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Summary of the International Consensus Symposium on Advances in the Diagnosis, Treatment and Prophylaxis of Cytomegalovirus Infection

Jan T.M. van der Meer[a,*], W. Lawrence Drew[b,c], Raleigh A. Bowden[d,e], George J. Galasso[f], Paul D. Griffiths[g], Douglas A. Jabs[h], Christine Katlama[i], Stephen A. Spector[j], Richard J. Whitley[k]

[a]*Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, University of Amsterdam, F5-162, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands*
[b]*Department of Clinical Microbiology and Infectious Diseases, University of California San Francisco, San Francisco, CA, USA*
[c]*Mt. Zion Medical Centre, University of Califiornia San Francisco, San Francisco, CA, USA*
[d]*Fred Hutchinson Cancer Research Center, Seattle, WA, USA*
[e]*University of Washington, Seattle, WA, USA*
[f]*National Foundation for Biomedical Research, Bethesda, MD, USA*
[g]*Communicable Diseases Division, Royal Free Hospital Medical School, London, UK*
[h]*Departments of Ophthalmology and Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[i]*Hôpital Pitié Salpêtrière, Service de Maladies Infectieuses, Paris, France*
[j]*Department of Pediatrics and Infectious diseases, University of California San Diego, La Jolla, CA, USA*
[k]*Departments of Pediatrics and Microbiology, University of Alabama School of Medicine, Birmingham, AL, USA*

1. Introduction

Cytomegaloviruses (CMVs) are highly species-specific DNA viruses that are found universally throughout the world and that commonly infect many animals, including humans. Many genetically unique strains of human CMV (HCMV) continually circulate in the general population. In the vast majority of cases the initial infection is asymptomatic. Some persons experience a mononucleosis-like illness with fever and a mild hepatitis. However, HCMV can cause severe morbidity and mortality in congenitally infected newborns and immuno-compromised patients. As with other herpesviruses, primary CMV infection is followed by persistent infection. The site of virus latency is unknown. Infectious HCMV may be shed in the body fluids of any previously infected person, and is most likely to be found in semen, urine, saliva, cervical secretions and breast milk. The shedding of virus usually occurs without either detectable signs or symptoms. In persistent infection the excretion of virus is indefinite but intermittent and may reflect reactivation of latent infection and/or persistent low-level chronic infection. Prolonged excretion of virus characterizes certain forms of HCMV infection such as primary infection and infection in the immunocompromised host.

* Corresponding author. Tel.: +31 20 5664479; fax: +31 20 6918821; e-mail: j.meer@amc.uva.nl

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To discuss the state-of-the-art knowledge on diagnosis, treatment and prophylaxis of HCMV infections a consensus symposium was organized and convened by The Macrae Group (New York City, NY, USA) in Fort Myers, FL, USA on April 21–23, 1996. This article provides a review of the information presented at this symposium, and the consensus reached in certain areas based on this information.

Members of the consensus panel were W. Lawrence Drew, Raleigh A. Bowden, George J. Galasso, Paul D. Griffiths, Douglas A. Jabs, Christine Katlama and Stephen A. Spector.

The presenters at the symposium were Dan Kisner, ISIS Pharmaceuticals, USA; Henry H. Balfour, Jr., University of Minnesota, USA; Karen K. Biron, Burroughs Wellcome Co., USA; Raleigh Anne Bowden, Fred Hutchinson Cancer Research Center, USA; Carol Brosgart, East Bay AIDS Center, USA; Julie Cherrington, Gilead Sciences, USA; Sunwen Chou, Veterans Administeration Medical Center, USA; Clyde S. Crumpacker, Berth Israel Hospital, USA; Douglas T. Dieterich, NYU Medical Center, USA; W. Lawrence Drew, Mt. Zion Medical Center of UCSF, USA; Judith E. Feinberg, University of Cincinnati Medical Center, USA; A. Kirk Field, Hybridon, Inc., USA; Giuseppe Gerna, University of Pavia, Italy; Paul Griffiths, Royal Free Hospital Medical School, England; Phuc Le Hoang, Hôpital Pitié Salpêtière, France; Carlos Isada, Cleveland Clinic, USA; Douglas Jabs, The Johns Hopkins University, USA; Mark Jacobson, University of California, San Francisco, USA; M. Colin Jordan, University of Minnesota, USA; Christine Katlama, Hôpital Pitié Salpêtière, France; George Kemble, Aviron, USA; Earl R. Kern, University of Alabama at Birmingham, USA; Janice Kolberg, Chiron Corporation, USA; Jacob Lalezari, University of California, San Francisco, USA; Per Ljungman, Huddinge University Hospital, Sweden; Daniel Martin, Emory Clinic, USA; Edward Mocarski, Stanford University, USA; Brent Petty, The Johns Hopkins University, USA; Stanley A. Plotkin, Pasteur Merieux SV, France; Richard B. Pollard, University of Texas Medical Branch, USA; Firas M. Rahhal, University of California San Diego, USA; Stanley Riddell, Fred Hutchinson Cancer Research Center, USA; Robert H. Rubin, Massachusetts General Hospital, USA; Stephen A. Spector, University of California San Diego, USA; Kathleen E. Squires, University of Alabama, Birmingham, USA; Mary Jean Stempien, Roche Bioscience, USA; Richard J. Whitley, University of Alabama at Birmingham, USA; John Zaia, City of Hope National Medical Center, USA.

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2. Epidemiology

Humans are believed to be the only reservoir for HCMV and transmission occurs by person-to-person contact. Infection with HCMV is more widespread in the developing countries and in areas of lower socioeconomic conditions. However, closeness of contacts within population groups appears to be a more important factor for transmission than socioeconomic status per se: the spread of infection requires close or intimate contact with infected secretions. The prevalence of antibodies in adults ranges from 40 to 100% (Krech, 1973). In most developed countries infection increases steadily after infancy and 10–20% of children are infected before puberty (Britt and Alford, 1996). Congenital infection-transplacental or intrauterine transmission-is relatively uncommon and occurs in 0.3–2.2% of all live births. Perinatal transmission, i.e. horizontal transmission during and shortly after birth, occurs by virus transmitted from the uterine cervix and breast milk and is very common in developing countries. Infants and young children excrete HCMV in their urine and respiratory tract over a long period of time and they may be sources of infection for other children and adults: the virus

has been shown to spread in households and day care centers (Stagno and Cloud, 1994). After puberty, sexual transmission of CMV probably represents the most important mode of horizontal transmission (Handsfield et al., 1985). In sexually active male homosexuals there has been shown to be an extremely high seroprevalence of antibodies to HCMV (Drew et al., 1981; Collier et al., 1987). Reinfection is relatively common in sexually active populations and disease by coinfection with multiple strains has been detected in immunocompromised individuals (Drew et al., 1984; Spector et al., 1984). HCMV can also be transmitted via blood products and transplanted organs.

3. Virology

HCMV is a member of the Betaherpesvirinae subfamily of herpesviruses. Members of the Herpesviridae family are DNA viruses with a capsid, a tegument and an envelope. HCMV has the largest genome among the herpesviruses (230 kbp) encoding about 200 genes. The genome of the AD169 strain has been completely sequenced and contains 208 predicted open reading frames encoding more than 100 polypeptides (Chee et al., 1990). Recently, further genetic complexity has been described in wild type clinical strains (Cha et al., 1996). The genome can be divided into two segments referred to as long (L) and short (S) components (Ho, 1991). Both components are flanked by terminal repititive sequences. The junction between the L and S components is composed of internal repeat (IR) sequences, which are designated IR_L and IR_S when attached to the L and S components, repectively. The sequences at both ends of the genome are designated terminal repeats (TR). The sequences between the repeat regions are unique (U) sequences and are referred to as the unique long (U_L) and the unique short (U_S) regions. Viral replication reflects the expression of three categories of genes termed immediate early (IE) (α), early (β) and late (γ). The categories are defined biochemically and correspond with the time of appearance of either viral mRNA or protein in the infected cell.

