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Mini review

Summary of the II International Symposium on Cytomegalovirus

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Abstract

Human cytomegalovirus (HCMV) is a highly species-specific DNA virus belonging to the Betaherpesvirinae subfamily of the herpesviridae family. Like other herpesviruses, primary infection with HCMV is followed by persistence of the virus in a latent form. The sites of latency are still largely undefined, but they probably include bone marrow progenitor cells and peripheral blood monocytes. From these sites, the virus can reactivate, resulting in renewed shedding of the virus, or, in immunocompromized persons, development of disease. Humans are the only reservoir of HCMV and transmission occurs by person-to-person contact. Infection with HCMV is common. In most developed countries, HCMV seroprevalence steadily increases after infancy and 10–20% of children are infected before puberty. In adults, the prevalence of antibodies ranges from 40 to 100%. Although HCMV has a world-wide distribution, infection with HCMV is more common in the developing countries and in areas of low socioeconomic conditions, which is predominantly related to the closeness of contacts within these populations. Except for a mononucleosis-like illness in some persons, infection with HCMV rarely causes disease in immunocompetent individuals. However, HCMV can cause severe morbidity and mortality in congenitally infected newborns and immunocompromized patients, most notably transplant-recipients and HIV-infected persons. This article provides a review of the information presented at the Second International Symposium on Cytomegalovirus organized and convened by The Macrae Group (New York City, NY) in Acapulco, Mexico on 24–28 April 1998. During this

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symposium, the state-of-the-art knowledge on diagnosis, treatment and prophylaxis of HCMV infections were discussed, and, based on this information, attempts to highlight the future directions in basic and clinical research areas that need to be stimulated to facilitate advancement in prevention and treatment of CMV disease.

Keywords: Human cytomegalovirus; Diagnosis; Treatment; Prophylaxis

1. Introduction

Human cytomegalovirus (HCMV) is a highly species-specific DNA virus belonging to the Betaherpesvirinae subfamily of the herpesviridae family. Like other herpesviruses, primary infection with HCMV is followed by persistence of the virus in a latent form. The sites of latency are still largely undefined, but they probably include bone marrow progenitor cells and peripheral blood monocytes. From these sites, the virus can reactivate, resulting in renewed shedding of the virus, or, in immunocompromized persons, development of disease.

Humans are the only reservoir of HCMV and transmission occurs by person-to-person contact. Infection with HCMV is common. In most developed countries, HCMV seroprevalence steadily increases after infancy and 10–20% of children are infected before puberty. In adults, the prevalence of antibodies ranges from 40 to 100%. Although HCMV has a world-wide distribution, infection with HCMV is more common in the developing countries and in areas of low socioeconomic conditions, which is predominantly related to the closeness of contacts within these populations.

Babies and children can become infected during birth by passage through a contaminated uterine cervix, during the postnatal period through breast-feeding, or during early childhood by transmission from other children in nurseries or daycare centers. After puberty, salivary and sexual transmission represent the most important mode of HCMV infection. Congenital infection is relatively common, occurring in 1% of all live births, and can be associated with severe sequelae. It represents the most common infectious cause of mental retardation and hearing disturbances in children. HCMV can also be transmitted via bloodproducts and transplanted organs, posing a severe threat to transplant recipients.

Except for a mononucleosis-like illness in some persons, infection with HCMV rarely causes disease in immunocompetent individuals. However, HCMV can cause severe morbidity and mortality in congenitally infected newborns and immunocompromized patients, most notably transplant-recipients and HIV-infected persons. In the latter patient groups, CMV disease can either result from primary infection or reactivation of latent virus (Chou, 1987; Grundy et al., 1988).

In view of the continuing importance of HCMV as a cause of morbidity and mortality, a second international symposium on cytomegalovirus was organized and convened by The Macrae Group (New York City, NY) in Acapulco, Mexico on April 24–28, 1998. During this symposium, the state-of-the-art knowledge on diagnosis, treatment and prophylaxis of HCMV infections were discussed. This article provides a review of the information presented at this symposium, and, based on this information, attempts to highlight the future directions in basic and clinical research areas that need to be stimulated to facilitate advancement in prevention and treatment of CMV disease.

The chairs of the symposium were Brian Gazzard, Chelsea and Westminster Hospital, UK, and Stephen Spector, University of California San Diego, USA. The presenters at the symposium were Fausto Baldanti, Servicio di Virologia, IRCCS, Italy; William Britt, University of Alabama at Birmingham, USA; Carol Brosgart, East Bay AIDS Center, USA; Frans Cromme, Organon

Teknika, The Netherlands; Lisa Dunkle, Bristol-Myers-Squibb, USA; Judith Feinberg, University of Cincinatti Medical Center, USA; Kirk Field, University of Massachussets Medical Center, USA; Paul Griffiths, Royal Free Hospital School of Medicine, UK; Lisa Grillone, Isis Pharmaceuticals Inc., USA; Gary Holland, Jules Stein Eye Institute, UCLA, USA; Douglas Jabs, The Johns Hopkins University School of Medicine, USA; Thomas Jones, Wyeth-Ayerst Research, USA; Show Yi Kao, Roche Molecular Systems, USA; George Kemble, Aviron, USA; Earl Kern, University of Alabama at Birmingham, USA; Janice Kolberg, Chiron Corporation, USA; Jorge Leon, Digene, USA; Deborah Lewinsohn, Fred Hutchinson Cancer Center, USA; Robert Pass, University of Alabama at Birmingham, USA; Carlos Paya, Mayo Clinic, USA; Michael Polis, NIH, USA; Charles Robinson, Roche Global Development, USA; Deborah Spector; University of California San Diego, USA; Stephen Spector, University of California San Diego, USA.

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2. Diagnostics

Diagnostic methods for CMV infection and CMV-associated disease include isolation of the virus by culture, histology of biopsies, serologic methods, measurement of pp65 antigen in leucocytes, and detection of viral nucleic acids using molecular methods, particularly the polymerase chain reaction (PCR). A review of the various diagnostic methods is provided in the summary of the previous edition of this symposium (van der Meer et al., 1996).

In recent years, the use of highly sensitive molecular methods for detection of virus has gained increasing attention, especially for the purpose of identifying patients at high risk of developing CMV disease. Established CMV disease is clearly characterized by high levels of CMV in blood, or to lesser extent, in urine, which indicates an important role of virus load in the pathogenesis of disease (Kidd et al., 1993; Drouet et al., 1995; Rasmussen et al., 1995; Bowen et al., 1996; Cope et al., 1997a,b; Bowen et al., 1998). While the sensitivity of viral culture is relatively low, the correlation between virus detection and disease is especially obvious using more sensitive methods, such as antigen-detection or PCR methods. In addition, prospective studies in transplant recipients and HIV-infected patients have shown that the detection of pp65 antigenemia, or, most notably, PCR detection of CMV DNA in blood, and to a lesser extent in urine, are highly predictive of subsequent development and clinical outcome of disease (Marenzi et al., 1996; Bowen et al., 1997; Dodt et al., 1997; Manfredi et al., 1997; Rasmussen et al., 1997; Shinkai et al., 1997; Spector et al., 1998). Furthermore, quantitative analyses of CMV DNA levels have shown that high absolute levels of virus, as well as increases of virus load over time carry the worst prognoses (Cope et al., 1997a,b; Gor et al., 1998). Finally, the risk of CMV disease is decreased in patients showing a conversion of PCR positivity to negativity during prophylactic therapy.

