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Contents

I. Cytomegalovirus infection in transplantation

II. Suppressor T lymphocytes in allogeneic reactions and their implication in transplantation

III. Non-specific processes amplifying rejection: inflammation

IV. Histocompatibility in renal transplantation

V. Poster session

I. Cytomegalovirus infection in transplantation

VIRUS AND HOST FACTORS IN CYTOMEGALOVIRUS INFECTIONS

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Cytomegaloviruses (CMV) comprise a genus within the Herpesviridae family. They infect man and other animals, but the viruses are highly species-specific. Even in vitro, they grow only in human cells. Human cytomegalovirus is distributed throughout the world, but its most dangerous effects are seen in newborns or in patients receiving immunosuppressive therapy. Subclinical infection and even serious disease may occur in persons with high levels of CMV antibody; thus, cellular immunity seems to play an important role in controlling infection.

Infection with cytomegaloviruses is widespread. Antibody is found in 80% of individuals over 35 years of age. The prolonged shedding of virus in urine and saliva suggests a urine-hand-oral route of infection. The rate of virus excretion among institutionalized children is 10 times that in children of comparable age in the population at large, suggesting virus transmission by close contact. Infected mothers may transmit virus to the fetus or to the newborn infant. Infected donors may transmit virus by blood transfusion or organ transplantation. Reactivation of latent CMV infection can occur and frequently occurs during pregnancy.

PROPERTIES OF THE VIRUS

Morphologically, cytomegalovirus is indistinguishable from herpes simplex or varicella-zoster virus. The virion is enveloped. The nucleocapsid consists of a 64 nm core enclosed by a 110 nm icosahedral capsid containing 162 capsomeres and is surrounded by a membrane (see Figures 1 and 2). The enveloped virion is about 180 nm in diameter. An additional viral structure is known as "the dense body". It consists of a homogeneous electron-dense sphere enclosed in a double membrane identical to that of mature virions. It is found in the cytoplasmic vacuoles from which it derives its membrane. The dense bodies measure 250-500 nm in diameter.

The virus contains DNA with a molecular weight of 150×10^6. The virion contains over 30 structural proteins with molecular weights ranging from 11,000 to 290,000. Of these, 8 are glycosylated and make up part of the envelope. The dense bodies contain the virus proteins but lack DNA.

An antigenic heterogeneity exists among cytomegalovirus strains. The human viruses form an antigenic spectrum rather than falling into distinct serotype groups. A number of clinical isolates were studied by DNA-DNA reassociation kinetics; their genomes were found to share 80% homology but none were identical. Restriction endonuclease fingerprinting is proving useful in tracing strains in epidemiological investigations.

Cytomegalovirus is easily inactivated. It is more stable when suspended in distilled water than in saline, and it is relatively stable when stored at -90°C in the presence of 35% sorbitol.

VIRUS ISOLATION

The virus can be recovered from mouth swabs, urine, liver, adenoids, kidneys, and peripheral blood leukocytes by inoculation of human fibroblastic cell cultures. From one to two weeks are usually needed for cytologic changes to develop; they consist of small foci of swollen, rounded, translucent cells with large intranuclear inclusions (see Figures 3 and 4). Cell degeneration progresses slowly, and the virus concentration is much higher within the cell than in the fluid.

