Human cytomegalovirus (CMV) poses an important public health problem as it may cause serious morbidity and mortality in congenitally infected newborns and immunocompromised patients, most notably transplant recipients and HIV-infected persons. It is probably one of the most common infections known to humans and is characterized by a self-limiting infection in healthy individuals. CMV infection is the single most frequent cause of infectious complications in the early period following kidney transplantation. Post-transfusion cytomegalovirus infection is of concern in the immunocompetent as well as in certain categories of immunocompromised individuals such as neonates, pregnant women, recipients of bone marrow and other organ transplants and individuals with immunodeficiency disorders. The emergence of AIDS in India has necessitated the establishment of reliable tests for diagnosis of cytomegalovirus infection as a damaged immune system permits cytomegalovirus reactivation. The magnitude of this problem in India and the various diagnostic modalities used have not been adequately investigated, and hence, CMV infection is still a major health problem warranting strong preventive measures. The ultimate goal of the prevention program is to develop a vaccine that can be administered to seronegative women of childbearing age to prevent primary infection during pregnancy.

**Key words:** Congenital, cytomegalovirus, latency, primary, recurrent

CMV is a virus of paradoxes and can be a potential killer or a silent companion lifelong. It is probably one of the most common infections known to humans and is characterized by a self-limiting infection in healthy individuals. Besides contact with seropositive mothers (passage through genital tract, breast milk, etc.), blood transfusion is the most important mode of perinatal/post-natal spread of CMV to neonates. Although CMV has a worldwide distribution, it is more common in developing countries. Seroprevalence varies greatly with a variety of epidemiological factors. In different parts of India, serological surveys have shown 80–90% prevalence of CMV IgG antibodies in women of childbearing age. Risk of seroconversion during pregnancy averages 2.0–2.5%. Gestational age has no influence on risk of intrauterine transmission; however, clinical consequence for infected offspring appears to be worse when infection takes place before 20 weeks. Due to latency and periodic reactivation, in utero transmission may follow either primary or recurrent infections. Primary infections are transmitted more frequently (15–50%) and are more likely to cause foetal damage than recurrent infections (0.15–1%). Ninety percent of congenitally infected infants are asymptomatic at birth, 5–17% of who develop symptoms such as sensorineural hearing loss, chorioretinitis or neurologic deficits usually during the first 2 years of life. Among 10% of symptomatic newborns, 20% die and 90% survivors develop severe sequelae. Mortality may be as high as 30% and more than 90% of the infants surviving CMV disease have late complications. Recurrent congenital CMV infections in children are less likely to be symptomatic or present with later sequelae, although recently, incidence of symptomatic infections in immune mothers has been shown to be similar in primary and recurrent infections. Studies regarding prevalence of birth defects being limited in India, the true frequency of recurrent infections remains to be determined; however, primary infection continues to be the major cause of congenital infections, with significant morbidity.

**CMV Diseases Among Transplant Recipients**

It is known that CMV remains latent in the leukocytes and gets reactivated as age advances. Multiple new strains also arise through recombination of existing strains. Both mechanisms could cause CMV disease in Indian transplant patients where seroprevalence is high. Immunosuppressive
therapy following transplantation can also enhance viral replication. Primary infection refers to reactivation of donor virus in CMV-seronegative recipient. Secondary infection occurs when endogenous latent virus is reactivated in a seropositive recipient and reinfection refers to reactivation of the virus of donor origin in a seropositive recipient. Sixty to 90% of all renal transplant candidates have latent CMV infections, but symptomatic infection occurs only in 20–60% of them.[9] CMV is the single most frequent cause of infectious complications in the early period following kidney transplantation, with the overall incidence of CMV infection and disease during the first 100 days post-transplantation being 60% and 25%, respectively, when no CMV prophylaxis or preemptive therapy is given.[10] Infection is defined as isolation of CMV, or detection of CMV proteins or nucleic acid, in any body fluid or tissue specimen. Disease is defined as detection of CMV in a clinical specimen, accompanied by either CMV syndrome with fever, muscle pain, leukopaenia and/or thrombocytopenia (other causes excluded), or by organ involvement, such as hepatitis, gastrointestinal ulceration, pneumonitis, retinitis, central nervous system disease, nephritis, myocarditis, cystitis or pancreatitis. The incidence of CMV infection is 50–75% when the recipient is CMV antibodies negative and the donor tests positive for CMV antibody. If serologic tests in both donor and recipient are negative, incidence of CMV disease is less than 5%. When the recipient tests positive, incidence of CMV infection, independent of CMV antibody status of the donor, is 25–40%.[11] Predictors of CMV disease following solid organ transplantation (SOT) include CMV mismatch, use of lymphocyte-depleting therapy and high doses of methylprednisolone. Risk factors for late-onset CMV disease include CMV D+/R- status and allograft rejection.[12] Considerable attention has been given to modulation of the immune system by CMV leading to opportunistic superinfections, allograft injury, acute rejection, chronic rejection and development of post-transplant lymphoproliferative disease.[13] Relapse occurs in 15–35% of SOT patients, the risk factors for which include D+/R- serostatus, disseminated CMV disease and repeated courses of antirejection therapy.[13] There is a paucity of data on CMV disease in renal transplant recipients from India. A study reported a four-fold increase in the incidence of CMV disease in renal transplant recipients after introduction of cyclosporine immunosuppression.[14] Another study showed use of DNAemia in detecting CMV infections among bone marrow transplant (BMT) recipients in which all recipients had high avidity CMV IgG (AI > 50%) confirming CMV reactivation or reinfection.[15]