In view of their excellent ability to identify patients at high risk of developing disease, the highly sensitive molecular methods for virus detection may play an increasingly important role in the management of CMV disease by serving as a marker for pre-emptive treatment (see Section 3.1). In addition, quantitative molecular assays, as well as the semi-quantitative antigenemia tests, allow for monitoring of treatment efficacy, and possibly, individualization of therapy (Einsele et al., 1991; Kuhn et al., 1994; Bowen et al., 1996; Spector et al., 1998).

To date, the vast majority of studies correlating CMV disease with detection of virus by molecular methods have used non-commercial in-house methods. Although the optimal use of these methods in clinical practice is being studied intensely, standardization of detection methods, especially for quantitative assays, is clearly desirable. Several commercial molecular assays for quantitation of CMV DNA or CMV mRNA, including PCR-, signal amplification-, NASBA-, and hybrid cap-

ture assays, are on the verge of becoming widely available. The relative benefits of these assays clearly require further clinical evaluation. Finally, it remains to be evaluated whether further increases in the sensitivity of virus detection are needed for optimal management of CMV disease. Even in immunocompetent individuals, reactivation of latent virus may be associated with renewed low-level shedding of virus, which indicates that ultrasensitive detection of virus does not necessarily result in improved predictive values for disease. It needs to be analyzed whether there exists a certain threshold level of virus load below which there is no increased risk of developing CMV disease.

3. Treatment and prevention strategies

3.1. Treatment strategies

In the management of CMV disease, four different strategies can be distinguished (Griffiths, 1997). Beside antiviral treatment of manifest disease, these include prophylactic, suppressive, and pre-emptive treatment, which are aimed at prevention of disease. In prophylaxis, treatment is started in the absence of detectable virus or disease, which is aimed at prevention of CMV infection or reactivation in patients at risk of subsequently developing disease. In suppressive and pre-emptive treatment strategies, therapy is started in the absence of manifest disease, but in the presence of detectable virus. These strategies are aimed at limiting treatment to individuals at higher risks of developing CMV disease. The distinction between suppressive and pre-emptive treatment is based on the site of virus detection. Suppressive treatment is started when virus is detectable in peripheral sites, such as urine or throat-washings, while pre-emptive treatment is based on the detection of virus systemically, such as in blood or in broncho-alveolar lavage fluid. As reviewed in the previous section, the detection of viremia by sensitive methods, such as PCR or antigen detection, carries the highest predictive value for development of disease.

The relative benefits of the four strategies in specific patient populations, i.e. in congenital CMV disease, transplant patients and HIV-infected patients, will be considered below. In general, however, the choice of the clinical strategy depends on the risk of CMV disease and the toxicity of the drug. For example, in a population with a high expected incidence of CMV disease, prophylactic treatment may be the optimal strategy with acceptance of a certain degree of toxicity. However, if the general risk of CMV disease in a population is low, a prophylactic strategy may result in overtreatment and toxicity which is not acceptable. In this case, identification of patients at higher risk of developing CMV disease would be preferable. In general, the more stringent these patients are identified, the higher the degree of acceptable toxicity can be. For example, in view of the relatively low predictive value for CMV disease of peripheral virus detection, acceptable toxicity should be low in case of suppressive treatment. Conversely, higher degrees of toxicity may be acceptable in case of pre-emptive treatment. Beside reducing exposure to the toxic effects of a drug, pre-emptive treatment strategies also potentially decrease costs. However, formal cost-benefit analyses are needed to evaluate whether this is indeed the case.

3.2. Antiviral drugs

Currently available treatment options for CMV infections include the CMV DNA polymerase-inhibitors ganciclovir, foscarnet and cidofovir. Although these agents potently inhibit CMV replication, they exhibit toxicity and require intravenous administration to obtain therapeutic drug levels, both of which limit their use for long-term treatment. Although an oral formulation of ganciclovir is also available, the bio-availability is low, which appears to restrict its use to prevention purposes and maintenance treatment of CMV retinitis in HIV-infected persons (Drew et al., 1995; Oral Ganciclovir European and Australian Cooperative Study Group, 1995; Spector et al., 1996; Gane et al., 1997). For the treatment of CMV retinitis, local administration of antiviral agents into the eye has also been used (see Section 6.2).

For prophylaxis, orally administered agents with minimal toxicity clearly are desirable. Although the anti-CMV activity of acyclovir is insufficient for treatment of manifest disease, high-dose prophylactic treatment with this drug has been shown to confer some benefit in select patient groups (Rubin and Tolkoff-Rubin, 1993). By achieving drug levels similar to intravenous treatment, the use of valacyclovir, the valine-ester prodrug of acyclovir, may improve the prophylactic or pre-emptive efficacy of acyclovir (Feinberg et al., 1998). The development of a similar valine-ester prodrug of ganciclovir (valganciclovir) may represent a valuable addition to the treatment arsenal, both for treatment of disease and for pre-emptive therapy. Drug levels achieved with a single daily dose of 900 mg valganciclovir are comparable to levels obtained with intravenous ganciclovir at dosages of 5 mg/kg/day. While clearly far more convenient, it may thus be expected that the efficacy and safety of once or twice daily oral dosing with valganciclovir will be comparable to intravenous treatment. Several studies are ongoing in transplant recipients and HIV-infected patients to evaluate whether this is indeed the case, both for pre-emptive treatment as well as for treatment of CMV retinitis.

Although considerable advances in the treatment and prevention of CMV disease have been achieved with the currently available agents, there clearly is room for novel drugs with improved efficacy. Several new antiviral agents are currently in clinical development, including lobucavir, adefovir-dipivoxil (bis pom-PMEA), and antisense oligonucleotides.

Lobucavir is a nucleoside analogue with activity against a broad-spectrum of viruses, including herpes simplex virus (HSV), varicella zoster virus, hepatitis B virus (HBV) and CMV (Tenney et al., 1997). Its good oral bio-availability and long half-life should permit convenient oral dosing schemes, although there seems to be substantial pharmacokinetic inter-subject variation. Although reversible hepatic enzyme elevations may be observed, lobucavir is generally well tolerated without evidence of myelosuppression. While a moderate beneficial effect on CMV viruria and virus load in semen has been observed in a small uncontrolled study in HIV-infected persons, further evaluation of its clinical efficacy in the management of CMV disease is ongoing.