A large number of HCMV proteins have been identified and classified according to their genetic origin (Mocarski, 1993; Spaete et al., 1994). The capsids of HCMV are composed of a relatively simple set of proteins. Pre-B capsids are present in the nucleus of infected cells and contain at least three major protein species: pUL86, pUL46 and M_R 12 K. After processing pre-B capsids become B capsids. These capsids have in addition a protein (pUL80a) that is probably involved in DNA packaging and/or nucleocapsid envelopment. B capsids become filled with DNA and mature into C capsids (Lee et al., 1988). Antibodies to pUL86, pUL46 and pUL80a do not exhibit virus-neutralizing activity and are unable to bind to either extracellular virus or the surface of virus-infected cells (Rasmussen, 1990; Britt, 1991). The region between the capsid and the envelope is called the tegument. HCMV tegument is formed by at least seven proteins of M_rs 212 K, 150 K, [71 K and 65 K], [28 K and 58 K], and 67 K. The function of these proteins is unclear; they may play a role in viral gene regulation, modification of host cell metabolism and the envelopment process. With the exception of p212, each tegument protein is phosphorylated. Tegument proteins do not seem to induce the production of monoclonal or polyclonal antibodies with either neutralizing activity or the capacity to bind virus-infected cells. The HCMV envelope glycoproteins are presumed to be involved in attachment to cellular receptors, fusion with the plasma membrane, penetration, virion assembly and egress of progeny virus from the cell. The envelope proteins are of interest because of their importance as antigens for both humoral and cellular immune response. One of the major envelope glycoproteins is referred to as the gB complex (gpUL55). This complex is a major target for virus-neutralizing antibody in the virus as well as in complexes isolated from viral membrane preparations and has become the leading subunit vaccine candidate. The envelope glycoprotein referred to as gH (gpUL75) also has been shown to be a target for the human immune response as well as being capable of inducing complement-independent neutralizing antibodies in mice and guinea pigs (Rasmussen et al., 1991; Rasmussen et al., 1988). gH requires co-expres-

sion of gh (gpUL115). A third glycoprotein complex (gc II) has been described (Kari et al., 1986). Neutralizing antibodies that react with the complex are complement independent. CMV specified components that appear on the cell surface during productive infection are being studied. Some of these virus-specified neoantigens on the surface of infected cells may be targets for a host's immune defense. The envelope glycoproteins gB and gH can also be detected on the surface of virus-infected cells and can be recognized by HCMV antibody-positive human serum.

4. Immune responses to CMV in humans

Virus neutralizing antibodies are produced during HCMV infection but how they are involved in protection is unclear. It is not yet known which of the HCMV glycoproteins are the most important antigens in humans for virus neutralizing antibody production. The gB homologue is immunodominant as compared to gH and human neutralizing monoclonal antibodies to gB have been generated. Numerous clinical observations have shown the importance of cellular immunity in HCMV. The delayed hypersensitivity response and functioning T lymphocytes are probably critical for keeping latent infection in check. Recovery from HCMV disease is correlated with the development of HCMV-specific cytotoxic T lymphocytes (CTL). However, the major viral target antigens to which the response is directed are ill-defined, though they may comprise viral structural elements. The HCMV tegument protein pp65 has been identified as a significant target antigen for CD8 + class I major histocompatibility complex (MHC)-restricted HCMV-specific CTL (McLaughlin-Taylor et al., 1994). Recognition of pp65 on target cells occurs prior to the onset of viral gene expression and persists throughout the duration of the replicative cycle. Recognition in the absence of viral gene expression suggests that abundant viral protein enters the normal trafficking pathway upon viral penetration and is readily made available to MHC molecules for presentation at the cell surface. Thus pp65 specific CTL may represent an important effector population for early control and limitation of HCMV infection and disease. In the setting of bone marrow transplantation, CTL of donor origin can be propagated in vitro and passively administered to recipients.

5. Animal models

Human CMV is highly species specific, it does not infect other animal species, and can be propagated only in cells of human origin. Therefore, surrogate animal models are used in which viruses of other species are used in a particular animal, e.g. the rat, the mouse and the guinea pig. There are two animal models from which insight in pathophysiology can be gained. One is the rhesus monkey model and the second model that has generated considerable information regarding congenital CMV is the guinea pig. The mouse model may be the most useful model for evaluating antivirals, as explained by Earl Kern (University of Alabama at Birmingham); their drug sensitivity is closer to HCMV than that of the guinea pig. The guinea pig CMVs are much more resistant to some drugs which are effective in HCMV. Animal models probably are not appropriate for the study of viral vaccines but may be useful in the preclinical evaluation of new drugs. A large number of compounds can be evaluated for activity. Dose range and toxicity studies can be done, and pharmacokinetics can be studied. The murine CMV model, as well as the rat, and to a lesser extent, the guinea pig have been demonstrated to be predictive for the efficacy of monotherapy in human studies (Kern, 1991). However, animal model systems have significant limitations and can not be viewed as precise models of HCMV infection in humans as is demonstrated by the antagonistic interactions in an animal model of a combination of GCV and foscarnet, which contrasts with the observed synergy in vitro and in humans (Freitas et al., 1989; Dieterich et al., 1992; Studies of Ocular Complications of AIDS Research Group, 1996). Recently, severe combined immunodeficient mice (SCID-hu) implanted with human fetal tissues under the kidney capsule, were found to support

HCMV replication and this model may be appropriate for this species-specific virus (Mocarski et al., 1993; Brown et al., 1995). When conjoint implants of human fetal thymus and liver were inoculated with a low-passage-number isolate of HCMV, strain Toledo, consistent high-level viral replication was detected and continued for up to 9 months. The species specificity of HCMV was preserved in this model such that virus was detected in the human conjoint thymus/liver implant but not in surrounding mouse tissues. Treatment of infected animals with ganciclovir reduced viral replication, thereby demonstrating the value of this system for evaluating antiviral therapies. This animal model opens the way for a range of investigations not previously possible with HCMV. A SCID mouse retinal implant model is being developed. In this model, fragments of human fetal retina tissue are implanted in the anterior chamber of the eye of SCID mice. A week after engraftment the fragments are infected with 10^3–10^4 pfu of HCMV laboratory strain AD169. The grafts can be monitored for 60 days and become well differentiated. The advantage of this model is the possibility of sequential sampling. The real potential of the model is not known at this moment. Additional animal models in which HCMV can be used are badly needed.

