J. Clin. Microbiol.* **17:**605–609, 1983.

Gretch, D. R., Stinski, M. F. Transcription of the human cytomegalovirus glycoprotein gene family in the short unique component of the viral genome. *Virology* **174:**522–532, 1990.

Gretch, D., Gehrz, R., Stinski, M. Characterization of a human cytomegalovirus glycoprotein complex (gCI). *J. Gen. Virol.* **69:**1205–1215, 1988a.

Gretch, D., Kari, B., Rasmussen, L., *et al.* Identification and characterization of three distinct families of glycoprotein complexes in the envelopes of human cytomegalovirus. *Virology* **62:**875–881, 1988b.

Gretch, D., Kari, B., Gehrz, R., Stinski, M. A multigene family encodes the human cytomegalovirus glycoprotein complex gCII (gp47–52 complex). *J. Virol.* **62:**1956–1962, 1988c.

Griffith, B. P., Hsiung, G. D. Cytomegalovirus infection in guinea pigs. IV. Maternal infection at different stages of gestation. *J. Infect. Dis.* **141:**787–793, 1980.

Griffith, B. P., Lucia, H. L., Bia, F. J., Hsiung, G. D. Cytomegalovirus induced mononucleosis in guinea pigs. *Infect. Immun.* **35:**857–863, 1981.

Griffith, B. P., McCormick, S. R., Booss, J., Hsiung, G. D. Inbred guinea pig model of intrauterine infection with cytomegalovirus. *Am. J. Pathol.* **122:**112–119, 1986.

Griffiths, P. D., Grundy, J. The status of CMV as a human pathogen. *Epidemiol. Inf.* **100:**1–15, 1988.

Griffiths, P. D., Buie, K. J., Heath, R. B. A comparison of complement fixation, indirect immunofluorescence for viral late antigens and anticomplement immunofluorescence tests for the detection of CMV specific serum antibodies. *J. Clin. Pathol.* **31:**827–831, 1978a.

Griffiths, P. D., Stagno, S., Reynolds, D. W., Alford, C. A. A longitudinal study of the serological and virological status of 18 women infected with CMV. *Arch. Virol.* **58:**111–118, 1978b.

Griffiths, P. D., Stagno, S., Pass, R. F., *et al.* Infection with cytomegalovirus during pregnancy; Specific IgM antibodies as a marker of recent primary infection. *J. Infect. Dis.* **146:**647–653, 1982.

Griffiths, P. D., Panjwani, D. D., Stirk, P. R., *et al.* Rapid diagnosis of cytomegalovirus infection in immunocompromised patients by detection of early antigen fluorescent foci. *Lancet* **2:**1242–1245, 1984.

Grob, J. P., Grundy, J. E., Prentice, H. G., *et al.* Immune donors can protect marrow-transplant recipients from severe cytomegalovirus infection. *Lancet* **1:**774–776, 1987.

Grompels, U., Munson, A. The properties and sequence of glycoprotein H of herpes simplex type 1. *Virology* **153:**230–247, 1986.

Grundy, J. E., Shearer, G. M. The effect of cytomegalovirus infection on the host response to foreign and hapten-modified self histocompatibility antigens. *Transplantation* **37:**485–490, 1984.

Grundy, J. E., Shanley, J. D., Shearer, G. M. Augmentation of graft-versus-host reaction by cytomegalovirus infection resulting in interstitial pneumonitis. *Transplantation* **39:**548–553, 1985.

Grundy, J., McKeating, J., Ward, P., Sanderson, A., Griffiths, P. A. 2 microglobulin enhances the infectivity of cytomegalovirus and when bound to the virus enables class I HLA molecules to be used as a virus receptor. *J. Gen. Virol.* **68:**793–803, 1987a.

Grundy, J. E., Shanley, J. D., Griffiths, P. D. Is cytomegalovirus intersistial pneumonitis in transplant recipients an immunopathological condition? *Lancet* **2:**996–999, 1987b.

Grundy, J. E., Super, M., Lui, S., *et al.* The source of cytomegalovirus infection in seropositive renal allograft recipients is frequently the donor kidney. *Transplant. Proc.* **19:**2126–2128, 1987c.

Grundy, J. E., McKeating, J. A., Sanderson, A. R., Griffiths, P. D. Cytomegalovirus and β_2 microglobulin in urine specimens. *Transplantation* **45:**1075–1079, 1988a.

Grundy, J., Ayles, H., McKeating, J., *et al.* Enhancement of class I HLA antigen expression by cytomegalovirus: Role in amplification of virus infection. *J. Med. Virol.* **25:**483–495, 1988b.

Grundy, J. E., Super, M., Berry, N. J., *et al.* Symptomatic cytomegalovirus infection in seropositive kidney recipients: Reinfection with donor virus rather than reactivation of recipient virus. *Lancet* **2:**132–135, 1988c.

Gui, X., Rinaldo, C. R., and Ho, M. Natural killer cell activity in renal transplant recipients receiving cyclosporine. *Infect. Immun.* **41**:965–970, 1983.

Gupta, P., St. Joer, S., Rapp, R. Comparison of the polypeptides of several strains of human cytomegalovirus. *J. Gen. Virol.* **34**:447–454, 1977.

Gurevich, I., Cunha, B. A. Non-parenteral transmission of cytomegalovirus in a neonatal intensive care unit. *Lancet* **2**:222–224, 1981.

Haahr, S., Moller-Larsen, A., Andersen, H. K., Spencer, E. S. Cell-mediated and humoral immune responses to herpes simplex virus and cytomegalovirus in renal transplant patients. *J. Clin. Microbiol.* **10**:267–274, 1979.

Habermann, R. T., Williams, F. P., Jr., Fite, G. L. Inclusion bodies associated with viral diseases of man and other animals. *J. Am. Vet. Med. Assoc.* **137**:171–176, 1960.

Hackman, R. C., Myerson, D., Meyers, J. D., *et al.* Rapid diagnosis of cytomegaloviral pneumonia by tissue immunofluorescence with a murine monoclonal antibody. *J. Infect. Dis.* **151**:325–329, 1985.

Haller, O., Kiessling, R., Orn, A., Wigzell, H. Generation of natural killer cells. An autonomous function of the bone marrow. *J. Exp. Med.* **145**:1411–1416, 1977.

Halliburton, I. W., Andrew, J. C. DNA polymerase in pseudorabies virus infected cells. *J. Gen. Virol.* **30**:145–148, 1976.

Hamilton, J. D., Seaworth, B. J. Transmission of latent cytomegalovirus in a murine kidney tissue transplantation model. *Transplantation* **39**:290–296, 1985.

Hamilton, J. D., Fitzwilliam, J. F., Cheung, K. S., *et al.* Viral infection–homograph interactions in a murine model. *J. Clin. Invest.* **62**:1303–1312, 1978.

Hamilton, J. R., Overall, J. C., Jr. Synergistic infection with murine CMV and *Pseudomonas aeruginosa* in mice. *J. Infect. Dis.* **137**:775–782, 1978.

Hamilton, J. R., Overall, J. C., Jr., Glasgow, L. A. Synergistic effect on mortality in mice with murine cytomegalovirus and *Pseudomonas aeruginosa, Staphylococcus aureus* or *Candida albicans* infections. *Infect. Immun.* **14**:982–989, 1976.

Hamilton, T. A., Adams, D. O. Molecular mechanisms of signal transduction in macrophages. *Immunol. Today* **8**:151, 1987.

Hamparian, V. V., Hilleman, M. R., Ketler, A. Contributions to characterization and classification of animal viruses. *Proc. Soc. Exp. Biol. Med.* **112**:1040–1050, 1963.

Handsfield, H. H., Chandler, S. H., Caine, V. A., *et al.* Cytomegalovirus infection in sex partners: Evidence for sexual transmission. *J. Infect. Dis.* **151**:344–348, 1985.

Haneberg, B., Bertnes, E., Haukenes, G. Antibodies to CMV among personnel at a children's hospital. *Acta Paediatr. Scand.* **69**:407–409, 1980.

Hanshaw, J. B. Clinical significance of cytomegalovirus infection. *Postgrad. Med.* **35**:472–480, 1964.

Hanshaw, J. B. Congenital and acquired cytomegalovirus infection. *Pediatr. Clin. North Am.* **13**:279–293, 1966a.

Hanshaw, J. B. Cytomegalovirus complement-fixing antibody in microcephaly. *N. Engl. J. Med.* **275**:476–479, 1966b.

Hanshaw, J. B. *Cytomegaloviruses. Virology Monograph No. 3.* Springer, New York, 1968.

Hanshaw, J. B. Congenital cytomegalovirus infection: Laboratory methods of detection. *J. Pediatr.* **75**:1179–1185, 1969.

Hanshaw, J. B. Congenital cytomegalovirus infection: A fifteen year perspective. *J. Infect. Dis.* **123**:555–561, 1971.

Hanshaw, J. B., Weller, T. H. Urinary excretion of cytomegalovirus by children with generalized neoplastic disease. *J. Pediatr.* **58**:305–311, 1961.

Hanshaw, J. B., Betts, R. F., Simon, G., Boynton, R. C. Acquired cytomegalovirus infection (Association with hepatomegaly and abnormal liver-function tests). *N. Engl. J. Med.* **272**:602–609, 1965.

Hanshaw, J. B., Steinfeld, H. J., White, C. J. Fluorescent-antibody test for cytomegalovirus macroglobulin. *N. Engl. J. Med.* **279**:566–570, 1968.

Hanshaw, J. B., Niederman, J. C., Chessin, L. N. Cytomegalovirus macroglobulin in cell-associated herpesvirus infections. *J. Infect. Dis.* **125**:304–306, 1972.

Hanshaw, J. B., Scheiner, A. P., Moxley, A. W., *et al.* School failure and deafness after "silent" congenital cytomegalovirus infection. *N. Engl. J. Med.* **295**:468–470, 1976.

Haraszti, A. Cytomegalia infantum. *Acta Morphol. Acad. Sci. Hung.* **6**:241–254, 1955.

Hardy, A. M., Wajszczuk, C. P., Suffredini, A. F., *et al. Pneumocystis carinii* pneumonia in renal transplant recipients treated with cyclosporine and steroids. *J. Infect. Dis.* **149**:143–147, 1984.

Harnden, D. G., Elsdale, T. R., Young, D. E., Ross, A. The isolation of cytomegalovirus from peripheral blood. *Blood* **30**:120–125, 1967.

Harnett, G. B., Shellam, G. R. Variation in murine cytomegalovirus replication in fibroblasts from different mouse strains *in vitro:* Correlation with *in vivo* resistance. *J. Gen. Virol* **62**:39–47, 1982.

Harris, A. I., Meyer, R. J., Brody, E. A. Cytomegalovirus-induced thrombocytopenia and hemolysis in an adult. *Ann. Intern. Med.* **83**:670–671, 1975.

Harris, J. P., Woolf, N. K., Ryan, A. F., *et al.* Immunologic and electrophysiological response to cytomegaloviral inner ear infection in the guinea pig. *J. Infect. Dis.* **150**:523–530, 1984.

Hashiro, G. M., Horikami, S., Loh, P. C. Cytomegalovirus isolations from cell cultures of human adenocarcinomas of the colon. *Intervirology* **12**:84–88, 1979.

Hawley, D. A., Schaeffer, J. F., Schulz, D. M., Muller, J. Cytomegalovirus encephalitis in acquired immunodeficiency syndrome. *Am. J. Clin. Pathol.* **80**:874–877, 1983.

Hayes, K., Danks, D. M., Gibas, H., Jack, I. Cytomegalovirus in human milk. *N. Engl. J. Med.* **287**:177–178, 1972.

Hayes, K., Alford, C., Britt, W. Antibody response to virus-encoded proteins after cytomegalovirus mononucleosis. *J. Infect. Dis.* **156**:615–621, 1987.

Haymaker, W., Girdany, B. R., Stephens, J., *et al.* Cerebral involvement with advanced periventricular calcification in generalized cytomegalic inclusion disease in the newborn. *J. Neuropathol. Exp. Neurol.* **13**:562–586, 1954.

Head, H., Campbell, A. W. The pathology of herpes zoster and its bearing on sensory localization. *Brain* **23**:353–523, 1900.

Hedley-Whyte, E. T., Craighead, J. E. Generalized cytomegalic inclusion disease after renal homotransplantation—report of a case with isolation of virus. *N. Engl. J. Med.* **272**:473–475, 1965.

Heieren, M. H., van der Woude, F. J., Balfour, H. H., Jr. Cytomegalovirus replicates efficiently in human kidney mesangial cells. *Proc. Natl. Acad. Sci. U.S.A.* **85**:1642–1646, 1988.

Heilbronn, R., Jahn, G., Burkle, A., *et al.* Genomic localization, sequence analysis, and transcription of the putative human cytomegalovirus DNA polymerase gene. *J. Virol.* **61**:119–124, 1987.

Heineman, T., Gong, M., Sample, J., Kieff, E. Identification of the Epstein–Barr virus gp85 gene. *J. Virol.* **62**:1101–1107, 1988.

Helenius, A., Morein, B., Fries, E., *et al.* Human (HLA-A and HLA-B) and murine (H-2K and H-2D) histocompatibility antigens are cell surface receptors for Semliki-forest virus. *Proc. Natl. Acad. Sci. U.S.A.* **75**:3846–3850, 1978.

Helgestrand, E., Eriksson, B., Johansson, N. G., *et al.* Trisodium phosphonoformate, a new antiviral compound. *Science* **201**:819–821, 1978.

Helgestrand, E., Flodh, H., Lernestedt, J.-O., *et al.* Trisodium phosphonoformate: Antiviral activities, safety evaluation and preliminary clinical results. In L. H. Collier and J. Oxford (eds.), *Developments in Antiviral Therapy.* Academic Press, London, 1980, pp. 63–83.

Henle, W., Henle, G., Scriba, M., *et al.* Antibody responses to the Epstein–Barr virus and cytomegalovirus after open-heart and other surgery. *N. Engl. J. Med.* **282**:1068–1074, 1970.

Henle, W., Henle, G., Horwitz, C. Epstein–Barr virus specific diagnostic tests in infectious mononucleosis. *Hum. Pathol.* **5**:552–565, 1974.

Henle, W., Henle, G., Horwitz, C. A. Infectious mononucleosis and Epstein–Barr virus-associated malignancies. In E. H. Lennette and N. J. Schmidt, (eds.), *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections,* 5th ed. American Public Health Association, Washington, 1979, pp. 441–470.

Henninghausen, L., Fleckenstein, B. Nuclear factor 1 interacts with five DNA elements in the promoter region of the human cytomegalovirus major immediate early gene. *EMBO J.* **5**:1367–1371, 1986.

Henry, K., Cantrill, H., Fletcher, C., *et al.* Use of intravitreal ganciclovir (dihydroxypropoxy-

methylguanine) for cytomegalovirus retinitis in a patient with AIDS. *Am. J. Ophthalmol.* **103:**17–23, 1987.

Henry, S. C., Klotman, M. E., Greene, R. C., *et al.* Detection of murine cytomegalovirus DNA in latently infected mice by *in vitro* enzymatic amplification. In *Herpesvirus Symposium, Irvine, California, June 1988,* 1988.

Henson, D., Strano, A. J. Mouse cytomegalovirus. *Clin. J. Path.* **68:**183–195, 1972.

Henson, D., Siegel, S. E., Fuccillo, D. A., *et al.* Cytomegalovirus infections during acute childhood leukemia. *J. Infect. Dis.* **126:**469–481, 1972a.

Henson, D., Strano, A. J., Slotnik, M., Goodheart, C. Mouse cytomegalovirus: Isolation from spleen and lymph nodes of chronically infected mice. *Proc. Soc. Exp. Biol. Med.* **140:**802–806, 1972b.

Herberman, R. *Natural Cell-Mediated Immunity against Tumors,* Academic Press, New York, 1980, p. 1321.

Hermiston, T. W., Malone, C. L., Witte, P. R., *et al.* Identification and characterization of the human cytomegalovirus immediate-early region 2 gene that stimulates gene expression from an inducible promoter. *J. Virol.* **61:**3214–3221, 1987.

Hermiston, T. W., Malone, C. L., Stinski, M. F. Human cytomegalovirus immediate early t protein region involved in negative regulatory of a major immediate early enhancer containing promoter regulatory region. *J. Virol.* **64:**3532–3536, 1990.

Herrera, G. A., Alexander, R. W., Cooley, C. F., Luke, R. G., Kelly, D. R., Curtis, J. J., Gockerman, J. P. Cytomegalovirus glomerulopathy: A controversial lesion. *Kidney Int.* **29:**725–733, 1986.

Hersman, J., Myers, J. D., Thomas, E. D., *et al.* The effect of granulocyte transfusions upon the incidence of cytomegalovirus infection after allogeneic marrow transplantation. *Ann. Intern. Med.* **96:**149–152, 1982.

Hildebrandt, R. J., Sever, J. L., Anderson, B. Preservation of infectious cytomegalovirus. *Proc. Soc. Exp. Biol. Med.* **129:**504–506, 1968.

Hilgers, F., Hilgers, J. An immunofluorescence technique with counterstain on fixed cells for the detection of antibodies to human herpesviruses; antibody patterns in patients with Hodgkin's disease and nasopharyngeal carcinoma. *Intervirology* **7:**309–327, 1976.

Hirai, K., Furukawa, T., Plotkin, S. A. Induction of DNA polymerase in WI-38 and guinea pig cells infected with human cytomegalovirus. *Virology* **70:**251–255, 1976.

Hirsch, M. S., Black, P. H., Tracy, G. S., *et al.* Leukemia virus activation in chronic allogeneic disease. *Proc. Natl. Acad. Sci. U.S.A.* **67:**1914–1917, 1970a.

Hirsch, M. S., Sisman, B., Allison, A. C. Macrophages and age-dependent resistance to herpes simplex virus in mice. *J. Immunol.* **104:**1160–1165, 1970b.

Hirsch, M. S., Phillips, S. M., Solnik, C., *et al.* Activation of leukemia viruses by graft versus host and mixed lymphocyte reactions *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* **69:**1069–1072, 1972.

Hirsch, M. S., Schooley, R. T., Cosimi, B., *et al.* Effects of interferon-alpha on cytomegalovirus reactivation syndromes in renal-transplant recipients. *N. Engl. J. Med.* **308:**1489–1494, 1983.

Ho, M. Virus infections after transplantation in man. *Arch. Virol.* **55:**1–24, 1977.

Ho, M. Role of specific cytotoxic lymphocytes in cellular immunity against murine cytomegalovirus. *Infect. Immun.* **27:**767–776, 1980.

Ho, M. *Cytomegalovirus: Biology and Infection,* first ed. Plenum Press, New York, 1982.

Ho, M. Infection and organ transplantation. In S. Gelman (ed.), *Anesthesia and Organ Transplantation.* W. B. Saunders, Philadelphia, 1987, pp. 49–60.

Ho, M., Armstrong, J. A. Interferon. *Annu. Rev. Microbiol.* **29:**131–161, 1975.

Ho, M., Ashman, R. B. Development *in vitro* of cytotoxic lymphocytes against murine cytomegalovirus. *Aust. J. Exp. Biol. Med. Sci.* **57:**425–428, 1979.

Ho, M., Dowling, J. N. Cytomegalovirus infection in transplant and cancer patients. In R. W. Remington and M. N. Swartz (eds.), *Current Clinical Topics in Infectious Diseases.* McGraw-Hill, New York, 1979, pp. 45–67.

Ho, M., Dummer, J. S. Risk factors and approaches to infections in transplant recipients. In G. J. Mandell, R. G. Douglas, and J. E. Bennett (eds.), *Principles and Practice of Infectious Diseases,* 3rd ed. Churchill, Livingston, New York, New York, 1990, pp. 2284–2291.

Ho, M., Kono, Y., Breinig, M. K. Tolerance to the induction of interferons by endotoxin and virus: Role of a humoral factor. *Proc. Soc. Exp. Biol. Med.* **119:**1227–1232, 1965.

Ho, M., Suwansirikul, S., Dowling, J. N., *et al.* The transplanted kidney as a source of cytomegalovirus infections. *N. Engl. J. Med.* **293:**1109–1112, 1975.

Ho, M., Dowling, J. N., Armstrong, J. A., *et al.* Factors contributing to the risk of cytomegalovirus infection in patients receiving renal transplants. *Yale J. Biol. Med.* **49:**17–26, 1976.

Ho, M., Miller, G., Atchison, R. W., *et al.* Epstein–Barr virus infections and DNA hybridization studies in post-transplantation lymphoma and lymphoproliferative lesions: Role of primary infection. *J. Infect. Dis.* **152:**876–886, 1985.

Ho, M., Jaffe, R., Miller, G., *et al.* The frequency of EBV infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation* **45:**719–727, 1988.

Hodgkin, P. D., Scalzo, A. A., Swaminathan, N., *et al.* Murine cytomegalovirus binds reversibly to mouse embryo fibroblasts: Implications for quantitation and explanation of centrifugal enhancement. *J. Virol. Methods* **22:**215–230, 1988.

Holland, G. N., Sakamoto, M. J., Hardy, D., *et al.* Treatment of cytomegalovirus retinopathy in patients with Acquired Immunodeficiency Syndrome. *Arch. Ophthalmol.* **104:**1794–1800, 1986.

Holland, G. N., Sidikaro, Y., Kreiger, A. E., *et al.* Treatment of cytomegalovirus retinopathy with ganciclovir. *Opthal.* **94:**815–23, 1987.

Holmes, A. R., Rasmussen, L., Merigan, T. C. Factors affecting the interferon sensitivity of human cytomegalovirus. *Intervirology* **9:**48–55, 1978.

Hope-Simpson, R. E. The nature of herpes zoster: A long-term study and a new hypothesis. *Proc. R. Soc. Med.* **58:**9–20, 1965.

Hopp, T. P., Woods, K. R. Prediction of protein antigenic determinants from amino acid sequences. *Proc. Natl. Acad. Sci. U.S.A.* **78:**3823–3828, 1981.

Horwitz, C. A., Henle, W., Henle, G., *et al.* Heterophile-negative infectious mononucleosis and mononucleosis-like illnesses. *Am. J. Med.* **63:**947–957, 1977.

Howard, R. J., Miller, J., Najarian, J. S. Cytomegalovirus-induced immune suppression. II. Cell-mediated immunity. *Clin. Exp. Immunol.* **18:**119–126, 1974.

Howard, R. J., Mattsson, D. M., Seidel, V., Balfour, H. H., Jr. Cell-mediated immunity to murine cytomegalovirus. *J. Infect. Dis.* **138:**597–604, 1978.

Hsia, K., Spector, D. H., Lawrie, J., Spector, S. A. Enzymatic amplification of human cytomegalovirus sequences by polymerase chain reaction. *J. Clin. Microbiol.* **27:**1802–1809, 1989.

Hsiung, G. D., Fishman, H. R., Fong, C. K. Y., Green, R. H. Characterization of a cytomegalo-like virus isolated from spontaneously degenerated equine kidney cell cultures. *Proc. Soc. Exp. Biol. Med.* **130:**80–84, 1969.

Hsiung, G. D., Tenser, R. B., Fong, C. K. Y. Comparison of guinea pig cytomegalovirus and guinea pig herpes-like virus: growth characteristics and antigenic relationship. *Infect. Immun.* **13:**926–933, 1976.

Hsiung, G. D., Choi, Y. C., Bia, F. J. Cytomegalovirus in guniea pigs. I. Viremia during acute primary and chronic persistent infection. *J. Infect. Dis.* **138:**191–196, 1978.

Huang, E. S. Human cytomegalovirus. III. Virus-induced DNA polymerase. *J. Virol.* **16:**298–310, 1975a.

Huang, E. S. Human cytomegalovirus. IV. Specific induction of virus-induced DNA polymerase activity and viral DNA replication by phosphonacetic acid. *J. Virol.* **16:**1560–1665, 1975b.

Huang, E. S. The role of CMV infection in Kaposi's sarcoma, in A. E. Friedman and L. J. Laubstein (ed.) "AIDS The Epidemic of Kaposi's Sarcoma and Opportunistic Infection" Masson. New York, 1984.

Huang, E. S., Pagano, J. S. Replication of cytomegalovirus DNA in human lymphoblastoid cells. In *WHO International Agency for Research on Cancer Publication No. 11, Oncogenesis and Herpesviruses,* II. World Health Organization, Geneva, 1974, pp. 475–482.

Huang, E. S., Roche, R. K. Cytomegalovirus DNA and adenocarcinoma of the colon: Evidence for latent viral infection. *Lancet* **1:**957–960, 1978.

Huang, E. S., Chen, S. T., Pagano, J. S. Human cytomegalovirus. I. Purification and characterization of viral DNA. *J. Virol.* **12:**1473–1781, 1973.

Huang, E. S., Kilpatrick, B. A., Huang, Y. T., Pagano, J. S. Detection of human cytomegalovirus and analysis of strain variation. *Yale J. Biol. Med.* **49:**29–43, 1976.

Huang, E. S., Kilpatrick, B. A., Lakeman, A., Alford, C. A., Jr. Genetic analysis of a cytomegalovirus-like agent isolated from human brain. *J. Virol.* **26:**718–723, 1978.

Huang, E. S., Alford, C. A., Jr., Reynolds, D. W., *et al.* Molecular epidemiology of CMV infections in women and their infants. *N. Engl. J. Med.* **303:**958–962, 1980a.

Huang, E. S., Huong, S. M., Tegmeier, G. E., Alford, C. A., Jr. Cytomegalovirus: Genetic variation of viral genomes. *Ann. N.Y. Acad. Sci.* **354:**332–346, 1980b.

Huang, Y. T., Huang, E. S., Pagano, J. S. Antisera to human cytomegaloviruses prepared in the guinea pig: Specific immunofluorescence and complement fixation tests. *J. Immunol.* **112:**528–532, 1974.

Huart, J., Baume, D., Jouet, J. Specific anti-cytomegalovirus immunoglobulins in the prevention of cytomegalovirus infections in bone marrow allografts. *Ann. Med. Interne (Paris)* **138:**372–374, 1987.

Hudson, J. B., Misra, V., Mosmann, T. R. Cytomegalovirus infectivity: Analysis of the phenomenon of centrifugal enhancement of infectivity. *Virology* **72:**235–243, 1976.