Adefovir-dipivoxil is a prodrug of adefovir (PMEA) with 40% oral bioavailability and a long half-life, which permits once-daily oral dosing. Adefovir is a nucleotide-monophosphate analogue with in vitro activity against a wide range of viruses, including HIV, HBV, HSV and CMV (Xiong et al., 1997). In vitro, the activity of adefovir against HCMV is similar to ganciclovir, and it is also active against HCMV isolates with reduced susceptibility to ganciclovir, foscarnet and/or cidofovir. Specific mutations which confer resistance to the drug have been identified in the DNA polymerase gene (UL54), which do not confer cross-resistance to the other respective drugs. Adefovir-dipivoxil is generally well tolerated, although the drug has been associated with reduced serum carnitine levels, which may require supplementation. In vivo, moderate reductions in semen CMV DNA load have been observed within a substudy of a clinical trial evaluating its activity against HIV-1 infection (Deeks et al., 1997). Two large scale clinical studies are currently ongoing in HIV-infected patients, with primary endpoints being its effect on CMV disease and survival.

Antisense oligonucleotides complementary to RNA transcripts of immediate-early genes, which act by inhibiting gene expression, provide a novel mechanism of inhibition of viral replication (Pari et al., 1995; Anderson et al., 1996). In vitro, these agents show activity against CMV, and, not surprisingly in view of the different mechanism of action, they are also effective against ganciclovir-resistant strains. A small number of antisense oligonucleotides are currently in clinical development, both for systemic therapy, as well as for intravitreal treatment. They seem to be well tolerated, both systemically and intravitreally, although intravitreal administration may be associated with adverse effects, such as increased intraocular pressure and inflammation. Clinical evaluation of these compounds is ongoing.

3.3. Viral drug resistance

As is the case for other viruses, most notably HIV, incomplete suppression of viral replication during chronic antiviral treatment predisposes to the development of viral drug resistance and consequent drug-failure. Indeed, the emergence of CMV strains with reduced susceptibility to ganciclovir, foscarnet and/or cidofovir during long-term therapy has been documented, and has been associated with increases in virus load in blood or urine and clinical failure (Erice et al., 1989; Knox et al., 1991; Sarasini et al., 1995; Wolf et al., 1995; Boivin et al., 1996; Alain et al., 1997; Bowen et al., 1998; Smith et al., 1998).

Genetic analysis of ganciclovir-resistant CMV isolates has shown that resistance to this drug is conferred by specific mutations in the UL97 gene, encoding for the viral phosphotransferase responsible for the initial phosphorylation of ganciclovir, as well as in the UL54 gene, which encodes for the viral DNA polymerase (Chou et al., 1995; Erice et al., 1997). Since foscarnet does not require phosphorylation for its activity, and cidofovir is already effectively monophosphorylated, resistance to these drugs is conferred by mutations in the UL54 gene only (Baldanti et al., 1996). High level, but not low level, ganciclovir-resistant CMV variants exhibit reduced susceptibility to cidofovir, and studies have shown that, while low level ganciclovir resistance is conferred by UL97 changes, mutations in the UL54 gene confer higher levels of resistance to ganciclovir and cross-resistance to cidofovir (Chou et al., 1997; Erice et al., 1997; Smith et al., 1997).

Since the development of drug resistance is driven by ongoing viral replication in the presence of selective drug pressure, prevention of resistance obviously requires optimal suppression of virus replication. In this light, there is concern regarding the long-term use of oral ganciclovir, since the suboptimal drug levels achieved with this formulation may facilitate the development of resistance. The issue of drug resistance clearly provides a potentially important rationale for continuing efforts to identify new potent antiviral drugs, including agents with novel mechanisms of action, and the use of combined chemotherapy.

3.4. Immunotherapy

The importance of cellular immunity in controlling CMV infection or reactivation, is evidenced by the fact that development of severe disease is almost invariably restricted to individuals with compromised cell-mediated immunity. This cell-mediated immunity includes class I major histocompatibility complex (MHC I)-restricted CD8+ cytotoxic T lymphocytes (CTL) and MHC II-restricted CD4+ T helper lymphocytes. In transplant-recipients and HIV-infected persons, the development of disease, e.g. pneumonitis or retinitis, is clearly correlated with absent or dimished CMV-specific CD8+ CTL responses and CD4+ lymphocyte responses. The major viral antigens to which CTL responses are directed appear to be tegument proteins,especially pp65 (McLaughlin-Taylor et al., 1994; Riddell and Greenberg, 1997). Recognition of this protein, which is abundantly present at entry of the virus, occurs prior to the onset of viral gene expression and persists at all stages of viral replication. Viral immune evasion strategies during active viral replication, involving MHC I down-regulation by early gene products, may explain the relative lack of significant CTL responses to other proteins (Riddell and Greenberg, 1997). In addition, pp65 seems to selectively inhibit presentation of immediate early gene products.

Considering the importance of cell-mediated immunity, the transfer of CMV-specific CD8+ CTL-clones, derived from seropositive donors and expanded in vitro, may prove valuable in the management of CMV disease (Riddell et al., 1992). Indeed, in a small uncontrolled phase I study in bone marrow transplant-recipients, this strategy has shown promising results, i.e. persistent reconstitution of CTL responses similar to immunocompetent persons, a suggestion of effective prevention of viremia and CMV disease, and minimal toxicity (Walter et al., 1995). In a currently ongoing phase II study, BMT recipients are also being treated with CMV-specific CD4+ T helper-clones, in addition to CD8+ CTL-clones. In view of the tropism of HIV, the use of CD4+ T cell clones obviously does not seem appropriate in HIV-infected persons. However, using intracel-

lular immunization strategies, CMV-specific CD4+ T cell clones have been generated, which are not permissive for productive HIV-infection. The efficacy of such genetically manipulated CD4+ T cell-clones in restoring CMV-specific immunity in HIV-infected patients is the subject of ongoing studies.

While cellular immunity is clearly important, several lines of evidence also indicate a potential role of humoral immunity in preventing or modulating CMV disease. The major targets for neutralizing antibodies are the envelope glycoproteins gB, and to lesser extent gH. Antibodies do not fully protect against infection since reinfection of seropositive persons does occur, as well as maternal-fetal transmission in seropositive pregnant women. Studies in animal models suggest that, rather than controlling infection, antibodies seem to aid in viral clearance and reduce dissemination of the virus (Jonjic et al., 1994). In humans, the preconceptual presence of maternal antibodies clearly reduces the rate of congenital infection and modulates the severity of congenital CMV disease (Fowler et al., 1992). The risk of intra-uterine transmission of virus appears to be dependent on the level of maternal neutralizing antibodies. In HIV-infected patients with CMV retinitis, higher levels of neutralizing antibodies have been associated with slower progression rates (Boppana et al., 1995).

In view of the potential efficacy of antibodies in preventing or modulating disease, passive immune prophylaxis, using either intravenous immunoglobulins or CMV-specific hyperimmune serum, has been extensively studied in variety of patient populations. However, while immunoglobulin-prophylaxis seems to reduce the severity of disease in some solid organ transplant-settings, the efficacy has been limited and seems inferior to antiviral treatment strategies. Since the level of antibodies appear to be important for their antiviral activity, the reason for the apparent limited efficacy may, in part, be secondary to the attainment of insufficient antibody titers (Bachmann et al., 1997).