Transfusion-Associated CMV Infection

Implementation of stringent donor eligibility criteria and more sensitive methods of viral genome detection have virtually eliminated transfusion-associated infection in developed countries; however, in developing countries like India, the risk is as yet considerable. Post-transfusion CMV infection is of concern in the immunocompetent as well as in certain categories of immunocompromised individuals such as neonates, pregnant women, recipients of bone marrow and other organ transplants and individuals with immunodeficiency disorders.[16] Perinatal/post-natal infections may be associated with protracted interstitial pneumonitis or developmental anomalies. Pneumonia, hepatitis, thrombocytopenia and anaemia associated with CMV infection may compound the problem. Although documented, little information is available regarding the risk of transmission of CMV following exchange transfusion in neonates in India. One study reported that 11.5% babies developed post-transfusion CMV infection though none exhibited any clinical manifestations.[17] Another study reported 95% of donor blood units to be positive for IgG whereas none were positive for IgM antibodies, indicating past exposure to infection.[18] It would be superfluous to screen voluntary blood donors in India due to the high seroprevalence of CMV among them in contrast with the West where it ranges from 38 to 75%.[18] Further studies need to be carried out in the Indian context regarding the transmission of transfusion-associated CMV before guidelines on routine screening for CMV in voluntary blood donors are laid down in the light of existing knowledge from various studies.

CMV Coinfection in Patients with HIV/AIDS

In most people with a fully functional immune system, initial infection with CMV may cause a mild flu-like illness, later, the virus remaining dormant. A damaged immune system permits CMV reactivation. A synergistic effect may worsen the immunologic profile and could potentially translate into a more rapid disease progression as transactivation of HIV-1 gene expression and release of a range of different cytokines by CMV-infected cells could activate the latent HIV proviral DNA. The risk of CMV is highest when CD4 counts are below 50 cells/µL and is rare with more than 100 cells/µL. Clinical diagnosis can be difficult and often requires the virus to be detected in biopsy samples. Up to 30% of people develop retinitis and an additional 5–10% develop disease in other organs.[19] Chorioretinitis most commonly occurs with CD4 counts < 50 cells/µL and accounts for 80–90% of CMV disease in patients with AIDS.[20] CMV can be found in lungs but this rarely seems to cause problems, usually in combination with Pneumocystis carinii pneumonia. CMV can also affect the gastrointestinal system and the peripheral nerves. Encephalopathy and cauda equina syndrome have also been linked to CMV. In developing countries where the HIV/AIDS pandemic is rapidly unfolding, the magnitude of CMV retinitis is as yet not known as it is largely undiagnosed and untreated. Yet, it has been estimated that between 5 and 25% of HIV-infected patients in the developing world can be expected to develop this blinding
disorder at some point during the course of their illness.\textsuperscript{[21]} A few reports based on clinical or autopsy findings suggest that a significant percentage of Indian patients with HIV and AIDS show evidence of active CMV infection. A study reported 32.4\% of patients of AIDS with CMV coinfection, and part of the symptoms were attributed to CMV.\textsuperscript{[19]} Biswas \textit{et al.} showed that ocular involvement was seen in 40\% and CMV retinitis was the commonest HIV-related ophthalmic lesion (17\%).\textsuperscript{[22]} In yet another study, the prevalence of CMV infection (7\%) fell within the range (4–26\%) reported by others. The emergence of AIDS in India has necessitated the establishment of reliable tests for diagnosis of CMV.

