Cytomegalovirus

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Introduction

Cytomegalovirus is a member of the Herpesviridae family of viruses and a common viral pathogen. Other members of this herpes family of viruses are herpes simplex I (HSV-I), herpes simplex II (HSV-II), varicella zoster virus (VZV), Epstein-Barr Virus (EBV), and human herpesvirus types 6, 7, and 8 (HHV-6, HHV-7, HHV-8). Evidence of CMV infection is found in all population groups and geographical locations. Nearly 100% of persons in developing countries and 50-75% in developed countries are infected with CMV. Nearly all CMV-infected persons are asymptomatic and may transmit disease without knowledge of dissemination. Infection with CMV causes few if any problems for healthy individuals; but, may produce a mononucleosis-like syndrome. However, CMV infections in newborns and immunocompromised or immunodeficient individuals may produce a variety of disease processes, that in some instances may be life-threatening (Brecher, 2002; McClatchey, 1994; Oshiro, 1999; Pass, 2002; Stevens, 1996; Turgeon, 1996).

CMV shares with other herpes viruses the characteristics of cell association, latency, and reactivation and gives rise to two forms of infection: 1) primary infection that results upon patient’s first encounter with the organism; and, 2) secondary infection that results with a reactivation of the same virus or a new infection with a different strain of CMV. CMV is the most common cause of congenital infection in the United States. Cytomegalovirus can be found in saliva, tears, semen, urine, cervical secretions, blood, and breast milk for months to years subsequent to initial infection (Oshiro, 1999; Pass, 2002).

There are a number of laboratory test procedures that may be used to aid in the diagnosis of CMV infection. These include culture techniques, cytologic techniques, serological methods, DNA probes, and polymerase chain reaction (PCR) (Brecher, 2002; Stevens, 1996).

Fortunately, most infections are self-limiting and require no treatment. Ganciclovir, foscarin, and cidofovir are the only medications that can be used for treatment of cytomegalovirus (Oshiro, 1999).

Epidemiology

CMV may be spread by oral, respiratory, or venereal routes. The virus can be transmitted by direct contact with infected secretions as well as via organ transplants, bone marrow transplants, and blood transfusions. Additionally, congenital infection has been documented via transplacental viral spread. Perinatal infection may be acquired as the fetus passes through the birth canal or through maternal colostrum and breast milk (Oshiro, 1999; Stevens, 1996; Turgeon, 1996). Most adults become infected with CMV via the venereal route and most children become infected via respiratory tract secretions (Rosen & Ablon, 1997).

In the United States, the congenital CMV infection rate is approximately 0.5-1.5%. It has been documented that CMV can be transmitted to the fetus even when the mother was infected several years prior to conception and possesses antibodies to CMV. The explanation given for this anomalous occurrence is that it could be the result of reactivation of the latent virus during pregnancy, chronic infection, or reinfection with a new strain of CMV. Children born to adolescent females are 3-7 times more likely to acquire congenital CMV infection than children born to post-adolescent females (Pass, 2002).

Mothers who breastfeed their infants strongly influence the spread of postnatal CMV infection. Cytomegalovirus was detected in breast milk more than 30 years ago. Research also indicates a high frequency of reactivation of CMV in breastfeeding mothers. In a study involving preterm babies, researchers found that 96% of the seropositive mothers exhibited CMV reactivation and 37% of these same mothers transmitted CMV to their offspring with a mean incubation time of 42 days (Hampecht et al., 2001). In one study, breast milk samples were analyzed and cytomegalovirus was isolated in 14% to 44% of the samples (Binda et al., 2001). Where breastfeeding is widely practiced and there is a high rate of seropositivity in the mothers, most infants are exposed to and acquire CMV during the first year of life (Pass, 2002).

Cytomegalovirus is also commonly spread in child care facilities from one child to another and from children to their caregivers. CMV, in this setting, is usually spread by hand contact as well as contact with various fomites (any substance, other than food, that can harbor and transmit infectious organisms) in the child care setting. Not only can children pass cytomegalovirus to each other, they can also pass the virus on to pregnant women, thus putting offspring at a high risk of acquiring a congenital cytomegalovirus infection. Studies have been conducted throughout the United States in various child care centers to determine the frequency of transmission from child to child and children to adults. Rates of cytomegalovirus excretion among children in group...
child care centers have averaged 15% to 25%, but even higher rates have been isolated in toddler-aged children and selected child care centers. Children in child care centers and their parents have an increased risk of acquiring a CMV infection when compared to children and parents who are not involved with child care centers (Bale et al., 1999).

