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Cytomegalovirus infection after organ transplantation: an update with special emphasis on renal transplantation

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Abstract. Cytomegalovirus infections are still the most important infectious complications after organ transplantation. Besides historical notes this review will deal with new aspects concerning the epidemiology of the CMV, diagnostic modalities of CMV infection, the delicate counterbalance between the immune system and the CMV, as well as the symptomatology of this infection. Furthermore, aspects like prophylaxis and new, promising therapeutic regimes for treatment of infection will be dealt with. Although this update is applicable for all types of solid organ transplantation, emphasis will be on renal transplantation.

Key words: Kidney transplantation, cytomegalovirus infection - Cytomegalovirus infection, in kidney transplantation - Cytomegalovirus infection and rejection.

More than 25 years ago, Weller and associates [194, 195] introduced the term "cytomegalovirus" (CMV) because of the characteristic cytomegaly observed in cells infected with this virus. Six years earlier, in 1954, the virus was isolated for the first time from the salivary glands of mice in tissue culture [161]. Yet, probably the first description of the virus was given by Jesionek and coworkers in 1904 [85] and by Löwenstein in 1907 [99], who found cytomegalic cells in autopsy material from children. In the last 20 years, an abundance of new information about the characteristics of this virus has emerged from new laboratory techniques, numerous studies in animal models, and clinical studies in immunosuppressed patients.

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Biologically, CMV is a DNA virus belonging to the herpes virus group that also includes varicella virus, herpes zoster virus, herpes simplex virus (HSV), and Epstein-Barr virus (EBV). It has a double-stranded DNA core, an icosahedral symmetry of its capsid, composed of 162 capsomeres, and a surrounding envelope. Cytomegalovirus is the largest member of the human herpes family. Physically, CMV is approximately 200 nm in diameter, making it one of the largest animal viruses. There are a number of different strains of CMV. All have specific characteristics, including a strong propensity for cell association and lability, a tendency to remain latent, and possibly the potential for inducing malignancy [2, 145].

After a primary infection (patients with no previous CMV infection), the CMV remains dormant in a variety of cells throughout the body and is capable of being reactivated under certain conditions, such as immunosuppression, allograft rejection, and humoral changes, as during pregnancy. Under these circumstances, CMV emerges from its latent state and is capable of causing clinical disease again (reactivation; secondary infections) [2, 145]. Cytomegalovirus is known to reside in splenic B cells, salivary gland tissue, the prostate, testes, and probably in macrophages and peripheral blood leukocytes [2, 81]. The transplanted kidney is also an established source of latent CMV [69, 82].

Epidemiology of cytomegalovirus infections

Cytomegaloviruses are ubiquitous agents that frequently infect both animals and humans [2, 63]. Epidemiological studies have shown that antibodies to CMV are prevalent in adults throughout the world, even in the remote and isolated Tiryo Indians of

Brazil, who essentially lack antibodies to measles and influenza virus [16]. Seropositivity for CMV among normal populations varies considerably, the reported range being 28%-100% [90]. Its prevalence greatly depends on age, race, socioeconomic, and geographic factors, Asian and African countries being those with the highest rate of occurrence. In West European countries, about 50% of the blood donor population between 18 and 65 years of age is seropositive, but it is estimated that only 1%-12% of all donors are actually infectious [18]. There is no clear difference in the prevalence of CMV antibodies between the sexes [63], although Luby and Shasby [100] were able to demonstrate a significantly higher prevalence of antibodies to CMV in women than in men in a nonwhite population of a well-defined, poor community in Dallas. There is no relationship to occupation per se [63], and even for personnel working in a dialysis unit there seems to be no increased risk for acquiring CMV infection [185].

In a normal population, the great majority of CMV infections are essentially asymptomatic, although they can cause serious disease, including neonatal infections, due to an immune system that is still immature, and a mononucleosis-like syndrome in young adults. While in neonatal forms of CMV disease infection appears to be transmitted via the infected cervix and breast milk of the mother, close contact, especially in underdeveloped countries and among lower socioeconomic groups where there is crowding and poor sanitation, blood transfusions, and venereal transmission are probably important factors for acquisition of CMV infections in later life [63].

In contrast with the predominantly asymptomatic CMV infections in the normal immunocompetent host, CMV infections after organ transplantation often cause overt, and sometimes even fatal, disease. Cytomegalovirus is, in fact, the single most important infectious agent complicating the course of renal allograft recipients. Incidence of CMV infections has been reported to be as high as 43%-92% [7, 61].

This great variation in incidence of CMV infections after organ transplantation quoted in the literature is most likely caused by differences in both immunosuppressive regimens and number of patients studied, differences in sensitivity of laboratory techniques, and differences in criteria used for the diagnosis of CMV infection. Furthermore, there is inevitably variety in populations studied (e.g., geographical, racial, and socioeconomic differences). Determinants in the high incidence of CMV infection in the transplant population are immunosuppression and blood transfusions, as well as serostatus of the organ donor. Host factors that may be involved will be discussed later.

As for the causative role of immunosuppressive drugs in the development of CMV infections, it is important to stress that corticosteroids per se may not play a major role. Prior to 1966, when corticosteroids alone were utilized for immunosuppression, CMV infection was virtually unknown [145]. Only after the introduction of azathioprine, cyclophosphamide, and antilymphocyte preparations did CMV become a frequent problem in organ transplantation [81, 145]. In another patient population, patients with rheumatic diseases, Dowling et al. [48] made the same observations. There are many reports indicating that the use of antilymphocyte (ALS) and antithymocyte (ATG) preparations greatly increases the risk of CMV viremia and clinical illness attributable to CMV [116, 123, 148, 149], a risk that also extends to patients treated with cyclosporin A as the main immunosuppressive drug [96]. There is not enough data at present to determine whether the use of the new immunosuppressive agent OKT3 (a monoclonal anti-T-cell antibody) also increases the risk of CMV infection post-transplantation, although a high incidence of active CMV infection has been reported [35]. In a recent report by Oh and associates [119], an increased risk of serious infections, particularly with opportunistic pathogens (*Listeria monocytogenes* and *Nocardia*), was found when OKT3 was used for steroid-resistant rejection when compared with the use of conventional antirejection treatment (another high steroid regime and/or ATG). Yet, the incidence of CMV infections in both groups was exceptionally low (1 out of 23 patients in both groups), something which might be related to the fact that they used only the insensitive complement-fixing antibody assay for establishing the diagnosis of CMV infection.