\textbf{Diagnosis of CMV Infection}

By far, the major role in transmitting CMV to the foetus is played by primary infection of the mother during pregnancy and hence in this respect, diagnosis of primary infection during pregnancy is a major task of the diagnostic virology laboratory. The diagnosis of primary infection is ascertained when seroconversion is documented, i.e., the \textit{de novo} appearance of virus-specific IgG in the serum of a pregnant woman who was previously seronegative. Such an approach is feasible only when seronegative women are identified and prospectively monitored. IgM detection in a pregnant woman is likely to be a reliable marker of primary infection; as it can reveal various clinical situations as related with acute phase of primary infection, convalescent phase of primary infection or persistence of IgM antibody. In pregnant women, detection of IgM antibody may be related to a primary infection occurring during pregnancy when the IgM titre falls sharply in sequential blood samples. Presence of low, slowly decreasing levels of IgM may indicate a primary infection initiated some months earlier and possibly before pregnancy. More recently, enzyme-linked immunosorbent assays (ELISAs) have been more widely used in both the indirect and the capture ELISA format, with either labelled antigen or antibody. The indirect ELISA shows the following potential sources of error when performed on whole serum: (i) competitive inhibition due to the presence of specific IgG, (ii) interference due to rheumatoid factor of the IgM class (IgM-RF) or to IgM-RF reactive only with autologous antigen or antibody. The indirect ELISA format, with either labelled antigen or antibody, shows the following potential sources of error when performed on whole serum: (i) competitive inhibition due to the presence of specific IgG, (ii) interference due to rheumatoid factor of the IgM class (IgM-RF) or to IgM-RF reactive only with autologous complexed IgG and (iii) interference due to IgM antibody reactive with cellular antigens. All these interfering factors could be readily eliminated by mixing serum samples with anti-human \gamma-chain serum. A recent study reported 18.75\% of babies with congenital anomalies to be positive for CMV IgM antibodies using \mu-capture ELISA, none of the mothers of whom were positive for IgM antibodies although all were positive for IgG antibodies, indicating primary infection in the past or reactivation/reinfection with a different strain of CMV in early pregnancy.\textsuperscript{[31]} Virus-specific IgG antibodies of low avidity are produced during the first months after onset of infection and subsequently a maturation process occurs by which antibodies of higher avidity are generated. Avidity levels are reported as the avidity index, expressing the percentage of IgG bound to antigen following treatment with denaturing agents such as 6M urea. Thus, the presence of high IgM levels and a low avidity index are highly suggestive of a recent (less than 3 months) primary CMV infection. A recent study reported that an avidity index above 65\% during the first trimester of pregnancy could reasonably be considered a good indicator of past CMV infection.\textsuperscript{[23]} One of our previous studies shows that an IgG avidity assay could be used in combination with an IgM ELISA for monitoring pregnant women for primary CMV infection.\textsuperscript{[24]}

\textbf{Diagnosis of recurrent infection} can be accomplished by virus isolation or antigen or DNA detection in clinical samples other than blood in absence of concomitant serologic and virologic markers of primary infection, i.e., CMV-specific IgM, low-avidity IgG, absence of neutralizing antibodies and absence of the virus and viral markers in blood. Following primary infection, CMV can be recovered from multiple body fluids such as saliva, urine and vaginal secretions for a variable period of time; however, virus shedding may also occur during reactivation and reinfection and hence does not allow differentiation between primary and non-primary infections. During the last decade, several methods have been developed to detect and quantify CMV in blood. The most widely used assays include determination of viraemia, antigenaemia, DNAemia, leuko–DNAemia, or RNAemia, and the search for the presence of circulating cytomegalic endothelial cells (CEC) in blood. In immunocompromised patients, the presence of CMV viraemia is commonly associated with a high risk of developing CMV disease. Thus, its determination represents a useful parameter for initiation of antiviral treatment, monitoring the efficacy of antiviral treatment and detection of treatment failure due to emergence of a drug-resistant CMV strain. The antigenaemia assay detects and quantifies peripheral blood leukocytes, mostly polymorphonuclear leukocytes and, to a much lesser extent, monocytes/macrophages, which are positive for the CMV lower matrix phosphoprotein pp65. Experience obtained with transplant recipients has shown that antigenaemia becomes positive earlier than viraemia but later than DNAemia at the onset of infection, and it becomes negative later than viraemia but earlier than DNAemia in the advanced stage of a systemic infection. High antigenaemia levels are often associated with CMV disease, the assay being widely used for monitoring CMV infections and antiviral therapy. Detection and quantification of CMV DNA in blood has become a major diagnostic tool for transplant recipients. For this purpose, two major approaches have been used: PCR and hybridization techniques. In immunocompromised patients, CMV DNA quantification has been shown to be useful for follow-up of disseminated infections and evaluation of the efficacy of antiviral treatment. In addition, it is also useful for the diagnosis and evaluation of the local effect of antiviral therapy at specific body sites, such as the eye and the nervous
system. Finally, a special application concerns its use for pre-natal diagnosis of CMV infection and for the quantification of viral DNA in amniotic fluid samples. Detection of CMV transcripts in blood is currently considered a marker of CMV replication in vivo and late viral transcripts in particular are considered to better reflect active CMV replication and dissemination. The term endotheliemia was introduced to indicate CMV-infected CEC in the peripheral blood of immunocompromised patients. More recently, CECs have been studied in haematopoietic stem cell transplant recipients, AIDS patients with disseminated CMV infection and foetuses and newborns with symptomatic congenital CMV infection.