Approximately 50% of blood donors possess antibodies to CMV; however, estimates are that currently less than 1% of the cellular components collected from these donors are able to transmit the virus. Post-transfusion CMV infection is not usually of any clinical consequence to immunocompetent patients and use of CMV-reduced-risk blood is not warranted. However, there is a likelihood of severe disease in immunocompromised/immunosuppressed patients for whom CMV-reduced-risk blood should be chosen. Patients for whom CMV-reduced-risk blood should be chosen include low-birth-weight premature babies born to seronegative mothers, seronegative pregnant women, recipients of intravenous transfusion, seronegative recipients of hematopoietic progenitor cells, seronegative recipients of any organ transplant, and seronegative AIDS patients (Brecher, 2002).

Transfusion-acquired cytomegalovirus infections may cause not only a mononucleosis-like syndrome, but also hepatitis and an increase in rejection of transplanted organs. Three types of cytomegalovirus infections are possible in blood transfusion recipients: a primary infection, a reactivated infection, or re-infection by a different strain of cytomegalovirus. A primary infection occurs when an unexposed recipient is transfused blood that has been collected from a donor who has either active or latent cytomegalovirus infection. Signs of this kind of infection include an immediate antibody response, presence of cytomegalovirus in the blood and urine, and eventual seroconversion. A reactivated infection can occur when a recipient is transfused with blood from a donor with a positive or negative cytomegalovirus antibody. A reinfection by a different strain of cytomegalovirus can occur if a strain of cytomegalovirus is found in the donor’s blood that is different from the one found in the recipient. In this type of infection, viral shedding and a critical antibody response is noted (Turgeon, 1996).

Cytomegalovirus has also been found in patients with suppressed immune systems. In immunosuppressed patients, such as organ transplant recipients or acquired immunodeficiency syndrome (AIDS) patients, diffuse pneumonitis is a frequent opportunistic infection associated with death in these patients (McClatchey, 1994). Cytomegalovirus has also been implicated in cases involving the vanishing bile duct syndrome (extrahepatic biliary atresia) subsequent to liver transplantation (Tarr, Haas & Christie, 1996).

### Types of CMV Infection

There are two basic types of cytomegalovirus infections: acquired or congenital. By the time an individual reaches adulthood, most have experienced asymptomatic infection with CMV. Since CMV can persist in the latent state, active infections can result related to a number of conditions including pregnancy, immunosuppression, and following organ or bone marrow transplantation. Low birth weight infants are also at a high risk for CMV infection via transfusion of blood products infected with CMV. Leukodepleted blood products and intravenous immunoglobulin may successfully protect these individuals from CMV infection. Nosocomial transmission from infected patients to health care workers has not been documented; however, good handwashing techniques seem to offer the best measure for preventing transmission (Turgeon, 1996).

The second type of cytomegalovirus infection is congenital infection that is transmitted from an infected mother to her baby. The most serious cases of cytomegalovirus infection are associated with preterm infants who acquire cytomegalovirus from their mothers (Maschmann, Hamprecht, Dietz, Jahn & Speer, 2001; Temple, Pass & Boll, 2000). Congenital cytomegalovirus usually affects the nervous system. Estimations show that at birth approximately 7% of infected newborns who have clinical signs of congenital cytomegalovirus infection, exhibit signs and symptoms such as microcephaly, splenomegaly, petechiae, jaundice, or retinitis. Symptomatic congenital cytomegalovirus infection often leads to mental retardation, seizures, legary, paralysis, damage to sensory organs, and can possibly cause hearing loss in infected newborns (Temple, Pass & Boll, 2000). Other findings in newborns with congenital cytomegalovirus infection are chorioretinitis, deafness, intracranial calcifications, seizures, and smallness for gestational age (Oshiro, 1999).