6. Clinical syndromes

6.1. Congenital and perinatal syndromes

Transplacental or intrauterine transmission occurs in about 30–40% of the pregnant women with a primary HCMV infection. The infection gives rise to clinical disease at birth in about 10% of infected infants (Raynor, 1993). Of these, approximately 10–20% die. Fulminant congenital HCMV infection is characterized by jaundice, hepatosplenomegally, a petechial rash and multiple organ involvement. Microcephaly, cerebral calcifications, mental retardation, deafness, motor disability and chorioretinitis are also seen. Late onset hearing loss, bilateral in almost half of the cases, has been reported in 17% of all congenital and 14% of all subclinical congenital HCMV infections (Stagno et al., 1977). Recurrent maternal infection of immune women leads to congenital HCMV infection in about 0.2–1% of the infants. Congenital infection due to recurrent disease is far less severe, and less than 10% of affected infants have long-term sequelae (Fowler et al., 1992). In postnatally acquired HCMV disease, diffuse visceral or central nervous system involvement is rare. When clinical manifestations are apparent they are most often nonspecific and are frequently respiratory in nature. Premature infants who acquire HCMV infection may develop pneumonia that can be associated with prolonged respiratory disease (Sawyer et al., 1987). Subtle sequelae of perinatal infections may still be discovered after long-term follow up.

6.2. Syndromes in healthy adults

Primary infection with HCMV in the healthy adult is often asymptomatic but may be manifested by a mononucleosis-like syndrome in less than 1:1000 (Horwitz et al., 1986).

6.3. Syndromes in transplant recipients

The two major risk factors for the development of HCMV infection in transplant recipients are preexisting HCMV antibody seropositivity of either the organ donor or the recipient, and host immunosuppression. The majority of morbidity for HCMV disease in solid organ and in allogenic bone marrow transplantation is found in the recipient seronegative, donor seropositive group (Ho et al., 1975; Smiley et al., 1985; Meyers et al., 1986; Appereley and Goldman, 1988; Winston et al., 1990; Wingard et al., 1988). The severity of HCMV disease also correlates with the degree of immunosuppression in the transplant setting. Primary infection is infection in a subject without antibodies to HCMV before transplantation; secondary infection is infection in a seropositive patient. The latter consists of reactivation or superinfection. The sources for primary infections or superinfection are mostly transplanted tissues or organs and, to a lesser degree, transfused blood (Wertheim et al., 1983; Bowden et al., 1986; Grundy et al., 1988; Bowden, 1995). In solid

organ transplant recipients, an immunosuppressive regimen that includes OKT3 antibodies increases infections (Singh et al., 1988; Johnson et al., 1994; Portela et al., 1995). Bone marrow transplant recipients are severely immunocompromised by radiation and cytotoxic drugs received before transplantation. Recovery of virus-specific T-cell immunity depends on the in vivo proliferation of T cells derived from the donor marrow. HCMV disease in these patients correlates with the deficiency of cytotoxic T lymphocytes specific for HCMV (Reusser et al., 1991). Infection also occurs in the recipients of autologous bone marrow transplants, but symptomatic HCMV infections are infrequent. Reactivation may occur in any seropositive patient and is mostly accounted for by the immunosuppression essential for maintenance of the graft (Ho, 1977). After organ transplantation allograft reactions may occur. Both the host-vs-graft disease and the graft-vs-host disease (GVHD) have been shown to affect virus infections (Wu et al., 1975; Dowling et al., 1977; McCarthy et al., 1992). In bone marrow transplant patients, acute GVHD significantly increases the risk of HCMV infection and of subsequent HCMV disease (Meyers et al., 1986). In contrast, HCMV infection does not increase the risk of acute or chronic GVHD. In some studies, HCMV infection was associated with the development of chronic rejection (Pouteil-Noble et al., 1993).

Patients receiving immunosuppressive chemotherapy for malignancies are usually not immunosuppressed for a long enough period of time to develop HCMV disease from primary infection or reactivation of a latent infection.

The risk for and the manifestations of HCMV disease are associated with the organ of transplant, e.g. HCMV pneumonia has been a major problem in bone marrow transplant patients and in lung transplant recipients, whereas HCMV hepatitis is quite common after liver transplantation. After transplantation, HCMV disease from primary infections occurs typically in the second or third month. The incubation period for secondary HCMV infections is somewhat longer. Primary infections are more likely to be symptomatic than secondary infections. HCMV infection may produce protean clinical manifestations but the most common clinical presentation is a febrile mononucleosis. Often leucopenia and atypical lymphocytes are present. Abnormalities of the liver function tests are common but severe hepatitis is unusual. HCMV infection may be associated with transient thrombocytopenia and hemolytic anemia. More extensive organ involvement may be evident. Interstitial pneumonia is the most common serious complication in transplant recipients. It is defined by the presence of tachypnea, hypoxemia, and interstitial pulmonary infiltrates on chest X-rays and the absence of bacterial or fungal infection, pulmonary edema or hemorrhage, or other obvious cause. Evidence from studies in man and the murine model of CMV show that virus replication in the lung is unrelated to the development of pathological effects, and that a host immune response is required for the induction of pneumonitis (Grundy et al., 1987; Tanaka et al., 1994).

6.4. Syndromes in persons infected with the human immunodeficiency virus (HIV)

Individuals, especially gay men, infected with HIV are nearly always HCMV seropositive and often develop symptomatic reactivation disease as immunocompromise progresses. Patients who have low immune reactivity to HCMV antigens in vitro are at a higher risk for the development of HCMV retinitis (Schrier et al., 1995). Retinitis is the most common manifestation of HCMV disease, it occurs in up to 45% of patients when the CD4 count falls below 50/mm^3 and accounts for 85% of HCMV end-organ disease in HIV-patients. It presents usually unilaterally, although it may progress to the contralateral retina, with painless, progressive visual loss and results in blindness (Bowen et al., 1996). HCMV gastroenteritis is usually manifested by submucosal ulcerations-although mucosal friability, erosions, hemorrhage, and plaque-like pseudomembranes may be observed-and can occur anywhere in the gastrointestinal tract from the esophagus to the rectum (Goodgame, 1993). Patients with esophageal disease complain about dysphagia and odynophagia. Diarrhea in association with ab-

dominal pain and fever is the most frequent symptom complex in patients with HCMV colitis which is manifested mainly in the cecum. In HIV infected patients, HCMV can also cause discrete mass lesions of the gastrointestinal tract (Rich et al., 1992; Laguna et al., 1993). HCMV (ventriculo)meningoencephalitis is characterized by non-specific neurological signs and is often accompanied by retinitis (Bylsma et al., 1995). Some patients have presented with mass lesions mimicking lymphoma which at biopsy turned out to be HCMV (Moulignier et al., 1996). Myeloradiculitis or polyradiculopathy by HCMV is manifested by rapidly progressive paraplegia and incontinence. In autopsies of AIDS patients there are often signs of HCMV disease in the adrenal glands although clinical evidence of Addison's disease is rare. Life-threatening HCMV pneumonitis in AIDS patients is seldom seen although HCMV may be cultured from bronchoalveolar lavage fluid (Millar et al., 1990).

7. Diagnostics

Active cytomegalovirus infection is diagnosed by isolation of the virus or by demonstrating its presence by immunologic or molecular techniques. However, HCMV persists in the body in a state of true latency or, as an asymptomatic productive infection in which there is excretion of infectious virus in body fluids. Therefore, isolation or identification of the virus does not prove an etiologic role of HCMV in disease and a definitive diagnosis of HCMV disease can only be made with careful assessment of a number of factors, e.g. histologic evidence of disease, characteristic syndrome and the exclusion of other etiologies. There is great need for diagnostic tests that predict disease in specific patient populations, discriminating infection from disease and, allowing for the monitoring of therapy.