The gold standard for diagnosis of congenital CMV infection is represented by virus isolation in human fibroblasts in the first 2 weeks of life, because subsequent virus excretion may represent neonatal infection acquired in the birth canal or following exposure to breast milk or blood products. Urine and saliva are clinical samples of choice. A pilot study concluded that to comprehensively establish CMV association in suspected congenital infection, both, nested PCR on urine and blood and IgM detection should be done, IgM detection in neonates having a value only in the absence of blood or blood product administration. Another study reported that the standard diagnostic test for congenital infection with CMV is viral culture within the first 3 weeks of life and where this is not feasible, there may be a limited role for CMV IgM detection, bearing in mind the causes of false positives and false negatives (>35%), especially in asymptomatic infected infants and when maternal primary infection occurs late in pregnancy. Another study from India analysed ELISA for CMV antibodies in CMV-DNA PCR-positive and -negative patients and concluded that ELISA has no diagnostic significance in the detection of CMV activation, although it may help in the differential diagnosis of CMV infection in the paediatric age group. Yet another Indian study, PCR for the immediate early gene of CMV, pp65 antigenaemia assay and IgM ELISA assay were correlated to detect the presence of CMV coinfection in AIDS and the authors concluded that PCR is a powerful tool for detection of CMV in blood and is superior to the antigenaemia assay. Clinical samples currently used for pre-natal diagnosis are foetal blood drawn by cordocentesis and amniotic fluid obtained by amniocentesis. Because of its high sensitivity and absolute specificity, isolation of CMV from amniotic fluid has been recognized as the reference for pre-natal diagnosis. No assay for detection of virus or virus components in foetal blood is sensitive enough to significantly improve pre-natal diagnosis of intrauterine transmission of the virus; however, tests performed on foetal blood are confirmatory of results achieved on amniotic fluid.

Immunocompromised individuals, including people living with HIV/AIDS, those receiving therapy for malignancies and transplant recipients, are on the rise in India. Although antiviral prophylaxis has led to a reduction of both morbidity and mortality of CMV disease in recent years, the toxicity associated with currently available antiviral agents remains a significant problem. Hence, efforts aimed at establishing cost-effective and rapid techniques for the diagnosis and typing of CMV to identify patients prior to the onset of disease thereby focusing antiviral treatment to patients at risk for disease only acquires greater significance in India. This strategy has been termed “preemptive” or “early” therapy. Although 60–100% of renal transplant recipients develop CMV infection, only 20–30% have symptomatic infection and it has been suggested that differences in virulence, pathogenicity, progression and severity of disease in immunocompromised individuals, including transplant recipients, may be attributed to variations between CMV strains. Appropriate antiviral therapy is based on a knowledge of the viral load as well as a quantification of the initial amount of CMV and the rate of increase between the last negative and the first positive PCR sample, which are independent risk factors for CMV disease. Evidence that CMV dissemination in blood is a significant risk factor for the progression from infection to disease comes from studies with allogeneic marrow transplant recipients, which showed that viraemia was highly predictive for the development of CMV disease. In solid-organ transplant recipients and HIV-infected patients, this association also exists but viraemia is somewhat less predictive of disease. The availability of diagnostic assays for the early identification of reactivated CMV replication has considerably improved the clinical management of these patients thus reducing the risk of CMV disease and allowing preemptive treatment as an alternative to universal prophylaxis. Early assays for CMV quantitation included the traditional plaque assay and the determination of the 50% tissue culture infective dose. These assays and modified tissue culture-based methods are hampered due to time-consuming procedures, poor reproducibility and/or a relatively low sensitivity. The determination of pp65 antigenaemia, consisting of direct staining of polymorphonuclear cells with monoclonal antibodies directed against the lower matrix protein pp65 (UL83) has long been the reference test for monitoring CMV reactivation. On technical grounds, however, pp65 antigenaemia, although less expensive, is often difficult to perform in leukopenic samples and suffers from major pitfalls such as labor-intensive manual procedure, need for immediate sample processing, lack of standardization, subjective and highly skilled interpretation of results and false-negative results because of low-level expression of the pp65 antigen on white blood cells in a small number of patients with definite CMV disease. Nevertheless, numerous studies indicate that the antigenaemia assay provides a good estimate of the systemic CMV burden, which correlates well with results obtained by quantitative PCR of leukocytes and plasma. Antigenaemia assays do not rely on the presence of replicating virus and are more sensitive than the conventional and shell vial cell cultures.
However, they are less sensitive than techniques based on molecular amplification and have inherent variability resulting from the lack of standardization.