### Pathogenesis

Viruses are strict intracellular parasites that reproduce only within host cells. Steps in viral replication include: 1) attachment; 2) penetration; 3) uncoating; 4) macromolecular synthesis; 5) assembly; and, 6) release. Attachment involves recognition of a suitable host and the joining of glycoprotein spikes from the virus to carbohydrate receptors of the host cell. The viral envelope fuses with the host cell membrane and penetrates the cell. The virus then sheds its coat which is necessary before viral DNA or RNA can be delivered to the manufacturing site. Macromolecular synthesis involves viral transcription with the production of messenger RNA (mRNA) and immediately, early, and late viral proteins. Early proteins are nonstructural components such as enzymes, while late proteins are structural proteins. Assembly involves the coupling of structural proteins, genome,
and enzymes into virions. Viral envelopes are acquired via budding from the host cell membrane. The virus completes assembly with the acquisition of the envelope. Release of intact virions occurs following cell lysis or by budding from the cytoplasmic membrane. These virions enter other cells and complete the cycle again or become latent and reside within the cell until some precipitating event occurs to reactivate them (Baron, Peterson, & Finegold, 1994).

CMV is a very complex opportunistic pathogen possessing 20 times more genetic material than the human immunodeficiency virus (HIV) and coding for the production of more than 100 different proteins. When CMV gains entrance to the body, it proceeds to take up residence within the nucleus of the host cell where replication occurs. Viral replication involves the expression of immediate-early, early, and late classes of genes. The viral envelope forms as an assembled nucleocapsid bud from the inner surface of the nuclear membrane (Murray, Baron, Pfaller, Tenover, & Yolken, 1999).

CMV disease is an anomaly in humans and occurs when the individual possesses defective T-cell immunity. When the immune system is intact, infection is usually asymptomatic and the virus enters a variety of cells in the body (particularly neutrophilic leukocytes) and becomes latent (clinically silent) and may remain in this state for years before being reactivated. The mechanisms of reactivation are yet to be discovered. Histologic studies have demonstrated the presence of CMV in salivary glands, kidney, pancreas, adrenals, lung, liver, eye, ear, placenta, gastrointestinal tract, heart, ovaries, skin, blood vessels, and brain. CMV has also been found associated with the central nervous system and the virus has been detected in association with neurons, glia, ependyma, choroids plexus, meninges, and vascular endothelium (Pass, 2002).

Virus infection of healthy persons usually manifests itself as a self-limited heterophile-negative, mononucleosis-like syndrome. Symptoms include fever, sore throat, lymphocytosis, malaise, myalgia, fatigue, diarrhea, rash, lymphadenopathy, pharyngitis, hepatosplenomegaly, viremia, viuria, and hepatitis. There is evidence that the symptoms are produced by cytotoxic lymphocytes that attempt to eliminate the CMV-infected cells (Brecher, 2002; Turgeon, 1996). Infrequent symptoms include interstitial pneumonitis, gastrointestinal ulceration, arthralgias, meningocerehalitis, and retinitis (Turgeon, 1996). CMV infection itself is immunosuppressive as indicated by an increased number of T suppressor cells (CD8+) and a decreased number of T helper cells (CD4+) (Rosen & Ablon, 1997; Turgeon, 1996).

CMV infection in the newborn, immunodeficient, and immunocompromised may produce life-threatening consequences including oculocutaneous disease, pneumonia, neurologic disorders, febrile illness, and hepatitis. Clinical findings in newborns with symptomatic congenital CMV infection include petechiae, jaundice, hepatomegaly and elevated liver enzymes, thrombocytopenia, microcephaly, intracranial calcifications, sensorineural hearing loss, mental retardation, and cerebral palsy (Pass, 2002).

Most CMV infections in immunocompromised patients are due to reactivation of the CMV and these are usually asymptomatic. When primary infection occurs, it is usually associated with disease. The major sources of CMV for these patients are blood products and transplanted organs. Clinical manifestations of CMV infection correlate with degree of immunologic impairment (Pass, 2002).

**Immunologic Response**

Normal humans produce both IgM and IgG antibodies in response to most pathogens. Following interaction with the pathogen, a multitude of activities take place that ultimately result in antibody production. Initially, there is a lag phase in which no antibody is detected, probably due to the sensitivity of the detection procedures. This is followed by a rapid increase in the amount of antibody produced known as the log phase. Antibody production reaches a plateau and continues for a period of time and then begins the decline phase. The initial antibody produced to this primary infection is IgM. Over a short period of time, the cells that were producing IgM switch to producing IgG (isotype switching). The binding sites on the antibody molecules bind to the offending pathogen and help to clear the pathogen from the body. The second encounter with the same pathogen will induce the same sort of response; however the lag phase is shortened and the intensity of the IgG response is increased. In immunocompromised or immunodeficient patients, the IgM antibody response may be delayed or not occur at all (Brecher, 2002; Stevens, 1996; Turgeon, 1996).