Hunninghake, C. W., Monick, M. M., Liu, B., Stinski, M. F. The promoter–regulatory region of the major immediate-early gene of human cytomegalovirus responds to T-lymphocyte stimulation and contains functional cyclic AMP-response elements. *J. Virol.* **63:**3026–3033, 1989.

Hutchinson, N. I., Tocci, M. J. Characterization of a major early gene from the human cytomegalovirus long inverted repeat: Predicted amino acid sequence of a 30 kDa protein encoded by the 1.2 kb mRNA. *Virology* **155:**172–182, 1986.

Hutchinson, N. I., Sondermeyer, R. T., Tocci, M. J. Organization and expression of the major genes from the long inverted repeat of the human cytomegalovirus genome. *Virology* **155:**160–171, 1986.

Hutt-Fletcher, L. M., Balachandran, N., Elkins, M. H. B cell activation by cytomegalovirus. *J. Exp. Med.* **158:**2171–2176, 1983.

Hymes, K. B., Cheung, T., Greene, J. B., *et al.* Kaposi's sarcoma in homosexual men—A report of eight cases. *Lancet* **2:**598–600, 1981.

Ikram, H. Cytomegalovirus infections in hemodialysis centers. *Clin. Nephrol.* **15:**1–4, 1981.

Irmiere, A., Gibson, W. Isolation and characterization of a noninfectious virion-like particle released from cells infected with human strains of cytomegalovirus. *Virology* **130:**118–133, 1983.

Irmiere, A., Gibson, W. Isolation of human cytomegalovirus intranuclear capsids, and demonstration that the B-capsid assembly protein is also abundant in noninfectious enveloped particles. *J. Virol.* **56:**227–283, 1985.

Ironside, A. G., Tobin, J. O'H. Cytomegalovirus infection in the adult. *Lancet* **2:**615–616, 1967.

Isom, H. C. Stimulation of ornithine decarboxylase by human cytomegalovirus. *J. Gen. Virol.* **42:**265–278, 1979.

Isom, H. C., Gao, M., Wighahl, B. L. Characterization of guinea pig cytomegalovirus DNA. *J. Virol.* **49:**426–436, 1984.

Jackson, L. An intracellular protozoan parasite of the ducts of the salivary glands of the guinea-pig. *J. Infect. Dis.* **26:**347–350, 1920.

Jacobs, J. P., Jones, C. M., Baille, J. P. Characteristics of a human diploid cell designated MRC-5, *Nature* **227:**168–170, 1970.

Jacobson, M. A., Mills, J. Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Clinical findings, diagnosis, and treatment. *Ann. Intern. Med.* **108:**585–594, 1988.

Jahn, G., Kouzarides, T., Mach, M., *et al.* Map position and nucleotide sequence of the gene for the large structural phosphoprotein of human cytomegalovirus. *J. Virol.* **61:**1358–1367, 1987a.

Jahn, G., Scholl, B. C., Traupe, B., *et al.* The two major structural phosphoproteins (pp65 and pp150) of human cytomegalovirus and their antigenic properties. *J. Gen. Virol.* **68:**3127–3137, 1987b.

Janssen, H. P., Van Loon, A. M., Meddens, M. J., *et al.* Immunological detection of cytomegalovirus early antigen on monolayer inoculated with urine specimens by centrifugation and cultured for 6 days as alternative to conventional virus isolation. *J. Clin. Microbiol.* **26:**1313–1315, 1988.

Jariwalla, R. J., Aurelian, L., Ts'o, P. O. P. Tumorigenic transformation induced by a specific fragment of DNA from herpes simplex virus type 2. *Proc. Natl. Acad. Sci. U.S.A.* **77**:2279–2283, 1980.

Jariwalla, R. J., Razzaque, A., Lawson, S., Rosenthal, L. J. Tumor progression mediated by two cooperating DNA segments of human cytomegalovirus. *J. Virol.* **63**:425–428, 1989.

Jason, J., Lui, K.-J., Ragni, M. V., Hessol, N. A., Darrow, W. W. Risk of developing AIDS in HIV-infected cohorts of hemophilic and homosexual men. *J.A.M.A.* **261**:725–727, 1989.

Jean, J. H., Yoshimara, N., Furukawa, T., Plotkin, S. A. Intracellular forms of the parental human cytomegalovirus genomes at early stages of the infective process. *Virology* **86**:281–286, 1978.

Jeang, K. T. *Molecular Analysis of Cytomegalovirus Colburn Gene Expression.* Ph.D. Thesis, Johns Hopkins University, Baltimore, 1984.

Jeang, K. T., Hayward, G. S. A cytomegalovirus DNA sequence containing tracts of tandemly repeated CA dinucleotides hybridizes to highly repetitive dispersed elements in mammalian cell genomes. *Mol. Cell. Biol.* **3**:1389–1402, 1983.

Jeang, K. T., Rawlins, D. R., Rosenfeld, P. J., *et al.* Multiple tandemly repeated binding sites for cellular nuclear factor 1 that surround the major immediate-early promoters of simian and human cytomegalovirus. *J. Virol.* **61**:1559–1570, 1987.

Jenson, A. B., Rosenberg, H. S., Notkins, A. L. Pancreatic islet cell damage in children with fatal viral infections. *Lancet* **2**:354–358, 1980.

Jerne, N. K., Nordin, A. A. Plaque formation in agar by single antibody-producing cells. *Science* **140**:405, 1963.

Jesionek, Kiolemenoglou. Ueber einen Befund von protozoen-artigen Gebilden in den Organen eines hereditar-luetischen Foetus. *Munch. Med. Wochenschr.* **51**:1905–1907, 1904.

Joassin, L., Reginster, M. Elimination of nonspecific cytomegalovirus immunoglobulin M activities in the enzyme-linked immunosorbent assay by using anti-human immunoglobulin G. *J. Clin. Microbiol.* **23**:576–581, 1986.

Johnson, K. P., Connor, W. S. Guinea pig cytomegalovirus: Transplancental transmission. *Arch. Virol.* **59**:263–269, 1979.

Johnson, R. T. The pathogenesis of herpes virus encephalitis. II. A cellular basis for the development of resistance with age. *J. Exp. Med.* **120**:359–374, 1964.

Joncas, J. H., Menezes, J., Huang, E. S. Persistence of CMV genome in lymphoid cells after congenital infection. *Nature* **258**:432–424, 1975.

Joncas, J. H., Wills, A., McLaughlin, B. Congenital infection with cytomegalovirus and Epstein–Barr virus. *Can. Med. Assoc. J.* **17**:1417–1418, 1977.

Joncas, J. H., Alfieri, C., Leyritz-Wills, M., *et al.* Simultaneous congenital infection with Epstein–Barr virus and cytomegalovirus. *N. Engl. J. Med.* **304**:1399–1403, 1981.

Jones, J. F., Minnich, L. L., Jeter, W. S., Pritchett, R. F., Fulginiti, V. A., Wedgwood, R. J. Treatment of childhood combined Epstein–Barr virus/cytomegalovirus infection with oral bovine transfer factor. *Lancet* **II**:122–124, 1981.

Jones, K. A., Kadonaga, J. T., Luciw, P. A., Tjian, R. Activation of the AIDS retrovirus promoter by the cellular transcription factor Sp1. *Science* **232**:755, 1986.

Jones, N. D., Rigby, P. W., Ziff, E. B. Transacting protein factors and the regulation of eukaryotic transcription: Lessons from studies on DNA tumor viruses. *Genes Dev.* **2**:267–281, 1988.

Jordan, M. C. Interstitial pneumonia and subclinical infection after intranasal inoculation of murine cytomegalovirus. *Infect. Immun.* **21**:275–280, 1978.

Jordan, M. C. Adverse effects of cytomegalovirus vaccination in mice. *J. Clin. Invest.* **65**:798–803, 1980.

Jordan, M. C. Spontaneous activation of murine cytomegalovirus from murine spleen explants. *J. Clin. Invest.* **70**:762–768, 1982.

Jordan, M. C., Mar, V. L. Spontaneous activation of latent cytomegalovirus from murine spleen explants: role of lymphocytes and macrophages in release and replication of virus. *J. Clin. Invest.* **70**:762–768, 1982.

Jordan, M. C., Rousseau, W. E., Noble, G. R., *et al.* Association of cervical cytomegaloviruses with venereal disease. *N. Engl. J. Med.* **288**:932–934, 1973a.

Jordan, M. C., Roussear, W. E., Stewart, J. A., *et al.* Spontaneous cytomegalovirus mononucleosis. *Ann. Intern. Med.* **79:**153–160, 1973b.

Jordan, M. C., Shanley, J. D., Stevens, J. G. Immunosuppression reactivates and disseminates latent murine cytomegalovirus. *J. Gen. Virol.* **37:**419–423, 1977.

Jung, M., Krech, U. Lyophilization of human cytomegalovirus. In *International Conference on Cytomegalovirus Infections, St. Gall,* 1970.

Jung, M., Price, P. C., Kistler, G. S., Krech, U. Immunoprecipitation studies with antigens of human cytomegalovirus. *Z. Immunitaetsforsch.* **145:**191–198, 1973.

Kaariainen, L., Klemola, E., Paloheimo, J. Rise of cytomegalovirus antibodies in an infectious-mononucleosis-like syndrome after transfusion. *Br. Med. J.* **2:**1270–1272, 1966a.

Kaariainen, L., Paloheimo, J. A., Klemola, E., Makela, T., Koivuniemi, A. Cytomegalovirus mononucleosis: Isolation of the virus and demonstration of subclinical infections after fresh blood transfusion in connection with open-heart surgery. *Ann. Med. Exp. Fenn.* **44:**297–301, 1966b.

Kamada, M., Komori, A., Chiba, S., Nakao, T. A prospective study of congenital cytomegalovirus infection in Japan. *Scand. J. Infect. Dis.* **15:**227–232, 1983.

Kane, R. C., Rousseau, W. E., Noble, G. R., *et al.* Cytomegalovirus infection in a volunteer blood donor population. *Infect. Immun.* **117:**719–723, 1975.

Kanich, R. E., Craighead, J. E. Cytomegalovirus infection and cytomegalic inclusion disease in renal homotransplant recipients. *Am. J. Med.* **40:**874–882, 1966.

Kanich, R. E., Craighead, J. E. Human cytomegalovirus infection of cultured fibroblasts. I. Cytopathologic effects induced by an adapted and a wild strain. II. Viral replicative sequence of a wild and an adapted strain. *Lab. Invest.* **27:**263–272, 1972.

Kantor, G. L., Goldberg, L. S., Johnson, B. L., Jr., Derechin, M. M., Barnett, E. V. Immunologic abnormalities induced by post-perfusion cytomegalovirus infection. *Ann. Intern. Med.* **73:**553–558, 1970.

Kaposi, K., Rice, G. P. A. Cytomegalovirus infection of peripheral blood mononuclear cells: Effects on interleukin-1 and -2 production and responsiveness. *J. Virol.* **62:**3603–3607, 1988.

Kari, B., Gehrz, R. Isolation and characterization of a human cytomegalovirus glycoprotein containing a high content of O-linked oligosaccharides. *Arch. Virol.* **98:**171–188, 1988.

Kari, B., Lussenhop, N., Goertz, R., *et al.* Characterization of monoclonal antibodies reactive to several biochemically distinct human cytomegalovirus glycoprotein complexes. *J. Virol.* **60:**345–352, 1986.

Kauffman, C. A., Linnemann, C. C., Jr., Alvira, M. M. Cytomegalovirus encephalitis associated with thymoma and immunoglobulin deficiency. *Am. J. Med.* **67:**724–728, 1979.

Kavin, H., Jonas, R. B., Chowdhury, L., Kabins, S. Acalculous cholecystitis and cytomegalovirus infection in the acquired immunodeficiency syndrome. *Ann. Intern. Med.* **104:**53–54, 1986.

Keay, S., Rasmussen, L., Merigan, T. C. Syngeneic monoclonal anti-idiotype antibodies that bear the internal image of a human cytomegalovirus neutralization epitope. *J. Immunol.* **140:**944–948, 1988.

Keil, G. M., Ebeling-Keil, A., Koszinowski, U. H. Temporal regulation of murine cytomegalovirus transcription and mapping of viral RNA synthesized at immediate early times after infection. *J. Virol.* **150:**784–795, 1984.

Keil, G. M., Fibi, M. R., Koszinowski, U. H. Characterization of the major immediate-early polypeptides encoded by murine cytomegalovirus. *J. Virol.* **54:**422–428, 1985.

Keil, G. M., Ebeling-Keil, A., Koszinowski, U. H. Sequence and structural organization of murine cytomegalovirus immediate-early gene 1. *J. Virol.* **61:**1901–1908, 1987.

Keir, H. M., Gold, E. Deoxyribonucleic acid nucleotidyl transferase and deoxyribonuclease from cultured cells infected with herpes simplex virus. *Biochim. Biophys. Acta* **72:**263–276, 1963.

Keller, P., Davison, A., Lower, R., *et al.* Identification and sequence of the gene encoding gpIII, a major glycoprotein of varicella–zoster virus. *Virology* **157:**526–533, 1987.

Keller, R., Peitchel, R., Goldman, J. N., Goldman, M. An IgG-Fc receptor induced in cytomegalovirus-infected human fibroblasts. *J. Immunol.* **116:**772–777, 1976.

Kelsey, D. K., Overall, J. C., Jr., Glasgow, L. A. Correlation of the suppression of mitogen responsiveness and the mixed lymphocyte reaction with the proliferative response to viral antigens of splenic lymphocytes from cytomegalovirus-infected mice. *J. Immunol.* **121**:464–470, 1978.

Kemble, G. W., McCormick, A. L., Pereira, L., Mocarski, E. S. A cytomegalovirus protein with properties of herpes simplex virus ICP8: Partial purification of the polypeptide and map position of the gene. *J. Virol.* **61**:3143–3151, 1987.

Kennedy, P. G., Newsome, D. A., Hess, J., *et al.* Cytomegalovirus but not HTLV-III detected by *in situ* hybridization in retinal lesions in patients with AIDS. *Br. Med. J.* **19**:293–162–164, 1986.

Kerbel, R. S. Herpes virus induction of Fc receptors. *Nature* **263**:192, 1976.

Kestelyn, P., Van de Perre, P., Rouvroy, D., *et al.* A prospective study of the opthalmologic findings in the acquired immune deficiency syndrome in Africa. *Am. J. Opthal.* **100**:230–238, 1985.

Kieff, E. D., Hoyes, B., Bachenheimer, S. L., Roizman, B. Genetic relatedness of type 1 and 2 herpes simplex viruses. *J. Virol.* **9**:738–745, 1972.

Kilpatrick, B. A., Huang, E. S. Human cytomegalovirus genome: Partial denaturation map and organization of genome sequences. *J. Virol.* **24**:261–276, 1977.

Kilpatrick, B. A., Huang, E. S., Pagano, J. S. Analysis of cytomegalovirus genomes with restriction endonucleases HinD III and EcoR-1. *J. Virol.* **18**:1095–1105, 1976.

Kim, K. S., Sapienza, V., Carp, R. I. Comparative studies of the Smith and Raynaud strains of murine cytomegalovirus. *Infect. Immun.* **10**:672–674, 1974.

Kim, K. S., Sapienza, V., Carp, R. I. Comparative characteristics of three cytomegaloviruses from rodents. *Am. J. Vet. Res.* **36**:1495–1950, 1975.

Kim, K. S., Sapienza, V., Carp, R. I., Moon, H. M. Analysis of structural proteins of purified murine cytomegalovirus. *J. Virol.* **17**:906–915, 1976a.

Kim, K. S., Sapienza, V., Carp, R. I., Moon, H. M. Analysis of structural polypeptides of purified human cytomegalovirus. *J. Virol.* **20**:604–611, 1976b.

Kim, K. S., Moon, H. M., Sapienza, V., Carp, R. I. Complement-fixing antigen of human cytomegaloviruses. *J. Infect. Dis.* **135**:281–288, 1977.

Kim, K. S., Sapienza, V. J., Chen, C. M. J., *et al.* Production and characterization of monoclonal antibodies specific for a glycosylated polypeptide of human cytomegalovirus. *J. Clin. Microbiol.* **18**:331–343, 1983.

Kimmel, N., Friedman, M. G., Sarov, I. Detection of human CMV-specific IgG antibodies by a sensitive solid-phase radioimmunoassay and by a rapid-screening test. *J. Med. Virol.* **5**:195–203, 1980.

King-Lewis, P. A., Gardner, S. D. Congenital cytomegalic inclusion disease following intrauterine transfusion. *Br. Med. J.* **2**:603–605, 1969.

Kirmani, N., Ginn, R. K., Mittal, K. K., Manischewitz, J. F., Quinnan, G. V., Jr. Cytomegalovirus-specific cytotoxicity mediated by non-T lymphocytes from peripheral blood of normal volunteers. *Infect. Immun.* **34**:441–447, 1981.

Kit, S., Sheppard, M., Ichimura, H., *et al.* Nucleotide sequence changes in thymidine kinase gene of herpes simplex virus type 2 clones from an isolate of a patient treated with acyclovir. *Antimicrob. Agents Chemother.* **31**:1483–1490, 1987.

Klages, S., Rueger, B., Jahn, G. Multiplicity dependent expression of the predominant phosphoprotein pp65 of human cytomegalovirus. *Virus Res.* **12**:159–168, 1989.

Klatt, E. C., Shibata, D. Cytomegalovirus infection in the acquired immunodeficiency syndrome. *Arch. Pathol. Lab. Med.* **112**:540–544, 1988.

Kleinman, D., Sarov, I., Insler, V. Infection of endometrial cells with human cytomegalovirus. *Gynecol. Obstet. Invest.* **17**:89–95, 1984.

Klemola, E. Hypersensitivity reactions to ampicillin in cytomegalovirus mononucleosis. *Scand. J. Infect. Dis.* **2**:29–31, 1970.

Klemola, E. Cytomegalovirus infection in previously healthy adults. *Ann. Intern. Med.* **79**:267–268, 1973.

Klemola, E., Kaariainen, L. Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. *Br. Med. J.* **2**:1099–1102, 1965.

Klemola, E., Kaariainen, L., von Essen, R., *et al.* Further studies on cytomegalovirus mononucleosis in previously healthy individuals. *Acta Med. Scand.* **182:**311–322, 1967.

Klemola, E., von Essen, R., Wager, O., *et al.* Cytomegalovirus mononucleosis in previously healthy individuals. *Ann. Intern. Med.* **71:**11–19, 1969.

Klemola, E., von Essen, R., Henle, G., Henle, W. Infectious-mononucleosis-like disease with negative heterophile agglutination test. Clinical features in relation to Epstein–Barr virus and cytomegalovirus antibodies. *J. Infect. Dis.* **121:**608–614, 1970.

Klemola, E., Stenstrom, R., von Essen, R. Pneumonia as a clinical manifestation of cytomegalovirus infection in previously healthy adults. *Scand. J. Infect. Dis.* **4:**7–10, 1972.

Klintmalm, G., Lonnqvist, B., Oberg, B., *et al.* Intravenous foscarnet for the treatment of severe cytomegalovirus infection in allograft recipients. *Scand. J. Infect. Dis.* **17:**157–163, 1985.

Klotman, M. E., Starnes, D., Hamilton, J. D. The source of murine cytomegalovirus in mice receiving kidney allografts. *J. Infect. Dis.* **152:**1192–1196, 1985.

Klotman, M. E., Henry, S. C., Hamilton, J. D. Determinants of the source of cytomegalovirus in murine renal allograft recipients. *Transplantation* **44:**636–639, 1987.

Knapp, A. B., Horst, D. A., Eliopoulos, G., *et al.* Widespread cytomegalovirus gastroenterocolitis in a patient with acquired immunodeficiency syndrome. *Gastroenterology* **85:**1399–1402, 1983.

Knez, V., Stewart, J. A., Ziegler, D. W. Cytomegalovirus specific IgM and IgG response in humans studied by radioimmunoassay. *J. Immunol.* **117:**2006–2013, 1976.

Knipe, D. M., Quinlan, M. P., Spang, A. E. Characterization of two conformational forms of the major DNA-binding protein encoded by herpes simplex virus 1. *J. Virol.* **44:**736–741, 1982.

Knopf, K.-W. Properties of the herpes simplex virus DNA polymerase and characterization of its associated exonuclease activity. *Eur. J. Biochem.* **98:**231–244, 1979.

Knowles, W. A. *In-vitro* cultivation of human cytomegalovirus in thyroid epithelial cells. *Arch. Virol.* **50:**119–124, 1976.

Knox, C. E., Pass, R. F., Reynolds, D. W., Stagno, S., Alford, C. A. Comparative prevalence of subclinical cytomegalovirus and herpes simplex virus infections in the genital and urinary tracts of low-income, urban women. *J. Infect. Dis.* **140:**419–422, 1979.

Kono, Y. An interferon-like substance produced in cells infected with Newcastle disease virus and its properties. *Natl. Inst. Anim. Health Q. (Tokyo)* **2:**183–188, 1962.

Koszinowski, U. H., Reddehase, J., Keil, G. M., Schickedanz, J. Host immune response to cytomegalovirus: Products of transfected viral immediate-early genes are recognized by cloned cytolytic T lymphocytes. *J. Virol.* **61:**2054–2058, 1987a.

Koszinowski, U. H., Keil, G. M., Schwartz, H., *et al.* A nonstructural polypeptide encoded by immediate-early transcription unit 1 of murine cytomegalovirus is recognized by cytolytic T lymphocytes. *J. Exp. Med.* **166:**298–294, 1987b.

Koszinowski, U. H., Keil, G. M., Volkmer, H., *et al.* The 89,000-M_r murine cytomegalovirus immediate-early protein activates gene transcription. *J. Virol.* **58:**59–66, 1986.

Kouzarides, T., Bankier, A., Satchwell, S., *et al.* Sequence and transcription analysis of the human cytomegalovirus DNA polymerase gene. *J. Virol.* **61:**125–133, 1987.

Kouzarides, T., Bankier, A., Satchwell, S., *et al.* An immediate early gene of human cytomegalovirus encodes a potential membrane glycoprotein. *Virology* **165:**151–164, 1988.

Krech, U. Complement-fixing antibodies against cytomegalovirus in different parts of the world. *Bull. W.H.O.* **49:**103–106, 1973.

Krech, U.. Jung, M. The development of neutralizing antibodies in guinea pigs following immunization with human cytomegalovirus. *Arch. Ges. Virusforsch.* **28:**248–250, 1969.

Krech, U., Jung, M., Jung, F. *CMV Infections of Man.* S. Karger, Basel, 1971a, p. 124.

Krech, U., Jung, M., Sonnabend, W. A study of complement-fixing immunofluorescent, and neutralizing antibodies in human cytomegalovirus infections. *Z. Immunitaetforsch. Exp. Lin. Immunol.* **141:**411–429, 1971b.

Krech, U., Konjajev, Z., Jung, M. Congenital cytomegalovirus infection in siblings from consecutive pregnancies. *Helv. Paediatr. Acta* **26:**355–362, 1971c.

Kreel, I., Zaroff, L. I., Canter, J. W., *et al.* Syndrome following total body perfusion. *Surg. Gynecol. Obstet.* **111**:317–332, 1960.

Kreth, H. W., ter Meulen, V., Eckert, G. Demonstration of HLA restricted killer cells in patients with acute measles. *Med. Microbiol. Immunol.* **165**:203–214, 1979.

Krugman, R. D., Goodheart, C. R. Human cytomegalovirus. Thermal inactivation. *Virology* **23**:290–291, 1964.

Kubanek, B., Ernst, P., Ostendorf, P., *et al.* Preliminary data of a controlled trial of intravenous hyperimmune globulin in the prevention of cytomegalovirus infection in bone marrow transplant recipients. *Transplant. Proc.* **17**:468–469, 1985.

Kumar, A., Nankervis, G. A., Cooper, A. R., *et al.* Acquisition of cytomegalovirus infection in infants following exchange transfusion: A prospective study. *Transfusion* **20**:327–331, 1980.

Kumar, M. L., Nankervis, G. A. Experimental congenital infection with cytomegalovirus: A guinea pig model. *J. Infect. Dis.* **138**:650–654, 1978.

Kumar, M. L., Nankervis, G. A., Gold, E. Inapparent congenital cytomegalovirus infections. A follow-up study. *N. Engl. J. Med.* **288**:1370–1372, 1973.

Kumar, M. L., Gold, E., Nankervis, G. A. Risk of acquired cytomegalovirus infection in infants of maternal CMV excretors. *Pediatr. Res.* **9**:512–512, 1975.

Kusne, S., Dummer, J. S., Singh, N. Infections after liver transplantation. *Medicine* **67**:132–143, 1988.

Kuttner, A. G. Further studies concerning the filtrable virus present in the submaxillary glands of guinea pigs. *J. Exp. Med.* **46**:935–956, 1927.

Kuttner, A. G., Wang, S. H. The problem of the significance of the inclusion bodies found in the salivary glands of infants, and the occurrence of inclusion bodies in the submaxillary glands of hamsters, white mice, and wild rats (Peiping). *J. Exp. Med.* **60**:773–791, 1934.

Labowskie, R. J., Edelman, R., Rustigian, R., Bellanti, J. A. Studies of cell-mediated immunity to measles virus by *in vitro* lymphocyte-mediated cytotoxicity. *J. Infect. Dis.* **129**:233–239, 1974.

LaFemina, R. L., Hayward, G. S. Structural organization of the DNA molecules from human cytomegalovirus. In B. N. Fields and R. Jaenisch (eds.), *Animal Virus Genetics.* Academic Press, New York, 1980, pp. 39–55.

LaFemina, R., Hayward, G. S. Constitutive and retinoic acid inducible expression of cytomegalovirus immediate-early genes in human tetracarcinoma cells. *J. Virol.* **58**:434–440, 1986.

Lakeman, A. D., Osborn, J. E. Size of infectious DNA from human and murine CMV. *J. Virol.* **30**:414–416, 1979.

Lamb, S. B., Stern, H. Cytomegalovirus mononucleosis with jaundice as presenting sign. *Lancet* **2**:1003–1007, 1966.