3.5. Vaccines

The most important rationale for development of a CMV vaccine is prevention of congenital CMV disease by preventing primary maternal infection. As will be summarized below, prevention of congenital infection is complicated by the fact that maternal infection usually passes unnoticed. In addition, prevention of maternal primary infection by limiting exposure to the virus is complicated by the fact that primary infection in children, representing the most important source of maternal infection, is followed by prolonged shedding of the virus in multiple body fluids, allowing for ample opportunities to spread infection. Sexual activity, which is another important risk factor for maternal infection, also is not easily amenable for preventive measures.

As mentioned in the previous section, the presence of maternal antibodies before conception clearly is effective in reducing the rate of congenital infection and preventing severe disease (Fowler et al., 1992). It has been estimated that vaccination of CMV seronegative mothers would prevent 80–85% of congenital CMV disease. Beside its potential use for prevention of congenital disease, effective vaccination strategies may also be useful in specific high-risk adult populations, e.g. seronegative transplant recipients.

In initial efforts to identify an effective vaccine, the Towne strain of CMV has been used, which is a laboratory-adapted, attenuated virus variant, originally isolated from a newborn with congenital disease (Plotkin et al., 1975). Initial studies showed that vaccination with this strain is safe and partially protected healthy volunteers from direct challenge with a non-attenuated strain (Plotkin et al., 1989). However, humoral and cellular immune responses to the Towne-strain are lower when compared to natural infection. In seronegative transplant recipients, vaccination with the Towne strain reduced the severity of disease without affecting the infection rate (Plotkin et al., 1984, 1991, 1994). Similarly, seroconversion rates in vaccinated mothers of children in daycare centers were comparable to mothers receiving placebo (Adler et al., 1995).

Thus, while the Towne strain may confer limited efficacy in some settings, vaccination strategies with improved protective efficacy are desirable.

Beside the use of an attenuated live strain, potential strategies include the use of subunit vaccines. In view of the immunodominance for neutralizing antibodies of the gB envelope glycoprotein, this antigen may be useful for this purpose. Studies evaluating the use of gB subunit vaccination, either as a purified recombinant protein or in the context of a viral vector, are currently ongoing.

An alternative approach for vaccination is the development of recombinant viral chimeras. Genomic comparison of the attenuated Towne strain with the non-attenuated, virulent Toledo strain of CMV, showed an additional 13 kb DNA fragment in the latter (Quinnan et al., 1984; Cha et al., 1996). Hypothesizing that this fragment may contain important genes for virulence and/or immunogenicity, several chimeras of the Towne- and Toledo strain have been constructed (Kemble et al., 1996). Studies in SCID-hu mice indicated that, while the virulence of chimeric viruses was similar to the Towne-strain, the immunogenicity may be retained. Therefore, these chimeric viruses may potentially confer improved protective efficacy, while retaining the safety of the Towne-strain. Phase I studies evaluating the safety and immunogenicity of these chimeras are planned.

Finally, DNA vaccination provides an elegant vaccination approach, in which immunogenic proteins are synthesized by host cells after intracellular delivery of genes encoding these proteins. The potential advantages of DNA vaccination include the lack of an infectious agent or live vector, the ease of production, manipulation and storage of DNA vaccines, and the potentially long-lasting responses to DNA vaccination. This strategy is currently undergoing evaluation in animal models.

4. Congenital HCMV infection

4.1. Clinical syndrome

Maternal primary HCMV infection occurs in approximately 2% of pregnancies, and results in transmission to the fetus in 30–40% of cases. Congenital infection following maternal primary infection gives rise to clinical disease at birth in about 7% of infected infants, of whom approximately 10% die of the consequences. While recurrent HCMV infection in pregnant women with immunity acquired before pregnancy can also result in congenital infection, clinical disease or severe sequelae seldom develop, indicating, as noted before, that the presence of maternal antibodies offers substantial protection to the fetus.

In a varying range of severity, symptomatic congenital HCMV disease is manifested by petechiae, jaundice, hepatosplenomegaly, chorioretinitis, microcephaly, and neurologic abnormalities, which may include encephalopathy, cerebral palsy, seizures, and sensorineural hearing deficits. In surviving children, the clinical outcome is mainly determined by the neurologic sequelae. Congenital HCMV infection is the most common infectious cause of mental retardation and deafness. Following symptomatic congenital HCMV infection, progressive hearing loss develops in up to 60% of cases and mental retardation in 35–50% of patients. The vast majority of infected infants who are asymptomatic at birth, remain free of sequelae. However, since progressive hearing deficits may develop in 7–15% of these children, serial audiologic examinations during childhood are also strongly advised in congenitally infected children who were asymptomatic at birth (Williamson et al., 1992; Fowler et al., 1997). Whether asymptomatic infants are at increased risk of mental retardation remains unclear, and requires large cohort studies which are currently in progress.

Beside maternal primary infection and symptomatic disease of the newborn, risk factors for severe neurologic sequelae include the presence of apparent neurological abnormalities or chorioretinitis at birth, and cerebral CT or MRI scan abnormalities. A varying range of CT and MRI scan abnormalities are seen in approximately two thirds of infants with symptomatic disease, and include periventricular calcifications, ventricular dilatation, pachygyria and cerebral atrophy (Boesch et al., 1989; Sugita et al., 1991). Gestational age at infection is not an absolute predictor

of neonatal disease. While the risk of severe neurological sequelae in the newborn is substantially higher in case of first trimester infections, severe complications can also occur in infants infected during later stages of pregnancy (Steinlin et al., 1996). Finally, the presence of detectable virus in the blood or urine at birth appears to be associated with a poor clinical outcome (Stagno et al., 1975; Barbi et al., 1996). The potential use of molecular methods for detection of virus in predicting the risk of neurological complications remains to be investigated.

4.2. Management of congenital HCMV infection

Limiting life- or organ-threatening disease, preventing progressive loss of hearing and optimizing long-term neurological development clearly seem goals of antiviral treatment. However, in contrast to HCMV disease in transplant-patients and HIV-infected persons, there has been little progress in the field of antiviral therapy or prevention of congenital CMV disease. To date, one dose-comparative study evaluating the efficacy and tolerance of a 6-week course of intravenous ganciclovir has been performed in congenitally infected infants at high risk of neurological sequelae, i.e. newborns with intracranial calcifications, retinitis, or hearing loss (Whitley et al., 1997). This study showed that the toxicity of ganciclovir is manageable in this patient group, with most babies being able to complete the full 6-week course of intravenous ganciclovir at dosages up to 12 mg/kg per day. Although the majority of infants demonstrated a virologic effect of treatment, i.e. a decline in viruria, the clinical efficacy of ganciclovir in congenital HCMV infection remains to be proven in controlled studies.