During the primary infection, IgM antibodies against immediate early and early antigens are produced and persist for 2-3 months. The IgM antibody disappears and does not reappear upon reactivation. IgG antibodies appear shortly subsequent to IgM antibodies and persist for 3-4 months and disappear. Antibody to late antigens may persist following recovery and may be detected for several years of life. Only IgG antibodies are formed during reactivated infection (Stevens, 1996).

**Diagnosis**

There are several characteristic but nonspecific findings in cytomegalovirus infection. A complete blood count (CBC) usually exhibits a leukocytosis with a characteristic lymphocytosis that includes greater than 20% variant lymphocytes. In infants with cytomegalovirus infection, the most common laboratory finding is a low platelet count (thrombocytope-
nia). Also clinical tests may show abnormal liver function (Turgeon, 1996).

There are a variety of tests that can be used to detect cytomegalovirus. Laboratory testing methods for cytomegalovirus vary from culture and cytologic techniques to deoxyribonucleic acid (DNA) probes and serological methods. The detection of antibodies is a good indicator of active or very recent infection, although some antibodies may not develop until a week or more after the patient shows clinical signs. The presence of IgM antibodies to cytomegalovirus indicates an acute or primary infection. The presence of IgG antibodies to cytomegalovirus is indicative of a recent, reactivated, or past infection (Murray et al., 1999; Stevens, 1996; Turgeon, 1996).

A single serum specimen can be of value in screening for evidence of past infection and identifying individuals at risk for CMV infection such as organ donors, blood donors, and bone marrow donors. When testing a single specimen, tests to detect both IgG and IgM forms should be performed. If both are negative, there is no infection. If only IgM or both IgG and IgM are present, infection is present. The presence of only IgG antibodies indicates a past infection in most cases. However, for diagnosis of recent infection, acute and convalescent sera collected at least two weeks apart should be tested. The acute sample should be collected as soon as possible following expression of symptoms and tested along with the convalescent sample. A recent infection would be indicated by a four-fold rise in antibody titer from the acute to convalescent samples; although, in some patients titers may not rise for months after the acute infection or they may never rise. In suspected congenital infections, both maternal and infant sera should be tested. The presence of IgM antibody in the infant indicates an in utero infection since maternal IgM antibodies are unable to cross the placenta. Presence of IgG in infant serum indicates either placental transport of maternal antibodies or fetal production (Sheehan, 1997).

Histologic examination of biopsy, autopsy, peripheral blood, and urine samples for cytomegalic cells may be used to suggest CMV infection. Cytomegalic cells are large cells with basophilic intranuclear inclusions with the appearance of an “owl’s eye.” CMV can infect cells without producing this morphologic effect; thus, a negative procedure does not rule out CMV infection. Additionally, the presence of the morphologic abnormality only suggests CMV infection and should be confirmed with another method. Exfoliative cytology techniques may also be used to identify characteristic enlarged cells with prominent inclusions. Several fresh samples should be tested since cells disintegrate rapidly and may be shed intermittently (Murray et al., 1999).

Cytomegalovirus early and late viral antigens in infected cells can be directly detected in tissues obtained by biopsy or at autopsy. The cells are usually prepared using cytospin techniques. Immunofluorescent techniques are rapid but not as sensitive as shell vial culture techniques (Murray et al., 1999; Stevens, 1996).

The CMV antigenemia assay allows direct detection and quantitation of CMV from peripheral blood leukocytes and has been shown to be sensitive, specific, rapid, and relatively simple to perform. This assay allows detection of CMV and viral load quantitation before the onset of symptoms. This allows differentiation of CMV disease from asymptomatic infection, evaluation of efficacy of antiviral therapy, and detection of CMV in cerebrospinal fluid (CSF) of AIDS patients with central nervous system infection (Murray et al., 1999).

Electron microscopy has been used to detect CMV in urine and oral specimens in congenital infections. The method is relatively insensitive but is rapid and can be used with contaminated specimens unsuitable for culture techniques (Murray et al., 1999).