Recently, a highly sensitive and specific PCR method that is not influenced by the white blood cell count in peripheral blood or by pp65 antigen expression has been used to diagnose CMV reactivation early after BMT. In real-time PCR (also called kinetic PCR), the accumulation of the PCR products is monitored continuously during the PCR run, compared with the end-point measurements that quantitate the final PCR product. The most commonly used detection methods in the virus diagnostic assays are based on the use of specific fluorogenic oligoprobes that rely on fluorescence resonance energy transfer (FRET), which is the interaction of two fluorescent dyes. The COBAS AMPLICOR CMV MONITOR assay (Roche Diagnostics, Pleasanton, CA, USA) was designed to detect CMV DNA by amplifying a segment of the CMV DNA polymerase gene UL54 within a turnaround time of approximately 3–4 h. With the LightCycler system (Roche Molecular Biochemicals, Indianapolis, IN, USA), results can be obtained rapidly (within 30–40 min); the system offers automation of PCR by precise air-controlled temperature cycling and provides continuous monitoring of amplicon development by a fluorometer (FRET) in a closed system. In addition, the LightCycler assay can analyze more samples per run (32 compared with 24) more quickly (240 min compared with 460 min) than the COBAS AMPLICOR CMV MONITOR assay. When clinical samples collected during episodes of CMV infection were analyzed using the LightCycler system, the results obtained closely correlated with those obtained with the COBAS AMPLICOR CMV MONITOR assay.[34] The NucliSens assay (Organon Teknika Diagnostics, Boxtel, The Netherlands), an isothermal nucleic acid amplification reaction assay, detects the presence of CMV late-mRNA pp67. Nevertheless, early experience suggests that this assay is less sensitive than DNA amplification assays and antigenaemia assays for detection of CMV infection. The Digene Hybrid Capture CMV DNA assay (Digene Corporation, Silver Spring, MD, USA) is a rapid, qualitative, signal-amplified solution hybridization assay that utilizes RNA probes that bind to the DNA target followed by antibodies directed to RNA–DNA hybrids as well as a sensitive chemiluminescence detection system. The utility of the assay in predicting the occurrence of CMV disease and its utility in monitoring the response to antiviral therapy is currently being investigated. The development of real-time methodology and the availability of automated real-time instruments have further simplified quantitative PCR assays and reduced the turnaround time needed for the test. In one study, using real-time-automated PCR as an alternative to the conventional PCR to detect CMV reactivation after BMT, it was possible to detect CMV infection at a very early stage compared with conventional detection methods, and real-time-automated PCR proved to be more time saving than the antigenaemia assay.[35] Another study has shown that quantitative detection by duplex real-time automated PCR system has a higher sensitivity than the COBAS Amplicor CMV Monitor test system and the reproducibility of both test systems is acceptable, but imperfect, and an international reference standard is needed.[36] Real-time PCR methods for CMV DNA quantitation could offer a convenient alternative for monitoring CMV replication that is less influenced by the quality of sample and more suited to standardization and automation when compared with antigen detection tests. In addition, real-time PCR requires a small volume of plasma (200 µL) as compared to 3–5 ml of whole blood required for antigenaemia, and can be performed on neutropenic patients. Although several studies have already addressed this issue, the correlation between antigenaemia results and real-time PCR, as well as the clinical significance of both the assays, requires further evaluation. Another analysis concluded that the sensitivity of the real-time PCR in detecting CMV infection was considerably higher than that of pp65 determination and that pp65 antigenaemia, despite being less sensitive for the diagnosis of active CMV infection when compared with CMV DNA load, showed a better qualitative and quantitative correlation with the presence of symptoms. Only at the onset of active replication was the DNA load able to discriminate patients at risk of developing CMV disease; hence, both tests can be useful for the prospective monitoring of immunocompromised subjects and clinically relevant cut-off levels should be identified for specific groups of patients and assays, and prospectively validated for their predictive value over the development of clinical symptoms of infection.[37] There is substantial evidence that a weekly measurement of the systemic CMV load during the first 3 months after transplant is useful to predict CMV disease in SOT recipients. Although good data is available for renal, liver and heart transplant recipients using the CMV antigenaemia assay, more data is needed to define breakpoints for CMV DNA in various patient populations using molecular methods. Prospective longitudinal studies are needed to further determine the utility of the systemic CMV DNA load for predicting the clinical outcome as well as the frequency of viral load determination and the viral load threshold associated with clinical progression. Quantification of CMV will undoubtedly play an important role in the future not only in identifying patients at high risk for disease but also in assessing the response to antiviral treatment. Transplant recipients are now monitored routinely for the CMV viral load at many centres and HIV-infected individuals are likely to benefit from such an approach as well.[31]