Complicating treatment of congenital CMV infection is the fact that the neurologic sequelae of congenital HCMV infection are progressive during childhood. In addition, the onset of hearing loss, and in some cases retinitis, can be delayed by several months. While the role of host-derived immunopathological mechanisms in the development of the neurological sequelae, especially the loss of hearing, still needs to be defined, it is clear that viral replication persists during the first several years of life. In view of these observations it may be doubted that a short course of antiviral treatment during the first few weeks of life can significantly prevent or modulate the complications of congenital HCMV infection; long-term therapy, possibly of several years duration, may thus be needed, and requires drugs with a favorable long-term safety-profile. Indicative of the fact that a short course of treatment may not be sufficient to alter the natural course of infection is the observation in the aforementioned ganciclovir-study that viruria immediately resurged after withdrawal of therapy (Whitley et al., 1997).

Beside treatment of the newborn, intra-uterine treatment of the fetus may be warranted to prevent neurodevelopmental abnormalities. In the primary development of the brain, neurons develop from the germinal matrix during the first 8–20 weeks of pregnancy, followed by migration to the cortex at 24–26 weeks, and further organization of the neurons from 26 weeks to post-term. Astrocytes and subsequently oligodendrocytes develop at 20 weeks, and begin myelination at week 26, which also continues until post-term. With organization of neurons and myelination still ongoing, the central nervous system thus is not fully developed at birth, and introduction of antiviral treatment at this time could therefore preserve some of the functions of the brain. However, it is obvious that abnormalities in the primary development of the brain during early pregnancy cannot be prevented by postnatal treatment. In view of the neurodevelopmental stages, it is not surprising that congenital infection during the first trimester of pregnancy carries the highest risk of severe neurological sequelae. Investigating the precise neuropathogenesis of CMV clearly is difficult in humans, especially since the exact gestational time of infection is often unknown. For this reason, studies have been performed in rhesus macaques, which are invariably CMV-seropositive at reproductive ages, in which the fetuses of pregnant animals were infected directly in utero at defined time-points in pregnancy (Tarantal et al., 1998). These studies showed that macroscopic morphological abnormalities of the brain were observed in newborn animals infected between the first and second trimester, but not in monkeys

infected after this time. Interestingly, around this time, the placenta begins to transmit maternal antiviral antibodies to the fetus, which may play a role in preventing further neurodevelopmental abnormalities.

While intra-uterine treatment seems important in preventing neurological sequelae of congenital infection, especially during the first trimester, a major problem is that approximately 95% of primary HCMV infections in pregnant women occur unnoticed. Furthermore, since the maternal-fetal transmission rate is only 30–40%, treating all maternal primary infections would result in considerable overtreatment with a potentially toxic agent.

Prevention of primary infection in pregnant women clearly represents the ideal strategy for preventing the morbidity and mortality of congenital HCMV disease. The most important risk factor for maternal primary infection is the age of the mother, especially in lower socioeconomic areas; teen-mothers are at extremely high risk of primary HCMV infection with seroconversion rates of up to 35% (Fowler et al., 1993). It is estimated that prevention of teen-pregnancies would result in an approximately 50% decrease of the incidence of congenital CMV disease. In addition, pregnant women exposed to young children, either in daycare centers or in households, also have an increased risk of primary infection. Finally, sexual activity represents an important risk factor. Unfortunately, in practice, neither of these risk factors are easily amenable for successful prevention purposes. Therefore, the only reasonable hope for prevention of primary infection in pregnant women lies within the development of an effective vaccine, which should aggressively be pursued.

5. CMV disease in transplant recipients

5.1. Clinical syndromes

Although the incidence of CMV disease following solid organ- or bone marrow transplantation is declining in the era of prophylactic or preemptive antiviral therapy, HCMV still is a major cause of morbidity and mortality in transplant recipients.

The most important risk factor for the development of CMV disease in solid organ transplant (SOT) recipients is donor seropositivity coupled with seronegativity of the recipient (D + R −), potentially resulting in primary HCMV infection in the recipient. While secondary infection in a seropositive SOT recipient, i.e. reactivation or superinfection, may also lead to CMV disease during immunosuppression, the risk is substantially smaller and disease generally is less severe. The risk of CMV disease is also dependent on the immunosuppressive regimen used. A regimen that includes OKT3 antibodies carries a high risk of CMV disease development (Portela et al., 1995). Finally, intercurrent bacterial infections, development of fulminant hepatitis after liver transplantation and the occurrence of graft-versus-host disease (GVHD) in bone marrow transplant recipients are all associated with an increased risk of CMV disease after transplantation (Meyers et al., 1986; Smyth et al., 1991; Mutimer et al., 1997). Interestingly, sero-epidemiologic studies in seropositive (D + R +) SOT recipients have suggested that co-infection with human herpesvirus-6 (HHV-6), which is an immunosuppressive virus in vitro, and possibly in vivo, represents an additional risk factor for the development of CMV disease, and may account for a substantial portion of D + R + recipients who develop CMV disease in the absence of other risk factors, such as OKT3 treatment (Dockrell et al., 1997).

When coupling all risk factors, two pathogenic mechanisms for the development of CMV disease can be deduced, i.e. absent immunity and/or immunosuppression (D + R −, OKT3 treatment), and reactivation of latent virus, which may be enhanced secondary to interactions with other viruses (perhaps HHV-6 and others), or increased cytokine production ('cytokine-storm') during bacterial infection, GVHD, or OKT3 treatment. As reviewed previously, established CMV disease is associated with high levels of CMV in blood or urine, indicating the importance of virus load in the pathogenesis of CMV disease. Since most aforementioned epidemiological risk factors are probably associated with increased viral replica-

tion, the detection of high viral burdens in blood or urine most likely represents the common denominator of most of these risk factors. Using molecular methods of viral nucleic acid detection, further studies relating epidemiological risk factors to virus load are currently in progress.

In bone marrow transplant (BMT) recipients, the most important disease manifestations are CMV pneumonia, and to a lesser extent gastrointestinal disease. In D + R − SOT recipients, CMV disease is usually associated with the organ of transplant, e.g. hepatitis after liver transplantation or pneumonia after lung transplantation, suggesting that viral reactivation and subsequent development of disease is, at least initially, restricted to the transplanted organ. CMV reactivation may, in a similar manner, also account for the development of atherosclerosis and coronary vasculopathy in the transplanted heart, and restenosis after coronary angioplasty or atherectomy (Zhou et al., 1996). Beside its role as a direct cause of disease, CMV may also have a number of indirect effects which influence post-transplantation morbidity and mortality. While still controversial, CMV may play a role in causing acute or chronic rejection of the transplanted organ. In addition, the immunosuppressive effects of the virus may enhance the risk of other opportunistic infections. Observations suggesting an increased risk of fungal infections in CMV-infected patients have been reported (Wagner et al., 1995; George et al., 1997). Although it is difficult to exclude the effects of immunosuppressive therapy, results of epidemiologic studies suggested an enhanced risk of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in CMV-infected transplant recipients (Badley et al., 1996).

5.2. Management of CMV disease in transplant recipients

While several antiviral agents are currently available or in clinical development (see Section 3), treatment of established CMV disease in transplant patients often has only limited benefit. In part, this limitation may be due to the fact that suppression of viral replication does not necessarily reverse secondary pathological processes which may have been triggered by CMV. For example, the pulmonary damage of CMV pneumonitis largely seems to be caused by the cell-mediated immune response evoked by CMV, rather than by the direct effects of the virus itself (Grundy et al., 1987; Milburn et al., 1990). This mechanism may explain why established CMV pneumonitis in BMT- or lung transplant recipients generally responds poorly to anti-CMV treatment.