Molecular dot blot hybridization techniques have been described for detection of CMV. This method is rapid and easy to read by light microscopy but lacks sensitivity (Murray et al., 1999).

The polymerase chain reaction (PCR) is the most widely used molecular method for the detection and quantification of CMV DNA and mRNA. The procedure is sensitive and specific and when a variety of primers are used, a number of different clinical isolates can be detected indicating that that strain variability does not limit the use of PCR. The major drawback to PCR is with the inability to distinguish between active disease, asymptomatic infection, or latency. CMV antigenemia is considered to be the best predictor for CMV disease (Murray et al., 1999).

CMV can also be grown in culture on human fibroblasts. Tubes are examined for dead or dying cells (cytopathic effect (CPE)) daily for 5 days and bi-weekly for 4 weeks. Thus the process is not very rapid (Murray et al., 1999).

The spin-amplification shell vial assay is a rapid method for detection of CMV in clinical specimens. This technique amplifies the virus in cell cultures following low-speed centrifugation and detects antigens produced early in the replication cycle of CMV utilizing monoclonal antibodies. Low titer are amplified and detected within 24 hours (McClatchey, 1994; Murray et al., 1999).

**Treatment**

Currently there are only a few drugs that can be taken as a form of treatment for a cytomegalovirus infection including ganciclovir, foscarnet, and cidofovir. However, these agents are far from ideal in terms of expense, ease of administration, toxicity, and pharmacodynamics. But, their use has substantially reduced the burden of CMV disease in immunocompromised patients. No antiviral agent has been approved for treatment of congenital CMV (Oshiro,
Prevention techniques for cytomegalovirus are very simple. Since cytomegalovirus can be transmitted through infected body fluids, all individuals (especially those at risk) should practice good personal hygiene. Good personal hygiene includes practicing good hand washing, not sharing food utensils, and not kissing infected people on the mouth. Transfusion-acquired CMV can be prevented by limiting transfusion of at-risk patients to CMV negative blood products or removing leukocytes and platelets from blood prior to transfusion (leukoreduction) (Bass, 2002).

Research on the development of a CMV vaccine is promising. However, there is no approved vaccine at present (Oshiro, 1999).

References


Questions for STEP Participants

1. Which of the following is NOT a member of the Herpesviridae family of viruses?
   A. Cytomegalovirus
   B. Epstein-Barr virus
   C. Human herpesvirus type 6
   D. Rotavirus

2. The MOST common cause of congenital infection in the United States is:
   A. CMV
   B. HSV
   C. EBV
   D. HBV

3. Via which of the following routes do MOST adults acquire CMV infection?
   A. Respiratory secretions
   B. Sexual contact
   C. Breastfeeding
   D. Hand contact

4. Which of the following procedures allows direct detection and quantitation of CMV antigens in the blood?
   A. Polymerase chain reaction
   B. CMV antigenemia assay
   C. Spin-amplification shell vial assay
   D. Fibroblast cell culture for cytopathic effect assay

5. CMV infection would LEAST likely be transmitted via:
   A. Transfusion of Leukoreduced RBC
   B. Bone marrow transplant from seropositive donor
   C. Contact with fomites in a childcare setting
   D. Breastfeeding practices

6. Which of the following individuals would be LEAST susceptible to CMV infection?
   A. A 28-year-old gay college student with AIDS
   B. A baby born to an adolescent female
   C. A 21-year-old male college student
   D. An acute leukemia patient

7. Which of the following steps in viral replication involves the production of virions?
   A. Attachment
   B. Penetration
   C. Macromolecular synthesis
   D. Assembly

8. Which of the following acute and convalescent sera results would NOT indicate an active CMV infection?
   A. Acute 10 Convalescent 40
   B. Acute 20 Convalescent 20
   C. Acute 20 Convalescent 80
   D. Acute 20 Convalescent 100

9. Which of the following findings indicates that CMV itself is immunosuppressive?
   A. Leukocytosis with lymphocytosis
   B. Leukopenia with lymphocytosis
   C. Increased CD8+ cells, decreased CD4+ cells
   D. Decreased CD8+ cells, increased CD4+ cells

10. The phase of antibody production in which there is the greatest amount of antibody produced is the:
    A. Lag
    B. Log
    C. Plateau
    D. Decline