Lamberson, H. V., Jr., McMillan, J. A., Weiner, L. B., *et al.* Prevention of transfusion-associated cytomegalovirus (CMV) infection in neonates by screening blood donors for IgM to CMV. *J. Infect. Dis.* **157**:820–822, 1988.

Landini, M. P., Michelson, S. Human cytomegalovirus proteins. *Prog. Med. Virol.* **35**:152–185, 1988.

Landini, M. P., Mirolo, G., Coppolecchia, P., *et al.* Serum antibodies to individual cytomegalovirus structural polypeptides in renal transplant recipients during viral infection. *Microbiol. Immunol.* **30**:683–695, 1986.

Landini, M., Severi, B., Badiali, L., *et al.* Structural components of human cytomegalovirus: *in situ* localization of the major glycoprotein. *Intervirology* **27**:154–160, 1987a.

Landini, M., Severi, B., Furlini, G., DeGiorgi, L. Human cytomegalovirus structural components: Intracellular and intraviral localization of p28 and p65–69 by immunoelectron microscopy. *Virus Res.* **8**:15–23, 1987b.

Landini, M. P., Rossier, E., Schmitz, H. Antibodies to human cytomegalovirus structural polypeptides during primary infection. *J. Virol. Methods* **22**:309–317, 1988.

Landini, M., Mirolo, G., Re, M., *et al.* Antibody reactivity to cytomegalovirus structural polypeptides in subclinical and clinical human immunodeficiency virus infections. *Eur. J. Clin. Microbiol.* **8**:159–163, 1989.

Lang, D. J. The association of indirect inguinal hernia with congenital cytomegalic inclusion disease. *Pediatrics* **38:**913–916, 1966.

Lang, D. J. Cytomegalovirus infections in organ transplantation and posttransfusion, an hypothesis. *Arch. Ges. Virusforsch.* **37:**365–377, 1972.

Lang, D. J. The epidemiology of cytomegalovirus infections: Interpretation of recent observations. In S. Krugman and A. A. Gershon (eds.), *Infection of the Fetus and the Newborn Infants.* Alan R. Liss, New York, 1975, pp. 35–45.

Lang, D. J., Cheung, K. S. Effectiveness of acycloguanosine and trifluorothymidine as inhibitors of cytomegalovirus infection *in vitro*. *Am. J. Med.* **735:**49–53, 1982.

Lang, D. J., Hanshaw, J. B. Cytomegalovirus infection and the post-perfusion syndrome—(Recognition of primary infections in four patients). *N. Engl. J. Med.* **280:**1145–1149, 1969.

Lang, D. J., Kummer, J. F. Demonstration of cytomegalovirus in semen. *N. Engl. J. Med.* **287:**756–758, 1972.

Lang, D. J., Kummer, J. F. Cytomegalovirus in semen: Observations in selected populations. *J. Infect. Dis.* **132:**472–473, 1975.

Lang, D. J., Montagnier, L., Latarjet, R. Growth in agarose of human cells infected with cytomegalovirus. *J. Virol.* **14:**327–332, 1974.

Lang, D. J., Ebert, P. A., Rodgers, B. M., *et al.* Reductions of post-perfusion cytomegalovirus infections following the use of leucocyte depleted blood. *Transfusion* **17:**391–395, 1977.

Lang, D. M., Scolnick, E. M., Willerson, J. T. Association of cytomegalovirus infection with the post-perfusion syndrome. *N. Engl. J. Med.* **278:**1147–1149, 1968.

Lange, C. de. Ueber einen merkwuerdigen Nierenbefund. *Virchows Arch.* **237:**276–280, 1922.

Langenhuysen, M. M. A. C., The, T. H., Nieweg, H. O., Kapsenberg, J. G. Demonstration of IgM cytomegalovirus-antibodies as an aid to early diagnosis in adults. *Clin. Exp. Immunol.* **6:**387–393, 1970.

Langenhuysen, M. M. A. C., Cazemier, T., Houwen, G., *et al.* Antibodies to Epstein–Barr virus, cytomegalovirus and Australia antigen in Hodgkin's disease. *Cancer* **34:**262–267, 1974.

Lanza, R. P., Cooper, D. K., Cassidy, M. J., Barnard, C. N. Malignant neoplasms occurring after cardiac transplantation. *J.A.M.A.* **249:**1746–1748, 1983.

LaQuaglia, M. P., Tolkoff-Rubin, N. E., Dienstag, J. L., Cosimi, A. B., Herrin, J. T., Kelly, M., Rubin, R. H. Impact of hepatitis on renal transplantation. *Transplantation* **32:**504–507, 1981.

Larder, B. A., Kemp, S. D., Darby, G. Related functional domains in virus DNA polymerases. *EMBO J.* **6:**169–175, 1987.

Larder, B. A., Darby, G. Virus drug-resistance: mechanisms and consequences. *Antiviral Res.* **4:**1–42, 1984.

LaRocco, M., Mortenson, J., Davis, B. G., Moore, D. G. Reactivity of serologic tests for the detection of antibody specific to cytomegalovirus. *Am. J. Clin. Path.* **86:**354–356, 1986.

Law, K. M., Wilton Smith, P., Farrar, G. H. A murine monoclonal antibody recognizing a single glycoprotein within a human cytomegalovirus virion envelope glycoprotein complex. *J. Med. Virol.* **17:**255–266, 1985.

Lawrence, H. S. Summation. In M. S. Ascher, A. A. Gotlieb, and C. H. Kirkpatrick (eds.), *Transfer Factor—Basic Properties and Clinical Applications.* Academic Press, New York, 1976, pp. 741–753.

Lawson, C. M., Grundy, J. E., Shellam, G. R. Delayed-type hypersensitivity responses to murine cytomegalovirus in genetically resistant and susceptible strains of mice. *J. Gen. Virol.* **68:**2379–2388, 1987.

Laychock, A. M., Geder, L., Sanford, E. J., Rapp, F. Immune response of prostatic cancer patients to cytomegalovirus-infected and transformed human cells. *Cancer* **42:**1766–1771, 1978.

Leach, F. S., Mocarski, E. S. Regulation of cytomegalovirus late-gene expression: Differential use of three start sites in the transcriptional activation of ICP36 gene expression. *J. Virol.* **63:**1783–1791, 1989.

Lee, C. K., Knipe, D. M. An immunoassay for the study of DNA-binding activities of herpes simplex virus protein ICP8. *J. Virol.* **54:**731–738, 1985.

Lee, F. K., Nahmias, A. J., Stagno, S. Rapid diagnosis of CMV infection in infants by electron microscopy. *N. Engl. J. Med.* **299:**1266–1270, 1978.

Lee, J., Irmiere, A., Gibson, W. Primate cytomegalovirus assembly: Evidence that DNA packaging occurs subsequent to B capsid assembly. *Virology* **167:**87–96, 1988.

Lee, J. Y.-Y. Cytomegalovirus infection involving the skin in immunocompromised hosts. A clinicopathologic study. *Am. J. Clin. Pathol.* **92:**96–100, 1989.

Lehner, R., Meyer, H., Mach, M. Identification and characterization of human cytomegalovirus gene coding for a membrane protein that is conserved among human herpesviruses. *J. Virol.* **63:**3792–3800, 1989.

Lehner, T., Wilton, J. M. A., Shillitoe, E. J. Immunological basis for latency recurrences, and putative oncogenicity of herpes simplex virus. *Lancet* **2:**60–62, 1975.

Leinbach, S. S., Reno, J. M., Lee, L. F., *et al.* Mechanism of phosphonoacetate inhibition of herpesvirus-induced DNA polymerase. *Biochemistry* **15:**426–430, 1976.

Leinikki, P., Heinonen, K., Pettay, O. Incidence of cytomegalovirus infections in early childhood. *Scand. J. Infect. Dis.* **4:**1–5, 1972.

Leinikki, P., Granstrom, M. L., Santavuori, P., Pettay, O. Epidemiology of cytomegalovirus infections during pregnancy and infancy. A prospective study. *Scand. J. Infect. Dis.* **10:**165–171, 1978.

Lemaster, S., Roizman, B. Herpes simplex virus phosphoproteins. II. Characterization of the virion protein kinase and of the polypeptides phosphorylated in the virion. *J. Virol.* **35:**798–811, 1980.

Lennette, E. H., Schmidt, N. J. *Diagnostic Procedures for Viral and Rickettsial Infection.* 5th ed. American Public Health Association, New York, Washington, 1979, pp. 399–439.

Lennette, E. T., Lennette, D. A. Immune adherence hemagglutination—alternative to complement fixation serology. *J. Clin. Microbiol.* **7:**282–285, 1978.

Leonard, J. C., Tobin, J. O'H. A report from various centers: Polyneuritis associated with cytomegalovirus infection. *Q. J. Med.* **40:**435–442, 1971.

Leonidas, J. C., Beatty, E. C., Wenner, H. A. Menetrier disease and cytomegalovirus infection in childhood. *Am. J. Dis. Child.* **126:**806–808, 1973.

Leport, C., Harzic, M., Pignon, J. M., *et al.* Benign cytomegalovirus mononucleosis in non-aids, HIV-infected patients. *Lancet* **2:**214, 1987.

Lerner, P. I., Sampliner, J. E. Transfusion-associated cytomegalovirus mononucleosis. *Ann. Surg.* **185:**406–410, 1977.

Leung, F., Fam, A. G., Osoba, D. Kaposi's sarcoma complicating corticosteroid therapy for temporal arteritis. *Am. J. Med.* **71:**320–322, 1981.

Levin, M. J., Parkman, R., Oxman, M. N., *et al.* Proliferative and interferon response by peripheral blood mononuclear cells after bone marrow transplantation in humans. *Infect. Immun.* **20:**678–684, 1978.

Levin, M. J., Rinaldo, C. R., Jr., Leary, P. L., Zaia, J. A., Hirsch, M. S. Immune response to herpes virus antigens in adults with acute cytomegalovirus mononucleosis. *J. Infect. Dis.* **140:**851–857, 1979.

Levine, P. H., Ablashi, D. V., Berard, C. W., *et al.* Elevated antibody titers to Epstein–Barr virus in Hodgkin's disease. *Cancer* **27:**416–421, 1971.

Levinsohn, E. M., Foy, H. M., Kenny, G. E., *et al.* Isolation of cytomegalovirus from a cohort of 100 infants throughout the first year of life. *Proc. Soc. Exp. Biol. Med.* **132:**957–962, 1969.

Levy, E., Sarov, I. Determination of IgA antibodies to human cytomegalovirus by enzyme linked immunosorbent assay (ELISA). *J. Med. Virol.* **6:**249–257, 1980.

Levy, R. M., Bredesen, D. E., Rosenblum, M. L. Neurological manifestations of the acquired immunodeficiency syndrome (AIDS): Experience at UCSF and review of the literature. *J. Neurosurg.* **62:**475–495, 1985.

Lewis, R. A., Watkins, L., St. Jeor, S. Enhancement of deoxyguanosine kinase activity in human lung fibroblast cells infected with human cytomegalovirus. *Mol. Cell. Biochem.* **65:**67–71, 1984.

Lindsley, M. D., Torpey, D. J. III, Rinaldo, C. R. R., Jr. HLA-DR-restricted cytotoxicity of cytomegalovirus-infected monocytes mediated by Leu-3-positive T cells. *J. Immunol.* **136:**3045–3051, 1986.

Linnemann, C. C., Jr., Kaufmann, C. A., First, M. R., *et al.* Cellular immune response to cytomegalovirus infection after renal transplantation. *Infect. Immun.* **22:**176–180, 1978.

Lipschuetz, B. Untersuchungen uber die Aetiologie der Krankheiten der Herpes genitalis. *Arch. Dermatol. Syph.* **136:**428–482, 1921.

Little, P. J., al-Khader, A., Farthing, C. F., Bunuan, H. Kaposi's sarcoma in a patient after renal transplantation. *Postgrad. Med. J.* **59:**325–326, 1983.

Liu, Y., Kari, B., Gehrz, R. Human immune responses to major human cytomegalovirus glycoprotein complexes. *J. Virol.* **62:**1066–1070, 1988.

LiVolsi, V. A., Merino, M. J. Cytomegalovirus infection of ovarian thecoma. *Arch. Pathol. Lab. Med.* **103:**653–654, 1979.

Lobdell, D. H. 'Ring' granulomas in cytomegalovirus hepatitis. *Arch. Pathol. Lab. Med.* **111:**881–882, 1987.

Loeken, M., Bikel, I., Livingston, D. M., Brady, J. Transactivation of RNA polymerase II and III promoters by SV40 small t antigen. *Cell* **55:**1171–1177, 1988.

Loh, L., Hudson, J. B. Murine cytomegalovirus infection in the spleen and its relationship to immunosuppression. *Infect. Immun.* **32:**1067–1072, 1981.

Loh, L., Hudson, J. B. Murine cytomegalovirus-induced immunosuppression. *Infect. Immun.* **36:**89–95, 1982.

Lopez, C., Simmons, R. L., Mauer, S. M., *et al.* Association of renal allograft rejection with virus infections. *Am. J. Med.* **56:**280–289, 1974a.

Lopez, C., Simmons, R. L., Park, B. H., *et al.* Cell-mediated and humoral immune responses of renal transplant recipients with cytomegalovirus infections. *Clin. Exp. Immunol.* **16:**565–673, 1974b.

Löwenstein, C. Ueber protozoenartige Gebilde in den Organen von Kindern. *Zentralbl. Allg. Pathol.* **18:**513–518, 1907.

Luban, N. L., Williams, A. E., Macdonald, M. G., *et al.* Low incidence of acquired cytomegalovirus infection in neonates transfused with washed red blood cells. *Am. J. Dis. Child.* **141:**416–419, 1987.

Lubon, H., Ghazal, P., Henninghausen, L., *et al.* Cell-specific activity of the modulator region in the human cytomegalovirus major immediate-early gene. *Mol. Cell. Biol.* **9:**1342–1345, 1989.

Luby, J. P., Brunett, W., Hull, A. R., *et al.* Relationship between cytomegalovirus and hepatic function abnormalities in the period after renal transplant. *J. Infect. Dis.* **129:**511–518, 1974.

Lui, W. Y., Chang, W. K. Cytomegalovirus mononucleosis in Chinese infants. *Arch. Dis. Child.* **47:**643–646, 1972.

Lussenhop, N., Goertz, R., Wabuke-Bunoti, M., *et al.* Epitope analysis of human cytomegalovirus glycoprotein complexes using murine monoclonal antibodies. *Virology* **164:**362–372, 1988.

Lussier, G. Cerebral calcification, cytomegalovirus infection. *Am. J. Pathol.* **80:**555–558, 1975.

Luthardt, T., Siebert, H., Losei, I., *et al.* Cytomegalovirus Infection bei Kindern mit Blutaustauschtransfusion im Neugeborenalter. *Klin. Wochenschr.* **49:**81–86, 1971.

Mach, M., Utz, U., Fleckenstein, B. Mapping of the major glycoprotein gene of human cytomegalovirus. *J. Gen. Virol.* **67:**1461–1467, 1986.

Macher, A. M., Reichert, C. M., Straus, S. E., *et al.* Death in the AIDS patient: Role of cytomegalovirus. *N. Engl. J. Med.* **85:**1454, 1983.

Mackowiak, P., Marling-Cason, M., Smith, J., Luby, J. Antibody-mediated bacterial adhesion to cytomegalovirus induced Fc receptors. *J. Clin. Invest.* **73:**987–991, 1984.

Maher, P., O'Toole, C. M., Wreghitt, T. G., *et al.* Cytomegalovirus infection in cardiac transplant recipients associated with chronic T cell subset ratio inversion with expansion of a Leu-7$^+$T$_S$$^-C^+$ subset. *Clin. Exp. Immunol.* **62:**515–524, 1985.

Mahieux, F., Gray, F., Fenelon, G., *et al.* Acute myeloradicultitis due to cytomegalovirus as the initial manifestation of AIDS. *J. Neurol. Neurosurg. Psychiatry* **52:**270–274, 1989.

Malone, C. L., Vesole, D. H., Stinski, M. F. Transactivation of a human cytomegalovirus early promoter by gene products from immediate early gene IE2 and augmentation by IE1: Mutational analysis of the viral proteins. *J. Virol.* **64:**498–506, 1989.

Manischewitz, J. E., Quinnan, G. V., Jr. Antivirus antibody-dependent cell-mediated cytotoxicity during murine cytomegalovirus infection. *Infect. Immun.* **29:**1050–1054, 1980.

Mannini, A., Medearis, D. N., Jr. Mouse salivary gland virus infections. *Am. J. Hyg.* **73:**329–343, 1961.

Mäntyjäryi, R. Preparation of cytomegalovirus immune system serum in rabbits with alkaline-extracted virus. *Acta Pathol. Microbiol. Scand.* **72:**345–346, 1968.

Mao, J. C.-H., Robishaw, E. E., Overby, L. R. Inhibition of DNA polymerase from herpes simplex virus-infected Wi-38 cells by phosphonoacetic acid. *J. Virol.* **15:**1281–1283, 1975.

Mar, E., Patel, P., Huang, E. Human cytomegalovirus-associated DNA polymerase and protein kinase activities. *J. Gen. Virol.* **57:**149–156, 1981.

Mar, E. C., Cheng, Y. C., Huang, E. S. Effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine on human cytomegalovirus replication *in vitro*. *Antimicrob. Agents Chemother.* **24:**518–521, 1983.

Mar, E., Chiou, J., Cheng, Y., Huang, E. Human cytomegalovirus-induced DNA polymerase and its interaction with triphosphates of 1-(2′-deoxy-2′fluoro-β-D-arabinofluranosyl)-5-methyluracil, -5-idocytosine, and -5-methylcytosine. *J. Virol.* **56:**846–851, 1985a.

Mar, E. C., Chiou, J. F., Cheng, Y. C., *et al.* Inhibition of cellular DNA polymerase-alpha and human cytomegalovirus-induced DNA polymerase by the triphosphates of 9-(2-hydroxyethoxymethyl)guanine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine. *J. Virol.* **53:**776–780, 1985b.

Margileth, A. M. The diagnosis and treatment of generalized cytomegalic inclusion disease of the newborn. *Pediatrics* **15:**270–283, 1955.

Margulis, S. J., Honig, C. L., Soave, R., *et al.* Biliary tract obstruction in the acquired immunodeficiency syndrome. *Ann. Intern. Med.* **105:**207–210, 1986.

Marker, O., Volkert, M. Studies on cell-mediated immunity to lymphocytic choriomeningitis virus in mice. *J. Exp. Med.* **137:**1511–1525, 1973.

Marker, S. C., Howard, R. J., Groth, K. E., *et al.* A trial of vidarabine for cytomegalovirus infection in renal transplant patients. *Arch. Intern. Med.* **140:**1441–1444, 1980.

Marker, S. C., Howard, R. J., Simmons, R. L., *et al.* Cytomegalovirus infection: A quantitative prospective study of 320 consecutive renal transplants. *Surgery* **89:**660–671, 1981a.

Marker, S. C., Simmons, R. L., Balfour, H. H., Jr. Cytomegalovirus vaccine in renal allograft recipients. *Transplant. Proc.* **13:**117–119, 1981b.

Marks, J. R., Spector, D. H. Replication of the murine cytomegalovirus genome: Structure and role of the termini in the generation and cleavage of concatenates. *Virology* **162:**98–107, 1988.

Marmor, M. F., Egbert, P. R., Egbert, B. M., Marmor, J. B. Optic nerve head involvement with cytomegalovirus in adult with lymphoma. *Arch. Ophthalmol.* **96:**1252–1254, 1978.

Martin, S., Courtney, R., Fowler, G., Rouse, B. Herpes simplex virus type-1 specific cytotoxic T lymphocytes recognize virus nonstructural proteins. *J. Virol.* **62:**2265–2273, 1988.

Martin, W. J. II, Smith, T. F. Rapid detection of cytomegalovirus in bronchoalveolar lavage specimens by a monoclonal antibody method. *J. Clin. Microbiol.* **23:**1006–1008, 1986.

Martinez, J., Lahijani, R., St. Jeor, S. Analysis of a region coding for a 25 kilodalton virion protein. *J. Virol.* **63:**233–241, 1989.

Mason, E. O., Jr., Sough, M. A., Montgomery, J. R. Cord serum IgA in congenital cytomegalovirus infection. *J. Pediatr.* **89:**945–946, 1976.

Masur, H., Lane, H. C., Palestine, A. C., *et al.* Effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine on serious cytomegalovirus disease in eight immunosuppressed homosexual men. *Ann. Intern. Med.* **104:**41–44, 1986.

Mathews, R. E. F. Classification and nomenclature of viruses. Third report of the International Committee on Taxonomy of Viruses. *Intervirology* **12:**132–296, 1979.

Matsumoto, Y., Sugano, T., Miyamoto, C., Masuho, Y. Generation of hybridomas producing human monoclonal antibodies against human cytomegalovirus. *Biochem. Biophys. Res. Commun.* **137:**273–280, 1986.

Matthews, T., Boehme, R. Antiviral activity and mechanism of action of ganciclovir. *Rev. Infect. Dis.* **10:**S490–S494, 1988.

May, A. G., Betts, R. F., Freeman, R. F., Andrus, C. H. An analysis of cytomegalovirus infection and HLA antigen matching on the outcome of renal transplantation. *Ann. Surg.* **187:**110–117, 1978.

Mayo, D. R., Armstrong, J. A., Ho, M. Reactivation of murine cytomegalovirus by cyclophosphamide. *Nature* **267:**721–723, 1977.

Mayo, D. R., Armstrong, J. A., Ho, M. Activation of latent murine cytomegalovirus infection: Cocultivation, cell-transfer and the effect of immunosuppression. *J. Infect. Dis.* **138:**890–896, 1978.

McAllister, R. M., Straw, R. N., Filbert, J. E., Goodheart, C. R. Human CMV Cytochemical observation of intracellular lesion development correlated with viral synthesis and release. *Virology* **19:**521–531, 1963.

McAllister, R. M., Wright, H. T., Jr., Tasem, W. M. Cytomegalovirus inclusion disease in newborn twins. *J. Pediatr.* **64:**278–281, 1964.

McBride, W. D. Antigenic analysis of polioviruses by kinetic study of serum neutralization. *Virology* **7:**45–58, 1959.

McCandless, A. E., Davis, C., Hall, E. G. Bone changes in congenital cytomegalic inclusion disease. *Arch. Dis. Child.* **50:**160–162, 1975.

McCordock, H. A., Smith, M. G. Intranuclear inclusions. Incidence and possible significance in whooping cough and in a variety of other conditions. *Am. J. Dis. Child.* **47:**771–779, 1934.

McCordock, H. A., Smith, M. G. The visceral lesions produced in mice by the salivary gland virus of mice. *J. Exp. Med.* **63:**303–310, 1936.

McCracken, G. J., Jr. Immunoglobulin concentrations in newborn infants with congenital cytomegalic inclusion disease. *Pediatrics* **36:**933–937, 1965.

McCracken, G. J., Jr., Luby, J. P. Cytosine arabinoside in the treatment of congenital cytomegalic inclusion disease. *J. Pediatr.* **80:**488–495, 1972.

McCracken, G. J., Jr., Shinefield, H. R., Cobb, K., *et al.* Congenital cytomegalic inclusion disease. A longitudinal study of 20 patients. *Am. J. Dis. Child.* **117:**522–539, 1969.

McDevitt, H. O., Bodmer, W. F. Histocompatibility antigens, immune responsiveness and susceptibility to disease. *Am. J. Med.* **52:**1–8, 1972.

McDonough, S. H., Spector, D. H., Spector, D. H. Transcription in human fibroblasts permissively infected by human cytomegalovirus strain AD169. *Virology* **125:**31–46, 1983.

McDonough, S. H., Straprans, S. I., Spector, D. H. Analysis of the major transcripts encoded by the long repeat of human cytomegalovirus strain AD169. *J. Virol.* **53:**711–718, 1985.

McEnery, G., Stern, H. Cytomegalovirus infection in early infancy. Five atypical cases. *Arch. Dis. Child.* **45:**669–673, 1970.

McFarland, H. F. *In vitro* studies of cell-mediated immunity in an acute viral infection. *J. Immunol.* **113:**173–180, 1974.

McGavran, M. H., Smith, M. G. Ultrastructural, cytochemical, and microchemical observations on cytomegalovirus (salivary gland virus) infection of human cells in tissue culture. *Exp. Mol. Pathol.* **4:**1–10, 1965.

McHugh, T. M., Cassavant, C. H., Wilber, J. C., Stites, D. P. Comparison of six methods for the detection of antibody to cytomegalovirus. *J. Clin. Microbiol.* **22:**1014–1019, 1985.

McKeating, J. A., Griffiths, P. D., Grundy, J. E. Cytomegalovirus in urine specimens has host, β_2 microglobulin bound to the viral envelope: A mechanism of evading the host immune response? *J. Gen. Virol.* **68:**785–792, 1987.

McMichael, A. J., Ting, A., Zweerink, H. J., Askonas, B. A. HLA restriction of cell mediated lysis of influenza virus-infected human cells. *Nature* **270:**524–526, 1977.

McVay, M. A., Adler, S. P. Immunologic evidence for frequent age-related cytomegalovirus reactivation in seropositive immunocompetent individuals. *J. Infect. Dis.* **160:**1–10, 1989.

Medearis, D. N., Jr. Cytomegalovirus inclusion disease. *Pediatrics* **19:**467–482, 1957.

Medearis, D. N., Jr. Observations concerning human cytomegalovirus infection and disease. *Bull. Johns Hopkins Hospital* **114:**181–211, 1964a.

Medearis, D. N., Jr. Mouse cytomegalovirus infection. II. Observations during prolonged infections. *Am. J. Hyg.* **80:**103–112, 1964b.

Medical Research Council (MRC) Working Party on Post-transfusion Hepatitis. Report to the MRC Blood Transfusion Research Committee. Post-transfusion hepatitis in a London hospital: Results of a two-year prospective study. *J. Hyg. (Camb.)* **73:**173–188, 1974.

Meijer, H., Bruggeman, C. A., Dormans, P. H. J., van Boven, C. A. P. A. Human cytomegalovirus induces a cellular deoxyguanosine kinase, also interacting with acyclovir. *FEMS Microbiol. Lett.* **25:**283–287, 1984.