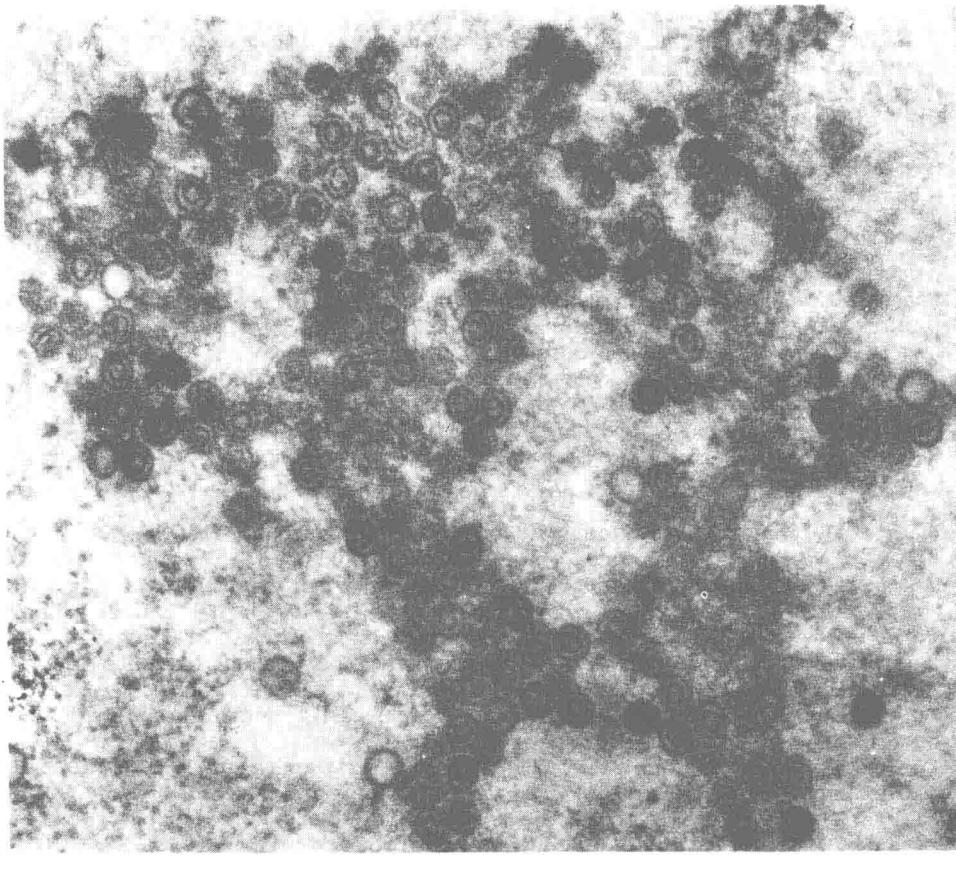

Figure 1. Electron micrograph of an ultrathin section of a human fibroblast infected with human cytomegalovirus. The virus is in the process of being replicated in the cell nucleus. Each virus particle contains a core and an outer membrane. Some of the virus particles contain their DNA genetic material, as evidenced by their dark centers.