7.1. Serology

Diagnosis of infection by serology, i.e. immunoglobulin IgG HCMV antibody testing, requires conversion from antibody negative to positive. The presence of IgM antibody is not a completely reliable indication of an acute infection. During an active infection IgM antibodies may be negative, or these antibodies may persist for such a long time that their presence is not diagnostic. Moreover, IgM antibody may be elevated during reactivation of CMV, and thus its presence does not definitely indicate primary infection.

7.2. Histology

CMV produces characteristic cytological changes, the infected cells are enlarged and contain intranuclear inclusions: the typical 'owl-eye' cells. The presence of these cells in tissue samples stained by, e.g. haematoxylin and eosin, is a specific but insensitive method of diagnosing infection and may be suggestive of HCMV disease in a patient with a compatible clinical picture. Fresh or frozen tissue sections may be examined using fluorescein-conjugated monoclonal antibodies against CMV.

7.3. Culture, shell vial assay and DEAFF

A sensitive and specific—but slow—diagnostic method is the demonstration of viral cytopathic effect in conventional cell culture. The main advantage of this method is the possibility to characterize the virus further, e.g. for antiviral drug testing. A more rapid method is the shell vial assay for HCMV. This assay detects viral infection by the presence of the immediate early antigen. Such antigens may be demonstrated directly or after 24–48 h incubation of tissue or body fluid cultures in 'shell vials' by enzyme-linked or fluorescence methods using monoclonal antibodies directed against the α and β proteins of CMV (Martin and Smith, 1986). In Europe this technique is termed DEAFF (Detection of Early Antigen Fluorescent Foci) (Griffiths et al., 1984). Cocktails of monoclonal antibodies have been found to be more successful than single monoclonals. With the use of the shell vial procedure, active HCMV infection can be recognized more reliably than by specific IgM antibody (Smith and Shelley, 1988). For maximum detection of HCMV

in shell vial cell cultures, at least three vials should be inoculated with blood specimens, and two vials should be used for urine, tissue, and BAL samples (Paya et al., 1988; Patel et al., 1995b). Virus isolation by the shell vial culture may fail to detect HCMV infection, especially in blood.

7.4. pp65 antigenemia

A sensitive and specific method to detect the presence of HCMV in the bloodstream is the identification of the pp65 KD tegument phosphoprotein (Van der Bij et al., 1988). This structural CMV antigen is expressed in the nucleus of circulating polymorphonuclear leucocytes which probably pick up pp65 via cell-to-cell contact with late-stage CMV infected endothelial cells or virions released into plasma (Grefte et al., 1996). The test is rapid and has the advantage of being quantitative: the level of viraemia can be expressed as the ratio of positive cells to the total number of leucocytes examined, usually 200 000 peripheral blood leucocytes. This makes it possible to monitor HCMV activity, including the efficacy of antiviral therapy, over a period of time in a given patient. However, the test is subjective and quite labor intensive and will have to compete with semi-automated, objective, DNA based assays.

7.5. Polymerase chain reaction

Polymerase chain reaction (PCR) can be used to detect viral DNA in tissues, blood leucocytes, plasma, serum, and other body fluids including cerebrospinal fluid, broncho-alveolar lavage and urine (Smith and Dunstan, 1993). In heart and lung transplant recipients and AIDS patients, the sensitivity of the qualitative HCMV PCR has been demonstrated to be superior to the detection of antigenemia or culture of virus. The high sensitivity of the PCR may be a disadvantage, since it may be difficult to discriminate an active infection from a latent infection. The sensitivity of the PCR may be lowered appropriately by reducing the number of DNA amplifications cycles, by testing in cell free blood samples, or by testing for the presence of HCMV-specific mRNA's by reverse transcriptase PCR (Patel et al., 1995a). Quantification of viral load with the PCR is still difficult although progress has been made (Fox et al., 1995). Recent data have indicated that quantification of HCMV DNA in plasma can be useful for identification of patients at highest risk for developing CMV disease (Spector et al., 1992; Wolf and Spector, 1993).

7.6. Hybrid capture™

In this assay the CMV isolate is inoculated in 96-well culture plates. At 2–6 days post-infection, the cells are lysed and the released DNA is hybridized with the CMV probe cocktail. The resultant RNA-DNA hybrids are captured and detected with a chemiluminescent substrate. The intensity of the light emitted is proportional to the amount of target DNA in the specimen. Preliminary data show that this assay has a high sensitivity and can be done quickly. A clinical trial, comparing this assay with the results of cell culture and antigenemia is in progress.

7.7. Branched DNA assay

This is an assay for the direct quantation of CMV in which the target is not amplified but the signal generated from the target is amplified. Because the assay is a signal amplification, anticontamination procedures are not required and one advantage of the assay is its ease of use.

8. Therapy

8.1. Systemic therapy for HCMV retinitis

Treatment options for newly diagnosed HCMV retinitis are intravenous ganciclovir, 5 mg/kg b.i.d., or intravenous foscarnet, 60 mg/kg t.i.d. or 100 mg/kg b.i.d., for 14–21 days (Spector et al., 1993; Palestine et al., 1991; Katlama et al., 1992). This is followed by lifelong maintenance treatment with either intravenous ganciclovir, 5 mg/kg once daily for 7 days per week or 6–7 mg/kg once daily for 5 days per week, or intravenous foscarnet, 90–120 mg/kg once daily (Jacobson et al.,

1988b; Hall et al., 1991; Jacobson et al., 1993). Ganciclovir and foscarnet are equally effective in the treatment of CMV retinitis (Studies of Ocular Complications of AIDS Research Group, 1994). Compared with ganciclovir, the use of foscarnet is more frequently limited by the occurrence of toxic reactions although these rarely have long-term sequelae (Studies of Ocular Complications of AIDS Research Group, 1995, 1992).

Recently, oral ganciclovir, at a dose of 1000 mg t.i.d. was compared with intravenous ganciclovir, at dose of 5 mg/kg per day, for maintenance therapy in AIDS patients with newly diagnosed stable CMV retinitis (The Oral Ganciclovir European and Australian Cooperative Study Group, 1995; Drew et al., 1995). In all patients the disease was stabilized by 2–3 weeks of treatment with intravenous ganciclovir. Although clinical assessment of time-to-progression favored intravenous ganciclovir, masked assessment of fundus photographs showed no statistically significant differences between intravenous and oral ganciclovir. Therefore, oral ganciclovir should be used only in patients who do not have sight threatening retinitis for whom the risk of progression of retinitis is balanced by the benefit associated with avoiding daily intravenous infusions. Also, there is concern that viral resistance to the oral drug will develop faster than to the intravenous preparation because of the lower serum levels obtained although there was no evidence for this in the 20 week study. A study is underway in which three dose regimes of oral ganciclovir, 1000 mg t.i.d., 1500 mg t.i.d. and 2000 mg t.i.d., are compared for maintenace therapy of CMV retinitis.