Meiselman, M. S., Cello, J. P., Margaretten, W. Cytomegalovirus colitis. Report of the clinical, endoscopic, and pathologic findings in two patients with acquired immune deficiency syndrome. *Gastroenterology* **88:**171–175, 1985.

Melnick, J. L., Midulla, M., Wimberly, I., *et al.* A new member of the herpesvirus group isolated from South American marmosets. *J. Immunol.* **92:**596–601, 1964.

Melnick, J. L., Lewis, R., Wimberly, I., *et al.* Association of cytomegalovirus (CMV) infection with cervical cancer: Isolation of CMV from cell cultures derived from cervical biopsy. *Intervirology* **10:**115–119, 1978.

Melnick, J. L., Petrie, B. L., Dreesman, G. R., *et al.* Cytomegalovirus antigen within human arterial smooth muscle cells. *Lancet* **2:**644–647, 1983.

Mercer, J. A., Wiley, C. A., Spector, D. H. Pathogenesis of murine cytomegalovirus infection: Identification of infected cells in the spleen during acute and latent infection. *J. Virol.* **62:**987–997, 1988.

Mercer, R. D., Luse, S., Guyton, D. H. Clinical diagnosis of generalized cytomegalic inclusion disease. *Pediatrics* **11:**502–514, 1953.

Merten, D. F., Goodling, C. A. Skeletal manifestation of congenital cytomegalic inclusion disease. *Radiology* **95:**333–334, 1970.

Meyer, H., Bankier, A. T., Landini, M. P., *et al.* Identification and procaryotic expression of the gene coding for the highly immunogenic 28-kilodalton structural phosphoprotein (pp28) of human cytomegalovirus. *J. Virol.* **62:**2243–2250, 1988.

Meyers, J. D. Cytomegalovirus infection following marrow transplantation: risk, treatment, and prevention. *Birth Defects* **20:**101–117, 1984.

Meyers, J. D. Prevention of cytomegalovirus infection after marrow transplantation. *Rev. Infect. Dis.* **2:**S1691–S1705, 1989.

Meyers, J. D., McGuffin, R. W., Neiman, P. E., *et al.* Toxicity and efficacy of human leucocyte interferon for treatment of cytomegalovirus pneumonia after marrow transplantation. *J. Infect. Dis.* **141:**555–562, 1980a.

Meyers, J. D., Leszczynski, J., Zaia, J. A., *et al.* Prevention of cytomegalovirus infection after marrow transplant with CMV immune globulin (CMVig). In *20th Interscience Conference on Antimicrobial Agents and Chemotherapy.* American Society of Microbiology, New Orleans, 1980b.

Meyers, J. D., Flournoy, N., Thomas, E. D. Cytomegalovirus infection and specific cell-mediated immunity after marrow transplant. *J. Infect. Dis.* **142:**816–824, 1980c.

Meyers, J. D., Flournoy, N., Thomas, E. D. Nonbacterial pneumonia after allogeneic marrow transplantation: A review of ten years' experience. *Rev. Infect. Dis.* **4:**1119–1132, 1982a.

Meyers, J. D., McGuffin, R. W., Bryson, Y. J., *et al.* Treatment of cytomegalovirus pneumonia after marrow transplant with combined vidarabine and human leukocyte interferon. *J. Infect. Dis.* **146:**80–84, 1982b.

Meyers, J. D., Wade, J. C., McGuffin, R. W., *et al.* The use of acyclovir for cytomegalovirus infections in the immunocompromised host. *J. Antimicrob. Chemother.* **12:**181–193, 1983.

Meyers, J. D., Flournoy, N., Thomas, E. D. Risk factors for cytomegalovirus infection after human marrow transplant. *J. Infect. Dis.* **153:**478–488, 1986.

Meyers, J. D., Reed, E. C., Shepp, D. H., *et al.* Acyclovir for prevention of cytomegalovirus infection and disease after allogenic marrow transplantation. *N. Engl. J. Med.* **318:**70–75, 1988.

Meyerson, D., Hackman, R. C., Meyers, J. D. Diagnosis of cytomegalovirus pneumonia by in situ hybridization. *J. Infect. Dis.* **150:**272–277, 1984.

Michalski, F., Hsiung, G. D. Malignant transformation of hamster embryo cells following infection with bovine herpesvirus (infectious rhinotracheitis virus). *Proc. Soc. Exp. Biol. Med.* **148:**891–896, 1975.

Michelson, S., Horodniceanu, F., Kress, M., Tardy-Panit, M. Human cytomegalovirus-induced immediate early antigens: Analysis in sodium dodecyl sulfate-polyacrylamide gel electrophoresis after immunoprecipitation. *J. Virol.* **32:**259–267, 1979a.

Michelson, S., Cabau, N., Boue, A., Horodniceanu, F. Comparison of occurrence of antibodies to human cytomegalovirus as demonstrated by immunofluorescence and indirect hemagglutination techniques. *J. Clin. Microbiol.* **9:**149–151, 1979b.

Michelson, S., Tardy-Panit, M., Barzu, O. Properties of a human cytomegalovirus-induced protein kinase. *Virology* **134:**259–268, 1984.

Michelson-Fiske, S., Arnoult, J., Febvre, H. Cytomegalovirus infection of human lung epithelial cells *in vitro*. *Intervirology* **5:**354–363, 1975.

Michelson-Fiske, S., Horodniceanu, F., Guillon, J. C. "Immediate early" antigens in human cytomegalovirus infected cells. *Nature* **270:**615–617, 1977.

Middeldorp, J., Tegzess, A., Jongsma, J., *et al.* Immunity to human cytomegalovirus (HCMV). I. Humoral immune responses to HCMV-specific membrane antigens (CMV-MA) in normal and immunocompromised donors during acute and latent CMV infections. In S. Plotkin, S. Michelson, J. Pagano, and F. Rapp (eds.), *CMV: Pathogenesis and Prevention of Human Infection.* Alan R. Liss, New York, 1984, pp. 441–445.

Midulla, M., Balducci, L., Iannetti, P., Businco, L., Rezza, E., Fois, A. Infantile spasms and cytomegalovirus infection. *Lancet* **2:**377, 1976.

Miller, N., Hutt-Fletcher, L. A monoclonal antibody to glycoprotein gp85 inhibits fusion but not attachment of Epstein–Barr virus. *J. Virol.* **62:**2366–2372, 1988.

Miller, W., Flynn, P., McCullough, J., *et al.* Cytomegalovirus infection after bone marrow transplantation: An association with acute graft versus host disease. *Blood* **67:**1162–1167, 1986.

Miller, W. J., Provost, P. J., McAleer, W. J., *et al.* Specific immune adherence assay for human hepatitis A. Application to diagnostic and epidemiologic investigations. *Proc. Soc. Exp. Biol. Med.* **149:**254–261, 1975.

Mills, J., Jacobson, M. A., O'Donnell, J. J., *et al.* Treatment of cytomegalovirus retinitis in patients with AIDS. *Rev. Infect. Dis.* **10:**S522–S531, 1988.

Mims, C. A., Gould, J. Splenic necrosis in mice infected with cytomegalovirus. *J. Infect. Dis.* **137:**587–591, 1978.

Mims, C. A., Gould, J. Infection of salivary glands, kidneys, adrenals, ovaries and epithelia by murine CMV. *J. Med. Microbiol.* **12:**113–122, 1979.

Minars, N., Silverman, J. F., Escobar, M. R., Martinez, A. J. Fatal cytomegalovirus inclusion disease. *Arch. Dermatol.* **113:**1569–1571, 1977.

Minder, W. H. Die Aetiologie der Cytomegalia infantum. *Schweiz. Med. Wochenschr.* **83:**1180–1182, 1953.

Minson, A. C., Darby, G. B. Hybridization techniques. In R. Colin (ed.), *New Developments in Practical Virology.* Alan R. Liss, New York, 1982, pp. 185–229.

Mintz, L., Drew, W. L., Miner, R. C., Braff, E. H. Cytomegalovirus infections in homosexual men. *Ann. Intern. Med.* **99:**326–329, 1983.

Mirchamsy, H., Rapp, F. A new overlay for plaquing animal viruses. *Proc. Soc. Exp. Biol. Med.* **129:**13–17, 1968.

Mirkovic, R., Werch, J., South, M. A., Benyesh-Melnick, M. Incidence of cytomegaloviremia in blood-bank donors and in infants with congenital cytomegalic inclusion disease. *Infect. Immun.* **3:**45–50, 1971.

Mirolo, G., Baldassarri, B., Ripalti, A., *et al.* Antibody response to individual cytomegalovirus structural proteins in different groups of subjects. *Eur. J. Clinical Microbiol.* **6:**207–210, 1987.

Mocarski, E. S., Roizman, B. Site-specific inversion sequence of herpes simplex virus genome: Domain and structural features. *Proc. Natl. Acad. Sci. U.S.A.* **78:**7047–7051, 1981.

Mocarski, E. S., Roizman, B. Structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA. *Cell* **31**:89–97, 1982.

Mocarski, E. S., Stinski, M. F. Persistence of the cytomegalovirus genome in human cells. *J. Virol.* **31**:761–775, 1979.

Mocarski, E. S., Post, L. E., Roizman, B. Molecular engineering of the herpes simplex virus genome: Insertion of a second L–S junction into the genome causes additional genome inversions. *Cell* **22**:243–255, 1980.

Mocarski, E. S., Deiss, L. P., Frenkel, N. Nucleotide sequence of a novel U_S–*a* junction present in a defective herpes simplex virus genome. *J. Virol.* **55**:140–146, 1985a.

Mocarski, E. S., Pereira, L., Michael, N. Precise localization of genes on large animal virus genomes: Use of lambda gt11 and monoclonal antibodies to map the gene for a cytomegalovirus protein family. *Proc. Natl. Acad. Sci. U.S.A.* **82**:1266–1270, 1985b.

Mocarski, E. S., Liu, A. C., Spaete, R. R. Structure and variability of the a sequence in the genome of human cytomegalovirus (Towne strain). *J. Gen. Virol.* **68**:2223–2230, 1987.

Mocarski, E. S., Pereira, L., McCormick, A. L. Human cytomegalovirus ICP22, the product of the HWLF1 reading frame, is an early nuclear protein that is released from cells. *J. Gen. Virol.* **69**:2613–2621, 1988.

Moller, G. Natural killer cells. *Immunol. Rev.* **44**:3–208, 1979.

Monif, G. R. G., Egan, E. A. II, Held, B., Eitzman, D. V. The correlation of maternal cytomegalovirus infection during varying stages in gestation with neonatal involvement. *J. Pediatr.* **80**:17–20, 1972.

Monif, G. R. G., Daicoff, G. I., Flory, L. F. Blood as a potential vehicle for the cytomegaloviruses. *Am. J. Obstet. Gynecol.* **126**:445–448, 1976.

Montgomery, R. L., Youngblood, L. A., Medearis, D. N., Jr. Recovery of cytomegalovirus from the cervix in pregnancy. *Pediatrics.* **49**:524–531, 1972.

Morahan, P. Macrophage nomenclature: Where are we going? *J. Reticuloendothel. Soc.* **27**:223–245, 1980.

Morahan, P. S., Morse, S. S., McGeorge, M. B. Macrophage extrinsic antiviral activity during herpes simplex virus infection. *J. Gen. Virol.* **46**:291–300, 1980.

Morgan, C., Rose, H. M., Holden, M., Jones, E. P. Electron microscopic observations on the development of herpes simplex virus. *J. Exp. Med.* **110**:643–656, 1959.

Morgello, S., Cho, E. S., Nielsen, S., *et al.* Cytomegalovirus encephalitis in patients with acquired immunodeficiency syndrome. *Hum. Pathol.* **18**:289–297, 1987.

Morris, G. E., Coleman, R. M., Best, J. M., *et al.* Persistence of serum IgA antibodies to herpes simplex, varicella-zoster, cytomegalovirus, and rubella virus detected by enzyme-linked immunosorbent assays. *J. Med. Virology* **16**:343–349, 1985.

Moskowitz, L. B., Hensley, G. T., Chan, J. C., *et al.* The neuropathology of acquired immune deficiency syndrome. *Arch. Pathol. Lab. Med.* **108**:867–872, 1984.

Mosmann, T. R., Hudson, J. B. Some properties of the genome of murine cytomegalovirus. *Virology* **54**:135–149, 1973.

Moss, A. R., Bacchetti, P., Osmond, D., *et al.* Seropositivity for HIV and the development of AIDS or AIDS related condition: Three year follow-up of the San Francisco General Hospital cohort. *Br. Med. J.* **296**:745–750, 1988.

Mueller, J. Ueber die protozoenartige Gibilde in den Harnkanaelchen epithelien Neugeborener. *Virchows Arch.* **238**:481–494, 1922.

Mueller, N., Hinkula, J., Wahren, B. Elevated antibody titers against cytomegalovirus among patients with testicular cancer. *Int. J. Cancer* **41**:399–403, 1988.

Muller, M., Misra, V., Chantler, J. K., Hudson, J. B. Murine cytomegalovirus gene expression during non-productive infection in G_0 phase 3T3 cells. *Virology* **90**:279–287, 1978.

Müller-Stamou, A., Senn, H. J., Emody, G. Epidermolysis in a case of severe cytomegalovirus infection. *Br. Med. J.* **3**:609–610, 1974.

Murph, J. R., Bale, J. F., Murray, J. C., Stinski, M. F. Cytomegalovirus transmission in a midwest day care center: Possible relationship to child care practices. *J. Pediatr.* **109**:35–39, 1986.

Murray, H. W. Uveitis and the immunologically compromised host. *N. Engl. J. Med.* **299:**1077–1078, 1978.

Murray, H. W., Hillman, J. K., Rubin, B. Y., *et al.* Patients at risk for aids-related opportunistic infections. Clinical manifestations and impaired gamma interferon production. *N. Engl. J. Med.* **313:**1504–1510, 1985.

Murray, J. F., Felton, C. P., Garay, S. M., *et al.* Pulmonary complications of the acquired immunodeficiency syndrome. Report of a National Heart, Lung and Blood Institute workshop. *N. Engl. J. Med.* **310:**1682–1688, 1984.

Musiani, M., Zerbini, M., Zauli, D., *et al.* Impairment of cytomegalovirus and host balance in elderly subjects. *J. Clin. Pathol.* **41:**722–725, 1988.

Myers, E. N., Stool, S. Cytomegalic inclusion disease of the inner ear. *Laryngoscope* **78:**1904–1915, 1968.

Myerson, D., Hackman, R. C., Nelson, J. A., *et al.* Widespread presence of histologically occult cytomegalovirus. *Hum. Pathol.* **15:**430–439, 1984.

Myllylo, G. A., Vaheri, A., Vesikari, T., Penttinen, K. Interaction between human blood platelets, viruses and antibodies. IV. Post-rubella thrombocytopenic purpura and platelet aggregation by rubella antigen–antibody interaction. *Clin. Exp. Immunol.* **4:**323–333, 1969.

Nabel, G., Baltimore, D. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* **326:**711–713, 1987.

Naeye, R. L. Cytomegalic inclusion disease. The fetal disorder. *Am. J. Clin. Pathol.* **47:**738–744, 1967.

Nagington, J. Cytomegalovirus antibody production in renal transplant patients. *J. Hyg. (Camb.)* **69:**645–660, 1971.

Najarian, J. S., Fryd, D. S., Strand, M., *et al.* A single institution, randomized, prospective trial of cyclosporine versus azathioprine–antilymphocyte globulin for immunosuppression in renal allograft recipients. *Ann. Surg.* **201:**142–157, 1985.

Nakao, T., Chiba, S., Ohsaki, M., Hanazono, H. Cytomegalovirus mononucleosis. *Lancet* **2:**1153–1153, 1967.

Nakoneczna, I., Kay, S. Fatal disseminated cytomegalic inclusion disease in an adult presenting with a lesion of the gastrointestinal tract. *Am. J. Clin. Pathol.* **47:**124–128, 1967.

Nankervis, G. A., Cox, F. C., Kumar, M. L. A prospective study of maternal cytomegalovirus and its effect on the fetus. *Pediatr. Res.* **7:**148, 1973.

Naraqi, S., Jackson, G. G., Johasson, O., Rubenis, M. Search for latent cytomegalovirus in renal allografts. *Infect. Immun.* **19:**669–703, 1978.

Navia, B. A., Jordan, B. D., Price, R. W. The AIDS dementia complex: I. Clinical features. *Ann. Neurol.* **19:**517–524, 1986a.

Navia, B. A., Cho, E. S., Petito, C. K., Price, R. W. The AIDS dementia complex: II. Neuropathology. *Ann. Neurol.* **19:**525–535, 1986b.

Neff, B. J., Weibel, R. E., Buynak, E. B., *et al.* Clinical and laboratory studies of live cytomegalovirus vaccine AD-169. *Proc. Soc. Exp. Biol. Med.* **160:**32–37, 1979.

Neiman, P. E., Reeves, W., Ray, G., *et al.* A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogeneic bone marrow grafts. *J. Infect. Dis.* **136:**754–767, 1977.

Nelson, D. S. Immune adherence. *Adv. Immunol.* **3:**131–180, 1963.

Nelson, J. A., Groudine, M. Transcriptional regulation of the human cytomegalovirus major immediate-early gene is associated with induction of DNase I-hypersensitive sites. *Mol. Cell. Biol.* **6:**452–461, 1986.

Nelson, J. A., Fleckenstein, B., Galloway, D. A., McDougall, J. K. Transformation of NIH 3T3 cells with cloned fragments of human cytomegalovirus strain AD169. *J. Virol.* **43:**83–91, 1982.

Nelson, J. A., Fleckenstein, B., Jahn, G., *et al.* Structure of the transforming region of human cytomegalovirus AD169. *J. Virol.* **49:**109–115, 1984.

Nelson, J. A., Reynolds-Kohler, C., Smith, B. A. Negative and positive regulation by a short segment in

the 5′-flanking region of the human cytomegalovirus major immediate-early gene. *Mol. Cell. Biol.* **7**:4425–4429, 1987.

Nelson, J. A., Reynolds-Kohler, C., Oldstone, M. B. A., Wiley, C. A. HIV and HCMV coinfect brain cells in patients with AIDS. *Virology* **165**:286–290, 1988.

Newball, H. H., Brahim, S. A. Chronic airway disease in patients with Sjogren's syndrome. *Am. Rev. Respir. Dis.* **115**:295–304, 1977.

Niebojewski, R. A., Aguilar-Torres, F. G., Rytel, M. W. Applications of counterimmunoelectrophoresis in rapid detection of cytomegalovirus antibodies. *Am. J. Clin. Pathol.* **68**:343–346, 1977.

Nielsen, C. M., Hansen, K., Andersen, H. M. K., *et al.* Vestergaard, B. F. An enzyme labelled nuclear antigen immunoassay for detection of cytomegalovirus IgM antibodies in human serum: Specific and non-specific reactions. *J. Med. Virol.* **22**:67–76, 1987.

Nielsen, S. L., Petito, C. K., Urmacher, C. D., Posner, J. B. Subacute encephalitis in acquired immune deficiency syndrome: A postmortem study. *Am. J. Clin. Pathol.* **82**:678–682, 1984.

Nielsen, S. L., Ronholm, E., Sorensen, I., *et al.* Improvement of serological diagnosis of neonatal cytomegalovirus infection by simultaneously testing for specific immunoglobulins E and M by antibody-capture enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **25**:1406–1410, 1987.

Nigida, S. M., Falk, L. A., Wolfe, L. G., *et al.* Experimental infection of marmosets with a cytomegalovirus of human origin. *J. Infect. Dis.* **132**:582–586, 1975.

Nishiyama, Y., Maeno, K., Yoshida, S. Characterization of human cytomegalovirus-induced DNA polymerase and the associated 3′-to-5′ exonuclease. *Virology* **124**:221–231, 1983.

Notkins, A. L. Immune mechanisms by which the spread of viral infections are stopped. *Cell. Immunol.* **11**:478–483, 1974.

Nowak, B., Gmeiner, A., Sarnow, P., Levine, A. J., Fleckenstein, B. Physical mapping of human cytomegalovirus genes: Identification of DNA sequences coding for a virion phosphoprotein of 71 kDa and a viral 65 kDa polypeptide. *Virology* **134**:91–102, 1984a.

Nowak, B., Sullivan, C., Sarnow, R., *et al.* Characterization of monoclonal antibodies and polyclonal immune sera directed against human cytomegalovirus virion proteins. *Virology* **132**:325–338, 1984b.

Numazaki, K., Goldman, H., Wong, I., Wainberg, M. A. Viral infection of human islets of langerhans. Replication of human cytomegalovirus in cultured human fetal pancreatic islets. *Am. J. Clin. Patho.* **90**:52–57, 1988.

Numazaki, Y., Yano, N., Morizuka, T., *et al.* Primary infection with human cytomegalovirus: Virus isolation from healthy infants and pregnant women. *Am. J. Epidemiol.* **91**:410–417, 1970.

O'Donnell, M. E., Elias, P., Lehman, I. R. Processive replication of single-stranded DNA templates by the herpes simplex virus-induced DNA polymerase. *J. Biol. Chem.* **262**:4252–4259, 1987a.

O'Donnell, M. E., Elias, P., Funnell, B. E., Lehman, I. R. Interaction between the DNA polymerase and single-stranded DNA-binding protein (infected cell protein 8) of herpes simplex virus 1. *J. Biol. Chem.* **262**:4260–4266, 1987b.

O'Grady, J. G., Sutherland, S., Harvey, F., *et al.* Cytomegalovirus infection and donor/recipient HLA antigens: Interdependent cofactors in pathogenesis of vanishing bileduct syndrome after liver transplantation. *Lancet* **2**:302–305, 1988.

Oill, P. A., Fiala, M., Schofferman, J., *et al.* Cytomegalovirus mononucleosis in a healthy adult. Association with hepatitis, secondary Epstein–Barr virus antibody response and immunosuppression. *Am. J. Med.* **62**:413–417, 1977.

Olding, L. B., Jensen, F. C., Oldstone, M. B. A. Pathogenesis of cytomegalovirus infection. I. Activation of virus from bone marrow-derived lymphocytes by *in vitro* allogeneic reaction. *J. Exp. Med.* **141**:561–572, 1975.

Olding, L. B., Kingsbury, D. T., Oldstone, M. B. A. Pathogenesis of cytomegalovirus infection. Distribution of viral products, immune complexes and autoimmunity during latent murine infection. *J. Gen. Virol.* **33**:267–280, 1976.

Oldstone, M. B. A., Perrin, L. H. Assay of cell-mediated immunity and immune complexes in viral

infection. In E. H. Lennette and N. J. Schmidt (eds.), *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, 5th ed. American Public Health Association, Washington, 1979, pp. 209–255.

Olshevsky, U., Becker, Y. Herpes simplex virus structural proteins. *Virology* **40:**948–960, 1970.

Olson, L. C., Ketusinha, R., Mansuwan, P., Snitbhan, R. Respiratory tract excretion of cytomegalovirus in Thai children. *J. Pediatr.* **77:**499–504, 1970.

Onorato, I. M., Morens, D. M., Martone, W. J., Stansfield, S. K. Epidemiology of cytomegalovirus infections: Recommendations for prevention and control. *Rev. Infect. Dis.* **7:**479–497, 1985.

Opelz, G., Sengar, D. P. S., Mickey, M. R., Terasaka, P. I. Effect of blood transfusions on subsequent kidney transplants. *Transplant. Proc.* **5:**253–259, 1973.

O'Reilly, R. J., Reich, L., Gred, J., *et al.* A ramdomized trial of intravenous hyperimmune globulin for the prevention of cytomegalovirus infection following bone marrow transplantation. *Transplant. Proc.* **15:**1405–1411, 1983.

Osborn, J. E., Medearis, D. N., Jr. Suppression of interferon and antibody and multiplication of Newcastle disease virus in cytomegalovirus infected mice. *Proc. Soc. Exp. Biol. Med.* **124:**347–353, 1967.

Osborn, J. E., Walker, D. L. Enhancement of infectivity of murine cytomegalovirus *in vitro* by centrifugal inoculation. *J. Virol.* **2:**853–858, 1968.

Osborn, J. E., Walker, D. L. Virulence and attenuation of murine cytomegalovirus. *Infect. Immun.* **3:**228-236, 1970.

Osborn, J. E., Blazkovec, A. A., Walker, D. L. Immunosuppression during acute murine cytomegalovirus infection. *J. Immunol.* **100:**835–844, 1968.

Ossowski, L., Quigley, J. P., Reich, E. Fibrinolysis associated with oncogenic transformation: Morphological correlates. *J. Biol. Chem.* **249:**4312–4320, 1974.

O'Toole, C. M., Gray, J. J., Maher, P., Wregitt, T. G. Persistent excretion of cytomegalovirus in heart transplant patients correlates with inversion of the ratio of T helper/T suppressor–cytotoxic cells. *J. Infect. Dis.* **153:**1160–1162, 1986.

Otto, S. M., Sullivan-Tailyour, G., Malone, C. L., Stinski, M. F. Subcellular localization of the major immediate early protein (ID1) of human cytomegalovirus at early times after infection. *Virology* **162:**478–482, 1988.

Ozawa, T., Stewart, J. A. Immune-complex glomerulonephritis associated with cytomegalovirus infection. *Am. J. Clin. Pathol.* **72:**103–107, 1979.

Pachl, C., Probert, W., Hermsen, K., *et al.* Nucleotide sequence of the CMV (Towne) gH gene and identification of glycoprotein p86 as the gH gene product. *Virology* **169:**418–426, 1989.

Pagano, J. S. Infections with cytomegalovirus in bone marrow transplantation: Report of a workshop from the National Institutes of Health. *J. Infect. Dis.* **132:**114–120, 1975.

Pagano, J. S., Huang, E. S. The application of RNA-DNA cytohybridization to viral diagnostics. In E. Kurstak and R. Morisset (eds.), *Viral Immunodiagnosis*. Academic Press, New York, 1974, pp. 279–299.

Pahwa, S., Kaplan, M., Fikrig, S., *et al.* Spectrum of human t-cell lymphotropic virus type III infection in children. Recognition of symptomatic, asymptomatic, and seronegative patients. *J.A.M.A.* **255:**2299–2305, 1986.