There are relatively few studies on the incidence and severity of CMV infections in cyclosporin A (CyA)/prednisolone-treated patients, and the results are somewhat controversial. A decreased incidence of CMV infection in CyA-treated patients has been reported when compared with conventionally treated patients [azathioprine (Aza)/prednisolone; 19].

The same experience has been reported by Najarian et al. [116]. However, in the Aza/prednisolone-treated group, ALG was also added to the immunosuppressive regimen prophylactically in the early post-transplant period. On the other hand, Bia et al. [11] observed similar rates of CMV infections, as well as overt CMV disease, in CyA/prednisolone-treated and Aza/prednisolone-treated patients. When ATG was given for steroid-resistant rejection, an increase in incidence of CMV infection was demonstrated as

well as more severe CMV disease, especially in the Aza/prednisolone-treated patients. None of the patients in the Aza/prednisolone-treated group was symptomatic without additional treatment with ATG, while the incidence of symptomatic CMV infection in the CyA/prednisolone group with ATG did not differ from that without ATG. Patients who are at risk for primary infection (seronegative recipient/seropositive donor combination) and who are treated with CyA as the main immunosuppressive drug continue to have considerable morbidity and mortality, whereas patients who have antibodies prior to transplantation may have less CMV disease when treated with CyA than those treated with Aza [191, 192]. From these data it can be concluded that the net state of immunosuppression most likely determines the incidence and severity of CMV infection after organ transplantation, particularly in patients at risk for primary infection.

The role of blood transfusions given to allograft recipients, as a main risk factor for contracting CMV infection, is debatable. Although organ transplant recipients are frequently transfused and the risk of acquiring an active CMV infection following blood transfusion has been recognized for more than 18 years [91], it is unlikely that in modern transplantation programs post-transfusion CMV accounts for more than 10% of the actual CMV infections. This is mainly due to the policy, adopted by the majority of transplant centers, of giving patients only leukocyte-depleted or frozen blood when transfused after transplantation, since it is well established that a significant reduction of post-transfusion CMV can be obtained when only leukocyte-depleted blood is used [91, 92].

There is increasing evidence that, in kidney transplantation, the donor kidney is, in a large majority of cases, the source of virus in episodes of CMV infections post-transplantation [69, 144]. This was confirmed in a rat model by Bruning and coworkers [22], who showed that CMV could be transferred by kidney transplantation.

With the aid of biotechnology techniques such as DNA restriction enzyme analysis, which permits the characterization of each isolate of CMV [33, 69, 196], it becomes increasingly apparent that such a transmission of CMV is evident in most cases, even in seropositive recipients who have received a seropositive kidney. Grundy et al. [71] further reported that reinfection with exogenous virus (strain from seropositive kidney donor) in seropositive recipients gave more symptomatic CMV disease than with reactivation of latent (endogenous) virus of the recipient. Another possibility is that transmission of CMV by the donor kidney may be caused by CMV-infected donor lymphocytes or monocytes carried in the graft, which may be activated post-transplantation [62]. Thus, aside from primary infections and secondary infections (reactivation), it is now possible to define a third major pattern of infection: superinfections [144].

Diagnosis of cytomegalovirus infection

The diagnosis of CMV infection can be substantiated by one or more of the following:

1. Electron microscopic detection of typical CMV virions
2. Histologic or cytologic detection of typical CMV cytopathology
3. Detection of CMV antigen in tissues or blood cells
4. Detection of CMV genome
5. Isolation of virus
6. Demonstration of a virus-directed humoral immune response (serology) [50].

Electron microscopic techniques have been advocated for diagnosing CMV infection (e.g., to detect CMV virions in urine) but are nowadays seldom used because they are rather insensitive and time-consuming [50].

As for histologic and/or cytologic detection of CMV, it should be stressed that the microscopic hallmark of CMV infection is the large (cytomegalic) 25–35 μm cell containing a large central, basophilic intranuclear inclusion [2, 50, 175]. The inclusion is referred to as the "owl's eye" because it is separated from the nuclear membrane by a halo that can readily be demonstrated with Papanicolaou's or hematoxylin-eosin stains. However, this method is also rather insensitive since cytomegalic cells do not appear before the last stages of CMV infection; moreover, histologic examination of a small piece of tissue (e.g., obtained by transbronchial lung biopsy or liver biopsy) is prone to sample errors and, thus, to false-negative results [50].

With the introduction of immunofluorescence (IF) techniques with monoclonal antibodies directed against CMV antigens, including immediate-early antigens (IEA), early antigens (EA), which are present in earlier stages of infection, and late antigens (LA), an exciting new mode of detecting CMV antigens in tissue specimens has been developed. For instance, lung biopsy tissue [50] or cells obtained via fine needle aspiration biopsy of the allograft [180] are suitable for establishing the diagnosis of CMV

infection. Although advocated by the authors as a means of rapid diagnosis of CMV infection, these techniques may be hampered by the sometimes high background levels of fluorescence (autofluorescence), resulting in poor sensitivity [50]. However, Volpi et al. [187] reported good sensitivity using finely minced lung tissue obtained from open-lung biopsies or autopsies of patients with interstitial pneumonia to which a mixture of monoclonal antibodies to CMV was added.