In view of the limited benefit of treating overt disease, prophylactic antiviral treatment has generally been used during the past several years in the routine clinical management of transplant patients, while the use of pre-emptive treatment is gaining increasing attention. The optimal strategy for prevention of CMV disease in transplant patients, i.e. prophylactic versus pre-emptive treatment, as well as the choice of drug, probably will depend on the risk of post-transplantation CMV disease and the toxicity of treatment. In this respect, the situation is different for BMT recipients, compared to SOT patients.

5.2.1. Management of BMT recipients

In CMV seronegative BMT recipients, the most effective way to prevent CMV disease is to avoid exposure to exogenous sources of CMV by the selective use of CMV-seronegative donors of blood products and bone marrow, or by the use of filtered blood (Bowden et al., 1991; Miller et al., 1991). In CMV-seropositive recipients, several studies have evaluated the prophylactic use of intravenous ganciclovir. While these studies showed a reduced incidence of CMV disease in patients receiving ganciclovir, this benefit was not accompanied by a commensurate effect on the mortality due to the toxicity of ganciclovir in this patient group (neutropenia), which appeared to counterbalance the beneficial effects of treatment on CMV disease (Goodrich et al., 1993; Winston et al., 1993). Of note, the incidence of CMV disease in untreated BMT patients is approximately 15–35%, and universal prophylaxis would therefore result in considerable overtreatment with this toxic drug.

The problem of drug toxicity has been the incentive to identify and treat only patients at high risk of developing CMV disease, and it is in

BMT patients that pre-emptive treatment strategies were initiated. While initial studies evaluating the efficacy of pre-emptive treatment based on positive cultures of blood or urine showed significant reductions in the risk of CMV disease and mortality, improved efficacy was subsequently observed when treatment is based on more sensitive methods for detection of virus, i.e. detection of viral nucleic acids using PCR (Rubin, 1991; Einsele et al., 1995).

However, relative to the substantial reduction in the risk of CMV disease in BMT patients during the last years, the reduction in mortality rates has only been minor. Of special concern is the observation that, while the incidence of CMV disease during the first 100 days post-transplantation is reduced significantly during prophylactic or pre-emptive treatment, there is an increased risk of late CMV disease (>100 days post-transplantation) (Boeckh et al., 1996). Although the reason for this phenomenon is unknown, it was hypothesized that the potent suppression of viral replication by ganciclovir may prevent immunologic priming to the virus in BMT patients, which would result in an incapability by the patient to fight renewed viral replication after ganciclovir treatment is stopped. In view of this hypothesis, it was further argued that the use of less potent drugs, such as (val)acyclovir or oral ganciclovir, which would enable immunization while still preventing CMV disease, should be investigated. Of note, prophylactic treatment with acyclovir has indeed been shown to significantly reduce the incidence of CMV disease and associated death in BMT patients (Prentice et al., 1994).

Beside the use of antiviral drugs, several studies have evaluated the efficacy of immunoprophylaxis with either intravenous immunoglobulins (IVIG) or CMV antibody-enriched immunoglobulins (CMVIG) in BMT patients. While CMVIG has been shown to decrease the incidence of CMV infection after bone marrow transplantation, it does not seem to be effective in preventing CMV disease. In contrast, IVIG has no effect on the incidence of infection, but appears to significantly reduce the risk of CMV disease (Messori et al., 1994). The mechanism for the latter observation is unknown, but it has been hypothesized that the reduced risk of CMV disease results from an IVIG-mediated influence on acute GVHD (Sullivan et al., 1990).

An alternative form of immunoprophylaxis, which considering the importance of cell-mediated immunity in clearing active CMV infection seems more appropriate than immunoglobulin prophylaxis and indeed has shown promising results, is the adoptive transfer of CMV-specific CD8+ cytotoxic T cell-clones from the donor to the recipient (see Section 3.4) (Walter et al., 1995).

5.2.2. Management of SOT recipients

In SOT patients, the risk of developing CMV disease depends on the organ of transplant, with risks being relatively low after kidney transplantation, higher after liver- and heart transplantation, and highest in lung transplant patients. In addition, as mentioned previously, the incidence of CMV disease increases in the presence of certain risk factors, most notably D+R− transplantations and OKT3 treatment. The optimal strategy for prevention of CMV disease in SOT patients appears to be determined by the risk of CMV disease, and therefore on the type of transplantation and the absence or presence of risk factors for development of CMV disease. For example, prophylaxis with a short course of ganciclovir, and even prophylaxis with a less potent drug such as acyclovir, has been shown to reduce significantly the incidence of CMV disease in renal transplant recipients, including D+R− transplantations (Balfour et al., 1989; Brennan et al., 1997). However, in liver- or heart transplant patients, short-term ganciclovir treatment only reduced CMV disease in CMV-seropositive recipients, and not in seronegative patients at risk for primary infection (Merigan et al., 1992; Dunn et al., 1994). In liver transplant patients, reductions of CMV disease after D+R− transplantation have only been observed during prolonged prophylactic treatment (>4 weeks) with either oral or intravenous ganciclovir and/or during sequential therapy with ganciclovir and high doses of acyclovir (Winston et al., 1995; Badley et al., 1997; Gane et al., 1997; Seu et al., 1997). Similarly, the beneficial effects of immunoprophylaxis with CMV-specific hyperimmunoglobulins are su-

perior in renal transplant patients, compared to recipients of other organs.

Since, unlike BMT patients, toxicity of ganciclovir treatment does not pose a major problem in SOT patients, the need for pre-emptive treatment strategies is less urgent in the latter patient group. While reducing toxicity and costs also seem a worthwhile rationale for pre-emptive treatment in these patients, future studies evaluating this strategy should include formal cost-benefit analyses. Pre-emptive treatment in SOT patients could either be based on the presence of risk factors, such as OKT3 treatment, or, similar to BMT patients, on the detection of virus (antigenemia, PCR). Reductions in the risk of CMV disease in renal transplant recipients have been observed using either of these strategies (Singh et al., 1994; Hibberd et al., 1995; Gomez et al., 1996; Gotti et al., 1996). Preliminary results of an ongoing placebo-controlled study evaluating the efficacy of PCR-based pre-emptive treatment with oral ganciclovir in liver transplant recipients were presented. In this study, weekly blood samples were obtained during the post-transplantation period, and patients with a positive PCR were randomized to oral ganciclovir or placebo. Patients receiving OKT3 treatment and patients with active CMV infection as detected by blood culture were not randomized, but received treatment with intravenous ganciclovir. Although this study is still unblinded, a striking result was that none of 20 PCR-negative patients had developed CMV disease during follow-up, versus seven of 48 randomized PCR-positive patients, which exemplifies the potential use of PCR as a marker for pre-emptive treatment. Other important observations were that PCR and blood cultures became positive at the same time in all five patients who were excluded from randomization due to evidence of active CMV infection, and that all of these patients involved D + R − transplantations. These findings suggest that, in D + R − patients, the rate of viral replication may be so rapid that the lag-time between detection of virus by PCR and by blood culture disappears, which seems to argue against the use of pre-emptive treatment in such high-risk patients. Universal prophylaxis may represent the preferred strategy for prevention of CMV disease in high-risk SOT patients, most notably D + R − recipients and patients receiving OKT3 treatment. Conversely, PCR- or antigenemia-based pre-emptive treatment may be preferable in low-risk transplantations, e.g. non-D + R − or renal transplantations. For the purpose of either strategy, the use of the more novel oral agents, such as valganciclovir, requires further research.