Pak, C. Y., McArthur, R. G., Eun, H.-M., Yoon, J.-W. Association of cytomegalovirus infection with autoimmune type 1 diabetes. *Lancet* **1:**4, 1988.

Palestine, A. G., Rodrigues, M. M., Macher, A. M., *et al.* Opthalmic involvement in acquired immunodeficiency syndrome. *Ophthalmology* **91:**1092–1099, 1984.

Palestine, A. G., Stevens, G. S., Jr., Lane, C. H., *et al.* Treatment of cytomegalovirus retinitis with dihydroxy propoxymethyl guanine. *Am. J. Ophthalmol.* **101:**95–101, 1986.

Paloheimo, J. A., von Essen, R., Klemola, E., *et al.* Subclinical cytomegalovirus infections and cytomegalovirus mononucleosis after open-heart surgery. *Am. J. Cardiol.* **22:**624–630, 1968.

Pande, H., Baak, S., Riggs, A., *et al.* Cloning and physical mapping of a gene fragment coding for a 64-

kilodalton major late antigen of human cytomegalovirus. *Proc. Natl. Acad. Sci. U.S.A.* **81**:4965–4969, 1984.

Pande, H., Campo, K., Churchill, M., *et al.* Genomic localization of the gene encoding a 32-kDa capsid protein of human cytomegalovirus. *Virology* **167**:306–310, 1988.

Pande, H., Campo, K., Churchill, M. A., Zaia, J. A. Genomic locus for a 140 kDa structural protein (pp150) of human cytomegalovirus in strain Towne and AD169. *Virus Res.* **12**:11–18, 1989.

Pannuti, C. S., Vilas-Boas, L. S., Angelo, M. J. O., *et al.* Congenital cytomegalovirus infection. Occurrence in two socioeconomically distinct populations of a developing country. *Rev. Inst. Med. Trop. (Sao Paulo)* **27**:105–107, 1985.

Para, M., Baucke, R., Spear, P. Glycoprotein gE of herpes simplex virus type 1: Effects of anti-gE on virion infectivity and on virus-induced Fc-binding receptors. *J. Virol.* **41**:129–136, 1982.

Paradis, I. L., Grgurich, W. F., Dummer, J. S., *et al.* Rapid detection of cytomegalovirus pneumonia from lung lavage cells. *Am. Rev. Respir. Dis.* **138**:697–702, 1988.

Parham, D. M. Post-transplantation pancreatitis associated with cytomegalovirus (Report of a case). *Human Pathology* **12**:663–665, 1981.

Parslow, T. G., Jones, S. D., Bond, B., Yamamoto, K. R. The immunoglobulin octanucleotide: Independent activity and selective interaction with enhancers. *Science* **235**:1498–1501, 1987.

Paryani, S. G., Yeager, A. S., Hosford-Dunn, H., *et al.* Sequelae of acquired cytomegalovirus infection in premature and sick term infants. *J. Pediatr.* **107**:451–456, 1985.

Pass, R. F., Long, W. K., Whitley, R. J., *et al.* Productive infection with cytomegalovirus and herpes simplex virus in renal transplant recipients: Role of source of kidney. *J. Infect. Dis.* **137**:556–563, 1978.

Pass, R. F., Stagno, S., Myers, G. J., Alford, C. A. Outcome of symptomatic congenital cytomegalovirus infection: Results of long-term longitudinal follow-up. *Pediatrics* **66**:758–762, 1980.

Pass, R. F., Reynolds, D. W., Whelchel, J. D., *et al.* Impaired lymphocyte transformation response to cytomegalovirus and phytohemagglutinin in recipients of renal transplants: Association with antithymocyte globulin. *J. Infect. Dis.* **143**:259–265, 1981a.

Pass, R. F., Dworsky, M. E., Whitley, R. J., *et al.* Specific lymphocyte blastogenic responses in children with cytomegalovirus and herpes simplex virus infections acquired early in infancy. *Infect. Immun.* **34**:166–170, 1981b.

Pass, R. F., Hutto, S. C., Reynolds, D. W., Polhill, R. B. Increased frequency of cytomegalovirus infection in children in group day care. *Pediatrics* **74**:121–126, 1984.

Pass, R. F., Hutto, C., Ricks, R., Cloud, G. A. Increased rate of cytomegalovirus infection among parents of children attending day-care centers. *N. Engl. J. Med.* **314**:1414–1418, 1986.

Pass, R. F., Little, E. A., Stagno, S., *et al.* Young children as a probable source of maternal and congenital cytomegalovirus infection. *N. Engl. J. Med.* **316**:1366–1370, 1987.

Pattengale, P. K., Smith, R. W., Gerber, P. B-cell characteristics of human peripheral and cord blood lymphocytes transformed by Epstein–Barr virus. *J. Natl. Cancer Inst.* **52**:1081–1086, 1974.

Paulin, T., Ringden, D., Lonnquist, B., *et al.* The importance of pre bone marrow transplantation serology in determining subsequent cytomegalovirus infection. *Scand. J. Infect. Dis.* **18**:199–209, 1986.

Paya, C. V., Hermans, P. E., Wiesner, R. H., *et al.* Cytomegalovirus hepatitis in liver transplantation: Prospective analysis of 93 consecutive orthotopic liver transplantations. *J. Infect. Dis.* **160**:752–758, 1989.

Pearson, E. F. Cytoplasmic inclusions produced by the submaxilliary virus. *Am. J. Pathol.* **6**:261–274, 1930.

Pecego, R., Hill, R., Appelbaum, F. R., *et al.* Interstitial pneumonitis following autologous bone marrow transplantation. *Transplantation* **42**:515–517, 1986.

Peckham, C. S., Chin, K. S., Coleman, J. C., *et al.* Cytomegalovirus infection in pregnancy: Preliminary findings from a prospective study. *Lancet* **1**:1352–1355, 1983.

Peden, K., Mounts, P., Hayward, G. S. Homology between mammalian cell DNA sequences and human herpesvirus genomes detected by a hybridization procedure with a high-complexity probe. *Cell* **31:**71–80, 1982.

Penn, I. Kaposi's sarcoma in renal transplant recipients. *Transplantation* **27:**8–11, 1979.

Penttinen, K., Kaariainen, L., Myllylö, G. Cytomegalovirus antibody assay by platelet aggregation. *Arch. Ges. Virusforsch.* **29:**189–194, 1970.

Pereira, L., Hoffman, M., Gallo, D., Cremer, N. Monoclonal antibodies to human cytomegalovirus: Three surface membrane proteins with unique immunological and electrophoretic properties specify cross reactive determinants. *Infect. Immun.* **36:**924–932, 1982.

Pereira, L., Hoffman, M., Tatsuno, M., Dondero, D. Polymorphism of human cytomegalovirus glycoproteins characterized by monoclonal antibodies. *Virology* **139:**73–86, 1984.

Pereira, R. A., James, D. C. O., Stern, H. Correlation between cytomegalovirus infection and HLA-BW15. *Br. Med. J.* **2:**126–126, 1978.

Perham, T. G. M., Caul, E. C., Clarke, S. K. R., Gibson, A. G. F. Cytomegalovirus meningoencephalitis. *Br. Med. J.* **2:**50–50, 1971a.

Perham, T. G. M., Caul, E. W., Conway, P. J., Mott, M. G. Cytomegalovirus infection in blood donors. A prospective study. *Br. J. Haematol.* **20:**307–320, 1971b.

Peterson, P. K., Balfour, H. H., Jr., Marker, S. C., *et al.* Cytomegalovirus disease in renal allograft recipients: A prospective study of the clinical features, risk factors and impact on renal transplantation. *Medicine* **59:**283–300, 1980.

Peterson, P. K., Balfour, H. H., Jr., Fryd, D. S., *et al.* Risk factors in the development of cytomegalovirus-related pneumonia in renal transplant recipients. *J. Infect. Dis.* **148:**1121, 1983.

Petito, C. K., Cho, E.-S., Lemann, W., *et al.* Neuropathology of acquired immunodeficiency syndrome (AIDS): An autopsy review. *J. Neuropathol. Exp. Neurol.* **45:**635–646, 1986.

Petrie, B. L., Melnick, J. L., Adam, E., *et al.* Nucleic acid sequences of cytomegalovirus in cells cultured from human arterial tissue. *J. Infect. Dis.* **155:**158–159, 1987.

Pettavel, C. A. Ueber eigentuemliche herdfoermige Degeneration der Thyreoidea-Epithelien bei Purpura eines Neonatus. *Virchows Arch. (Pathol. Anat.)* **206:**1–8, 1911.

Phillips, C. A., Fanning, W. L., Gump, D. W., Phillips, C. F. Cytomegalovirus encephalitis in immunologically normal adults. Successful treatment with vidarabine. *J.A.M.A.* **28:**2299–2300, 1977.

Phipps, P. H., Gregoire, L., Rossier, E., Perry, E. Comparison of five methods of cytomegalovirus antibody screening of blood donors. *J. Clin. Microbiol.* **18:**1296–1300, 1983.

Pien, F. D., Smith, T. F., Anderson, C. F., *et al.* Herpesviruses in renal transplant patients. *Transplantation* **16:**489–495, 1973.

Pisano, G. Su di un reperto istologico raro in feto Scleromatoso. *Gazz. Osp. Clin.* **31:**249–250, 1910.

Pitchenik, A. E., Fischl, M. A., Dickinson, G. M., *et al.* Opportunistic infections and Kaposi's sarcoma among Haitians: Evidence of a new acquired immunodeficiency state. *Ann. Intern. Med.* **98:**277–284, 1983.

Pizzorno, M., O'Hara, P., Sha, L., *et al.* Transactivation and autoregulation of gene expression by the immediate-early region 2 gene products of human cytomegalovirus. *J. Virol.* **62:**1167–1179, 1988.

Plotkin, S. A., Furukawa, T., Zygraich, N., Huygelen, C. Candidate cytomegalovirus strain for human vaccination. *Infect. Immun.* **12:**521–527, 1975.

Plotkin, S. A., Farquhar, J., Hornberger, E. Clinical trials of immunization with the Towne 125 strain of human cytomegalovirus. *J. Infect. Dis.* **134:**470–475, 1976.

Plotkin, S. A., Smiley, M. L., Friedman, H. M., *et al.* Towne-vaccine-induced prevention of cytomegalovirus disease after renal transplants. *Lancet* **1:**528–530, 1984.

Plotkin, S. A., Drew, W. L., Felenstein, D., Hirsch, M. S. Sensitivity of clinical isolates of human cytomegalovirus to 9-(1,3-dihydroxy-2-propoxymethyl) guanine. *J. Infect. Dis.* **152:**833–844, 1985a.

Plummer, G. Comparative virology of the herpes group. *Prog. Med. Virol.* **9:**302–340, 1967.

Plummer, G., Benyesh-Melnick, M. A plaque reduction neutralization test for human cytomegalovirus. *Proc. Soc. Exp. Biol. Med.* **117:**145–150, 1964.

Plummer, G., Lewis, B. Thermoinactivation of herpes simplex virus and cytomegalovirus. *J. Bacteriol.* **89:**671–674, 1965.

Plummer, G., Goodheart, C. R., Henson, D., Bowling, C. P. A comparative study of the DNA density and behavior in tissue cultures of 14 different herpesviruses. *Virology* **39:**134–137, 1969.

Pollard, R. B., Rand, K. H., Arvin, A. M., Merigan, T. C. Cell-mediated immunity to cytomegalovirus infection in normal subjects and cardiac transplant patients. *J. Infect. Dis.* **137:**541–549, 1978.

Pollard, R. B., Egbert, P. R., Gallagher, J. G., Merigan, T. C. Cytomegalovirus retinitis in immunosuppressed hosts. I. Natural history and effects of treatment and adenine arabinoside. *Ann. Intern. Med.* **93:**655–664, 1980.

Pollard, R. B., Arvin, A. M., Gamberg, P., *et al.* Specific cell-mediated immunity and infections with herpes viruses in cardiac transplant recipients. *Am. J. Med.* **73:**679–687, 1982.

Popovic, M., Sarngadharan, M. G., Read, E., Gallo, R. C. Detection, isolation and continuous production of cytopathic human T lymphotropic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224:**497–500, 1984.

Porter, K. R., Starnes, D. M., Hamilton, J. D. Reactivation of latent murine cytomegalovirus from kidney. *Kidney Int.* **28:**922–925, 1985.

Postic, B., Dowling, J. N. Susceptibility of clinical isolates of cytomegalovirus to human interferon. *Antimicrob. Agents Chemother.* **11:**656–660, 1977.

Potenza, L., deGavaller, B., Feo, M., Mantilla, A. Cytomegalia en recien nacidos y martinatos. *Arch. Venezol. Puericult. Pediatr.* **18:**85–88, 1955.

Powell, R. D., Warner, N. E., Levine, P. S., Kirsner, J. B. Cytomegalic inclusion disease and ulcerative colitis. *Am. J. Med.* **30:**334–340, 1961.

Prawirohardjo, S. Een Onderzock Naarbet Voorkomen zan Zoogenaamde Protozoaire Cellen in den Organen van Kindern te Batavia. *Ned. Tidjschr. Geneeskd.* **82:**6218–6223, 1938.

Preiksaitis, J. K., Brown, L., McKenzie, M. The risk of cytomegalovirus infection in seronegative transfusion recipients not receiving exogenous immunosuppression. *J. Infect. Dis.* **157:**523–529, 1988.

Prentice, H. G. Use of acyclovir for prophylaxis of herpes infections in severely immunocompromised patients. *J. Antimicrob. Chemother.* **12**(Suppl B)**:**1593–1599, 1983.

Price, P., Winter, J. G., Nikoletti, S., *et al.* Functional changes in murine macrophages infected with cytomegalovirus related to H-2-determined sensitivity to infection. *J. Virol.* **61:**3602–3606, 1987.

Prince, A. M., Szumuness, W., Millian, S. J., *et al.* A serologic study of cytomegalovirus infections associated with blood transfusions. *N. Engl. J. Med.* **284:**1125–1131, 1971.

Pritchett, R. F. DNA nucleotide sequence heterogeneity between the Towne and AD 169 strains of cytomegalovirus. *J. Virol.* **36:**152–161, 1980.

Pullen, H., Wright, N., Murdoch, J. McC. Hypersensitivity reactions to antibacterial drugs in infectious mononucleosis. *Lancet* **2:**1176–1178, 1967.

Quinlan, M. P., Chen, L. B., Knipe, D. M. The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. *Cell* **36:**857–868, 1984.

Quinnan, G. V., Manischewitz, J. E. The role of natural killer cells and antibody-dependent cell-mediated cytotoxicity during murine cytomegalovirus infection. *J. Exp. Med.* **150:**1549–1554, 1979.

Quinnan, G. V., Jr., Manischewitz, J. E. Genetically determined resistance to lethal murine cytomegalovirus infection is mediated by interferon-dependent and -independent restriction of virus replication. *J. Virol.* **61:**1875–1881, 1987.

Quinnan, G. V., Manischewitz, J. E., Ennis, F. A. Cytotoxic T lymphocyte response to murine cytomegalovirus infection. *Nature* **273:**541–543, 1978.

Quinnan, G. V., Manischewitz, J. E., Ennis, F. A. Role of cytotoxic T lymphocytes in murine cytomegalovirus infection. *J. Gen. Virol.* **47:**503–508, 1980.

Quinnan, G. V., Kirmani, N., Esber, E., *et al.* HLA-restricted cytotoxic T lymphocyte and non-thymic cytotoxic lymphocyte responses to cytomegalovirus infections of bone marrow transplant recipients. *J. Immunol.* **126:**2036–2041, 1981.

Quinnan, G., Kirmani, N., Rook, A., *et al.* HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone-marrow-transplant recipients. *N. Engl. J. Med.* **307:**7–13, 1982.

Quinnan, G. V., Jr., Delery, M., Rook, A. H., *et al.* Comparative virulence and immunogenicity of the Towne strain and a nonattenuated strain of cytomegalovirus. *Ann. Intern. Med.* **101:**478–483, 1984.

Rabson, A. S., Edgcomb, J. H., Legallais, F. Y., Tyrrell, S. A. Isolation and growth of rat cytomegalovirus *in vitro*. *Proc. Soc. Exp. Biol. Med.* **131:**923–927, 1969.

Radsak, K., Mertensmaier, I., Kaiser, C., *et al.* Distinction of viral and host-derived glycopolypeptides induced by "early" functions of human cytomegalovirus. *Arch. Virol.* **85:**217–30, 1985.

Rager-Zisman, B., Grose, C., Bloom, B. R. Mechanism of selective nonspecific cell-mediated cytotoxicity of virus-infected cells. *Nature* **260:**369–370, 1976.

Rahman, A. A., Teschner, M., Sethi, K. K., Brandis, H. Appearance of IgG (Fc) receptor(s) on cultured human fibroblasts infected with human cytomegalovirus. *J. Immunol.* **117:**253–258, 1976.

Ralph, D. D., Springmeyer, S. C., Sullivan, K. M., *et al.* Rapidly progressive airflow obstruction in marrow transplant recipients. *Am. Rev. Respir. Dis.* **129:**641–44, 1984.

Ramirez, M. L., Virmani, M., Garon, C., Rosenthal, L. J. Defective virions of human cytomegalovirus. *Virology* **96:**311–314, 1979.

Ramsay, K. M., Djeu, J. Y., Rook, A. H. Decreased circulating large granular lymphocytes associated with depressed natural killer activity in renal transplant recipients. *Transplantation* **38:**351–356, 1984.

Ranchod, M., Bissell, M. Pulmonary alveolar proteinosis and cytomegalovirus infection. *Arch. Pathol. Lab. Med.* **103:**139–142, 1979.

Rand, K. H., Merigan, T. C. Cytomegalovirus, a not so innocent bystander. *J.A.M.A.* **240:**2470–2471, 1978.

Rand, K. H., Pollard, R. B., Merigan, T. C. Increased pulmonary superinfections in cardiac transplant patients undergoing primary cytomegalovirus infection. *N. Engl. J. Med.* **298:**951–953, 1978.

Randazzo, R. F., Hulette, C. M., Gottlieb, M. S., *et al.* Cytomegaloviral epididymitis in a patient with the acquired immune deficiency syndrome. *J. Urol.* **136:**1095–1097, 1986.

Rando, R. R., Pellett, P. E., Luciw, P. A., *et al.* Transactivation of human immunodeficiency virus by herpesviruses. *Oncogene* **1:**13, 1987.

Rao, N., Waruszewski, D. T., Armstrong, J. A., Atchison, R. W., Ho, M. Evaluation of antibomplement immunofluorescence test in cytomegalovirus infection. *J. Clin. Microbiol.* **6:**633–638, 1977.

Rasmussen, L. The Immune Response to Human Cytomegalovirus Infection. *Current Topics in Microbiol. Immunol.* Springer-Verlag, Berlin. (in press) December, 1989.

Rasmussen, L. E., Nelson, R. M., Kelsall, D. C., Merigan, T. C. Murine monoclonal antibody to a single protein neutralizes the infectivity of human cytomegalovirus. *Proc. Natl. Acad. Sci. U.S.A.* **81:**876–880, 1984.

Rasmussen, L., Mullenax, J., Nelson, R., Merigan, T. Viral polypeptides detected by a complement dependent neutralizing murine monoclonal antibody to human cytomegalovirus. *J. Virol.* **55:**274–280, 1985a.

Rasmussen, L. E., Mullenax, J., Nelson, M., Merigan, T. C. Human cytomegalovirus polypeptides stimulate neutralizing antibody *in vivo*. *Virology* **145:**186–190, 1985b.

Rasmussen, L., Nelson, M., Neff, M., Merigan, T. Characterization of different human cytomegalovirus glycoproteins which are targets for virus neutralizing antibody. *Virology* **163:**308–318, 1988.

Ravin, C. E., Smith, G. W., Adhern, M. J., *et al.* Cytomegaloviral infection presenting as a solitary pulmonary nodule. *Chest* **71:**220–222, 1977.

Raynaud, J., Antanasiu, P., Barreau, C., Jahkola, M. Adaptation d'un virus cytomegalie provenant du mole (*Apodemus sylvaticus*) sur differentes cellules heterologues y compris les cellules humanines. *C.R. Acad. Sci. (D) (Paris)* **269:**104–106, 1969.

Raynaud, J., Raynaud, A. Etude histologique des lesions des glandes salivaires observees chez quelques

expeces de campagnols: Apparentenant aux genres microtus et evotomys. *Ann. Inst. Pasteur* **73:**174–185, 1947.

Re, M., Landini, M., Coppolecchia, P., *et al.* A 28000 molecular weight human cytomegalovirus structural polypeptide studied by means of a specific monoclonal antibody. *J. Gen. Virol.* **66:**2507–2511, 1985.

Rector, E. J., Rector, L. E. Intranuclear inclusions in the salivary glands of moles. *Am. J. Pathol.* **10:**629–636, 1934.

Reddehase, M. J., Keil, G. M., Koszinowski, U. H. The cytolytic T lymphocyte response to the murine cytomegalovirus. I. Distinct maturation stages of cytolytic T lymphocytes constitute the cellular immune response during acute infection of mice with the murine cytomegalovirus. *J. Immunol.* **132:**482–489, 1984a.

Reddehase, M. J., Keil, G. M., Koszinowski, U. H. The cytolytic T lymphocyte response to the murine cytomegalovirus II. Detection of virus replication state-specific antigens by separate populations of *in vivo* active cytolytic T lymphocyte precursors. *Eur. J. Immunol.* **14:**56–61, 1984b.

Reddehase, M. J., Weiland, F., Munch, K., *et al.* Interstitial murine cytomegalovirus pneumonia after irradiation: Characterization of cells that limit viral replication during established infection of the lungs. *J. Virol.* **55:**264–273, 1985.

Reddehase, M. J., Mutter, W., Munch, K., *et al.* CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. *J. Virol.* **61:**3102–3108, 1987a.

Reddehase, M. J., Mutter, W., Koszinowski, U. *In vivo* application of recombinant interleukin 2 in the immunotherapy of established cytomegalovirus infection. *J. Exp. Med.* **165:**650–656, 1987b.

Reddehase, M. J., Jonjic, S., Weiland, F., *et al.* Adoptive immunotherapy of murine cytomegalovirus adrenalitis in the immunocompromised host: CD4-helper-independent antiviral function of CD8-positive memory T lymphocytes derived from latently infected donors. *J. Virol.* **62:**1061–1065, 1988.

Reed, E. C., Bowden, R. A., Dandliker, P. S., *et al.* Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. *Ann. Intern. Med.* **109:**783–788, 1988.

Reily, C. A., Kocoshis, S., Gryboskki, J. D., Klatskin, G. Portal hypertension following neonatal cytomegalovirus infection. *J. Pediatr.* **94:**1011–1011, 1979.

Reimer, C. B., Black, C. M., Phillips, D. J., *et al.* The specificity of fetal IgM: Antibody or antiantibody? *Ann. N.Y. Acad. Sci.* **254:**77–93, 1975.

Reinherz, E. L., O'Brein, C., Rosenthal, P., Schlossmann, S. F. The cellular basis for viral induced immunodeficiency: Analysis by monoclonal antibodies. *J. Immunol.* **125:**1269–1269, 1980.

Reller, L. B. Granulomatous hepatitis associated with acute cytomegalovirus infection. *Lancet* **1:**20–22, 1973.

Renvoize, E. B., Hembling, M. H., Pepper, M. D., Rajah, S. M. Possible association of Alzheimer's disease with HLA-BW15 and cytomegalovirus infection. *Lancet* **1:**1238–1238, 1979.

Reynolds, D. W. Development of early nuclear antigen in cytomegalovirus infected cells in the presence of RNA and protein synthesis inhibitors. *J. Gen. Virol.* **40:**475–480, 1978.

Reynolds, D. W., Stagno, S., Hosty, T. S., *et al.* Maternal cytomegalovirus excretion and perinatal infection. *N. Engl. J. Med.* **289:**1–5, 1973.

Reynolds, D. W., Stagno, S., Stubbs, G., *et al.* Inapparent congenital cytomegalovirus infection with elevated cord IgM levels. *N. Engl. J. Med.* **290:**291–296, 1974.

Reynolds, D. W., Dean, P. H., Pass, R. F., Alford, C. A., Jr. Specific cell-mediated immunity in children with congenital and neonatal cytomegalovirus infection and their mothers. *J. Infect. Dis.* **140:**493–499, 1979a.

Reynolds, D. W., Stagno, S., Alford, C. A., Jr. Laboratory diagnosis of cytomegalovirus infections. In E. H. Lennett and N. J. Schmidt (eds.), *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections.* American Public Health Association, Washington, 1979b, pp. 399–439.

Reynolds, D. W., Stagno, S., Alford, C. A., Jr. Congenital cytomegalovirus infection. *Teratogen Update: Environmentally induced birth defect risks* 93–95, 1986.

Ribbert, H. Ueber proozoanartige Zellen in der Niere eines syphilitischen Neugeborenen und in der Parotis von Kindern. *Zentrabl. Allg. Pathol.* **15:**945–948, 1904.

Rice, G. P. A., Schrier, R. D., Oldstone, M. B. A. Cytomegalovirus infects human lymphocytes and monocytes: Virus expression is restricted to immediate early antigen products. *Proc. Natl. Acad. Sci. U.S.A.* **81:**6134–6138, 1984.

Richardson, W. P., Colvin, R. B., Cheeseman, S. H., *et al.* Glomerulopathy associated with cytomegalovirus viremia in renal allografts. *N. Engl. J. Med.* **305:**57–63, 1981.

Rifkind, D. Cytomegalovirus infection after renal transplantation. *Arch. Intern. Med.* **116:**554–558, 1965.

Riggs, J. L., Cremer, N. E. Differentiation of cytomegalovirus antigens by their reactivity with various classes of human antibodies in the indirect fluorescent antibody test. *J. Clin. Microbiol.* **11:**88–93, 1980.

Rinaldo, C. R., Jr., Black, P. H., Hirsch, M. S. Interactions of cytomegalovirus with leucocyte from patients with mononucleosis due to cytomegalovirus. *J. Infect. Dis.* **136:**667–678, 1977.

Rinaldo, C. R., Jr., Levin, M. J., Carney, W. P., *et al.* Interactions of lymphocytes with cytomegalovirus. In M. R. Proffitt (ed.), *Virus–Lymphocyte Interactions: Implications for Disease.* Elsevier North-Holland, Amsterdam, 1979a, pp. 267–282.