**Treatment of CMV Infection**

Two different strategies, prophylaxis and preemptive therapy, are used for the prevention of symptomatic CMV infection following transplantation. In prophylaxis, antiviral drugs are administered before any evidence of the virus
and in preemptive therapy, antiviral drugs are administered when there is laboratory evidence of active but asymptomatic infection. The three antiviral drugs that are currently licensed for the treatment of CMV infection are ganciclovir (GCV), phosphonofomate (PFA) or foscarnet and cidofovir (CDV). GCV has been shown to be effective for prophylaxis and treatment of CMV infection in multiple studies. PFA, unlike GCV, does not require intracellular phosphorylation to inhibit DNA polymerase and, therefore, retains activity against most GCV-resistant strains of CMV. Intravenous PFA may be used under conditions of failure of GCV treatment, GCV resistance or excessive side effects such as leucopaenia. However, PFA is nephrotoxic, which limits its use in kidney transplantation. CDV acts directly on DNA polymerase and may be used as an alternative to PFA in case of GCV resistance, but experience with this agent in SOTs is still very limited. A promising new agent, valganciclovir, a prodrug of GCV, is as potent as intravenous GCV in induction therapy for newly diagnosed retinitis and has the advantages of oral bioavailability, once-daily dosing and reduced risk of development of resistance. Maribavir, an orally bioavailable benzimidazole compound not phosphorylated in cells, does not inhibit DNA polymerase but appears to interfere with DNA synthesis by blocking a virus-specific process on a new viral target. Passive immune prophylaxis seems to be inferior to antiviral therapy due to cost and heterogeneity of the preparation but is still used as an adjunct therapy with intravenous GCV in severe CMV infections. Although the efficacy of prophylaxis has been proven in many studies, there are still some controversial issues. It has been suggested that prophylaxis may only delay the development of CMV disease in some patients.[37] Long-term and/or suboptimal antiviral therapy may also induce resistant CMV strains.[38] Also, the fact that prophylaxis exposes all patients to potentially toxic drugs may diminish the utility of this approach.[37] In addition, although the prophylaxis strategy has been found as a cost-effective intervention against CMV,[39] the surveillance strategies may be less costly.[37] The advantage of preemptive therapy is the targeting of antiviral drugs only to those patients who have laboratory evidence of active CMV infection. This minimizes the number of patients who are exposed to antiviral drugs. Currently, the drug of choice for preemptive therapy is GCV.

Drug Resistance in CMV Infection

Although advances in antiviral therapy against CMV have improved outcomes from CMV disease in the immediate post-transplant setting, antiviral drugs have toxicities and emergence of antiviral resistance is a concern. After the more widespread use of antiviral drugs, resistant CMV strains have been reported in the transplant patient population.[40,41] Until recently, there have been only scattered reports of GCV resistance in transplant recipients. As reported in a study of 240 allograft recipients, subsets at special risk were CMV-seronegative patients who received an organ from a CMV-seropositive donor and those patients who required long periods of intense immunosuppression. On average, the patients in this report were treated with GCV for a mean of 190 days, which suggested that the duration of GCV exposure is also an important factor in the development of resistance.[42] In contrast, GCV resistance during antiviral treatment of CMV retinitis in adults with AIDS has been shown to occur frequently after more than 3 months of therapy, and resistance to PFA and CDV has been described after even longer durations of therapy.[43] Studies in AIDS patients made clear that GCV resistance develops in a slow but progressive manner. GCV-resistant CMV seems to infect immunocompromised patients who have either profound immunosuppression with very high viral loads or who have undergone long periods of therapy.[44] In the BMT setting, no report exists of CDV resistance and only very few about GCV and PFA resistance.[45] Nevertheless, there are indications that children with combined immune deficiencies after T-cell-depleted BMT are at a high risk of developing early GCV resistance.[46]