A clear disadvantage of IF methods is that they do not permit a study of the distribution of CMV-infected cells within the normal tissue architecture. Hackmann and coworkers [72] reported their experience with frozen tissue sections from 52 consecutive open-lung biopsies from patients with interstitial pneumonia using a single monoclonal antibody directed against CMV-LA. They concluded from this study that the sensitivity of this IF technique for diagnosing CMV pneumonia exceeded that of standard histology and was equal to viral tissue culture. However, in 7 of the 27 biopsy specimens found to be positive with IF, histologic findings did not give a diagnosis of CMV pneumonia, which raises the question of whether cases lacking histologic confirmation are true instances of CMV pneumonia [50].

Recently van der Bij and coworkers [12, 13, 183] developed a CMV antigenemia assay based on the detection of CMV-IEA in circulating blood leukocytes. Demonstration of CMV in blood samples is particularly important because CMV viremia is considered to be a marker of active infection and has been shown to correlate well with significant CMV disease [74, 145]. The assay was shown to be a rapid (processing time 3-5 h) and sensitive test and appeared to give positive results on an average of 9 days before there was serological evidence of CMV infection [13]. Also in our laboratory, van den Berg et al. (manuscript in preparation) recently found the same results in a prospective study of more than 200 consecutive renal transplant recipients and they demonstrated the usefulness of this assay even in patients with poor antibody response. This may be important since it is known that patients with mild disease mount a brisk antibody response, while those with progressive fatal infection may not respond at all ("wasting disease") [81, 158, 159]. Thus, this assay could be a useful marker for clinically relevant infection and may be helpful in clinical decision-making about the initiation and monitoring of antiviral therapy.

The use of DNA-DNA hybridization to detect CMV in clinical samples seems promising, but its usefulness still has to be evaluated in the clinical management of transplant recipients. Dot-spot hybridization and in situ hybridization have been described for diagnosing CMV infection in blood samples [173], in urine [34, 153], and in material obtained by biopsy [115]. Small quantities of CMV-DNA can also be detected in, for example, peripheral blood by DNA amplification using the polymerase chain reaction (PCR). In a longitudinal study of 76 patients, Jiwa et al. [86] showed that this assay can be an accurate and rapid marker of an active CMV infection after transplantation. However, as reported by others, an as yet unsolved problem could be the somewhat disappointing low specificity of this test (2nd International CMV-workshop, San Diego 1989).

Isolation of virus is a standard diagnostic method, originally based on the observation of CMV-specific cytopathogenic effect (CPE) [175]. These characteristic cells emerge after inoculation of the sample (urine, saliva, blood, biopsy homogenates, or material obtained by bronchoalveolar lavage fluid; BAL). A clear disadvantage of the classic inoculation culture is that it may not give positive results early in the course of infection. Cultures must be maintained for up to 6 weeks, since the characteristic cytopathogenic effect develops very slowly when titers are low [50]. Since this delay is not acceptable for the management of immunocompromised patients, attempts have been made to "accelerate" the test to make it more feasible in clinical practice.

With the aid of monoclonal antibodies directed against CMV-EA and -IEA, it has become possible to detect CMV-EA or CMV-IEA clearly before the development of the CMV-specific CPE (stadium of late antigen: LA) from within less than one day to 4-6 days after inoculation of any clinical specimen [42, 60, 65, 84, 103, 104, 151, 177]. Obviously, this "accelerated" isolation method has a great impact on the demonstration of virus in clinically highly relevant specimens such as blood [151] and BAL fluid [42, 103].

Serology is a technique widely used in clinical practice for substantiating the diagnosis of CMV infection and seroconversion. The development of CMV antibodies in an individual whose serum was antibody-negative before the infection is usually an excellent marker for primary infections. However, there are some pitfalls that have to be taken into account. Firstly, when a complement-fixing antibody assay (CF) is used, titers of antibodies can fluctuate considerably. In a longitudinal study by Waner et al. of CF titers in a blood donor population [189], a majority of the donors showed CF titers fluctuating between significant ($\geq$ 1:8) and undetectable ($\leq$ 1:4) values, implying that apparent seroconversion may not really reflect primary infection [50, 189]. Moreover, in the same study, up to 16-fold increases in CF antibody titers were observed in healthy individuals, something which may hamper correct interpretation

of a fourfold rise in antibodies as an indicator of reactivation of infection (secondary infection). Secondly, a clear disadvantage of serologic measurements in substantiating the diagnosis of CMV infection is the physiological response time of antibody synthesis during infection. This is, on the average, 1-2 weeks, which means that serology may not be helpful early in the course of infection. Thus, serology is not an early marker of infection. Thirdly, as mentioned above, serology may be unreliable under conditions of extreme immunosuppression, since these patients may not form antibodies at all [81, 158].

Complement-fixing antibody assays are not the most sensitive tests for establishing the diagnosis of CMV infection, and as many as 10%-20% of patients seronegative with CF prove to be positive in other assays [112, 128]. Immunofluorescence (IF) [182], enzyme-linked-immunosorbent assay (ELISA), and radioimmunoassay (RIA) have become methods of first choice in many laboratories because of their high sensitivities [36, 110, 124]. Moreover, with these assays it has become possible to detect CMV-specific IgM antibodies, while antibodies detected with CF are mainly of the IgG class. This may be helpful since IgM antibodies to CMV develop relatively early in the course of a primary infection [50]. Theoretically, CMV-specific IgM antibodies develop only during primary infection, but in fact they may reappear during reactivation of CMV [50, 124]. It is not known whether IgM antibodies formed during infection in a previously seropositive patient are sometimes indicative for superinfections with a new strain of CMV rather than reactivation.

Finally, it is important to stress that CMV infection is not synonymous with CMV disease, the latter being much more difficult to establish. A positive result for CMV on a given assay is not, in itself, definitive proof that CMV is the cause of the current symptoms of the patient. For example, it is known that patients may excrete CMV in their urine for up to 14 years after CMV infection without any symptoms [31]. Even in transplant recipients, isolation of CMV from a clinical specimen of, for example, the lungs does not necessarily confirm an etiological relationship with an existing disease [94, 118, 175]. Thus, a positive result from any test has to be judged in concert with other signs of CMV infection.