Combination therapy may provide better in vivo antiviral activity in suppressing CMV replication than previously reported with monotherapy regimens. In one study, patients who had completed a 14-day course of ganciclovir induction therapy were randomly assigned to an alternating or concurrent combination regimen of ganciclovir-foscarnet maintenance therapy (Jacobson et al., 1994). CMV was isolated from none of 21 patients who had urine cultured and from only 1 of 24 who had blood cultured while being treated during the study (median evaluation, 12 weeks). In another study, patients with relapsed CMV retinitis were randomized to induction with foscarnet at 90 mg/kg intravenously bid for 2 weeks, followed by maintenance at a dosage of 120 mg/kg per day; induction with ganciclovir at 5 mg/kg intravenously bid for 2 weeks followed by maintenance at 10 mg/kg per day; or continuation of previous maintenance therapy plus induction with the other drug (either ganciclovir or foscarnet) for 2 weeks followed by maintenance therapy with both drugs, ganciclovir at 5 mg/kg per day and foscarnet at 90 mg/kg per day (Studies of Ocular Complications of AIDS Research Group 1995, 1996). Comparison of retinitis progression, as evaluated in a masked fashion by fundus photograph reading showed that combination therapy was the most effective regimen. The median times to retinitis progression were: foscarnet group, 1.3 months; ganciclovir group, 2.0 months; and combination therapy group, 4.3 months ($P < .001$). Rates of visual field loss and retinal area involvement confirmed the time-to-progression results. No difference could be detected in visual acuity outcomes or survival. The improved response of recurrent retinitis with combination therapy versus monotherapy was offset by the decline in quality of life of the patients randomized to the combination therapy (reflecting the daily 4 h of infusion therapy required).

J. Lalezari from University of California San Francisco, reported two clinical studies on systemic treatment with the nucleotide analogue cidofovir (HPMPC). In the first study, 48 patients with peripheral, newly diagnosed and previously untreated CMV retinitis were randomized to immediate vs deferred treatment and followed with retinal photographs to determine time to progression. Patients on therapy received an induction course of 5 mg/kg intravenously once weekly for 2 weeks followed by maintenance therapy of 5 mg/kg once every other week. Patients also received oral probenecid: 2 grams 3 h before the infusion and 1 gram 2 and 8 h after the infusion. They received 1 l of intravenous saline hydration. Cidofovir doses were reduced for (+) proteinuria and discontinued for (++) proteinuria. The median time to retinitis progression was significantly delayed by cidofovir, 120 days for immediate treatment vs 22 days in the deferred therapy

group. In a second study, patients with relapsing retinitis despite standard doses of approved therapies, received an induction course of cidofovir of 5 mg/kg once weekly for 2 weeks. After this they were randomized to maintenance therapy of either 3 mg/kg or 5 mg/kg every other week. In this study patients received 2 l of i.v. saline hydration. The median time to progression of retinitis in the 3 mgr/kg ($n = 51$) and 5 mgr/kg ($n = 49$) group was 49 and 115 days, respectively. The percentage of patients who developed persistent proteinuria was 30%. None of the patients in both studies developed severe nephrotoxicity. There were four patients in the salvage protocol who had serious metabolic acidosis possibly due to renal loss of bicarbonate. Overall 50% of patients reported effects related to drug toxicity. Cidofovir has been recently approved for treatment of AIDS patients with CMV retinitis.

8.2. Intravitreal therapy for CMV retinitis

Intravitreous injection of ganciclovir was first reported in 1987 and since then there there have been several reports about its clinical use (Henry et al., 1987; Engstrom and Holland, 1995). Although there are no comparative studies to establish the efficacy of intravitreous injections for the treatment of CMV retinitis, it appears to be efficacious and similar in efficacy to intravenous therapy. Intravitreous injection has the advantage of high vitreous drug levels. The rate of retinal detachments with intraocular therapy is similar to the rate seen with other forms of parenteral therapy. The most commonly used regimen is ganciclovir, 200 μg 2 or 3 times weekly as induction therapy, and once a week as maintenance therapy, although doses as high as 2 mg have been given. Foscarnet has also succesfully been given by intravitreous injection at doses of 2400 μg per injection, using a similar frequency of administration as with ganciclovir (Diaz-Llopis et al., 1992, 1994). Arrest of the progression of CMV retinitis may be obtained with a single intravitreal injection of 20 μg cidofovir (Kirsch et al., 1995). Patients with acquired immune deficiency syndrome who had active CMV retinitis in at least one eye and no evidence of extraocular CMV disease received a 20 μg cidofovir trans pars plana injection and were treated with concomitant oral probenecid. Retreatments were carried out for progression of retinitis as determined by serial fundus photographs. The median time to retinitis progression after 8 repeat cidofovir injections was 63 days.

F. Rahhal from the University of California San Diego reported a short-term study of intravitreal cidofovir, 20 μg every 5 to 6 weeks for maintenance therapy of CMV retinitis. Patients received oral probenecid on the day of the injection. Group A were newly diagnosed patients, group B were patients who failed treatment with ganciclovir or foscarnet. In group A none of the 24 eyes had progression of disease. In group B, four of the 29 eyes had one reactivation episode each. Of the 35 patients, followed for a mean period of 15 weeks, 5 developed extra-ocular disease. In a second study, a single intravitreal injection of cidofovir of 10 μg was given, and no maintenance injections were given. The median time to progression was 45 days—statistically significantly worse than the time to progression with 20 μg injection—and 25% of the eyes had primary failure, suggestive that the 10 μg dose is less effective than the 20 μg dose.

An important development for local therapy is the use of an intraocular sustained-release implant containing ganciclovir. In a controlled trial, 26 patients with untreated CMV retinitis in the peripheral retina were assigned randomly to an intraocular ganciclovir device or deferred therapy (Martin et al., 1994). Median time to disease progression in the intraocular device group was 226 days, compared with 15 days for the deferred treatment group. This time-to-progression was four times longer than that seen in trials of intravenous ganciclovir, intravenous foscarnet or oral ganciclovir. A transient post operative reduction in visual acuity occurred in most patients, which in some patients lasted up to 28 days after implantation. As with all forms of local therapy, the device did not prevent contralateral ocular disease, which occurred in 50% at 6 months, and visceral disease which occurred in 31%. Studies of the combination of ganciclovir implant plus oral ganciclovir are underway.

8.3. Therapy for gastrointestinal disease

CMV gastrointestinal disease is an important complication in HIV infected patients, but there have been few studies addressing the response to currently available antiviral therapy, relapse rate without maintenance therapy, and long-term outcome. Both ganciclovir and foscarnet may be effective first-line treatment (Chachoua et al., 1987; Weber et al., 1987; Dieterich et al., 1988; Jacobson et al., 1988a; Reed et al., 1988b; Kaplan et al., 1989; Mayoral et al., 1991; Blanshard, 1992; Blanshard et al., 1995; Wilcox et al., 1995; Dieterich et al., 1993a). Patients who failed ganciclovir therapy may have a positive response to reinduction with foscarnet (Dieterich et al., 1993b). Maintenance therapy may not prevent progression of disease but appears to prevent retinitis. There are no data on therapy of gastrointestinal disease with ganciclovir in combination with foscarnet.

8.4. Therapy for central nervous system disease

Early diagnosis and treatment may be a major prognostic factor in CMV disease of the central nervous system (CNS) (Jacobson et al., 1988). The diagnosis of HCMV encephalitis is based on a compatible clinical picture and the detection of HCMV in cerebrospinal fluid (CSF). PCR for HCMV DNA appears to be a sensitive and specific diagnostic method for detection of HCMV CNS disease in AIDS (Cinque et al., 1992; Wolf and Spector, 1992). Neither ganciclovir nor foscarnet consistently halts progression of CNS CMV disease and higher doses of these drugs or combination therapy may be required to treat this disease entity effectively although there have been no comparative trials (Peters et al., 1992; Enting et al., 1992). The penetration of drugs through the blood brain barrier is of concern, as is the sensitivity of the virus to the administered drugs because CNS disease often occurs in patients on maintenance therapy for retinitis. At the meeting, C. Katlama from Paris reported preliminary results of an uncontrolled case-series of ganciclovir, in combination with foscarnet, for acute and maintenance therapy of CMV disease of the CNS in HIV infected patients. Inclusion criteria were: CD4 $< 50/mm^3$, encephalitis and/or myelitis, no other CNS disease, two positive PCR's of the CSF and/or CMV inclusion bodies in the biopsy. Patients were treated for 3–6 weeks with ganciclovir 5 mg/kg b.i.d. plus foscarnet 90 mg/kg b.i.d. In the maintenance phase the same drugs were administered at a reduced dose regimen. As of April 1996, 25 patients had been included in this ongoing study. Of these patients, 17 entered maintenance therapy, a number suggesting a reduction of mortality in the acute phase when compared to historical controls. Seven patients died during acute therapy. Of the patients on maintenance therapy, five have relapsed.