6. CMV disease in HIV-infected persons

6.1. Clinical syndromes

Ever since the beginning of the AIDS pandemic, HCMV has been a major cause of morbidity in HIV-infected individuals. As CMV seroprevalence rates are high among HIV-infected persons, especially in homosexual men, CMV disease is usually caused by reactivation of latent virus, rather than by primary infection. The development of CMV disease in HIV-infected persons is clearly correlated with the severity of immunodeficiency, occurring predominantly in patients with less than 50 CD4 + lymphocytes per mm^3 (Gallant et al., 1992). The most common and debilitating manifestation of CMV disease is retinitis, occurring in up to 30% of patients with CD4 + cell counts lower than 100 cells/mm^3, and accounting for approximately 80–90% of HCMV end-organ disease in HIV-patients (Gallant et al., 1992; Hoover et al., 1996). It usually begins unilaterally in the peripheral regions of the retina, and is manifested by a painless loss of visual acuity, which progresses as disease advances to the central retinal regions, and ultimately results in blindness. In the course of disease, involvement of the contralateral retina may also occur. Other, less frequently occurring manifestations of CMV disease include gastro-intestinal disease, most notably esophageal disease and colitis, and neurological disease, including meningo-encephalitis and polyradiculopathy. Although post-mortem studies have shown a high prevalence of CMV infection in adrenal glands, clinical evidence of Addison's disease during life is rare. In contrast to transplant recipients, HCMV pneumonitis is sel-

dom observed in HIV-patients. This difference may be explained by the immunopathological nature of HCMV pneumonitis and the inability of immunocompromized HIV-patients to mount a significant cell-mediated immune response (Grundy et al., 1987).

Since the introduction of highly active antiretroviral treatment (HAART), i.e. combined therapy with potent HIV reverse transcriptase- and protease inhibitors, two important changes in the occurrence of HIV-associated CMV disease have been observed. On one hand, secondary to improved suppression of HIV replication and associated maintenance of a relatively intact immune system, the rate of CMV retinitis is declining substantially. In addition, probably due to HAART-induced improvement of cell-mediated immunity, manifest retinitis seems easier to control than before. In fact, resolution of CMV retinitis during HAART has even been observed in the absence of anti-CMV treatment (Reed et al., 1997; Whitcup et al., 1997; Tural et al., 1998). On the other hand, a sudden onset of increased intraocular inflammation has also been observed during HAART (Jacobson et al., 1997). This phenomenon may be explained by an inflammatory response, upon HAART-induced restoration of the immune system, to viral proteins present in inactive retinal CMV lesions. By a similar mechanism, it has been expected that the incidence of CMV pneumonitis would increase in patients receiving HAART (Grundy et al., 1987). However, there has been no evidence of such an increase to date.

6.2. Management of CMV disease in HIV-patients

Controlling CMV retinitis poses a continuing challenge with only limited success. Although systemic high-dose induction therapy with currently available anti-CMV agents, including ganciclovir, foscarnet and cidofovir, is effective in controlling acute retinitis, relapses of disease almost invariably occur during chronic maintenance dosing. Improving suppression of viral replication by combined treatment with ganciclovir and foscarnet has been shown to prolong the progression-free interval (Studies of Ocular Complications of AIDS Research Group, 1996). However, in an intent to treat analysis, visual acuity outcomes in patients receiving combined treatment were similar compared to patients treated with monotherapy, while the quality of life in the former group of patients was substantially diminished due to long infusion times and increased toxicity.

Poor penetration of the drugs to the eye, resulting in inadequate drug levels intraocularly, represents the most important reason for the high progression rates during systemic treatment (Kuppermann et al., 1993; Arevalo et al., 1995). In part related to suboptimal drug levels, the development of drug-resistance is another potential factor in causing drug failure during chronic suppressive treatment (see Section 3.4).

The importance of drug levels is exemplified in a study comparing maintenance treatment with foscarnet at dosages of 90 and 120 mg/kg/day, which showed higher progression rates in the lower dose group (Jacobson et al., 1993). In addition, while masked assessment of fundus photographs in a study comparing intravenous and oral maintenance treatment with ganciclovir did not show statistically significant differences, clinical assessment of time-to-progression in this study clearly favored intravenous treatment with ganciclovir (Drew et al., 1995). The problem of insufficient intraocular drug levels during systemic therapy can be circumvented by direct intravitreal administration of anti-CMV agents. Several studies have evaluated the efficacy of intravitreal treatment with either ganciclovir, foscarnet or cidofovir (Ussery III et al., 1988; Heinemann, 1989; Cochereau-Massin et al., 1991; Diaz-Llopis et al., 1994; Kirsch et al., 1995; Hodge et al., 1996; Rahhal et al., 1996; Tognon et al., 1996; Taskintuna et al., 1997). In these studies, intravitreous injections with ganciclovir or foscarnet were performed 2–3 times weekly during the first 2 weeks (induction), followed by weekly injections. Intravitreal cidofovir can be given every 6 weeks. Although these studies were uncontrolled, the beneficial effects on CMV retinitis at least appeared to be similar to intravenous treatment. Although the therapeutic window of intravitreous cidofovir is relatively small, with a substantial risk

of uveitis at therapeutic dosages, intravitreous injection of the respective drugs is generally well tolerated. However, repeated injections into the eye has logistic problems, as well as an increased risk of infection. In this respect, an important development has been the use of an intra-ocular sustained-release ganciclovir implant, which achieves sufficient intra-ocular drug-concentrations for a period of up to 6–8 months (Martin et al., 1994; Musch et al., 1997). In a study comparing the use of this implant with intravenous ganciclovir treatment, the implant proved to be more effective in reducing the risk of CMV retinitis progression (Musch et al., 1997). Vitreous hemorrhage represents the most common post-operative complication of implant-procedures, the risk of which appears to increase with each subsequent implant-exchange through the same site (Martin et al., 1997).

The most important disadvantage of intravitreal treatment, as evidenced in controlled studies evaluating this strategy, is the lack of systemic protection against CMV, resulting in an increased risk of contralateral retinitis or visceral disease (Musch et al., 1997). Combining intravitreal and systemic treatment therefore seems appropriate. In a randomized clinical trial evaluating the efficacy of the ganciclovir implant in combination with either placebo, oral ganciclovir or intravenous ganciclovir, the risk of visceral disease or contralateral retinitis was lowest in patients treated with intravenous ganciclovir, followed by patients receiving oral ganciclovir.