Cytomegalovirus and immunity

Cytomegalovirus and the immune system exert reciprocal effects on each other. The host factors involved in the defense against the CMV are genetic make-up, nonspecific immunity, specific humoral factors, such as circulating antibodies, and specific cellular immune factors.

Most evidence for genetic control of susceptibility and resistance to CMV has emerged from animal studies. Diosi et al. [45] reported evidence for genetic transmission of susceptibility or resistance to murine CMV (MCMV). Using Swiss-strain mice and wild strain mice, they found that the wild strain inherited susceptibility to MCMV as an autosomal dominant trait and resistance as an autosomal recessive trait, controlled by a single pair of genes [45, 74]. Chalmer et al. [28] and Grundy et al. [66] confirmed these observations in the murine model. They found that susceptibility or resistance to the virus was controlled by genes of the H2 complex. In humans, an association between HLA-DR antigens and cellular immune response to infectious agents has been found [186]. Roenhorst et al. [140] demonstrated an increased incidence of active CMV infection in renal recipients positive for HLA-DRw6. They also found that recipients positive for HLA-DRw6 with secondary infections excreted infectious virus more often, and were more symptomatic, than HLA-DRw6-negative recipients.

As for immune reactivity after CMV infection, a variety of humoral and cell-mediated immune responses occur. These include production of IgG and IgM antibodies, responses by cytotoxic T cells, and activation of natural killer cells (NKs) and antibody-dependent killer cells (ADKs) [141].

Humoral immunity as a host defense to CMV is probably relatively ineffective since it exerts its effect outside the cell while CMV is a strongly cell-associated virus that usually remains inside the infected host cell. This may explain why patients who have circulating anti-CMV antibodies may experience clinically important CMV disease (reactivation) despite the presence of these antibodies [74, 81, 102, 141, 144, 160]. In an animal model the virus was neutralized in vitro by antibodies in the sera of acutely infected animals, but in vivo viremia and viruria occurred despite the presence of neutralizing antibodies to CMV [74]. Moreover, neutralizing antibodies may be detected in the saliva of infected patients despite the continuing excretion of CMV in the saliva of those patients [181].

On the other hand, humoral immunity may modify the severity of infection, and many reports support this possibility. Firstly, most authors agree that primary infections are more severe in transplant recipients than reactivated or recurrent infections [10, 29, 144, 179], something which also extends to living related transplants [193]. Secondly, patients who fail to develop antibodies or who lose detectable serum

antibody to CMV are likely to have overwhelming, and even fatal, infections [124, 144, 158, 159]. Thirdly, there is some evidence that prophylactic administration of CMV immunoglobulin to seronegative allograft recipients may be beneficial. Finally, although the virus is essentially an intracellular virus, virus-induced neoantigens expressed on the surface of CMV-infected cells (CMV membrane-antigens: CMV-MA) may serve as the primary target for host immunological humoral, as well as cellular, defense [8]. Middeldorp [109] found evidence for the importance of humoral immunity directed against CMV-MA when he found that the appearance of antibodies to CMV-MA was related to subsequent recovery from CMV disease.

Cellular host defense probably plays a pivotal role in the defense against CMV [74, 141, 145]. For instance, Rook and associates [142] demonstrated the importance of cytomegalovirus-specific cytotoxic lymphocytes during infection in renal transplant recipients. Clinically important CMV disease, including pneumonitis, pancreatitis, superinfections, and death, occurred exclusively among patients without a cytotoxic lymphocyte response. Furthermore, acute allograft dysfunction during CMV infection was experienced more frequently in patients without this specific cellular response, indicating that a CMV-specific cytotoxic response did not result in a renal immunopathological condition leading to graft dysfunction. In bone marrow transplant recipients, Quinnan and coworkers [133] also demonstrated the importance of certain subsets of cytotoxic lymphocytes in the defense against CMV in vivo. Since immunosuppressive drugs, especially high-dose steroids, can completely abrogate a cytotoxic T-cell response [142] and since CMV-specific cytotoxic responses can be detected early in the course of a CMV infection, the absence of such a response in the presence of infection might stress the need for early tapering of the immunosuppressive therapy during infection [141]. There is some evidence that cyclosporin A is less suppressive on CMV-specific cytotoxic T-cell responses than the more conventional immunosuppressive drugs [113]. This might be responsible, at least in part, for the diminished incidence of serious CMV infections after renal transplantation reported by some investigators when cyclosporin A is used as an immunosuppressive drug. During CMV infection, a persistent inversion in the ratio CD4+ (helper/inducer cells) to CD8+ (cytotoxic suppressor cells) circulating lymphocytes is found, and a very low CD4/CD8 ratio during infection was associated with a higher incidence of superinfections and glomerular damage during CMV infection [152].

Of the nonspecific factors involved in the defense against CMV, macrophages are believed to play an important role [74]. Although serum complement is activated during CMV infection [168], most likely via the alternative pathway of complement activation [171], its role in the defense against CMV is probably limited since in states of C-deficiencies recurrent, life-threatening, viral infections seldom occur [64]. On the other hand, anaphylatoxins formed during complement activation might be responsible for some of the protean manifestations of the CMV syndrome [41, 170, 171].

Another important feature of CMV infections involves the fact that CMV infection is an important contributor to the net state of immunosuppression present in a given transplant patient [83, 144, 146]. The immunosuppressive effect of the virus makes the patient prone to superinfections with opportunistic pathogens such as *Pneumocystis carinii, Aspergillus fumigatus,* and gram-negative microorganisms [144, 146].

CMV infection is also associated with suppressed monocyte function and monocyte-induced suppression of lymphocyte function [26]. The additional role of leukopenia, which is so frequently observed during infection in the net state of immunosuppression, remains unclear despite the finding that severe leukopenia during symptomatic CMV disease, lasting for more than 5 days, has been associated with high mortality due to superinfection [146]. The impaired cellular immunity found during a CMV infection is not confined to the period during which the patient suffers from CMV disease since it has been demonstrated that patients with a secondary infection have a depressed immune reactivity against alloantigens and CMV-infected targets of long duration [139].