Rinaldo, C. R., Jr., Stossel, T. P., Black, P. H., Hirsch, M. S. Polymorphonuclear leukocyte function during cytomegalovirus mononucleosis. *Clin. Immunol. Immunopathol.* **12:**331–334, 1979b.

Rinaldo, C. R., Jr., Carney, W. P., Richter, B. S., *et al.* Mechanisms of immunosuppression in cytomegaloviral mononucleosis. *J. Infect. Dis.* **141:**488–495, 1980.

Rinaldo, C. R., Jr., Hamoudi, W. H., DiBaiasio, R. L., *et al.* Cellular immune response and cytomegalovirus infection in renal transplant patients receiving cyclosporine. *Transplant. Proc.* **15:**2775, 1983.

Rinaldo, C. R., Jr., DeBiasio, R. L., Hamoudi, W. H., *et al.* Effect of herpesvirus infections on T-lymphocyte subpopulations and blastogenic responses in renal transplant recipients receiving cylosporine. *Clin. Immunol. Immunopathol.* **38:**366, 1986.

Rinaldo, C. R., Jr., Piazza, P., Wang, Y., *et al.* HIV-I-specific production of gamma interferon and modulation by recombinant interleukin 2 during early HIV-I infection. *J. Immunol.* **140:**3389–3393, 1988.

Ringden, O., Longvist, B., Paulin, T., *et al.* Pharmacokinetics, safety and preliminary clinical experiences using foscarnet in the treatment of cytomegalovirus infections in bone marrow and renal transplant recipients. *J. Antimicrob. Chemother.* **17:**373–387, 1986.

Ripalti, A., Landini, M., LaPlaca, M. A 46 KD polypeptide, present in purified human cytomegalovirus, is provided with DNAse activity and is antigenically related to a higher molecular weight, enzymatically inactive, cellular protein. *Microbiological* **11:**69–76, 1988.

Ripalti, A., Landini, M., Mocarski, E., LaPlaca, M. Identification and preliminary use of recombinant lambda gt11 fusion proteins in cytomegalovirus diagnosis. *J. Gen. Virol.* **70:**1247–1251, 1989.

Robbins, A., Dorney, D., Wathen, M., *et al.* The pseudorabies virus gII gene is closely related to the gB glycoprotein gene of herpes simplex virus. *J. Virol.* **61:**2691–2701, 1987.

Robson, G. S., MacKay, I. R. Generalized cytomegalovirus infection in a patient with lupoid hepatitis. *Aust. Ann. Med.* **18:**147–150, 1969.

Robson, L., Gibson, W. Primate cytomegalovirus assembly protein: Genome location and nucleotide sequence. *J. Virol.* **63:**669–676, 1988.

Roby, C., Gibson, W. Characterization of phosphoproteins and protein kinaseactivity of virions, noninfectious enveloped particles, and dense bodies of human cytomegalovirus. *J. Virol.* **59:**714–727, 1986.

Roder, J. C. The beige mutation in the mouse. I. A stem cell predetermined impairment in natural killer cell function. *J. Immunol.* **123:**2168, 1979.

Rodgers, B., Borysiewicz, L., Mondin, J., *et al.* Immunoaffinity purification of a 72 K early antigen of

human cytomegalovirus: Analysis of humoral and cell-mediated immunity to the purified polypeptide. *J. Gen. Virol.* **68:**2371–2378, 1987.

Rodgers, M. C., Scott, D. M., Mundin, J., Sissons, J. G. Monocyte derived inhibitor of interleukin 1 induced by human cytomegalovirus. *J. Virol.* **55:**527–532, 1985.

Roenhorst, H. W., Tegzess, A. M., Beelen, J. M., *et al.* HLA-DRw6 as a risk factor for active cytomegalovirus but not for herpes simplex virus infection after renal allograft transplantation. *Br. Med. J.* **291:**619–622, 1985.

Roizman, B. Provisional labels for herpesviruses. *J. Gen. Virol.* **20:**417–419, 1973.

Roizman, B. The organization of the herpes simplex virus genomes. *Annu. Rev. Genet.* **13:**25–57, 1979a.

Roizman, B. The structure and isomerization of herpes simplex virus genomes. *Cell* **16:**481–494, 1979b.

Roizman, B., Batterson, W. Herpesviruses and their replication. In B. N. Fields (ed.), *Virology*. Raven Press, New York, 1985, pp. 497–526.

Rola-Pleszczynski, M., Frenkel, L. D., Fuccillo, D. A., *et al.* Specific impairment of cell-mediated immunity in mothers of infants with congenital infection due to cytomegalovirus. *J. Infect. Dis.* **135:**386–391, 1977.

Rook, A. H. Interactions of cytomegalovirus with the human immune system. *Rev. Infect. Dis.* **10:**S460–S467, 1988.

Rook, A. H., Masur, H., Lane, H. C., *et al.* Interleukin-2 enhances the depressed natural killer and cytomegalovirus-specific cytotoxic activities of lymphocytes from patients with the acquired immune deficiency syndrome. *J. Clin. Invest.* **72:**398–403, 1983.

Rook, A. H., Quinnan, G., Fredrick, J., *et al.* Importance of cytotoxic lymphocytes during cytomegalovirus infection in renal transplant recipients. *Am. J. Med.* **76:**385–392, 1984a.

Rook, A. H., Manischewitz, J. F., Frederick, W. R., *et al.* Deficient, HLA-restricted, cytomegalovirus-specific cytotoxic T cells and natural killer cells in patients with the acquired immunodeficiency syndrome. *J. Infect. Dis.* **152:**627–630, 1984b.

Rosen, P., Hajdu, S. Cytomegalovirus inclusion disease at autopsy of patients with cancer. *Am. J. Clin. Pathol.* **55:**749–756, 1971.

Rosen, R., Armstrong, D., Rice, N. Gastrointestinal cytomegalovirus infection. *Arch. Intern. Med.* **132:**274–276, 1973.

Rosensheim, M. S., Farewell, V. T., Price, T. H., *et al.* The cost effectiveness of therapeutic and prophylactic leukocyte transfusion. *N. Engl. J. Med.* **302:**1058–1062, 1980.

Ross, R. The pathogenesis of atherosclerosis—an update. *N. Engl. J. Med.* **314:**488–500, 1986.

Rouse, B. T., Horohov, D. W. Immunosuppression in viral infections. *Rev. Infect. Dis.* **8:**850–873, 1986.

Rouse, B., Norley, S., Martin, S. Antiviral cytotoxic T lymphocyte induction and vaccination. *Rev. Infect. Dis.* **10:**16–33, 1988.

Rowe, W. P., Hartley, J. W., Waterman, S., *et al.* Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. *Proc. Soc. Exp. Biol. Med.* **92:**418–424, 1956.

Rubin, R. H., Cosimi, A. B., Tolkoff-Rubin, N. E., *et al.* Infectious disease syndromes attributable to cytomegalovirus and their significance among renal transplant recipients.*Transplantation* **24:**458–464, 1977.

Rubin, R. H., Tolkoff-Rubin, N. E., Oliver, D., *et al.* Multicenter seroepidemiologic study of the impact of cytomegalovirus infection on renal transplantation. *Transplantation* **40:**243–249, 1985.

Rubin, R. H., Wilson, E. J., Barrett, L. V., Meadearis, D. N. Primary cytomegalovirus infection following cardiac transplantation in a murine model. *Transplantation* **37:**306–310, 1984.

Rubin, R. H., Young, L. S. *Clinical Approach to Infection in the Compromised Host.* 2nd edition, New York: Plenum, N.Y., 1988.

Ruebner, B. H., Hirano, T., Slusser, R. J., Medearis, D. N., Jr. Human cytomegalovirus infection. *Am. J. Pathol.* **46:**477–496, 1965.

Ruebner, B. H., Miyai, K., Slusser, R. J., *et al.* Mouse cytomegalovirus infection. *Am. J. Pathol.* **44:**799–821, 1964.

Ruger, R., Bornkamm, G. W., Fleckenstein, B. Human cytomegalovirus DNA sequences with homologies to the cellular genome. *J. Gen. Virol.* **65:**1351–1364, 1984a.

Ruger, R., Burmester, G. R., Kalden, J. R., *et al.* Search for human cytomegalovirus DNA in Kaposi sarcomas and hematopoietic cells from homosexual men with AIDS or unexplained lymphadenopathy. *UCLA Symp. Mol. Cell. Biol.* **16:**127–137, 1984b.

Ruger, B., Klages, S., Walla, B., *et al.* Primary structure and transcription of the genes coding for the two virion phosphoproteins pp65 and pp71 of human cytomegalovirus. *J. Virol.* **61:**446–453, 1987.

Rundell, B. B., Betts, R. F. Interaction of cytomegalovirus immune complexes with host cells. *Infect. Immun.* **3:**658–665, 1981.

Ruskin, J., Remington, J. A. Toxoplasmosis in the compromised host. *Ann. Intern. Med.* **84:**193–199, 1976.

Ruyechan, W. T., Weir, A. C. Interaction with nucleic acids and stimulation of the viral DNA polymerase by the herpes simplex virus type 1 major DNA-binding protein. *J. Virol.* **52:**727–733, 1984.

Rytel, M. W., Kauffman, H. M. Clinical efficacy of adenine arabinoside in therapy of cytomegalovirus infections in renal allograft recipients. *J. Infect. Dis.* **133:**202–205, 1976.

Rytel, M. W., Aaberg, T. M., Dee, T. H., Heim, L. R. Therapy of cytomegalovirus retinitis with transfer factor. *Cell Immunol.* **19:**8–21, 1975.

Rytel, M. W., Aguilar-Torres, F. G., Balay, J., Heim, L. R. Assessment of the status of cell-mediated immunity in cytomegalvirus-infected renal allograft recipients. *Cell Immunol.* **37:**31–40, 1978.

Sabourin, C. L. K., Reno, J. M., Boezi, J. A. Inhibition of eukaryotic DNA polymerases by phosphonoacetate and phosphonoformate. *Arch. Biochem. Biophys.* **187:**96–101, 1978.

Sacrez, R., Fruhling, L., Korn, R., Juif, J. G., *et al.* Trois observations de maladie des inclusions cytomegaliques. *Arch. Fr. Pediatr.* **17:**129–142, 1960.

Sahud, M. A., Bachelor, M. M. Cytomegalovirus-induced thrombocytopenia. An unusual case report. *Arch. Intern. Med.* **138:**1573–1575, 1978.

Saigal, S., Lunyk, O., Larke, R. P. B., Chernesky, M. A. The outcome in children with congenital cytomegalovirus infection. *Am. J. Dis. Child.* **136:**896–901, 1982.

Saiki, R. K., Gelfand, D. H., Stoffel, S., *et al.* Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239:**487–491, 1988.

St. Clair, M. H., Furman, P. A., Lubbers, C. M., Elion, G. B. Inhibition of cellular ã and virally induced deoxyribonucleic acid polymerases by the triphosphate of acyclovir. *Antimicrob. Agents Chemother.* **18:**741–745, 1980.

St. Jeor, S. C., Albrecht, T. B., Funk, F. D., Rapp, F. Stimulation of cellular DNA synthesis by human cytomegalovirus. *J. Virol.* **13:**353–362, 1974.

St. Onge, G., Bezahler, G. H. Giant esophageal ulcer associated with cytomegalovirus. *Gastroenterology* **83:**127–130, 1982.

Sakuma, S., Furukawa, T., Plotkin, S. A. The characterization of IgG receptor induced by human cytomegalovirus. *Proc. Soc. Exp. Biol. Med.* **155:**168–172, 1977.

Salahuddin, S. Z., Ablashi, D. V., Markham, P. D., *et al.* Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* **234:**596–601, 1986.

Salahuddin, S. Z., Nakamura, S., Biberfeld, P., *et al.* Angiogenic properties of Kaposi's sarcoma-derived cells after long-term culture *in vitro*. *Science* **242:**430, 1988.

Saltzman, R. L., Quirk, M. R., Jordan, M. C. Disseminated cytomegalovirus infection. Molecular analysis of virus and leukocyte interations in viremia. *J. Clin. Invest.* **81:**75–81, 1988.

Sama, S. K., Bhargava, S., Nath, N. G., *et al.* Non-cirrhotic portal fibrosis. *Am. J. Med.* **51:**160–169, 1971.

Sanford, E. J., Geder, L., Laychock, A., *et al.* Evidence for the association of cytomegalovirus with carcinomas of the prostate. *J. Urol.* **118:**789–792, 1977.

Saraux, J. L., Lenoble, L., Toublanc, M., *et al.* Anaclulous cholecystitis and cytomegalovirus infection in a patient with AIDS. *J. Infect. Dis.* **155**(4):829, 1987.

Sarov, B., Naggan, L., Haikin, H., Sarov, I. Early acquisition of cytomegalovirus infection in kibbutz

children in comparison with urban and bedouin children in southern Israel. *Isr. J. Med. Sci.* **19:**913–915, 1983.

Sarov, I., Abody, K. The morphogenesis of human cytomegalovirus. Isolation and polypeptide characterization of cytomegalovirions and dense bodies. *Virology* **66:**464–473, 1975.

Sarov, I., Siqueira-Linhares, M., Chardonnet, Y., *et al.* Detection of Specific IgA antibodies in serum of kidney transplant patients with recurrent cytomegalovirus infection. *Intervirology* **15:**228–234, 1981.

Sarov, I., Levy, E., Aymard, M., *et al.* Detection of virus-specific IgA antibodies in serum of kidney transplant patients with recurrent cytomegalovirus infection by enzymeimmuno and radioimmunoassay techniques. *Clin. Exp. Immunol.* **48:**321–328, 1982.

Sawyer, M. H., Edwards, D. K., Spector, S. A. Cytomegalovirus infection and bronchopulmonary dysplasis in premature infants. *Am. J. Dis. Child.* **141:**303–305, 1987.

Schaeffer, H. J. Nucleosides, nucleotides and their biological applications. In: J. L. Rideout, D. W. Henry, L. M. Beacham III (eds.), *Nucleosides with Antiviral Activity.* New York: Academic Press, 1982, 1–17.

Schaeffer, J. H., Beauchamp, L., De Miranda, P., *et al.* 9-(2-Hydroxymethyl)guanine activity against viruses of the herpes group. *Nature* **272:**583–585, 1978.

Schechter, G. P., Whang-Pang, J., McFarland, W. Circulation of donor lymphocytes after blood transfusions in man. *Blood* **49:**651–656, 1977.

Scher, I., Ahmed, A., Strong, D. M., *et al.* X-linked B-lymphocyte immune defect in CBA/HN mice. *J. Exp. Med.* **141:**788–803, 1975.

Schmidt, N. J., Dennis, J., Lennette, E. H. Plaque reduction neutralization test for human cytomegalovirus based upon enhanced uptake of neutral red by virus-infected cells. *J. Clin. Microbiol.* **4:**61–66, 1976.

Schmidt, N. J., Emmons, R. W. *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections,* 6th ed. American Public Health Association, Washington, 1989, p. 1255.

Schmitz, H., Enders, G. Cytomegalovirus as a frequent cause of Guillain–Barré Syndrome. *J. Med. Virol.* **1:**21–27, 1977.

Schmitz, H., Haas, R. Erfahrungen mit der fluorescenzserologischen Bestimmung von Antikoerpern gegen Cytomegalie-virus. *Z. Med. Mikrobiol. Immunol.* **155:**283–296, 1970.

Schmitz, H., Haas, R. Determination of different cytomegalovirus immunoglobulins (IgG, IgA, IgM) by immunofluorescence. *Arch. Ges. Virusforsch.* **37:**131–140, 1972.

Schmitz, H., Haas, R. Zytomegalievirus-Erkrankungen bei Erwachsenen. *Deutsch. Med. Wochenschr.* **98:**649–653, 1973.

Schmitz, H., Kampa, D., Doerr, H. W., *et al.* IgM antibodies to cytomegalovirus during pregnancy. *Arch. Virol.* **53:**177–184, 1977.

Schneck, S. A. Neuropathological features of human organ transplantation: I. Probable cytomegalovirus infection. *J. Neuropathol. Exp. Neurol.* **24:**415, 1965.

Schneiderman, D. J., Cello, J. P., Laing, F. C. Papillary stenosis and sclerosing cholangitis in the acquired immunodeficiency syndrome. *Ann. Intern. Med.* **106:**546–549, 1987.

Schochetman, G., Ou, C.-Y., Jones, W. K. Polymerase chain reaction. *J. Infect. Dis.* **158:**1154–1157, 1988.

Schoenberger, L. B., Bregman, D. J., Sullivan-Bolyai, J. Z., *et al.* Guillain–Barre syndrome following vaccination in the national influenza immunization program. U.S.A., 1976–1977. *Am. J. Epidemiol.* **110:**105–123, 1979.

Schooley, R. T., Hirsch, M. S., Colvin, R. B., *et al.* Association of herpesvirus infections with T-lymphocyte-subset alterations. glomerulopathy, and opportunistic infections after renal transplantation. *N. Engl. J. Med.* **308:**307–313, 1983.

Schrier, R. D., Nelson, J. A., Oldstone, M. B. A. Detection of human cytomegalovirus in peripheral blood lymphocytes in a natural infection. *Science* **230:**1048–1051, 1985.

Schrier, R. D., Rice, G. P. A., Oldstone, M. B. A. Suppression of natural killer cell activity and T cell proliferation by fresh isolates of human cytomegalovirus. *J. Infect. Dis.* **153:**1084–1091, 1986.

Schwartz, J. N., Daniels, C. A., Klintworth, G. K. Lymphoid cell necrosis, thymic atrophy, and growth

retardation in newborn mice inoculated with murine cytomegalovirus. *Am. J. Pathol.* **79:**509–518, 1975.

Seifert, G. Weitere Untersuchungen zur Frage der Syntropie von interstitieller Pneumonie und Zytomegalie. *Zentralbl. Allg. Pathol.* **91:**445–450, 1954.

Seifert, G., Oehme, J. *Pathologie und Klinik der Cytomegalie.* Georg Thieme, Leipzig, 1957, p. 114.

Selgrade, M. K., Osborn, J. E. Role of macrophages in resistance to murine cytomegalovirus. *Infect. Immun.* **10:**1383–1390, 1974.

Selgrade, M. K., Ahmed, A., Sell, K. W., *et al.* Effect of murine cytomegalovirus on the *in vitro* responses of T and B cells to mitogens. *J. Immunol.* **116:**1459–1465, 1976.

Sell, M. S., Bale, J. F., Jr., Giller, R. H. T-lymphocyte subpopulations and function during murine cytomegalovirus infection. *Pediatr. Res.* **19:**583–587, 1985.

Sen, R., Baltimore, D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* **46:**705, 1986.

Sererat, M. N., Schifano, J. V., Lau, P., *et al.* Evaluation of cytomegalvirus (CMV) antibody screening tests for blood donors. *Am. J. Clin. Path.* **86:**523–526, 1985.

Serwadda, E., Mugerwa, R. D., Sewankambo, N. K., *et al.* Slim disease: A new disease in Uganda and its association with HTLV-III infection. *Lancet* **2:**849–852, 1985.

Severi, B., Landini, M. P., Musiani, M., Zerbini, M. A study of the passage of human cytomegalovirus from the nucleus to the cytoplasm. *Microbiologica* **2:**265–273, 1979.

Severi, B., Landini, M. P., Govoni, E. Human cytomegalovirus morphogenesis: An ultrastructural study of the late cytoplasmic phases. *Arch. Virol.* **98:**51–64, 1988.

Shanley, J. D. Modification of acute murine cytomegalovirus adrenal gland infection by adoptive spleen cell transfer. *J. Virol.* **61:**23–28, 1987.

Shanley, J. D., Jordan, M. C., Stevens, J. G. Modification by adoptive humoral immunity of murine cytomegalovirus infection. *J. Infect. Dis.* **143:**231–237, 1981.

Shanley, J. D., Pesanti, E. L., Nugent, K. M. The pathogenesis of pneumonitis due to murine cytomegalovirus. *J. Infect. Dis.* **156:**388–396, 1982.

Shanley, J. D., Via, C. S., Sharrow, S. O., Shearer, G. M. Interstitial pneumonitis during murine cytomegalovirus infection and graft-versus-host reaction. *Transplantation* **44:**658–662, 1987.

Shanley, J. D., Pomeroy, C., Via, C. S., Shearer, G. M. Interstitial pneumonitis during murine cytomegalovirus infection and graft-versus-host reaction: Effect of ganciclovir therapy. *J. Infect. Dis.* **158:**1391–1394, 1988.

Shapiro, S., Stone, D., Siskind, K., *et al.* Drug rashes with ampicillin and penicillins. *Lancet* **2:**969–972, 1969.

Shaw, S. B., Rasmussen, R. D., McDonough, S. H., *et al.* Cell-related sequences in the DNA genome of human cytomegalovirus strain AD169. *J. Virol.* **55:**843–848, 1985.

Shelby, J., Shanley, J. Transfer of murine cytomegalovirus by syngeneic skin grafts. *Transplantation* **44:**318–320, 1987.

Sheldon, P. J., Papamichail, M., Hemsted, E. H., Holborow, E. J. Thymic origin of atypical lymphocytes in infectious mononucleosis. *Lancet* **1:**1153–1155, 1973.

Shepp, D. H., Dandliker, P. S., de Miranda, P., *et al.* Activity of 9-[2-hydroxy-1-(hydroxymethyl) ethoxymethyl] guanine in the treatment of cytomegalovirus pneumonia. *Ann. Intern. Med.* **103:**368–373, 1985.

Shibata, D., Martin, W. J., Appleman, M. D., *et al.* Detection of cytomegalovirus DNA in peripheral blood of patients infected with human immunodeficiency virus. *J. Infect. Dis.* **158:**1185–1192, 1988.

Shorr, R. I., Longo, W. L., Oberley, T. D., *et al.* Cytomegalovirus-associated tubulointerstitial nephritis in an allogeneic bone marrow transplant recipient. *Ann. Intern. Med.* **107:**351–352, 1987.

Shuster, E. A., Beneke, J. S., Tegtmeier, G. F., *et al.* Monoclonal antibody for rapid laboratory detection of cytomegalovirus infections: Characterization and diagnostic application. *Mayo Clinic Proc.* **60:**577–585, 1985.

Signas, C., Katze, M. G., Persson, H., Philipson, L. An adenovirus glycoprotein binds heavy chains of class I transplantation antigens from man and mouse. *Nature* **299:**175–178, 1982.

Simmons, R. L., Weil, R., Tallent, M. B., *et al.* Do mild infections trigger the rejection of renal allografts? *Transplant Proc.* **2**:419–423, 1970.

Simmons, R. L., Matas, A. J., Rattazzi, L. C., *et al.* Clinical characteristics of the lethal cytomegalovirus infection following renal transplantation. *Surgery* **82**:537–546, 1977.

Singh, N., Dummer, J. S., Ho, M., *et al.* Infections with cytomegalovirus and other herpesviruses in 121 liver transplant recipients: Transmission by donated organ and the effect of OKT3 antibodies. *J. Infect. Dis.* **158**:124–131, 1988.

Sinha, S. K., Pauls, F. P. Cytomegalovirus complement-fixation antibody responses in Eskimo families. *Pediatrics* **48**:157–157, 1971.

Skolnik, P. R., Kosloff, B. R., Hirsh, M. S. Bidirectional interactions between human immunodeficiency virus type 1 and cytomegalovirus. *J. Infect. Dis.* **157**(3):508–514, 1988.

Smee, D. F. Interaction of 9-(1,3-dihydroxy-2-propoxymethyl)guanine with cytosol and mitochondrial deoxygua nosine kinases: Possible role in anticytomegalovirus activity. *Mol. Cell Biochem.* **69**:75–81, 1985.

Smee, D. F., Boehme, R., Chernow, M., *et al.* Intracellular metabolism and enzymatic phosphorylation of 9-(1,3-dihydroxy-2-propoxymethyl)guanine and acyclovir in herpes simplex virus-infected and uninfected cells. *Biochem. Pharmacol.* **34**:1049–1056, 1985.

Smiley, M. L., Mar, E-C., Huang, E-S. Cytomegalovirus infection and viral-induced transformation of human endothelial cells. *J. Med. Virol.* **25**:213–226, 1988.

Smiley, M. L., Wlodaver, C. G., Grossman, R. A., *et al.* The role of pretransplant immunity in protection from cytomegalovirus disease following renal transplantation. *Transplantation* **40**:157–161, 1985.

Smith, A. J., Wiedman, F. D. Infection of a still-born infant by an amebiform protozoan (*Entamoeba mortinatalium*, N.S.). *Univ. Penn. Med. Bull.* **23**:285–285, 1910.

Smith, A. J., Weidman, F. D. Further note upon the occurrence of *Entamoeba mortinatalium* as a human parasite. *Am. J. Trop. Dis. Prev. Med.* **2**:256–259, 1914.

Smith, D. R. A syndrome resembling infectious mononucleosis after open heart surgery. *Br. Med. J.* **1**:945–948, 1964.

Smith, J. D. Human cytomegalovirus: Demonstration of permissive epithelial cells and nonpermissive fibroblastic cells in a survey of human cell lines. *J. Virol.* **60**:583–588, 1986.

Smith, J. D., De Harven, E. Herpes simplex virus and human cytomegalovirus replication in WI 38 cells. I. Sequence of viral replication. *J. Virol.* **12**:919–930, 1973.

Smith, J. D., De Harven, E. Herpes simplex virus and human cytomegalovirus replication in WI 38 cells. II. An ultrastructural study of viral penetration. *J. Virol.* **14**:945–956, 1974.

Smith, J. D., De Harven, E. Herpes simplex virus and human cytomegalovirus replication in WI-38 cells. *J. Virol.* **26**:102–109, 1978.

Smith, J. D., De Harven, E. Localization of arginine-rich protein in cytomegalovirus dense bodies using phenanthrenequinone fluorescence. *Virology* **96**:335–338, 1979.

Smith, M. G. Propagation of salivary gland virus of the mouse in tissue cultures. *Proc. Soc. Exp. Biol. Med.* **86**:435–440, 1954.

Smith, M. G. Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. *Proc. Soc. Exp. Biol. Med.* **92**:424–430, 1956.