The high level of viral replication (due to primary infection), a prolonged antiviral therapy and administration of immunosuppressive drugs such as cyclosporine and corticosteroids as well as the conditioning regimen required for haemopoetic stem cell transplantation (and therefore an incompetent immune response with low CD4 and CD8 T-cell counts) are all factors that favour the emergence of resistant viruses during a course of therapy.[42] These factors are most prevalent in patients with primary infection, i.e., in the D+/R- population, in which resistance has most often been observed.[47] The mutations in both, the viral phosphotransferase gene (UL97) and the viral polymerase gene (UL54) may confer antiviral drug resistance in CMV. The mutations of the UL97-coding sequence, which may confer resistance only to GCV, occur mainly in the region including codons 460–607. The more rare mutations in the UL54-coding sequence may confer resistance to any or all of the three most commonly used drugs (GCV, PFA or CDV). Management of GCV-resistant CMV infections is difficult. Increased dosages of GCV in combination with CMV hyperimunoglobulin and reduction of immunosuppression or combination therapy with GCV and PFA have been used.[48] New drugs are also under development, many having different targets than the currently available DNA polymerase inhibitors,[49] but their suitability for clinical use will be seen in the near future. Recently, quantitative PCR has also proven its utility in the monitoring of response to antiviral treatment.[49]

The currently available methods for antiviral susceptibility testing rely on the suppression of virus growth in the presence of serial concentrations of antiviral drugs (phenotypic assays) or the determination of specific mutations that has been associated to confer resistance (genotypic assays).
Phenotypic Assays

Phenotypic methods assess the concentration of the drug that inhibits virus replication. Typically, the level of virus is plotted against the concentration of the drug that causes 50% inhibition of the virus in cell cultures. The phenotypic methods that have been employed include plaque reduction assay (inhibition of viral replication), ELISAs (inhibition of protein synthesis), flow cytometric fluorescence-activated cell sorter and DNA hybridization assays (inhibition of viral DNA synthesis). The plaque reduction assay is the standard method of antiviral susceptibility testing for CMV. The test is burdensome and lacks standardization. It requires the recovery of the virus in cell cultures followed by several passages to attain the necessary viral titres for the performance of the assay. Benchmark analysis of several strains of CMV has shown wide variability in results. Typically, these assays require at least 4 weeks to obtain results.[34]

Genotypic Assays

The significant problems with the use of phenotypic assays and the recognition that specific mutations in the UL54 and UL97 genes of CMV are associated with antiviral drug resistance have led to the development of molecular methods for the detection of the CMV mutants. UL97 encodes for the thymidine kinase that is essential for the initial phosphorylation of GCV into its active form. Accordingly, the functional consequence of these mutations is the inadequate intracellular phosphorylation of GCV into the GCV monophosphate form thus leading to GCV resistance. Because CDV and PFA do not require thymidine kinase, UL97 mutations do not confer resistance to these agents. These mutations can be detected by direct sequencing of the PCR products (combination of PCR-based amplification and sequencing for rapid diagnosis of GCV-resistant CMV strains). Mutations in the UL54 CMV DNA polymerase gene, the main target of all three antiviral drugs, could result in the resistance to any or all of the three drugs. Most UL54 mutations are accompanied by UL97 mutations; strains with double UL97 and UL54 mutations are believed to be highly resistant to GCV with possible cross-resistance to CDV and/or PFA.[50] The rapid thermocycling utilized by the automated PCR methods may help us attain the goal of real-time antiviral susceptibility testing. While the LightCycler assay may not detect the exact point mutation, it could serve as a screening method before gene sequencing can be performed. The antigenaemia test is useful for viral monitoring as a guide for preemptive therapy after organ transplantation. Persistence of high counts of antigenaemia may indicate inadequate antiviral therapy or emergence of resistance. Recurrence of positive antigenaemia after treatment of CMV disease can be a sign of relapse. Antiviral drug resistance in CMV is now an emerging concern in certain groups of CMV-infected patients and hence antiviral susceptibility testing will be a common occurrence during the upcoming years. Currently, the conventional phenotypic methods are not rapid or standardized enough to be of immediate clinical utility in CMV disease management and genotypic assays, although easily performed with modern molecular methods need optimization and clinical validation; thus, surrogate markers such as the failure of the viral load to decrease during antiviral treatment are used as indirect measures of antiviral resistance. Prudent use of antiviral drugs will hopefully limit the problem of drug resistance.