Relationship between cytomegalovirus and rejection

There is a definite and mutual relationship between CMV infection and rejection. There are many reports on the sequence in which infection and rejection occur. For example, Betts et al. [9] report that infection was followed by graft rejection, while others report that graft rejection preceded seroconversion and virus isolation [20, 98]. Although the exact sequence of events is still controversial, most data suggest that allograft rejection precedes active CMV infection. Therefore, rejection is probably important for the occurrence of primary infections or for the reactivation of latent virus [74]. The increased immunosuppression given during allograft rejection may be responsible for the occurrence of the infection,

but the continuous allogenic stimulation by the graft may also be important.

In the murine model, enhancement of MCMV infection was demonstrated during host versus graft reaction [203] and graft versus host reaction [49]. In another model, Olding et al. [120] found that MCMV could be activated from apparently uninfected murine spleen cells by cocultivation with allogenic, but not syngeneic, fibroblasts. These observations were recently confirmed by Bruning et al. in studies in the rat [23]. Therefore, allogenic stimulation by the graft might also play a role in the occurrence of CMV infection after allograft rejection in humans.

On the other hand, some exciting new data have emerged from recent reports that make the inverse sequence of events (i.e. infection followed by rejection) a real possibility. More than 18 years ago, Simmons et al. [157] suggested for the first time that CMV infection could be associated with an increased risk of allograft dysfunction and graft loss. They suggested the possibility of infection triggering allograft rejection. While the inevitable tapering of immunosuppressive therapy during an active CMV infection seems to be a reasonable explanation for an increased incidence of rejection episodes after infection, this might not be the only factor involved. Gaston and Waer [59] supposed, for example, that allograft rejection is initiated by virus-specific, MHC-restricted T lymphocytes. The effector T lymphocytes generated during CMV infection, whose primary specificity is for foreign antigens (those of the infecting agent) that they see in the context of self major histocompatibility complex (MHC) antigens, may mediate rejection because of their crossreactive recognition of allogenic MHC antigens ("nonself").

Furthermore, there is growing evidence that during a CMV infection an increased expression of HLA class I and II antigens on peripheral blood leukocytes as well as on cells of the graft may occur and, thus, may play a role in the process leading to allograft rejection [53, 70, 73, 198]. For example, von Willebrand et al. [198] demonstrated an upregulation of class II antigen display in the graft and suggested that gamma-interferon, produced as a response to the virus infection, could be responsible for the increased antigen presentation leading to allograft rejection following CMV infection. Recently, however, van Dorp et al. [47] demonstrated in an in vitro model of cultured venous endothelial cells from human umbilical cord that these cells, when infected with CMV (CMV AD 169 strains), express class I but not class II antigens on their surface. They were able to demonstrate that this expression was not due to soluble factors in the culture medium synthesized by the CMV-infected cells.

The enhanced expression of class I HLA antigens by CMV [47, 70] may not only be involved in the generation of allograft rejection but may also be important in amplification of virus infection [6, 70]. Beck and coworkers [6] demonstrated that CMV encodes a molecule similar to the MHC-class I antigens of higher eucaryotes. Grundy et al. [67] showed that CMV exists in vivo as β2M-coated particles and suggested that those β2M-coated virus particles may use class I HLA molecules as a virus receptor, thereby increasing the infectivity of the virus. The CMV class I-like encoded membrane glycoproteins may be involved in this binding process. When the β2M-coated CMV comes in close contact with a target cell, exchange or displacement of CMV-bound β2M with β2M bound to cellular HLA class I antigens may take place and trigger internalization of the virus by receptor-mediated endocytosis [6]. Some other fascinating data came from the observation by Funjinami and coworkers [58], who demonstrated sequence homology and immunologic crossreactivity of human CMV with HLA-DR β chain, which might thus also be involved in the rejection process. An alternative explanation for graft dysfunction, CMV glomerulopathy, as proposed by Richardson et al. [137], will be discussed in the following section.

Symptomatology

Despite the fact that many of the patients with laboratory signs of an active CMV infection have no clinical symptoms at all and despite new immunosuppressive regimens, CMV infection after organ transplantation still has a significant impact on graft and patient survival [11, 57, 61, 96, 126, 144, 146, 149, 192, 193]. As mentioned earlier, the most important factors involved in the liability for developing severe CMV disease are the serostatus of the recipient/donor pairs (the seropositive donor/seronegative recipient combination posing the greatest risk) and the net state of immunosuppression. With regard to the impact of CMV infections on graft and patient survival, Fryd and coworkers [57], in a prospective study of 276 renal allograft recipients, found a 1-year graft survival of 53% in patients with CMV disease as compared to 79% in patients without CMV disease. Patient survival at 1 year was 76% and 92%, respectively. Metselaar and associates [106] studied 73 cadaveric renal graft recipients for whom the serostatus of both the recipient and donor were known. A poor graft survival of 41% at 3 years after transplantation was found in CMV-negative recipients who had been transplanted with kidneys of seropositive donors, compared with an actuarial 3-

year graft survival of 72% in seronegative/seronegative pairs. In a large multicenter study by Rubin et al. [149] a worse outcome for patients at risk for primary CMV infection was demonstrated, especially when antilymphocyte preparations were used.

Of all patients who develop clinical manifestations of CMV infection as a result of primary, secondary, or superinfections, more than 90% do so in the period 1-6 months post-transplantation, and 60% of the febrile episodes during this period are due to CMV infections [90, 126, 144, 146, 179]. However, clinically important, even fatal, CMV infections may occur up to 2 years or more after transplantation [97]. When patients with a CMV infection are symptomatic, the symptoms may vary greatly. Most of the patients have a so-called self-limiting syndrome, consisting of fever (often spiking), arthralgia, leukopenia and/or thrombopenia, and abnormalities in liver enzymes [90, 144, 146]. With tapering of the immunosuppressive therapy, the great majority of patients recover completely from the syndrome.