8.5. Therapy for pneumonia

The most common problem in bone marrow transplant patients is CMV pneumonia. Criteria for the diagnosis and the initiation of therapy differ between centers. Some centers require symptoms combined with an abnormal radiograph and CMV detected in bronchoalveolar lavage (BAL) or lung specimen, whereas others accept symptoms with CMV viremia or CMV detection at any site (Ljungman et al., 1993). Diagnostic methods for the detection of CMV differ from center to center. However, standardized criteria for the diagnosis of CMV pneumonia are essential if different treatment regimes are to be compared. Neither therapy with ganciclovir alone or in combination with high-dose corticosteroids, nor therapy with intravenous cytomegalovirus immune globulin alone has had much impact on the overall survival of patients with CMV pneumonia after allogenic bone marrow transplantation (Shepp et al., 1985; Reed et al., 1986, 1987). The effect of high-dose intravenous immune globulin (IVIG) in addition to ganciclovir remains controversial (Emanuel et al., 1988; Reed et al., 1988a; Verdonck et al., 1989). While initially associated with improved survival, this combination does not appear to give much benefit in survival when compared to ganciclovir alone. There are little data available on the efficacy of foscarnet in the treatment of CMV pneumonia (Aschan et al., 1992). Combination therapy

of ganciclovir with foscarnet has not been published in bone marrow transplant patients.

8.6. Therapy for congenital CMV disease

A pharmacodynamic/kinetic study to determine feasibility of treating high-risk babies with evidence of congenital CMV infection was reported by R. Whitley, University of Alabama at Birmingham. Statistically significant predictors of neurologic sequelae are intracranial calcifications and evidence of CMV retinitis. Deafness is considered a predictor of long-term neurologic outcome as well. Included in the study were children with intracranial calcifications, CMV retinitis, or deafness. These children ($n = 42$) were treated for 6 weeks with ganciclovir at dosages of either 8 or 12 mg/kg per day. Within 2 weeks of the onset of therapy approximately 90% of the babies had resolution of thrombocytopenia, while the leucocyte count and liver function tests returned to normal. Only four children had to be removed from the study because of drug-associated toxicity. Babies who received the higher dose were more likely to clear the virus from their urine, throat and blood, than those that were on the lower dose. At long-term follow-up, at least 3 years, there seems to be neurological benefit for the children. These preliminary results remain to be proven in a controlled study. The conclusions from this trial were that ganciclovir at 12 mg/kg per day in divided doses can be administered without inordinate toxicity. A comparative controlled study is now underway to test the hypothesis that ganciclovir therapy improves the outcome of babies with congenital CMV infection.

9. Prophylaxis

9.1. Prophylaxis of CMV in HIV infected patients

Results of the first oral ganciclovir prophylaxis study (Syntex 1654) for CMV retinitis were presented by Dr. Spector from the University of California San Diego (Spector et al., 1996). This study was a randomized, double-blind, controlled trial of oral ganciclovir, 1000 mg t.i.d., versus placebo. Included were HIV seropositive individuals, with a CD4 count of less than 100/mm^3 plus an AIDS defining opportunistic infection, or a CD4 count of less than 50/mm^3, and positive CMV serology. Patients were seen at baseline and every 2 months and had a dilated eye examination by an ophthalmologist at these times. No fundus photographs were taken. Excluded were patients with a history of past CMV disease, previous treatment with anti-CMV drugs, persistent gastrointestinal disease, and a low leucocyte (< 750/mm^3) or thrombocyte count ($< 50\,000$/mm^3) or a creatinine clearance < 20 ml/min. The median CD4 cell count was about 20/mm^3 in both groups. The incidence of cytomegalovirus disease at 18 months was 39% in the placebo group ($n = 239$) and 20% in the ganciclovir group ($n = 486$) ($p < 0.0001$). The major difference was for CMV retinitis, where there was an incidence of 39% in the placebo group versus 18% in the ganciclovir group. The frequency of sight threatening zone I retinitis was higher in the placebo group than in the ganciclovir group. The protective efficacy of prophylaxis was not restricted to individuals with a CD4 cell count of under 50/mm^3. One potential complication of ganciclovir prophylaxis is the development of resistance, but preliminary data from this study suggest that this is not a major problem.

Dr. Brosgart of the East Bay AIDS Center presented the results of the CPCRA trial 023. This study was a randomized controlled trial of oral ganciclovir, 1000 mg t.i.d., versus placebo. Included were HIV infected patients with a history of CD4 cell count < 100/mm^3, positive CMV serology and no evidence of CMV disease. Patients were not seen by an ophthalmologist at entry. At every study visit patients were asked about visual symptoms. Any visual symptom required that the patient was sent to the ophthalmologist. Endpoint of the study was CMV disease. A total of 994 patients were included, 662 of which were assigned to oral ganciclovir and 332 to placebo. There was no statistically significant difference in CMV disease between placebo and ganciclovir groups. In a subanalysis of pa-

tients with CD4 < 50/mm^3 or CD4 < 100/mm^3 and an AIDS defining opportunistic infection, no effect of prophylaxis was found either.

Dr. Feinberg of the University of Cincinnati presented the ACTG 204 study, a randomized, double-blind, controlled trial of valaciclovir for prophylaxis of CMV end-organ disease in patients with advanced HIV. Included were patients with positive CMV serology, no evidence of CMV disease and CD4 < 100/mm^3. Patients were stratified according to CD4 cell count: < 50/mm^3 and > 50/mm^3 and then randomized in a 3:2:2 fashion to valaciclovir 2000 mg q.i.d., acyclovir 800 mg q.i.d. or acyclovir 400 mg b.i.d. Patients were seen by an ophthalmologist at entry and then every 6 months or when they had complaints. Retinal photographs were taken at diagnosis and then read by an independent ophthalmologist in a masked fashion. A total of 1227 patients was included. CMV endpoints were reached in 17.5% of the pooled acyclovir group versus 11.7% in the valaciclovir group. Retinitis was diagnosed in 13.5% of the former group and in 9.8% of the latter. In individuals with a positive blood PCR for CMV at baseline, there was a stronger protective effect than in patients with a negative PCR. There was a trend towards higher mortality in the valaciclovir group but this did not reach statistical significance ($p = 0.06$).

Dr. Katlama of Paris reported an ongoing randomized open-label, controlled trial (ANRS 022) to evaluate the efficacy of foscarnet compared to no treatment in HIV-infected patients with CD4 < 100/mm^3, two positive blood cultures for CMV, no evidence of CMV disease or previous anti-CMV treatment. Patients are randomized to foscarnet 100 mg b.i.d, 50 mg b.i.d. or no therapy. Preliminary results show a decrease in viremia under therapy and a return to pretreatment levels after discontinuation.