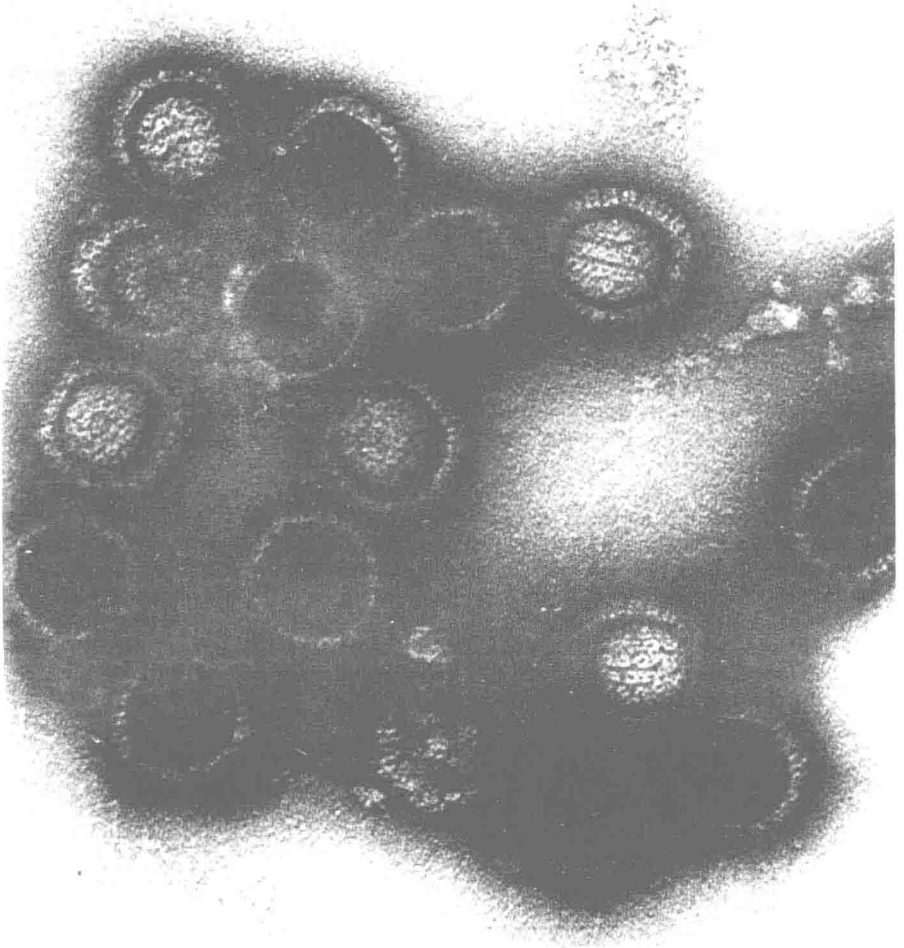

Figure 2. Electron micrograph of purified human cytomegalovirus at 200,000 times magnification. The particles with visible cores contain a full complement of DNA.

Human cytomegalovirus replicates in vitro only in human fibroblasts, although the virus is often isolated from epithelial cells of the host. Nonpermissive human epithelial cells, however, can be made permissive for cytomega lovirus by prior treatment of cells with IUDR. The virus can transform human and hamster cells in culture. Whether the virus is oncogenic in vivo is unknown, but it has been recovered from cervical cancer tissue.

In infected human fibroblasts, virus particles are assembled in the nucleus (Fig. 1). An envelope is acquired as the virus buds through the inner nuclear membrane or through the membrane of cytoplasmic vacuoles. The growth cycle of cytomegalovirus is slow, and infectious virus is cell-associated (Fig. 3), in contrast to herpes simplex virus.

ASPECTS OF CYTOMEGALOVIRUS INFECTIONS

In infants, the severe **cytomegalic inclusion disease** is congenitally acquired, probably as a result of primary infection of the mother during pregnancy. The virus can be isolated from the urine and milk of the mother at the time of birth of the infected baby, and typical cytomegalic cells, 25-40 µm in size, occur in the chorionic villi of the infected placenta.

As shown in Table 1, from 0.5 to 2.5% of newborn populations are congenitally infected with cytomegalovirus, although 95% of those infected have no apparent disease at birth (but later they may develop hearing loss and psychomotor disability). In the 5% with severe neonatal disease, the clinical syndrome may include signs of prematurity, jaundice with hepatosplenomegaly, thrombocytopenic purpura, pneumonitis, and central nervous system damage (microcephaly, periventricular calcification, chorioretinitis, optic atrophy, and mental or motor retardation). It has been estimated that one of every 1000 infants (in the U.S.A. this means more than 3000 per year) is seriously retarded as a result of infection with cytomegalovirus.

Elevated IgM antibody to cytomegalovirus or isolation of the virus from the urine occurs in up to 2.5% of apparently normal newborns. This high prevalence occurs in spite of the fact that women may already have cytomegalovirus antibody before becoming pregnant. The mother's level of immunity seems to be the critical factor in determining the severity of the infection in the newborn. In a recent study of 22 congenitally infected infants, all 14 infected neonates born from immune mothers had silent infections, but 3 of 8 neonates born from mothers undergoing a primary infection during pregnancy had severe cytomegalic inclusion disease.

TABLE 1. The annual disease burden resulting from congenital cytomegalovirus infection in the U.S.A.