Renal involvement during CMV infection is frequently observed, but the cause of it is controversial. The mutual relationship between CMV infection and allograft rejection as a possible cause of renal dysfunction during CMV infection has already been extensively discussed.

Alternatively, in 1981, Richardson et al. [137] described a distinctive pattern of glomerular injury in renal allografts that they associated with CMV viremia without relation to allograft rejection. The pathological features consisted of diffuse endothelial hypertrophy and necrosis, accompanied by accumulation of fine fibrillar webs of periodic acid-Schiff (PAS)-positive material and mononuclear cells that resulted in obliteration of the glomerular capillaries. Furthermore, fibrin and IgM as well as C3 were found by IF. No viral particles were detectable by electron microscopy or by IF using monoclonal antibodies directed against CMV-EA and -LA. Recently, the existence of a distinct CMV glomerulopathy has been disputed [17, 79]. Herrera and coworkers [79] state that the pathological condition that has been designated as CMV glomerulopathy probably represents rejection, either a peculiar antiendothelial type of rejection or a protracted, early, or partially resolved vascular type of rejection. It is also noteworthy that patients without an allograft who contract CMV do not develop lesions as described by Richardson et al. Boyce and associates [17] found a significant prevalence of "transplant glomerulopathy" in their renal transplant patients, but they could not demonstrate a correlation with CMV infection. They also suggested that since the condition correlated well with poor graft survival, "transplant glomerulopathy" is a manifestation of allograft rejection. On the other hand, Smith and Wehner [162, 190] were able to induce glomerulonephritis associated with azotemia and proteinuria in an animal model by injecting CMV intraperitoneally.

In those studies, CMV was only demonstrated by electron microscopy in mesangial cells [162, 190]. In this respect it is noteworthy that Heieren et al. [78] recently demonstrated that CMV efficiently replicates in cultured human kidney mesangial cells. Yet, in human pathology, CMV is seldom demonstrated in kidney cells, even in patients with disseminated disease, although Shorr and coworkers [156] were able to describe a case of tubulointerstitial nephritis during CMV infection where, in autopsy material, classic CPE was demonstrated around the kidney tubular epithelium.

Although seldom observed, an alternative explanation of renal impairment during CMV infection might be glomerulonephritis due to immune complexes formed during infection [122, 174]. Thus, although renal impairment during CMV infection is frequently observed, its cause remains rather obscure and may even be multifactorial.

Gastrointestinal symptoms during CMV infection are numerous. They include gastrointestinal ulcers that may bleed or perforate (i.e., esophageal, gastric, and colon ulcers), gastritis, and pancreatitis [3, 38, 55, 81, 126, 143, 144, 159] as well as (granulomatous) hepatitis [37, 117] and possibly pneumatosis intestinalis [167]. Inclusion bodies [126] and sometimes vasculitis [55] may be found at the site of the ulcers in the alimentary tract. It is important to note that gastrointestinal symptoms due to CMV can be present without other major symptoms of the infection. In this respect it is important to stress that CMV can be present in the gastroduodenal tract without symptoms. Franzin and coworkers [56] found evidence of CMV inclusion bodies in biopsies collected from gastroduodenal mucosa of patients with a renal allograft in 9 out of 20 cases. The presence of these CMV inclusion bodies was unrelated to viremia-induced or gastrointestinal symptoms at the time of endoscopy. Therefore, when gastrointestinal symptoms are present in a given patient, one single positive laboratory test consistent with CMV infection may not be enough to consider the symptoms present as CMV-induced. Other possible causes must first be excluded and positive laboratory signs for CMV infection have to be judged in concert with other signs of CMV infection. This also holds for the other numerous manifestations attributed to CMV: lymphadenopathy, rash, hepatosplenomegaly, conjunctivitis, pericarditis, myocarditis, encephalitis,

Guillain-Barré syndrome, and skin ulcerations associated with vasculitis [2, 46, 61, 76, 111, 144, 200].

Of the more common manifestations of CMV infection in the transplanted patient, pneumonia is the one that separates serious illness from more benign disease [126, 144], with a reported mortality of 48% rising to over 90% if assisted ventilation is required [126]. The most common form of CMV pneumonitis is a bilateral, symmetrical, interstitial process that affects predominantly the lower lobes of the lungs [146]. Alternatively, a more lobar pattern may be found, and even a solitary pulmonary nodule has been reported solely due to CMV [135]. Thus, the radiological presentation can be indistinguishable from other causes of pulmonary infiltrates, such as bacteria, fungi, or HSV. Attempts to make the proper diagnosis should, therefore, not be postponed and should be rather aggressive (BAL, transbronchial, or open-lung biopsy, together with extensive bacterial, viral, and fungal cultures) in order to initiate the proper treatment as soon as possible. It is also noteworthy that HSV and CMV are frequently present concomitantly in the same patient. If an asymptomatic patient with only laboratory signs of CMV infection and an oral infection with herpes simplex (labial HSV) has to be intubated for whatever reason (e.g., an operation) and later develops focal or multifocal pulmonary infiltrates, the possibility of herpes simplex pneumonia, via contiguous spread of the virus during intubation, has to be taken into account [134].

Peterson and coworkers [127] analyzed the risk factors in the development of CMV-related pneumonia in renal transplant recipients. They found that recipients of kidneys from seropositive donors had a more than threefold greater risk of developing CMV pneumonia than recipients of kidneys from seronegative donors. Moreover, an increased risk was found in patients who received ATG as immunosuppressive therapy. Clinically, patients with CMV pneumonia have a characteristic "subacute" presentation with symptoms developing over a period of several days, the first signs being a typical dry, hacking cough with an increasing respiratory rate. Otherwise, physical signs at that time are minimal. When progressive, symptoms evolve over a period of several days, eventually leading to respiratory failure [146].