9.2. Prophylaxis in solid organ transplantation

High-dose oral acyclovir, 800 mg q.i.d., administered for 4–6 months posttransplant have been shown to be moderately effective in preventing CMV disease in a variety of solid organ transplant settings (Rubin and Tolkoff-Rubin, 1993; Rubin, 1994).

Ganciclovir has been administered prophylactically to heart transplant recipients and has been shown to be effective in preventing disease in patients who were seropositive, but ineffective in seronegative patients at risk for primary infection (Merigan et al., 1992; Aguado et al., 1995). In renal transplant patients ganciclovir appears to be effective in seronegative patients receiving a seropositive kidney (Conti et al., 1994). Ganciclovir followed by high dose oral acyclovir, was more effective than acyclovir alone in liver transplant patients (Martin, 1994). Recently, ganciclovir has been shown to be superior to acyclovir or placebo in liver transplant recipients (Winston et al., 1995). Sequential therapy with ganciclovir and intravenous immunoglobulin, followed by a variety of doses of oral acyclovir was ineffective in preventing primary CMV disease in lung transplant recipients (Bailey et al., 1992). Intravenous immunoglobulin preparations are moderately effective in preventing CMV disease in organ transplant recipients.

9.3. Prophylaxis in bone marrow transplant (BMT) patients

The most effective way to prevent CMV disease in the CMV negative recipient is the avoidance of exposure to exogenous sources of CMV by selective use of CMV-seronegative donors of blood products and marrow (Bowden et al., 1991b; Miller et al., 1991). It is unneccessary to use CMV-negative blood products in a CMV-seropositive recipient. Another way to reduce the risk of CMV transmission in transplant recipients is by the use of blood filters (Bowden et al., 1989).

In a randomized controlled trial the effect of prophylactic intravenous ganciclovir initiated at engraftment, 5 mg/kg b.i.d. for 5 days and then once daily until day 100, was assessed in CMV-seropositive BMT recipients (Goodrich et al., 1993). CMV associated disease was significantly reduced at day 100 and day 180 post BMT. There was no difference in mortality rates between control and treatment groups. Patients in

the ganciclovir group who became neutropenic (30%) had an increased risk of bacterial infection and one patient died of sepsis. In another randomized, double-blind study in CMV-positive allogeneic BMT recipients, patients were assigned to either placebo or ganciclovir at a dose of 2.5 mg/kg t.i.d. for 1 week before transplantation and then at at dose of 6 mg/kg once a day for 5 consecutive days per week, after transplantation when the post-BMT neutrophil count reached 1×10^9/l (Winston et al., 1993). CMV infection developed in 56% of placebo patients versus 20% of ganciclovir patients ($P < 0.001$). CMV disease also occurred less often in the ganciclovir patients (10%) than in the placebo patients (24%) but this did not reach statistical significance ($P = 0.09$). In summary, prophylaxis with ganciclovir in BMT recipients reduces CMV infection but is not unequivocally associated with a positive outcome.

No controlled trials of the propylactic effect of foscarnet in transplant recipients have been reported. In a phase I–II study in recipients of allogeneic and autologous BMT, foscarnet was reported to reduce the occurrence of CMV infection significantly.

The efficacy of intravenous immunoglobulin (IVIG) or CMV antibody-enriched immunoglobulin (CMVIG) for prophylaxis of CMV infection and CMV disease in BMT recipients, has been the subject of several controlled studies (Bowden et al., 1986, 1991a). CMVIG has been shown to decrease the incidence of CMV viremia and infection but not of CMV disease. In contrast, IVIG does not change the incidence of CMV infection but does significantly lower the incidence of CMV disease (Messori et al., 1994). How IVIG exerts this influence is unknown but it has been hypothesized that the effect is the result of a modification of acute GvHD by IVIG (Sullivan et al., 1990).

In a recently published study the safety and immunologic effects of immunotherapy with clones of CD8+ cytotoxic T cells specific for CMV, were evaluated in recipients of allogeneic BMT (Walter et al., 1995). Fourteen patients each received four intravenous infusions of these clones from their donors beginning 30–40 days after marrow transplantation. Five of the recipients were CMV seropositive and nine were CMV seronegative. All donors were CMV seropositive. Neither CMV viremia nor CMV disease developed in any of the 14 patients. Further studies of the efficacy of this approach as prophylaxis against CMV are warranted.

9.4. Preemptive therapy for CMV in BMT recipients

Preemptive therapy is the treatment of active CMV infection as indicated by assays for active replication of virus before the occurrence of disease (Zaia, 1993). Different investigators use different definitions of disease, different diagnostic criteria, and different therapeutic strategies. Therefore, it is difficult to compare studies and to translate the results into clinical practice. Culture-based preemptive therapy with ganciclovir has been shown to reduce the incidence of CMV disease after BMT. However, culture techniques do not detect CMV in a substantial number of patients before the onset of CMV disease. PCR helps to identify those patients who will not develop CMV disease and narrows down the number of patients who eventually will suffer symptomatic CMV infection (Schmidt et al., 1994). In a prospective study, patients either received preemptive therapy based on polymerase chain reaction (PCR) technique or on culture assays (Einsele et al., 1995). In both groups, therapy was continued until clinical signs disappeared and PCR negativity was documented. Preemptive therapy based on PCR results reduced the incidence of CMV disease and CMV-related mortality. Additionally, stopping and withholding antiviral therapy in a PCR-negative patient proved to be safe and allowed reduction of the duration and side effects of antiviral therapy in a relatively small number of patients.

10. CMV vaccine

Towne strain of CMV was developed as an attenuated vaccine and has been tested in renal transplant recipients and mothers of children in day-care centers (Plotkin, 1994). Prior vaccination of transplant recipients did not prevent superin-

fection by CMV from seropositive kidney donors but did decrease the severity of subsequent CMV disease and increased the succesful outcome of the transplant. In mothers of children in day-care centers, vaccination with Towne strain did not protect against infection with HCMV from their child.

The envelope glycoprotein gB or UL55 has been shown to elicit B cell, helper T cell and cytotoxic T cell responses, suggesting that it may be useful as a subunit HCMV vaccine. But although peripheral blood mononuclear cells from all HCMV-seropositive donors proliferate in response to stimulation with whole HCMV, not all donors respond to purified recombinant gB (Curtsinger et al., 1994). Other candidates for a subunit vaccine include the gH glycoprotein or the use of a protein antigen combined with a suitable adjuvant. Inserted into a deletion mutant of adenovirus type 5, the gB gene was shown to be immunogenic in small animals (Marshall et al., 1990). Thus, complex subunit or vectored vaccines may be needed to elicit protective immune responses.

Another approach was reported by G. Kemble, Aviron, CA. This group has shown that the low passage, virulent Toledo strain of CMV compared to the attenuated, avirulent Towne strain has an additional 13 kbp DNA. The factors for virulence or greater immunogenicity might be present in this region. A recombinant Towne/Toledo virus has been constructed and the virulence of this virus was measured in the SCID-Hu model. The recombinant virus had replication levels almost identical to that of Towne, indicating that the virus was avirulent. It is expected that the recombinant strain retains all the safety features of Towne, while increasing its immunogenicity and that it will lead to a new generation of CMV vaccines.

11. New anti-CMV drugs

11.1. Benzimidazole

This drug is targeted at UL89, the most highly conserved open reading frame in the herpes virus and essential for replication. The drug is active against foscarnet- and ganciclovir-resistant strains, and has a good therapeutic index. Phase I clinical studies have been started with a derivative compound.