In conclusion, while advances in the treatment of CMV retinitis have been evident during the past decade, the development of treatment regimens with improved efficacy needs to continue to be pursued. Although the rate of CMV retinitis is declining in the HAART era, this decline should not lead to a relaxation of these efforts. A considerable proportion of HIV-infected patients do not have access to HAART, and it remains to be seen whether the beneficial effects of HAART will persist in the long term.

Several novel antiviral agents are currently in clinical development and may prove to be valuable additions to the anti-CMV treatment arsenal. In addition, the quest for additional potent antiviral agents and predictable animal models for evaluation of these agents should continue. For this purpose, the development of a SCID-hu mouse model containing human retinal implant tissue may prove to be a valuable tool (DiLoretto et al., 1994).

In view of the difficulty in controlling manifest retinitis and the fact that this disease ultimately may result in complete loss of vision, prevention of CMV retinitis should clearly be the ultimate goal of management. While prevention or restoration of immunodeficiency by HAART appears to confer beneficial effects in this respect, one should not rely on HAART for this purpose in view of the reasons mentioned above.

Antiviral treatment strategies aimed at prevention of CMV disease are facilitated by the development of potentially effective oral drugs, such as oral ganciclovir, valganciclovir, and other drugs under development. One study of prophylactic treatment with oral ganciclovir of CMV seropositive, severely immunocompromised (< 50 CD4+ lymphocytes/mm^3) showed that it reduced the risk of developing CMV disease by 50% (Spector et al., 1996). However, 74% of placebo-recipients in this study did not develop CMV disease during the 12-month study period, suggesting that prophylactic treatment was not (yet) needed in a large proportion of patients. This seems to argue for improved identification of patients at high risk of developing CMV disease and pre-emptive treatment. The relative benefit of preemptive treatment is supported by the results of a study comparing the prophylactic use of valacyclovir and acyclovir in a similar patient group (CMV seropositive, < 100 CD4+ lymphocytes/mm^3) (Feinberg et al., 1998; Griffiths et al., 1998). In this study, valacyclovir prophylaxis reduced the risk of CMV disease by 33% when compared to prophylaxis with acyclovir (Feinberg et al., 1998). However, the strongest beneficial effect on CMV disease was observed in patients who were PCR-positive in blood, and to a lesser extent in urine, prior to initiation of treatment (Griffiths et al., 1998). These findings suggest that, while valacyclovir does have activity as a prophylactic agent, it seems more effective in pre-emptive treatment strategies.

As mentioned previously, the detection of virus by molecular amplification methods, especially in blood, is highly predictive of retinitis development, and provides a valuable tool for pre-emptive treatment. Interestingly, instances of high levels of viremia preceding the development of retinitis, but undetectable levels at the time of diagnosis of retinitis, have also been observed. These cases suggest that, after viral reactivation and seeding to the eye, the retina may represent an independent reservoir of continued viral replication.

Beside its predictive value for CMV disease, the level of CMV DNA load also predicts survival in HIV-infected persons (Bowen et al., 1996; Spector et al., 1998). There is no significant correlation between CMV DNA- and HIV-1 RNA load in patients with advanced AIDS, indicating that the survival-effect of CMV DNA load is directly related to the increased risk of CMV disease, rather than to an interaction between CMV- and HIV-1 replication.

7. Conclusions and future directives

In recent years, the increasing knowledge on the pathogenesis, improved treatment options, and the development of sensitive diagnostic methods have greatly contributed to advances in the management of CMV infections. Sensitive molecular methods for detection and quantitation of CMV DNA load have enabled improved identification of patients at high risk of developing CMV disease, and are important tools for guiding treatment against CMV infections. Pre-emptive treatment strategies, based on PCR detection of virus, have shown promising efficacy in a number of settings, and are being used increasingly in the routine clinical care of select patient groups, e.g. BMT recipients. Prophylactic or pre-emptive treatment strategies are facilitated by the development of oral antiviral drugs with potential efficacy for these purposes, such as oral ganciclovir or valganciclovir. In addition, several novel antiviral drugs are currently in clinical development, including agents with novel mechanisms of action, which may prove valuable additions to the treatment arsenal. Likewise, immunologic approaches, most notably vaccines and transfer of CMV-specific CTL- or T helper cell clones, are potentially effective strategies for prevention of CMV disease.

While considerable progress in the field of diagnosis and treatment of CMV infections has thus been made, CMV disease continues to pose a severe health threat in select patient populations, and further research is required to determine the optimal strategy for management of CMV infections in individual patient groups. In this respect, important questions include the choice of prophylactic versus pre-emptive treatment, the choice of drug, and, for the purpose of identifying high-risk patients, the issues of which method for virus detection to use in which material and at which time-points.

For detection of CMV DNA, several methods have been used and additional new commercial assays are in clinical development. Clearly, standardization of virus detection methods is necessary, and studies attempting to address this issue are urgently needed.

The optimal preventive strategy, as well as the choice of drug, may depend on the clinical setting. In BMT patients, pre-emptive strategies with potent antiviral agents seem preferable, and a degree of toxicity should probably be accepted. However, the occurrence of late-onset CMV disease is of concern, and may require evaluation of less potent antiviral agents or alternative approaches, e.g. immunotherapy. In SOT patients, prophylaxis, rather than pre-emptive treatment may represent the optimal strategy in the presence of risk-factors for development of disease, most notably D + R − transplantations or OKT3 treatment. The same may be true for high-risk transplantations, such as lungs. In other situations, pre-emptive treatment may be preferable in view of the potential advantages concerning toxicity and costs.

In HIV-infected persons, high progression rates during treatment of established retinitis clearly argue for prevention of disease. Prophylactic, and especially pre-emptive treatment strategies have shown promising results to this extent. In the HAART era, the incidence of CMV retinitis is declining, while increased inflammation during

HAART has also been observed. Especially in view of the latter observation, persistence of virus in the retina and immune function in the eye represent interesting subjects for future research. Although the rate of retinitis is declining, efforts to improve the management of retinitis should not diminish, in view of the unknown long-term effects of HAART and the substantial numbers of people who do not have access to HAART.

Although congenital CMV infection represents the most common infectious cause of brain damage and hearing disturbances in children, progress in the field of managing congenital CMV disease clearly lags behind the other clinical settings. Potent antiviral treatment of infected newborns may provide some benefit and should be further evaluated. However, prevention of primary maternal infection remains the ultimate goal, for which purpose the development of an effective vaccine is warranted and should intensively be pursued.

In the pathogenesis of CMV infection and disease, the role of virus load has increasingly become clear. However, the role of the immune system, with all its complexity, is still poorly understood. A potentially valuable strategy to gain more insight in the immune control of CMV infection is to target research efforts at acute infection and the establishment of latency in immunocompetent individuals. Finally, the role of CMV in the pathogenesis of other disease entities, such as atherosclerosis and vasculopathy, requires continuing research efforts.

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