A brisk, rapid deterioration of the clinical situation must alert the clinician to the possibility of superinfection with opportunistic pathogens such as *Pneumocystis carinii, Aspergillus fumigatus, Candida albicans* or gram-negative microorganisms [144].

Although the exact pathogenesis of CMV pneumonitis is not known [154], some investigators suggest that it could be an immunologically mediated phenomenon rather than the result of direct viral damage to the lungs [68, 154]. For example, Grundy and associates [68] state that CMV pneumonitis is due to uncontrolled accumulation in the lungs by host T cells during infection. One other possible factor involved in the pathogenesis of CMV pneumonitis might be the serum complement C system, since C is activated during an active CMV infection [168, 171] and C-cleavage products, formed during activation, are thought to play an important role in the immunopathological aspects of several pulmonary afflictions, as reviewed by Stimler et al. [176] and Till et al. [184]. In our laboratory, van Son and coworkers [169, 170] showed in a prospective study of renal transplant patients that every patient with an active CMV infection has pulmonary dysfunction [measured as decreased diffusing capacity for carbon monoxide (KCO)], even patients with a normal chest roentgenogram and normal blood gas analysis. The pulmonary dysfunction found in these patients occurred concomitantly with the appearance of C-activation products like the anaphylactoid factor C3a des arg in the circulation [170], together with a sharp rise in serum angiotensin-converting enzyme (as a possible marker for pulmonary endothelial cell damage) [172], leading to the hypothesis that these findings were linked.

The formation of C-anaphylatoxins during CMV infection might, for instance, have caused damage to the pulmonary endothelial cells via chemotaxis and activation of polymorphonuclear cells and the formation of toxic oxygen radicals, thereby influencing the gas transport from the alveoli to the capillaries in the lungs [44, 172]. However, further research will be needed to prove this hypothesis. Another important, though rather infrequent and late, feature of the CMV syndrome post-transplantation is the CMV-induced chorioretinitis, especially in patients with long-lasting viremia [21, 105, 114, 144]. The hallmark of CMV retinitis is hemorrhagic retinal necrosis, which leads to irreversible, devastating ocular damage [105, 114] and, sometimes, to bilateral retinal detachment [21].

Finally, in 1977, Simmons and coworkers [159] described the characteristics of a lethal CMV syndrome - "wasting disease" - in which patients suffer an unrelenting, disseminating disease despite cessation of the immunosuppression. The clinical characteristics of the lethal CMV infection following transplantation are prostration, orthostatic hypotension, severe pulmonary dysfunction with undersaturation, hepatic dysfunction, muscle wasting, central nervous system depression, and severe gastrointestinal symptoms associated with bleeding from ulcers, ultimately leading

to death. Fortunately, with modern immunosuppressive regimens and improved techniques for early detection of CMV infection, the incidence of this lethal syndrome has decreased [144].

Prophylaxis and treatment of cytomegalovirus infections

As for the prophylaxis of CMV infections, one logical approach to preventing severe CMV disease after transplantation would be to identify patients at risk (seronegative recipients) and to transplant them only with organs from seronegative donors. While advocated by some authors [1, 106, 199], this "double matching" of HLA and serostatus might not always be feasible. In a situation where, for example, there is an increasing shortage of kidneys, as at Eurotransplant, this might merely increase the waiting time, especially for patients with a high percentage of circulating HLA antibodies [147]. In living related transplantation, a seropositive donor for a seronegative kidney recipient should also be accepted since the advantages of living related grafting far outweigh the disadvantages of CMV disease [149]. However, since CMV can cause serious morbidity in living related transplantation, when more than one equivalent donor is available, a seronegative recipient should, whenever possible, be transplanted with the kidney of a seronegative donor [193].

If only a seropositive donor is available, another approach could be to transfuse the seronegative recipient with the seropositive blood of the donor, i.e., donor-specific transfusion (DST) [193]. Since DST means transfusion with viable leukocytes, there is a reasonable chance of seroconversion of the recipient. One could postpone transplantation until this seroconversion has occurred, some 3-4 months after DST. In two studies [4, 129], active immunization of seronegative healthy volunteers and uremic candidates for transplantation by the live, attenuated Town strain of CMV elicited a humoral response, and antibodies to CMV could be demonstrated up to 3 years after vaccination. However, while in a study of Plotkin et al. [131] there was less severe CMV disease in the Town-vaccinated patients than in the nonvaccinated patients, another study showed that vaccination could not prevent severe, even fatal, CMV infection [4]. It is important to stress that vaccination only gives limited immunity to the Town strain, and given the fact that even natural infection does not confer immunity to superinfection with other strains of the CMV, it is unlikely that vaccination with a monovalent CMV vaccine can really be effective in preventing CMV disease [144]. High-titer, hyperimmune anti-CMV globulin preparations are polyclonal and derived from many donors, making them, at least theoretically, candidates to cover a broad group of naturally occurring strains of the CMV [144]. In an animal model, Rubin and associates [150] showed that administration of hyperimmune antimurine CMV antiserum provided mice with complete, long-lasting protection against a lethal challenge with murine CMV.

In a controlled study by Snydman and coworkers [164], a protective effect of CMV hyperimmune globulin was reported when given prophylactically to renal transplant patients during the first 4 months after transplantation. A significant reduction in serious CMV-associated disease was observed, even when patients were grouped according to therapy for transplant rejection. However, the rate of viral isolation was not influenced. In a recent open-label trial, they found comparable results [165]. The same conclusions have been reported in bone marrow grafting [40, 201] and in heart transplantation (Metselaar and Weimar, personal communication).

Other prophylactic strategies reported are of antiviral origin. Cheeseman and coworkers [30] and Hirsch and associates [80] showed in 1979 and 1983, respectively, that interferon-alpha of leukocyte origin, when given prophylactically to renal transplant recipients, decreased the incidence of clinical CMV disease as well as the incidence of superinfection with opportunistic pathogens. However, a strong correlation between administration of interferon-alpha and irreversible rejection has been noted [89], even when given in low doses and to patients treated with cyclosporin A, [88] something which may be related to the reported upregulation of MHC-antigen expression by interferon. Thus, prophylactic administration of interferon cannot be recommended.