Of 3,000,000 infants born annually in the U.S.A.		
0.5-2.5% are infected with cytomegalovirus		15,000-75,000 infections
Of these,	95% silent neonatal infections	14,000-71,000 inapparent
	5% severe neonatal disease	750-3,750 cases
Up to 25% may later develop hearing loss		3,500-17,500 cases
Up to 10% may later develop psychomotor disability		1,400-7,000 cases

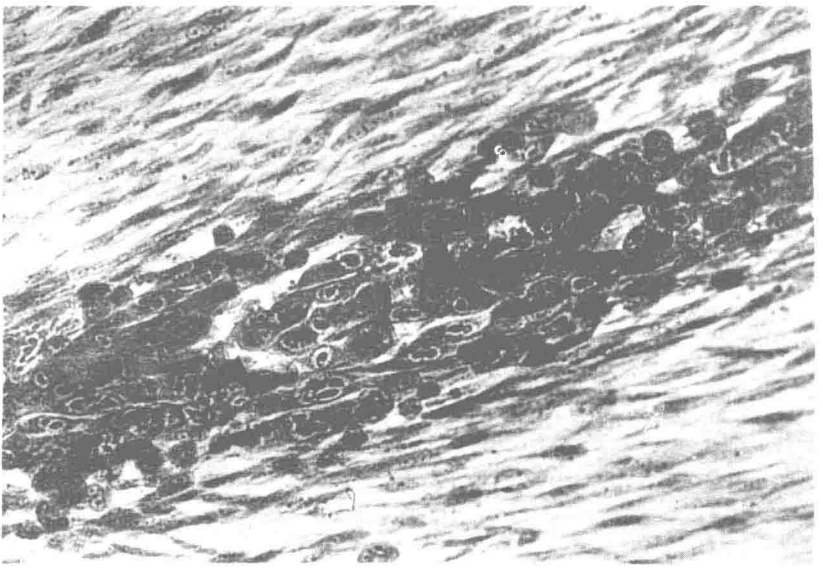

Figure 3. Human embryonic lung fibroblasts infected with cytomegalo virus. Stained with hematoxylin and eosin (X 260).

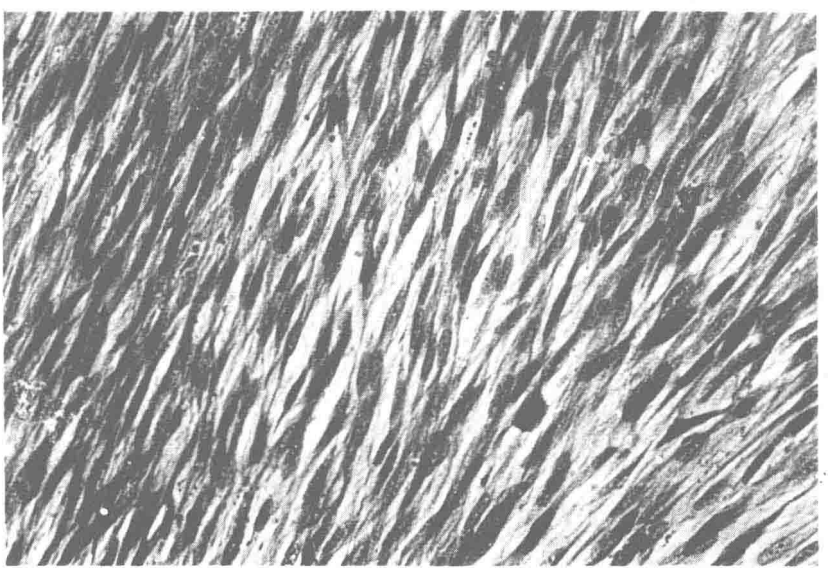

Figure 4. Uninfected human embryonic lung fibroblasts stained with hematoxylin and eosin (X 260).

Many women who had been infected with cytomegalovirus prior to their pregnancy excrete the virus from the cervix, particularly during the last trimester of pregnancy. This heightened excretion seems to be the result of a hormonal reactivation of latent virus or enhancement of a low-level chronic infection. At the time of delivery, infants pass through the infected birth canal and become infected, although they possess high titers of maternal antibody acquired transplacentally. These infants begin to excrete the virus in their urine at about 8-12 weeks of age. They continue to excrete the virus for several years, usually with no signs of a related illness.