Vaccines Development in CMV Infection

The impact of congenital CMV infection as a public health problem is not widely recognized and, when recognized, cannot be addressed adequately either by education or prevention. The economic costs to society associated with congenital CMV infection are considerable in terms of lifelong disability and requiring long-term residential care and extensive medical intervention. In a recent review of priorities for vaccine development, the Institute of Medicine ranked a vaccine to prevent CMV disease at the highest priority on the basis of the economic costs that would be avoided and the years of life and disability that would be saved by a successful vaccine. Because these costs are substantial, an effective vaccine could confer a lifetime of benefit for a newborn infant, by preventing neurological sequelae and other disabilities, and result in considerable cost savings for society. Potential benefits could include reduced CMV disease following transplantation, reduced use of antiviral therapy, prolonged graft survival and reduction in CMV-associated transplant complications, including graft versus host disease and fungal infections. Although the correlates of vaccine-engendered immunity that limit CMV disease are not fully understood, several CMV vaccines have been evaluated in pre-clinical and clinical studies.[51] Approaches to the development of a safe and effective CMV vaccine for the prevention of CMV diseases include (a) a live attenuated vaccine (Towne strain), (b) recombinant constructs of the attenuated Towne and the virulent Toledo CMV strains, (c) subunit glycoprotein B (gB) adjuvanted with MF59 to induce neutralizing antibodies, (d) pp65 peptide-based vaccines to induce cytotoxic T lymphocytes (CTL) for use in therapeutic vaccination, (e) canary pox–CMV recombinants, e.g., ALVAC–CMV (gB) and ALVAC-CMV (pp65) to induce neutralizing antibodies and CTL responses respectively, (f) DNA plasmids containing the genes for gB and pp65 and (g) dense bodies containing the key antigens. The attenuated Towne strain, gB/MF59, ALVAC–CMV (gB) and ALVAC–CMV (pp65) approaches have already been tested in clinical trials. Meanwhile, other approaches such as DNA peptide and dense body vaccines should enter Phase I trials.[53] However, vaccine developers are uncertain about the immunological basis of protection and the design and conduct of appropriate clinical trials. The various constraints for a successful vaccine efficacy trial are listed below.[52]
1. Definition of the desired clinical trial end point: prevention of infection or illness, infections in day care attendees, infections in mothers of day care attendees, viraemia in pregnancy, foetal infection or foetal disease and disease in adults, such as as transplant recipients.

2. Identification of sites and populations appropriate to the end point chosen, such as day care centres (for prevention of infections in infants) or CMV-negative women likely to become pregnant in the next year.

3. Standardization and validation of methods chosen to detect infection, such as virus isolation, PCR and/or serological testing.

4. Raising consciousness concerning the risks of CMV infection, particularly intrauterine infection, among physicians and prospective trial volunteers.

5. Important unanswered questions about congenital CMV infection: mechanisms by which CMV harms the foetus, the relationship of maternal viraemia (virus load and duration of viraemia) to foetal infection, role of immunopathology in foetal disease, mechanisms that permit or block placental transfer, characteristics of immune response, whether the protein specificity of the initial immune “burst” and the subsequent memory T cell repertoire induced by CMV vaccines should mimic the patterns that are observed with naturally acquired immunity, the use of animal models such as murine, guinea pig or rhesus CMV to explore virus–host interactions, how host age or genetic make up may alter the outcome of CMV infection, whether a novel strain of CMV is more likely to infect a person with pre-existing immunity due to CMV infection, will vaccination protect against all strains of CMV, protection afforded by prior infection, importance of strain heterogeneity and do the antigenic differences noted among strains mean that vaccines must be multivalent, etc. An increased awareness of the public health importance of congenital CMV infection will be required to foster the development and testing of CMV vaccines and to drive the industry and regulatory commitment required to solve this public health urgency.[1]

The facts presented in this article conclude beyond doubt that CMV infections cause considerable burden on society, especially in a developing country like ours, and we truly need to develop and implement consensus strategies for prevention of CMV infection, ideally through a vaccine.

References


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