Smith, T. F., Holley, K. E., Keys, T. F., Macasaet, F. F. Cytomegalovirus studies of autopsy tissue. *Am. J. Clin. Pathol.* **63**:854–858, 1975.

Snider, W. D., Simpson, D. M., Nielsen, S., *et al.* Neurological complications of acquired immune deficiency syndrome: Analysis of 50 patients. *Ann. Neurol.* **14**:403–418, 1983.

Snover, D. C., Hutton, S., Balfour, H. H., Bloomer, J. R. Cytomegalovirus infection of the liver in transplant recipients. *J. Clin. Gastroenterol.* **9**(6):659–665, 1987.

Snydman, D. R., Werner, B. G., Heinze-Lacey, B., *et al.* Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. *N. Engl. J. Med.* **317**:1049–1054, 1987.

Sodroski, J., Patarca, R., Rosen, C., *et al.* Location of the trans-activating region on the genome of human T-cell lymphotropic virus type III. *Science* **229**:74, 1985.

Sölt-Szöts, J. Virologische und serologische Untersuchungen beim Herpes zoster. *Arch. Klin. Exp. Dermatol.* **220:**105–128, 1964.

Somagyi, T., Colimon, R., Michelson, S. An illustrated guide to the structure of the human cytomegalovirus genome and a review of transcription data. *Prog. Med. Virol.* **33:**99–133, 1986.

Spaete, R. R., Frenkel, N. The herpes simplex virus amplicon: Analysis of cis-acting replication functions. *Proc. Natl. Acad. Sci. U.S.A.* **82:**694–698, 1982.

Spaete, R. R., Mocarski, E. S. The a sequence of the cytomegalovirus genome functions as a cleavage/packaging signal for herpes simplex virus defective genomes. *J. Virol.* **54:**817–824, 1985.

Spaete, R., Thayer, R., Probert, W., *et al.* Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. *Virology* **167:**207–225, 1988.

Spear, J. B., Kessler, H. A., Dworin, A., Semel, J. Erythema nodosum associated with acute cytomegalovirus mononucleosis in an adult. *Arch. Intern. Med.* **148:**323–324, 1988.

Spear, P. G. Glycoproteins specified by herpes simplex virus. In B. Roizman (ed.), *The Herpesviruses,* Vol. 3. Plenum Press, New York, 1985, pp. 315–356.

Spector, D. H., Vacquier, J. P. Human cytomegalovirus (strain AD169) contains sequences related to the avian retrovirus oncogene v-myc. *Proc. Natl. Acad. Sci. U.S.A.* **80:**3389–3393, 1983.

Spector, D. H., Shaw, S. B., Hock, L. J., *et al.* Association of human cytomegalovirus with Kaposi's sarcoma. In M. S. Gottlieb, and J. E. Groopman (eds.), *Acquired Immune Deficiency Syndrome.* Alan R. Liss, New York, 1984, pp. 109–126.

Spector, S. A. Transmission of cytomegalovirus among infants in hospital documented by restriction-endonuclease-digestion analysis. *Lancet* **1:**378–381, 1983.

Spector, S. A., Spector, D. H. Use of restriction endonuclease mapping to document nosocomial acquisition of cytomegalovirus by monozygotic twins. In *International Conference on Human Herpesviruses.* Atlanta, 1980.

Spector, S. A., Spector, D. H. Molecular epidemiology of cytomegalovirus infections in premature twin infants and their mother. *Pediatr. Infect. Dis.* **1:**405–409, 1982.

Spector, S. A., Rua, J. A., Spector, D. H., McMillan, R. Detection of human cytomegalovirus in clinical specimens by DNA–DNA hybridization. *J. Infect. Dis.* **150:**121–126, 1984a.

Spector, S. A., Hirata, K. K., Neuman, P. R. Identification of multiple cytomegalovirus strains in homosexual men with acquired immunodeficiency syndrome. *J. Infect. Dis.* **150:**953–956, 1984b.

Speel, L. F., Osborn, J. E., Walker, D. L. An immuno-cytopathogenic interaction between sensitized leucocytes and epithelial cells carrying a persistent noncytocidal myxovirus infection. *J. Immunol.* **101:**409–417, 1968.

Spencer, E. S. Clinical aspects of cytomegalovirus infection in kidney-graft recipients. *Scand. J. Infect. Dis.* **6:**315–323, 1974.

Spencer, E. S., Andersen, H. K. Clinically evident, non-terminal infections with herpesviruses and the wart virus in immunosuppressed renal allograft recipients. *Br. Med. J.* **3:**251–254, 1970.

Spencer, E. S., Andersen, H. K. Viral infections in renal allograft recipients treated with long-term immunosuppression. *Br. Med. J.* **2:**829–830, 1979.

Stagno, S., Reynolds, D. W., Lakeman, A., *et al.* Congenital cytomegalovirus infection: Consecutive occurrence due to viruses with similar antigenic compositions. *Pediatrics* **52:**788–794, 1973.

Stagno, S., Reynolds, D. W., Tsiantos, A., *et al.* Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natally acquired cytomegalovirus infections. *J. Infect. Dis.* **132:**568–577, 1975a.

Stagno, S., Reynolds, D. W., Tsiantos, A., *et al.* Cervical cytomegalovirus excretion in pregnant and nonpregnant women: Suppression in early gestation. *J. Infect. Dis.* **131:**522–527, 1975b.

Stagno, S., Volanakis, J. E., Reynolds, D. W., *et al.* Immune complexes in congenital and natal cytomegalovirus infections of man. *J. Clin. Invest.* **60:**838–845, 1977a.

Stagno, S., Reynolds, D. W., Huang, E. S., *et al.* Congenital cytomegalovirus: Occurrence in an immune population. *N. Engl. J. Med.* **296:**1254–1258, 1977b.

Stagno, S., Reynolds, D. W., Amos, C. S., *et al.* Auditory and visual defects resulting from symptomat-

ic and subclinical congenital cytomegaloviral and *Toxoplasma* infections. *Pediatrics* **59:**669–678, 1977c.

Stagno, S., Reynolds, D. W., Pass, R. F., Alford, C. A., Jr. Breast milk and the risk of cytomegalovirus infection. *N. Engl. J. Med.* **302:**1073–1074, 1980.

Stagno, S., Pass, R. F., Dworsky, M. E., *et al.* Congenital cytomegalovirus infection: The relative importance of primary and recurrent maternal infection. *N. Engl. J. Med.* **306:**945–949, 1982a.

Stagno, S., Pass, R. F., Thomas, J. P., *et al.* Defects of tooth structure in congenital cytomegalovirus infection. *Pediatrics* **69:**646–648, 1982b.

Stagno, S., Pass, R. F., Dworsky, M. E., Alford, C. A., Jr. Congenital and perinatal cytomegalovirus infections. In M. S. Amstey (ed.), *Virus Infection in Pregnancy.* Gruane & Stratton, New York, 1984, pp. 105–123.

Stagno, S., Tinker, M. K., Elrod, C., *et al.* Immunoglobulin M antibodies detected by enzyme-linked immunosorbent assay and radioimmunoassay in the diagnosis of cytomegalovirus infections in pregnant women and newborn infants. *J. Clin. Microbiol.* **21:**930–935, 1985.

Stagno, S., Pass, R. F., Cloud, G., *et al.* Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus and clinical outcome. *J.A.M.A.* **256:**1904–1908, 1986.

Stainier, P., Taylor, D. L., Kitchen, A. D., *et al.* Persistance of cytomegalovirus in mononuclear cells in peripheral blood from blood donors. *Brit. Med. J.* **299:**897–898, 1989.

Stalder, H., Ehrensberger, A. Microneutralization of cytomegalovirus. *J. Infect. Dis.* **142:**102–105, 1980.

Stannard, L., Rider, J., Farrar, G. Morphology and distribution of gp52 on extracellular human cytomegalovirus (HCMV) supports biochemical evidence that it represents the HCMV glycoprotein B. *J. Gen. Virol.* **70:**1553–1560, 1989.

Staprans, S. I., Spector, D. H. 2.2-Kilobase class of early transcripts encoded by cell-related sequences in human cytomegalovirus strain AD169. *J. Virol.* **57:**591–602, 1986.

Staprans, S. I., Rabert, D. K., Spector, D. H. Identification of sequence requirements and trans-acting functions necessary for regulated expression of a human cytomegalovirus early gene. *J. Virol.* **62:**3463–3473, 1988.

Starr, J., Gold, E. Congenital cytomegalovirus infection associated with low birth weight. *J. Pediatr.* **74:**815–816, 1969.

Starr, J. G., Calafiore, D., Casey, H. L. Experience with a human cytomegalovirus complement fixing antigen. *Am. J. Epidemiol.* **86:**507–512, 1967.

Starr, S. E., Allison, A. C. Role of T lymphocytes in recovery from murine cytomegalovirus infection. *Infect. Immun.* **17:**458–462, 1977.

Starr, S. E., Tolpin, M. D., Freidman, H. J., *et al.* Impaired cellular immunity to cytomegalovirus in congenitally infected children and their mothers. *J. Infect. Dis.* **140:**500–505, 1979.

Starr, S. E., Glazer, J. P., Friedman, H. M., *et al.* Specific cellular and humoral immunity following immunization with live Towne strain of cytomegalovirus vaccine. *J. Infect. Dis.* **143:**585–589, 1981.

Starzl, T. E., Klintmalm, G. B. G., Porter, K. A., *et al.* Liver transplantation with use of cyclosporin A and prednisone. *N. Engl. J. Med.* **305:**266–269, 1981.

Starzl, T. E., Fung, J., Venkataramman, R., *et al.* FK 506 for liver, kidney, and pancreas transplantation. *Lancet* **2:**1000–1004, 1989.

Steele, R. W., Hensen, S. A., Vincent, M. M., *et al.* A ^{51}Cr microassay technique for cell-mediated immunity to viruses. *J. Immunol.* **110:**1502–1510, 1973.

Stenberg, R. M., Stinski, M. F. Autoregulation of the human cytomegalovirus major immediate-early gene. *J. Virol.* **56:**676–682, 1985.

Stenberg, R. M., Thomsen, D. R., Stinski, M. F. Structural analysis of the major immediate early gene of human cytomegalovirus. *J. Virol.* **49:**190–199, 1984.

Stenberg, R. M., Witte, P. R., Stinski, M. F. Multiple spliced and unspliced transcripts from human cytomegalovirus immediate-early region 2 and evidence for a common initiation site within immediate-early region 1. *J. Virol.* **56:**665–675, 1985.

Stenberg, R. M., Depto, A. S., Fortney, J., Nelson, J. A. Regulated expression of early and late RNAs and proteins from the human cytomegalovirus immediate-early gene region. *J. Virol.* **63**:2699–2708, 1989.

Stern, H. Isolation of cytomegalovirus and clinical manifestations of infection at different ages. *Br. Med. J.* **1**:665–669, 1968.

Stern, H., Elek, S. D. The incidence of infection with cytomegalovirus in a normal population. *J. Hyg. (Camb.)* **63**:79–87, 1965.

Stern, H., Friedman, I. Intranuclear formation of cytomegalovirus inclusion disease virus. *Nature* **188**:768–770, 1960.

Stern, H., Tucker, S. M. Prospective study of cytomegalovirus infection in pregnancy. *Br. Med. J.* **2**:268–270, 1973.

Sterner, G., Agell, B. O., Sahren, B., Espmark, A. Acquired cytomegalovirus infection in older children and adults. *Scand. J. Infect. Dis.* **2**:95–103, 1970.

Stevely, W., Katan, M., Stirling, V., *et al.* Protein kinase activities associated with the virions of pseudorabies and herpes simplex virus. *J. Gen. Virol.* **66**:661–673, 1985.

Stevens, D. P., Barker, L. F., Ketcham, A. S., Meyer, H. M. Asymptomatic cytomegalovirus infection following blood transfusion in tumor surgery. *J.A.M.A.* **211**:1341–1344, 1970.

Stinski, M. F. Human cytomegalovirus: Glycoproteins associated with virions and dense bodies. *J. Virol.* **19**:594–609, 1976.

Stinski, M. F. Synthesis of proteins and glycoproteins in cells infected with human cytomegalovirus. *J. Virol.* **23**:751–767, 1977.

Stinski, M. F. Sequence of protein synthesis in cells infected by human cytomegalovirus: Early and late virus-induced polypeptides. *J. Virol.* **26**:686–701, 1978.

Stinski, M. F. Molecular biology of cytomegaloviruses. In B. Roizman (ed.), *The Herpesviruses.* Plenum Press, New York, 1983, pp. 67–113.

Stinski, M. The proteins of human cytomegalovirus. *Birth Defects* **20**:49–62, 1984.

Stinski, M. F., Roehr, T. J. Activation of the major immediate early gene of human cytomegalovirus by cis-acting elements in the promoter–regulatory sequence and by virus-specific trans-acting components. *J. Virol.* **55**:431–441, 1985.

Stinski, M. F., Mocarski, E. S., Thomsen, D. R. DNA of human cytomegalovirus: Size heterogeneity and defectiveness resulting from serial undiluted passage. *J. Virol.* **31**:231–239, 1979a.

Stinski, M. F., Mocarski, E. S., Thomsen, D. R., Urbanowski, M. L. Membrane glycoproteins and antigens induced by human cytomegalovirus. *J. Gen. Virol.* **43**:119–129, 1979b.

Stinski, M. F., Thomsen, D. R., Wathen, M. W. Structure and function of the human cytomegalovirus genome. In A. Nahmias, W. Dowdle, and R. Schinazi (eds.), *The Human Herpesviruses.* Elsevier/North-Holland, New York, 1980, pp. 72–84.

Stinski, M. F., Thomsen, D. R., Rodriguez, J. E. Synthesis of human cytomegalovirus-specified RNA and protein in interferon-treated cells at early times after infection. *J. Gen. Virol.* **60**:261–270, 1982.

Stinski, M. F., Thomsen, D. R., Stenberg, R. M., Goldstein, L. C. Organization and expression of the immediate early genes of human cytomegalovirus. *J. Virol.* **46**:1–14, 1983.

Stinski, M. F., Stenberg, R. M., Goins, W. F. Structure and function of the human cytomegalovirus immediate early genes. In F. Rapp (ed.), *Herpesvirus.* Alan R. Liss, New York, 1984, pp. 399–421.

Stocke, E., Popow-Kraupp, T., Heinz, F. X., *et al.* Potential of *in situ* hybridization for early diagnosis of productive cytomegalovirus infection. *J. Clin. Microbiol.* **26**:2536–2540, 1988.

Stow, N. D., McMonagle, E. C., Davison, A. J. Fragments from both termini of the herpes simplex virus type 1 genome contain signals required for the encapsidation of viral DNA. *Nucleic Acids Res.* **11**:8205–8220, 1983.

Strand, O. A., Hoddevik, G. M. The diagnostic significance of specific serum IgA detection in cytomegalovirus infections. *Arch. Virology* **82**:173–180, 1984.

Strauss, M. A clinical pathologic study of hearing loss in congenital cytomegalovirus infection. *Laryngoscope* **95**:951–962, 1985.

Strauss, M., Davis, G. L. Viral disease of the labyrinth: I. Review of the literature and discussion of the role of cytomegalovirus in congenital deafness. *Ann. Otol. Rhinol. Laryngol.* **82**:577–583, 1973.

Strayer, D. S., Phillips, G. B., Barker, K. H., *et al.* Gastric cytomegalovirus infection in bone marrow transplant patients: An indication of generalized disease. *Cancer* **48**:1478–1483, 1981.

Stribling, J., Weitzner, S., Smith, G. V. Kaposi's sarcoma in a renal allograft recipient. *Cancer* **42**:442–446, 1978.

Strickland, S., Mahdavi, V. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell* **15**:393–403, 1978.

Stringfellow, D. A., Glasgow, L. A. Hyporeactivity of infection: Potential limitation to therapeutic use of interferon-inducing agents. *Infect. Immun.* **6**:743–747, 1972.

Strong, D. J., Ahmed, A., Knudsen, R. C., *et al.* Cellular immunity to cytomegalovirus in a patient following bone marrow transplantation. *Transplantation* **26**:99–102, 1978.

Stulberg, C. S., Zuelzer, W. W., Page, R. H., *et al.* Cytomegalovirus infections with reference to isolations from lymph nodes and blood. *Proc. Soc. Exp. Biol. Med.* **123**:976–982, 1966.

Subietas, A., Deppisch, L. J., Astarloa, J. Cytomegalovirus ooophoritis: Ovarian cortical necrosis. *Hum. Pathol.* **8**:285–292, 1977.

Sullivan, M. P., Hanshaw, J. B., Cangir, A., Butler, J. J. Cytomegalovirus complement-fixation antibody levels of leukemic children. *J.A.M.A.* **206**:569–574, 1968.

Sullivan-Tailyour, G., Garnett, H. M. Plasma membrane proteins and glycoproteins induced by human cytomegalovirus infection of human embryonic fibroblasts. *J. Gen. Virol.* **67**:515–526, 1986.

Surawicz, C. M., Myerson, D. Self-limited cytomegalovirus colitis in immunocompetent individuals. *Gastroenterology* **94**:194–199, 1988.

Sutton, R. N. P., Darby, C. W., Gumpel, S. M. Cytomegalovirus infection in childhood leukemia. *Br. J. Haematol.* **20**:437–442, 1971.

Suwansirikul, S., Rao, N., Dowling, J. N., Ho, M. Clinical manifestations of primary and secondary cytomegalovirus infection after renal transplantation. *Arch. Intern. Med.* **137**:1026–1029, 1977.

Swack, N. S., Liu, O. C., Hsiung, G. D. Cytomegalovirus infections of monkeys and baboons. *Am. J. Epidemiol.* **94**:397–402, 1971.

Swack, N. S., Michalski, F. J., Baumgarten, A., Hsiung, G. D. Indirect fluorescent-antibody test for human cytomegalovirus infection in the absence of interfering immunoglobulin G receptors. *Infect. Immun.* **16**:522–526, 1977.

Sweet, G. H., Tegtmeier, G. E., Bayer, W. L. Antigens of human cytomegalovirus: Electronimmunodiffusion assay and comparison among strains. *J. Gen. Virol.* **43**:707–712, 1979.

Symmers, W. St. C. Generalized cytomegalic inclusion-body disease associated with pneumocystis pneumonia in adults. *J. Clin. Pathol.* **13**:1–21, 1960.

Tajima, T. An autopsy case of primary cytomegalic inclusion enteritis with remarkable hypoproteinemia. *Acta Pathol. Jpn.* **24**:151–162, 1974.

Tamashiro, J. C., Filpula, D., Freidmann, T., Spector, D. H. Structure of the heterogeneous L-S junction region of human cytomegalovirus strain AD169 DNA *J. Virol.* **52**:541–548, 1984.

Tamura, H. Acute ulcerative colitis associated with cytomegalic inclusion virus. *Arch. Pathol.* **96**:477–482, 1973.

Tamura, T., Chiba, S., Chiba, Y., Nakao, T. Virus excretion and neutralizing antibody response in saliva in human cytomegalovirus infection. *Infect. Immun.* **29**:842–845, 1980a.

Tamura, T., Chiba, S., Abo, W., *et al.* Cytomegalovirus-specific lymphocyte transformations in subjects of different ages with primary immunodeficiency. *Infect. Immun.* **28**:49–53, 1980b.

Tanaka, J., Ogura, T., Sato, H., Hatano, M. Establishment and biological characterization of an *in vitro* human cytomegalovirus latency model. *Virology* **161**:62–72, 1987.

Tanaka, J., Ogura, T., Iida, H., *et al.* Inhibitors of prostaglandin synthesis inhibit growth of human

cytomegalovirus and reactivation of latent virus in a productively and latently infected human cell line. *Virology* **163:**205–208, 1988.

Tanaka, S., Furukawa, T., Plotkin, S. A. Human cytomegalovirus stimulates host cell RNA synthesis. *J. Virol.* **15:**297–304, 1975.

Tarr, G. C., Armstrong, J. A., Ho, M. Production of interferon and serum hyporeactivity factor in mice with murine cytomegalovirus. *Infect. Immun.* **19:**903–907, 1978.

Taylor, D. L., Taylor-Robinson, D., Jeffries, D. J., Tyms, A. S. Characterization of cytomegalovirus isolates from patients with AIDS by DNA-restriction analysis. *Epidem. Inf.* **101:**483–493, 1988.

Taylor, R. J., Saul, S. H., Dowling, J. N., *et al.* Primary disseminated herpes simplex infection with fulminant hepatitis following renal transplantation. *Arch. Intern. Med.* **141:**1519–1521, 1981.

Tegtmeyer, P. J., Craighead, J. E. Infection of adult mouse macrophage *in vitro* with cytomegalovirus. *Proc. Soc. Exp. Biol. Med.* **129:**690–694, 1968.

Teixidor, H. S., Honig, C. L., Norsoph, E., *et al.* Cytomegalovirus infection of the alimentary canal: Radiologic findings with pathologic correlation. *Radiology* **163:**317–323, 1987.

Ten Bensel, R. W., St. Geme, J. W., Jr. A search for the reservoir of cytomegalovirus in salivary gland tissue. *J. Pediatr.* **72:**479–482, 1968.

Ten Napel, C. H. H., The, T. H. Acute cytomegalocirus infection and the host immune response. I. Development and maintenance of cytomegalovirus induced *in vitro* lymphocyte reactivity and its relationship to production of cytomegalovirus antibodies. *Clin. Exp. Immunol.* **39:**263–271, 1980.

Tevethia, M. J., Spector, D. J., Leisure, K. M., *et al.* Participation of two human cytomegalovirus immediate early gene regions in transcriptional activation of adenovirus promoters. *Virology* **161:**276–285, 1987.

Tevethia, S. S., Flyer, D. C., Tijian, R. Biology of simian virus 40 (SV40) transplantation antigen (TrAg). *Virology* **107:**13–23, 1980.

The, T. H., Langenhuysen, M. M. A. C. Antibodies against membrane antigens of cytomegalovirus infected cells in sera of patients with a cytomegalovirus infection. *Clin. Exp. Immunol.* **11:**475–482, 1972.

The, T. H., Klein, G., Langenhuysen, M. M. A. C. Antibody reactions to virus-specific early antigens (EA) in patients with cytomegalovirus (CMV) infection. *Clin. Exp. Immunol.* **16:**1–12, 1974.

The, T. H., Anderson, H. K., Spencer, E. S., Klein, G. Antibodies against cytomegalovirus-induced early antigens (CMV-EA) in immunosuppressed renal allograft recipients. *Clin. Exp. Immunol.* **28:**502–505, 1977.

Thomas, I. T., Soothill, J. F., Hawkins, G. T., Marshall, W. C. Transfer-factor treatment in congenital cytomegalovirus infection. *Lancet* **2:**1056–1057, 1977.

Thomas, W. A., Kim, D. N. Biology of disease. Atherosclerosis as a hyperplastic and/or neoplastic process. *Lab. Invest.* **48:**245–255, 1983.

Thomason, A. W. FK-506—How much potential? *Immunol. Today* **11:**6–9, 1990.

Thomsen, D. R., Stenberg, R. M., Goins, W. F., Stinski, M. F. Promoter–regulatory region of the major immediate early gene of human cytomegalovirus. *Proc. Natl. Acad. Sci. U.S.A.* **81:**659–663, 1984.

Thong, Y. H., Hensen, S. A., Vincent, M. M., *et al.* Use of cryopreserved virus-infected target cells in a lymphocytotoxicity ^{51}Cr release microassay for cell-mediated immunity to cytomegalovirus. *Infect. Immun.* **13:**643–645, 1976.

Tidy, H. L. Glandular fever and infectious mononucleosis. *Lancet* **2:**180–186, 1934.

Tilney, N. L., Collins, J. J. Jr., Wilson, R. E. Hemorrhagic pancreatitis. A fatal complication of renal transplantation. *N. Engl. J. Med.* **274:**1051–1057, 1966.

Tindall, R. S. A., Cloud, R., Luby, J., Rosenberg, R. N. Serum antibodies to cytomegalovirus in myasthemia gravis: Effects of thymectomy and steroids. *Neurology (Minneap.)* **28:**273–277, 1978.

Tobin, J. O'H., Warrell, M. J., Morris, P. J. Cytomegalovirus infection and renal transplantation, *Lancet* **1:**926–926, 1979.

Tocci, M. J., St. Jeor, S. C. Persistence and replication of the human cytomegalovirus genome in lymphoblastoid cells of B and T origin. *Virology* **96:**664–668, 1979.

Toghill, P. J., Bailey, M. E., Williams, R., *et al.* Cytomegalovirus hepatitis in the adult. *Lancet* **1**:1351–1354, 1967.

Tokumaru, T. A possible role if IgA-immunoglobulin in herpes simplex infection in man. *J. Immunol.* **97**:248–259, 1966.

Tolkoff-Rubin, N. E., Rubin, R. H., Keller, E. E., *et al.* Cytomegalovirus infection in dialysis patients and personnel. *Ann. Intern. Med.* **89**:625–628, 1978.

Tolpin, M. D., Stewart, J. A., Warren, D., *et al.* Transfusion transmission of cytomegalovirus confirmed by restriction endonuclease analysis. *J. Pediatr.* **6**:953–956, 1985.

Toorkey, C. B., Carrigan, D. R. Immunohistochemical detection of an immediate early antigen of human cytomegalovirus in normal tissues. *J. Infect. Dis.* **160**:741–751, 1989.

Townsend, C. T. R., Bolyard, E. H., Yolken, R. H., *et al.* Outbreak of Coxsackie A/gastroenteritis: A complication of bone-marrow transplantation. *Lancet* **1**:820–823, 1982.

Traswell, H. F., Reisner, R. K., Rabe, D. E., *et al.* Comparison of three methods for detecting antibody to cytomegalovirus. *Transfusion* **26**:285–289, 1986.

Trlifajova, J., Sourek, J., Seeman, J. A study on cytomegalovirus by the gel precipitation method. *Zentralbl. Bakteriol. [Orig. A]* **221**:433–438, 1972.

Trump, B. F., Arstila, A. U. Cell membranes and diseases processes. In B. F. Trump and A. U. Arstila (eds.), *Pathobiology of Cell Membranes,* Vol. 1. Academic Press, New York, 1975, pp. 1–103.