11.2. MSL-109

This product is a monoclonal antibody against the gH glycoprotein of CMV. A phase I/II study of MSL-109 in combination with either ganciclovir or foscarnet showed that the antibody is well tolerated and has favorable pharmacokinetics (Pollard, 1996). The agent is administered once every 14 days. In a pilot study of MSL-109 used as an adjunctive to ganciclovir therapy, the median time-to-progression of retinitis was 202 days, by clinician evaluation. No adverse events were observed and the agent did not induce antibodies. Two randomized, placebo-controlled, clinical trials evaluating the efficacy of MSL-109 in combination with standard therapy against CMV retinitis and BMT patients have been initiated.

11.3. Lobucavir

This is a nucleoside analog with activity against a wide range of viruses. Open-label dose escalating studies to evaluate in vivo antiviral efficacy, pharmacokinetics and safety, are underway. As of April 1996, patients have completed 28 days of up to 400 mg q.i.d. and the agent seems to be well tolerated.

11.4. Antisense

Inhibition of virus replication by using antisense oligonucleotides complimentary to the RNA of essential genes may be an effective antiviral therapy. UL36 and UL37 are immediate-early genes that are initially expressed 0–4 h post infection. The products of these genes are required for HCMV DNA replication. In vitro, an antisense phophorothiate oligonucleotide complementary to the intron-exon boundary of HCMV genes UL36 and UL37 (UL36ANTI) reduced the yield of infectious virus by 99% and inhibited DNA replication (Pari et al., 1995). The compound has shown

activity in a CMV strain resistant to ganciclovir as well as to a strain resistant to ganciclovir, foscarnet, and cidofovir.

12. Resistance

12.1. Resistance to ganciclovir

The initial ganciclovir phosphorylation is carried out by a viral phosphotransferase encoded by the UL97 gene. Specific mutations within the HCMV UL97 gene have been described in clinical CMV isolates resistant to ganciclovir. The most common mutations are on codons 460, 520, 591–595, 607 and there may be more than one mutation in an isolate. Chou et al., found an excellent correlation between resistance as determined by a plaque reduction assay and specific UL97 mutations directly in PMNL DNA and those in blood isolates recovered at the same time (Boivin et al., 1996). The mutations appeared within 3 months of cumulative ganciclovir therapy and were stable during prolonged therapy. One or two of these mutations were detected in all ganciclovir-resistant HCMV isolates but not in the ganciclovir-sensitive isolates. Emergence of these mutations was associated with an increasing CMV DNA burden in PMNL and MNL extracts in the patients with AIDS but not in the patients with leukemia. HCMV resistance to ganciclovir may also occur via mutations in viral DNA polymerase (gene region UL54). These mutations may occur alone or in addition to UL97 mutations. Isolates with resistent mutations at both sites are more resistant than those with only UL97 mutations. Persistent HCMV viremia or viruria during prolonged ganciclovir therapy should raise the possibility of a drug resistant HCMV mutant.

12.2. Resistance to foscarnet

Foscarnet does not require any previous phosphorylation and it blocks the viral DNA polymerase by a noncompetitive mechanism. It is assumed that a single mutation in the CMV polymerase gene could be responsible for the foscarnet-resistant strains exhibiting cross-resistance to other DNA polymerase inhibitors (Knox et al., 1991; Sarasini et al., 1995). However, in a recent study HCMV strains resistant to both foscarnet and ganciclovir were found to carry two amino acid changes, one in UL97 and the other in domain II of the DNA polymerase gene (UL54), which conferred ganciclovir- and foscarnet-resistance, respectively (Baldanti et al., 1996). The level of foscarnet resistance that correlates with poor clinical outcome is not currently known.

12.3. Resistance to cidofovir

Cidofovir is a nucleotide and in contrast to ganciclovir is phosphorylated in both infected and non-infected cells . Resistance to cidofovir occurs by changes in CMV DNA polymerase. No significant changes in IC_{50} values were found in 29 patients with AIDS who were treated with cidofovir during a short term phase I trial. In a study of isolates from several centers of patients who had received ganciclovir and/or foscarnet, cidofovir resistance was detected in CMV strains with high-level resistance to ganciclovir. Ten of ten isolates with high-level resistance to ganciclovir were also resistant to cidofovir, versus 0 of 12 isolates with low-level resistance to ganciclovir. Of eight isolates with resistance to foscarnet zero were resistant to cidofovir, as were zero of eight isolates with resistance to foscarnet and low-level resistance to ganciclovir. All of five isolates with resistance to foscarnet and high-level resistance to ganciclovir, were resistant to cidofovir. In summary, these results suggest that low level ganciclovir resistance, mediated by changes in UL97, may still be susceptible to cidofovir. In contrast, high level ganciclovir resistance, mediated by changes in DNA polymerase, are probably cross-resistant to cidofovir.

12.4. Resistance testing

DNA based assays, e.g. the Hybrid Capture™ assay developed by Digene and the b-DNA assay developed by Chiron are potentially useful for the screening of drug resistance and susceptibility. Currently, the plaque reduction assay is used for this purpose, but this assay is labor-intensive and

time-consuming. The results of DNA based assays, which correlate closely with those of the plaque reduction assay, are available more quickly but still require isolation of virus and passage to obtain sufficient virus.

13. Summary

CMV infection and CMV disease can be difficult to differentiate and the diagnosis is usually based on a compatible clinical picture and the results of a diagnostic test for CMV. The only exception to this rule is in HIV-infected patients where fundoscopy is sufficient to diagnose CMV retinitis. Of the current diagnostic tests, qualitative and quantitative PCR, branched DNA and Hybrid Capture™, are the most promising. The pp65 antigenemia assay has the disadvantage of being more labor-intensive than the DNA based tests.

Preliminary data show that a positive qualitative PCR in a HIV-infected patient has a predictive value for the development of CMV retinitis. However, of the patients positive by qualitative PCR, those with high viral loads in quantitative PCR were at the greatest risk of CMV disease. This might make it possible to identify with great certainty the patients who will go on to develop CMV retinitis, thereby decreasing the number of patients eligible for preemptive or prophylactic therapy and increasing the cost-benefit of this therapeutic measure. Quantative tests might also be useful in monitoring response to therapy, but randomized trials comparing the tests are needed.

Prophylactic antiviral agents should not be used in seronegative transplant recipients receiving organs from seronegative donors. In high-risk transplant recipients, ganciclovir should be used. CMV vaccines are useful for the protection of babies from CMV seronegative mothers against congenital CMV disease. It also may be useful in seronegative transplant recipients receiving a seropositive donor organ, although the benefit of chemo prophylaxis may surpass that of vaccine.

HIV-infected patients with CMV retinitis who relapse under either ganciclovir or foscarnet benefit from subsequent combination therapy, rather than switching to the other drug. However, the cost is high in terms of quality of life.

Intravitreal therapy for CMV retinitis is very efficacious, suggesting that drug delivery is a problem in systemic therapy. However, intravitreal therapy does not protect against the development of CMV retinitis in the contralateral eye or from CMV disease elsewhere. Therefore, systemic therapy should be added.

CMV disease of the CNS should be diagnosed early and treated agressively, possibly with combination therapy. A diagnosis of CMV disease should be based on a compatible clinical picture and the demonstration of CMV in CSF by DNA or antigen assays which are more sensitive than culture.

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