Breast milk is an important source of cytomegalovirus infection. In a Melbourne study, virus was isolated from the milk of 17 of 63 seropositive women: 6 of the 14 with and 11 of the 49 without viruria. Isolates were obtained as early as 2 days and as late as 10 weeks after delivery, but more often after the first week.

Acquired infection with cytomegalovirus is common and usually inapparent. In children, acquired infection may result in hepatitis, interstitial pneumonitis, or acquired hemolytic anemia. The virus is shed in the saliva and urine of infected individuals. After acquired infections, adults may shed virus for up to 4 weeks in saliva and up to 2 years in urine; in infants, salivary shedding may extend to several months and urinary shedding to several years.

Cytomegalovirus can cause an infectious mononucleosis-like disease without heterophil antibodies. **Cytomegalovirus mononucleosis** occurs either spontaneously or after transfusions of fresh blood during surgery **(postperfusion syndrome).** The incubation period is about 30-40 days. There is cytomegaloviruria and a rise of cytomegalovirus antibody. Cytomegalovirus has been isolated from the peripheral blood leukocytes of such patients. The postperfusion syndrome may be caused by cytomegalovirus harbored in the leukocytes of the blood donors.

Table 2. Summary of host responses to cytomegalovirus

I. Infection of the fetus
1. CMV inclusion disease
II. Infection of the newborn infant
1. congenital
2. acquired
III. Infection of children and adults
1. hepatitis, pneumonitis, hemolytic anemia
2. infectious mononucleosis
3. posttransfusion syndrome
4. posttransplantation syndrome
5. immunologic deficiency syndrome
6. cancer
a. CMV-transformed human cells in vitro
b. CMV isolated from cervical cancer and colon adenocarcinoma

Patients with malignancies or immunologic defects or those undergoing immunosuppressive therapy for organ transplantation may develop cytomegalovirus pneumonitis or hepatitis and occasionally generalized disease. In some of these patients a latent infection may be reactivated when host susceptibility to infection is increased by immunosuppression. In seronegative patients without evidence of previous cytomegalovirus infection, the virus may be transmitted exogenously. In a prospective study, 83% of seronegative patients who received kidneys from seropositive transplant donors developed infection. Thus, latently infected kidneys are the most likely source of virus. In such acquired cytomegalovirus infections, there seems to be an elevated risk of pulmonary complications due to the virus and concomitant fungal and bacterial pathogens.

A variety of host responses to cytomegalovirus infections may occur. They are listed in Table 2.

ANTIBODIES

Antibodies of IgM, IgG, and IgA classes develop in infected persons. Glycoprotein antigens that induce neutralizing antibody are located in the envelopes both of the virus and of the dense body. Antiserum prepared against viral glycoprotein not only neutralizes virus infectivity, but it also reacts with the membranes of infected cells in fluorescent antibody tests. However, the serum fails to react with the membranes of uninfected cells.

Complement-fixing and neutralizing antibodies occur in most human sera. In young children possessing antibodies, virus may be detected in the saliva and in the urine for many months or even years. Virus is not found in young children who lack antibody.

Infants infected during fetal life are born with antibody that continues to rise after birth in the presence of persistent virus excretion, regardless of whether the infection is associated with disease or is inapparent clinically.

In contrast to infants, previously infected mothers who excrete virus during pregnancy show little or no change in antibody titers as a result of the virus excretion. The infection is believed to be localized. The failure to isolate cytomegalovirus from leukocytes and throat secretions of these mothers or from their placentas or amniotic fluids supports this view.

TREATMENT AND CONTROL

There is no specific treatment. Neither immune gamma globulin nor DNA virus inhibitory drugs have had any effect.

Specific control measures are also not available. Isolation of newborns with generalized cytomegalic inclusion disease from other neonates is advisable.

Screening of transplant donors and recipients for cytomegalovirus antibody may prevent some transmissions of primary cytomegalovirus. The cytomegalovirus seronegative transplant recipient population represents a high-risk group for cytomegalovirus infections and should be an important target population for a safe vaccine as soon as one becomes available.