Tumilowicz, J. J., Gawlik, M. E., Powell, B. B. Replication of cytomegalovirus in human arterial smooth muscle cells. *J. Virol.* **56**:839–845, 1985.

Turtinen, L. W., Saltzman, R., Jordan, M. C., Haase, A. T. Interactions of human cytomegalovirus with leukocytes *in vivo:* Analysis by *in situ* hybridization. *Microbiol. Pathol.* **3**:287–297, 1987.

Tymus, A. S., Davis, J. M., Jeffries, D. J., Meyers, J. D. BWB759U, an analogue of acyclovir, inhibits human cytomegalovirus *in vitro. Lancet* **2**:294–295, 1984.

Tyzzer, E. E. The histology of the skin lesions in varicella. *Philipp. J. Sci.* **1**:349–372, 1906.

Umetsu, M., Chiba, Y., Horino, K., *et al.* Cytomegalovirus-mononucleosis in a newborn infant. *Arch. Dis. Child.* **50**:496–598, 1975.

Unkeless, J. C., Tobia, A., Ossowski, L., *et al.* An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. I. Chick embryo fibroblast cultures transformed by avian RNA tumor viruses. *J. Exp. Med.* **137**:85–111, 1973.

Utz, U., Britt, W., Vugler, L., Mach, M. Identification of a neutralizing epitope on glycoprotein gp58 of human cytomegalovirus. *J. Virol.* **63**:1995–2001, 1989.

Van den Berg, F. M., van Ooyen, A. J. J., Volkers, H., Walboomers, J. M. M. Heterogeneity in subregions of the terminal repeats of herpes simplex virus type 2 DNA. *Intervirology* **21**:96–103, 1984.

Van der Bij, W., Schirm, J., Torensma, R., *et al.* Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. *J. Clin. Microbiol.* **26**:2531–2535, 1988.

Van der Voort, L., Leij, L., The, T. Characterization of membrane antigens on human cytomegalovirus-infected fibroblasts recognized by human antibodies. *J. Virol.* **63**:1485–1488, 1989.

Van Es, A., Baldwin, W. M., Oljans, P. J., *et al.* Expression of HLA-DR on T lymphocytes following renal transplantation, and association with graft-rejection episodes and cytomegalovirus infection. *Transplantation* **37**:65–69, 1984.

Varmuza, S. L., Smiley, J. R. Signals for site-specific cleavage of HSV DNA: Maturation involves two separate cleavage events at sites distal to the recognition sequences. *Cell* **41**:793–802, 1985.

Verheyden, J. P. H. Evolution of therapy for cytomegalovirus infection. *Rev. Infect. Dis.* **10**:S477–489, 1988.

Via, C. S., Shanley, J. D., Weatherly, B. R., *et al.* Altered threshold for the induction of graft-versus-host immunodeficiency following murine cytomegalovirus infection. *Transplantation* **30**:298–302, 1988.

Vierling, J. M., Fennell, R. H. Histopathology of early and late human hepatic allograft rejection: Evidence of progressive destruction of interlobular bile ducts. *Hepathology* **5**:1076–1082, 1985.

Vietzke, W. M., Gelderman, A. H., Grimley, P. M., Valsamis, M. P. Toxoplasmosis complicating malignancy. *Cancer* **21**:816–827, 1968.

Vinh, L. T., Van Duc, T., Aicardi, J., Rossier, A., Thieffry, S. Association de toxoplasmose congenitale et de cytomegalie chez le nourisson. *Arch. Fr. Pediatr.* **27**:511–521, 1970.

Vinters, H. V., Kwok, M. K., Ho, H. W., *et al.* Cytomegalovirus in the nervous system of patients with the acquired immune deficiency syndrome. *Brain* **112**:245–268, 1989.

Vlazny, D. A., Kwong, A., Frenkel, N. Site-specific cleavage/packaging of herpes simplex virus DNA and the selective maturation of nucleocapsids containing full-length viral DNA. *Proc. Natl. Acad. Sci. U.S.A.* **79**:1423–1427, 1982.

Vogel, F. S., Pinkerton, H. Spontaneous salivary gland disease in chimpanzees. *Arch. Pathol.* **60**:281–285, 1955.

Volkmer, H., Bertholet, C., Jonjic, S., *et al.* Cytolytic T lymphocyte recognition of the murine cytomegalovirus nonstructural immediate-early protein pp89 expressed by recombinant vaccinia virus. *J. Exp. Med.* **166**:668–677, 1987.

Volpi, A., Britt, W. J. Serological heterogeneity of CMV isolates with a monoclonal antibody. *J. Infect. Dis.* **152**:648–649, 1985.

Von Glahn, W. C., Pappenheimer, A. M. Intranuclear inclusions in visceral disease. *Am. J. Pathol.* **1**:445–465, 1925.

Vonka, V., Benyesh-Melnick, M. Thermoinactivation of human cytomegalovirus. *J. Bacteriol.* **91**:221–226, 1966.

Vonka, V., Anisimova, E., Macek, M. Replication of cytomegalovirus in human epitheloid diploid cell line. *Arch. Virol.* **52**:153–169, 1976.

Von Willebrand, E. V., Pettersson, E., Ahonen, J., Hayry, P. CMV infection, class II antigen expression, and human kidney allograft rejection. *Transplantation* **42**:364–367, 1986.

Vortel, V. Ueber das Vorkommen von Encephalitis bei Saeuglingen und Kindern. *Frankfort Z. Pathol.* **67**:153–169, 1956.

Wade, J. C., McGuffin, R. W., Springmeyer, S. C., *et al.* Treatment of cytomegaloviral pneumonia with high-dose acyclovir and human leukocyte interferon. *J. Infect. Dis.* **148**:557–562, 1983.

Wade, J. C., Newton, B., Flournoy, N., Meyers, J. D. Oral acyclovir for prevention of herpes simplex virus reactivation after marrow transplant. *Ann. Intern. Med.* **100**:823–828, 1984.

Wadsworth, S., Hayward, G. S., Roizman, B. Anatomy of herpes simplex virus DNA. V. Terminally repetitive sequences. *J. Virol.* **17**:503–512, 1976.

Wager, O., Rasanen, J. A., Hagman, A., Klemonla, E. Mixed cryoimmunoglobulinanaemia in infectious mononucleosis and cytomegalovirus mononucleosis. *Int. Arch. Allergy Appl. Immunol.* **34**:345–362, 1968.

Wagner, H. Zur Kenntnis der "protozoenartigen Zellen" in den Organanen von Kindern. *Beitr. Pathol. Anat. Allg. Pathol.* **85**:145–164, 1930.

Wainwright, J. Cytomegalovirus infection in black children in Durban. *S. Afr. Med. J.* **48**:1408–1412, 1972.

Walker, B. D., Flexner, C., Paradis, T. J., *et al.* HIV-1 reverse transcriptase is a target for cytotoxic T lymphocytes in infected individuals. *Science* **240**:64–66, 1988.

Waltzer, W. C., Arnold, A. N., Anaise, D., *et al.* Impact of cytomegalovirus infection and HLA-matching on outcome of renal transplantation. *Transplant. Proc.* **19**:4077–4080, 1987.

Walzer, P. D., Perl, D. P., Krogstad, P. J., Rawson, P. G., Schultz, M. G. *Pneumocystis carinii* pneumonia in the United States. Epidemiologic, diagnostic and clinical features. *Ann. Intern. Med.* **80**:83–93, 1974.

Waner, J. L. Partial characterization of a soluble antigen preparation from cells infected with human cytomegalovirus: Properties of antisera prepared to the antigen. *J. Immunol.* **114**:1454–1457, 1975.

Waner, J. L., Biano, S. A. Antibody reactivity to human and vervet cytomegalovirus early antigen(s) in sera from patients with active cytomegalovirus infections and from asymptomatic donors. *J. Clin. Microbiol.* **9**:134–140, 1979.

Waner, J. L., Budnick, J. E. Three-day assay for human cytomegalovirus applicable to serum neutralization tests. *Appl. Microbiol.* **25**:37–39, 1973.

Waner, J. L., Weller, T. H. Behavior of human cytomegaloviruses in cell cultures of bovine and simian origin. *Proc. Soc. Exp. Biol. Med.* **145**:379–384, 1974.

Waner, J. L., Weller, T. H. Analysis of antigenic diversity among human cytomegalovirus by kinetic neutralization tests with high-titered rabbit antisera. *Infect. Immun.* **21**:151–157, 1978.

Waner, J. L., Weller, T. H., Kevy, S. V. Patterns of cytomegalovirus complement-fixing antibody activity: A longitudinal study of blood donors. *J. Infect. Dis.* **127**:538–543, 1973.

Waner, J. L., Hopkins, D. R., Weller, T. H., Alfred, E. N. Cervical excretion of cytomegalovirus: Correlation with secretory and humoral antibody. *J. Infect. Dis.* **136**:805–809, 1977.

Wang, N. S., Huang, S. H., Thurlbeck, W. M. Combined *Pneumocystis carinii* and cytomegalovirus infection. *Arch. Pathol.* **90**:529–535, 1970.

Ward, K. P., Galloway, W. H., Auchterlonie, I. A. Congenital cytomegalovirus infection and diabetes. *Lancet* **1**:497–497, 1979.

Ward, P. H., Lindsay, J. R., Warner, N. E. Cytomegalic inclusion disease affecting the temporal bone. *Laryngoscope* **75**:628–638, 1965.

Ware, A. J., Luby, J. P., Hollinger, B., *et al.* Etiology of liver disease in renal transplant patients. *Ann. Intern. Med.* **91**:364–371, 1979.

Wathen, M., Stinski, M. Temporal patterns of human cytomegalovirus transcription: Mapping the viral RNAs synthesized at immediate early, early, and late times after infection. *J. Virol.* **41**:462–477, 1982.

Wathen, M. W., Thomsen, D. R., Stinski, M. F. Temporal regulation of human cytomegalovirus transcription at immediate early and early times after infection. *J. Virol.* **38**:446–459, 1981.

Wayne, E. R., Burrington, J. D., Myers, D. N., *et al.* Bilateral eventration of the diaphram in a neonate with congenital cytomegalic inclusion disease. *J. Pediatr.* **83**:164–165, 1973.

Weber, P. C., Challberg, M. D., Nelson, N. J., *et al.* Inversion events in the HSV-1 genome are directly mediated in the viral DNA replication machinery and lack sequence specificity. *Cell* **54**:369–381, 1988.

Webster, A., Cook, D. G., Emery, V. C., *et al.* Cytomegalovirus infection and progression towards AIDS in haemophiliacs with human immunodeficiency virus infection. *Lancet* **2**:63–66, 1989.

Weigand, D. A., Burgdorf, W. H. C., Tarpay, M. M. Vasculitis in cytomegalovirus infection. *Arch. Dermatol.* **116**:1174–1176, 1980.

Weimar, W., Lameijer, L. D. F., Edy, V. G., Schellekens, H. Prophylactic use of interferon in renal allograft recipients. *Transplant Proc.* **11**:69–70, 1979.

Weiner, D., Gibson, W., Fields, K. Anti-complement immunofluorescence establishes nuclear localization of human cytomegalovirus matrix protein. *Virology* **147**:19–28, 1986.

Weinshenker, B. G., Wilton, S., Rice, G. P. A. Phorbol ester-induced differentiation permits productive human cytomegalovirus infection in a monocytic cell line. *J. Immunol.* **140**:1625–1631, 1988.

Weir, M. R., Irwin, B. C., Maters, A. W., *et al.* Incidence of cytomegalovirus disease in cyclosporine-treated renal transplant recipients based on donor/recipient pretransplant immunity. *Transplantation* **43**:187–193, 1987.

Weller, T. H. Serial propagation *in vitro* of agents producing inclusion bodies derived from varicella and herpes zoster. *Proc. Soc. Exp. Biol. Med.* **83**:340–346, 1953.

Weller, T. H. Cytomegalovirus: The difficult years. *J. Infect. Dis.* **122**:532–539, 1970.

Weller, T. H. The cytomegaloviruses: Ubiquitous agents with protean clinical manifestations. *N. Engl. J. Med.* **285**:203–214, 267–274, 1971.

Weller, T. H. Varicella and herpes zoster. In E. H. Lennette and N. J. Schmidt (eds.), *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections.* American Public Health Association, Washington, 1979, pp. 375–398.

Weller, T. H., Hanshaw, J. B. Virologic and clinical observations on cytomegalic inclusion disease. *N. Engl. J. Med.* **266**:1233–1244, 1962.

Weller, T. H., Macauley, J. C., Craig, J. M., Wirth, P. Isolation of intranuclear inclusion producing

agents from infants with illnesses resembling cytomegalic inclusion disease. *Proc. Soc. Exp. Biol. Med.* **94:**4–12, 1957.

Weller, T. H., Witton, H. M., Bell, E. J. The etiologic agents of varicella and herpes zoster. Isolation, propagation and cultural characteristics *in vitro*. *J. Exp. Med.* **108:**843–868, 1958.

Weller, T. H., Hanshaw, J. B., Scott, D. E. Serologic differentiation of viruses responsible for cytomegalic inclusion disease. *Virology* **12:**130–132, 1960.

Welsh, R. M. Cytotoxic cells induced during lymphocytic choriomeningitis virus infection of mice. I. Characterization of natural killer cell induction. *J. Exp. Med.* **148:**163–181, 1978.

Wentworth, B. B., Alexander, E. R. Seroepidemiology of infections due to members of the herpesvirus group. *Am. J. Epidemiol.* **94:**496–507, 1971.

Wentworth, B. B., Gloyd, P. W., Jr. Thermostability of cytomegalovirus strains. *Arch. Ges. Virusforsch.* **25:**255–262, 1968.

Westmoreland, D., Watkins, J. F. The IgG receptor induced by herpes simplex virus: Studies using radioiodinated IgG. *J. Gen. Virol.* **24:**167–178, 1974.

Westmoreland, D., St. Jeor, S., Rapp, F. The development of cytomegalovirus infected cells of binding affinity for normal human immunoglobulin. *J. Immunol.* **116:**1566–1570, 1976.

Weston, K. An enhancer element in the short unique region of human cytomegalovirus regulates the production of a group of abundant immediate early transcripts. *Virology* **162:**404–416, 1988.

Weston, K., Barrell, B. Sequence of the short unique region, short repeats, and part of the long repeats of human cytomegalovirus. *J. Mol. Biol.* **192:**177–208, 1986.

Wheeler, E. O., Turner, J. D., Scannell, J. G. Fever, splenomegaly and atypical lymphocytes: Syndrome observed after cardiac surgery utilizing pump oxygenator. *N. Engl. J. Med.* **266:**454–456, 1962.

Whelchel, J. D., Pass, R. F., Diethelm, A. G., *et al.* Effect of primary and recurrent cytomegalovirus infection upon graft and patient survival after renal transplantation. *Transplantation* **28:**443–446, 1979.

Whitley, R. J., Brasfield, D., Reynolds, D. W., *et al.* Protracted pneumonitis in young infants associated with perinatally acquired cytomegalovirus infection. *J. Pediatr.* **89:**16–22, 1976.

Whitley, R. J., Seng-Jaw, S., Dolin, R., *et al.* Collaborative Study Group. Adenine arabinoside therapy of biopsy-proven herpes simplex encephalitis. *N. Engl. J. Med.* **297:**289–294, 1977.

Wildy, P. Classification and nomenclature of virus. *Monogr. Virol.* **5:**1–81, 1971.

Wildy, P. Herpes: History and classification. In A. S. Kaplan (ed.), *The Herpesviruses*. Academic Press, New York, 1973, pp. 1–22.

Wildy, P., Russell, W. C., Horne, R. W. The morphology of herpesvirus. *Virology* **12:**204–222, 1960.

Wiley, C. A., Nelson, J. A. Role of human immunodeficiency virus and cytomegalovirus in AIDS encephalitis. *Am. J. Pathol.* **133:**73–81, 1988.

Wiley, C. A., Schrier, R. D., Denaro, F. J., *et al.* Localization of cytomegalovirus proteins and genome during fulminant central nervous system infection in an AIDS patient. *J. Neuropathol. Exp. Neurol.* **45:**127–139, 1986.

Wilhelm, J. A., Matter, L., Schopfer, K. The risk of transmitting cytomegalovirus to patients receiving blood transfusions. *J. Infect. Dis.* **154:**169–171, 1986.

Wilkinson, G., Akrigg, A., Greenaway, P. Transcription of the immediate early genes of human cytomegalovirus strain AD 169. *Virus Res.* **1:**101–116, 1984.

Willmott, F. E. Cytomegalovirus in female patients attending a VD clinic. *Br. J. Vener. Dis.* **51:**278–280, 1975.

Wilson, R. S. E., Morris, T. H., Rees, J. R. Cytomegalovirus myocarditis. *Br. Heart J.* **34:**865–868, 1972.

Wingard, J. R., Chen, D. Y.-H., Burns, W. H., *et al.* Cytomegalovirus infection after autologous bone marrow transplantation with comparison to infection after allogenic bone marrow transplantation. *Blood* **71:**1432–1437, 1988a.

Wingard, J. R., Mellits, E. D., Sostrin, M. B., *et al.* Interstitital pneumonitis after allogeneic bone marrow transplantation. *Medicine* **67:**175–186, 1988b.

Winston, D. J., Ho, W. G., Howell, C. L., *et al.* Cytomegalovirus infections associated with leukocyte transfusions. *Ann. Intern. Med.* **93**:671–675, 1980.

Winston, D. J., Pollard, R. B., Ho, W. G., *et al.* Cytomegalovirus immune plasma in bone marrow transplant recipients. *Ann. Intern. Med.* **97**:11–18, 1982.

Winston, D. J., Ho, W. G., Lin, C. H., *et al.* Intravenous immune globulin for prevention of cytomegalovirus infection and interstitial pneumonia after bone marrow transplantation. *Ann. Intern. Med.* **106**:12–18, 1987.

Winston, D. J., Ho, W. G., Champlin, R. E. Cytomegalovirus infection after allogenic bone marrow transplantation. *Rev. Infect. Dis.* **12**(Supp. 7), 5776–5792, 1990.

Wirth, T., Baltimore, D. Nuclear factor NF-k B can interact functionally with its cognate binding site to provide lymphoid-specific promoter function. *EMBO J.* **7**:3109–3113, 1988.

Wise, T. G., Manischewitz, J. E., Quinnan, G. V., *et al.* Latent cytomegalovirus infection of BALB/c mouse spleens detected by an explant culture technique. *J. Gen. Virol.* **44**:551–556, 1979.

Witherspoon, R. P., Storb, R., Mickelson, E. M., *et al.* Association of interstitial pneumonia and dimished *in vitro* lymphocyte blastogenesis in human marrow graft recipients. *Transplantation* **28**:412–414, 1979.

Wolfe, B. M., Cherry, J. D. Hemorrhage from cecal ulcers of cytomegalovirus infection: Report of a case. *Ann. Surg.* **177**:490–494, 1973.

Wong, T. W., Warner, N. E. Cytomegalic inclusion disease in adults. Report of 14 cases with review of literature. *Arch. Pathol.* **74**:403–494, 1962.

Woolf, N. K., Harris, J. P. Cochlear pathophysiology associated with inner ear immune responses. *Acta Otolaryngol.* **102**:353–364, 1986.

Woolf, N. K., Ochi, J. W., Silva, E. J., *et al.* Ganciclovir prophylaxis for cochlear pathophysiology during experimental guinea pig cytomegalovirus labyrinthitis. *Antimicrob. Agents Chemother.* **32**:865–872, 1988.

Wreghitt, T. G., Hakim, M., Gray, J. J., *et al.* Cytomegalovirus infections in heart and heart and lung transplant recipients. *J. Clin. Pathol.* **41**:660–667, 1988.

Wright, D. A., Spector, D. H. Post-transcriptional regulation of a class of human cytomegalovirus phosphoproteins encoded by an early transcription unit. *J. Virol.* **63**:3117–3127, 1989.

Wright, D. A., Staprans, S. I., Spector, D. H. Four phosphorproteins with common amino termini are encoded by human cytomegalovirus AD169. *J. Virol.* **62**:331–340, 1988.

Wright, H. T., Jr. Cytomegaloviruses. In A. S. Kaplan (ed.), *The Herpesviruses*. Academic Press, New York, 1973, pp. 353–388.

Wright, H. T., Jr., Goodheart, C. R., Lielausis, A. Human cytomegalovirus. Morphology by negative staining. *Virology* **23**:419–424, 1964.

Wu, B. C., Ho, M. Characteristics of infection of B and T lymphocytes from mice after inoculation with cytomegalovirus. *Infect. Immun.* **24**:856–864, 1979.

Wu, B. C., Dowling, J. N., Ho, M., *et al.* Enhancement of mouse cytomegalovirus infection during host versus graft reaction. *Science* **190**:56–58, 1975.

Wunderli, W., Kagi, M. K., Gruter, E., Auracher, J. D. Detection of cytomegalovirus in peripheral leukocytes by different methods. *J. Clin. Microbiol.* **27**:1916–1917, 1989.

Wyatt, J. P., Saxton, J., Lee, R. S., Pinkdrton, H. Generalized cytomegalic inclusion disease. *J. Pediatr.* **36**:271–294, 1950.

Wyatt, J. P., Hemsath, F. A., Soash, M. D. Disseminated cytomegalic inclusion disease in an adult (with primary refractory anemia and transfusional siderosis. Report of a case). *Am. J. Clin. Pathol.* **21**:50–55, 1951.

Wyhinny, G. J., Apple, D. J., Guastella, F. R., Vygantas, C. M. Adult cytomegalic inclusion retinitis. *Am. J. Ophthalmol.* **76**:773–781, 1973.

Xu-Bin, T., Ishida, K., Furukawa, T. Characterization of IgG Fc receptors induced by human cytomegalovirus. *J. Gen. Virol.* **70**:893–900, 1989.

Yaminishi, K., Rapp, F. Production of plasminogen activator by human and hamster cells infected with human cytomegalovirus. *J. Virol.* **31**:415–419, 1979.

Yamashiroya, H. M., Ghosh, L., Yang, R., Robertson, A. L. Herpesviridae in the coronary arteries and aorta of young trauma victims. *Am. J. Pathol.* **130**:71–79, 1988.

Yanagisawa, N., Toyokura, Y., Shiraki, H. Double encephalitis with herpes simplex virus and cytomegalovirus in an adult. *Acta Neuropathol.* **33**:153–165, 1975.

Yeager, A. S. Transfusion-acquired cytomegalovirus infection in newborn infants. *Am. J. Dis. Child.* **128**:478–483, 1974.

Yeager, A. S. Longitudinal, serological study of cytomegalovirus infections in nurses and in personnel without patient contact. *J. Clin. Microbiol.* **2**:448–452, 1975.

Yeager, A. Improved indirect hemagglutination test for cytomegalovirus using human O erythrocytes in lysine. *J. Clin. Microbiol.* **10**:64–68, 1979.

Yeager, A. S., Grumet, F. C., Hafleigh, E. B., *et al.* Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. *J. Pediatr.* **98**:281–287, 1981.

Yolken, R. H., Stopa, P. J. Comparison of seven enzyme immunoassay systems for measurement of cytomegalovirus. *J. Clin. Microbiol.* **11**:546–551, 1980.

Yoshino, K., Taniguchi, S. The appearance of complement-requiring neutralizing antibodies by immunization and infection with herpes simplex virus. *Virology* **22**:193–201, 1964.

Young, A. B., Reid, D., Grist, N. R. Is cytomegalovirus a serious hazard to female hospital staff? *Lancet* **1**:975–976, 1983.

Youtree, E. L., Bia, F. J., Griffith, B. P., Root, R. K. Neutrophil response and function during acute cytomegalovirus infection in guinea pigs. *Infect. Immun.* **36**:11–16, 1982.

Yow, M. D., Lakeman, A. D., Stagno, S., *et al.* Use of restriction enzymes to investigate the source of a primary cytomegalovirus infection in a pediatric nurse. *Pediatrics* **70**:713–716, 1982.

Zablotney, S. L., Wentworth, B. B., Alexander, E. R. Antigenic relatedness of 17 strains of human cytomegalovirus. *Am. J. Epidemiol.* **107**:336–343, 1978.

Zaia, J. A., Levin, M. J., Leszczynski, J., *et al.* Cytomegalovirus immune globulin: Production from selected normal donor blood.*Transplantation* **27**:66–67, 1979.

Zaia, J., Forman, S., Ting, Y., *et al.* Polypeptide-specific antibody response to human cytomegalovirus after infection in bone marrow transplant recipients. *J. Infect. Dis.* **153**:780–787, 1986.

Zaloga, G. P., Chernow, B., Eil, C. Hypercalcemia and disseminated cytomegalovirus infection in the acquired immunodeficiency syndrome. *Ann. Intern. Med.* **102**:331–333, 1985.

Zavada, V., Erban, V., Rezacova, D., Vonka, V. Thymidine-kinase in cytomegalovirus infected cells. *Arch. Virol.* **52**:333–339, 1976.

Zinkernagel, R. M., Doherty, P. C., Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis with a syngeneic or semiallogeneic system. *Nature* **248**:701–702, 1974.

Zinkernagel, R. M., Doherty, P. C. MHC-restricted cytotoxic T cell studies on the biologic role of the polymorphic major transplantation antigens determining T-cell restricting specificity, function and responsiveness. *Adv. Immunol.* **27**:51–177, 1979.

Zisbord, Z., Haimov, M., Schanzer, M., *et al.* Kaposi's sarcoma after kidney transplantation. Report of complete remission of cutaneous and visceral involvement. *Transplantation* **30**:383–384, 1980.

Zuelzer, W. W., Stulberg, C. S., Page, R. H., Teruya, J., Brough, A. J. Etiology and pathogenesis of acquired hemolytic anemia. *Transfusion* **6**:438–460, 1966.

Zur Hausen, H., Bornkamm, G. W., Schmidt, R., Hecher, E. Tumor initiators and promoters in the induction of Epstein–Barr virus. *Proc. Natl. Acad. Sci. U.S.A.* **76**:782, 1979.

Author Index

Senior authors only are listed in this Author Index. Boldface page numbers denote the first appearances of a particular work in the text, while subsequent numbers denote other appearances of the same work. Different works of the same author are separated by semicolons.

Subject Index