There is some evidence for acyclovir as prophylaxis for CMV disease. Meyers et al. [108] showed in bone marrow graft recipients and Balfour et al. [5] in renal transplant recipients that prophylactic treatment with high-dose oral acyclovir resulted in a significant reduction in the rate of CMV infections and disease. However, in the placebo-controlled study of Balfour and coworkers, neither a difference in the severity of CMV infection nor any in patient and graft survival could be demonstrated between the treated and placebo groups. In both studies the prophylactic treatment started on the day of transplantation and ended 30 days later.

Finally, it needs to be stressed that one of the major ways of preventing CMV disease is to avoid overimmunosuppression, especially in the patients at risk for primary infection. Together with the new techniques for rapid diagnosis of CMV infection,

this is the basic approach to preventing clinically important CMV disease.

The first, and probably most important, step towards treating patients with CMV disease is to taper the immunosuppression. Therapy with ALG, ATG, and OKT3, in particular, should be stopped without delay.

Treatment of CMV disease with acyclovir [130, 188], adenine-arabinoside [32, 101], or adenine-arabinoside in combination with interferon [107] have all been unsuccessful because of proven ineffectiveness and/or toxicity. New drugs, such as phosphonoformate (Foscarnet) and especially DHPG (9-[(1,3-dihydroxy-2propoxy)methyl]guanine), are much more promising. Klintmalm and colleages [87] demonstrated a favorable clinical reponse to intravenously administered Foscarnet in six immunosuppressed patients with life-threatening CMV infection. Ringdén and associates [138] also found considerable improvement in clinical and laboratory signs of CMV infection in bone marrow and renal transplant recipients, although they reported a significant mortality despite treatment with Foscarnet. Adverse effects, such as decreased values of hemoglobin, decreased renal function, and increased serum calcium levels, were reported in only a few patients in this study. Recently, however, Cacoub and coworkers [25] reported four cases of acute renal failure, which they attributed to the use of Foscarnet.

DHPG (ganciclovir) is a new antiviral drug which, like acyclovir, is a cogener of deoxyguanosine, a normal component of DNA [95]. Ganciclovir is activated by kinases of CMV-infected cells to its probably active triphosphate-derivate, which is an inhibitor and substrate for CMV-DNA polymerase [14]. In this respect it is established that in vitro ganciclovir is activated ten times more readily than acyclovir in CMV-infected cells and up to 100 times more when compared to uninfected cells. This may explain why ganciclovir, in contrast to acyclovir, is not only excellent at inhibiting CMV replication in vitro but also inhibits CMV at clinically achievable levels in vivo [95, 132]. It should be noted that, because of its structure, ganciclovir is, in contrast to acyclovir, not only incorporated in the viral DNA but also in the DNA of the host cell [197]. This may be relevant for the definition of the therapeutic index or ratio of efficacy to toxicity. Ganciclovir is metabolized like acyclovir and is excreted largely unchanged in the urine [166]. In patients with normal renal function, a biexponential decay of ganciclovir from plasma has been observed, with an initial distribution half-life ($t_{1/2}$) of 0.76 ± 0.67 h and a terminal elimination $t_{1/2}$ of 3.60 ± 1.40 h. Aside from glomerular filtration of ganciclovir, there is evidence that tubular secretion may also be involved in the clearance of ganciclovir, and the clearance of the drug was found to be substantially higher than the estimated creatinine clearance [166].

Hemodialysis has been shown to be very effective in eliminating ganciclovir from plasma [166]. In patients with renal insufficiency, the terminal elimination $t_{1/2}$ was found to be markedly increased, which makes adjustment of the dosage of the drug in case of renal function impairment necessary. Ganciclovir has been shown to be effective in treating CMV disease in immunosuppressed patients, especially in patients with the acquired immunodeficiency syndrome (AIDS), in patients with solid organ transplantation and, to a lesser extent, in patients with bone marrow transplants [27, 39, 51, 54, 93, 95, 125]. In patients with bone marrow grafts, the results of DHPG treatment for CMV pneumonia have generally been disappointing [136, 155, 202]. Recently, however, Crumpacker and associates [43] reported a somewhat higher survival rate of 38% in 21 bone marrow graft patients with well-documented CMV pneumonia who were treated with DHPG. They suggested that the more favorable outcome observed in the study group, when compared to previous studies, was possibly due to the somewhat lower dosage of DHPG (10 mg/kg per day), thereby avoiding profound neutropenia, and to the concomitant use of hyperimmune globulin in some of the survivors. Although the results thus far indicate that CMV pneumonia in bone marrow transplant recipients is somewhat refractory to treatment with ganciclovir, it was anticipated that in less severely immunocompromised patients, such as renal allograft recipients, the results might be more favorable. There are, however, only a few, as yet inconclusive, reports on relatively few patients about the effects of DHPG on CMV pneumonia in renal transplant recipients. Hecht and associates [77] reported 50% survival in four patients with CMV pneumonia after renal transplantation who were treated with DHPG. This treatment was started in both survivors within 4 days after the onset of the pneumonia, while one of the survivors also received hyperimmuneglobulin.

Harbison and coworkers [75] reported a 67% recovery from CMV pneumonia after treatment with DHPG; in four out of six cases, however, the pneumonia was not very well documented. Favorable outcome of DHPG-treated CMV pneumonia in renal transplant recipients has also been reported by Buhles et al. [24] and Stoffel et al. [178].

Snydman and associates [163] recently reported very encouraging results in renal graft recipients with well-documented (histologically and virologically) CMV pneumonia who were treated with DHPG.

Since none of the studies with this drug have been randomized or placebo-controlled, further data from well-documented cases are required before the efficacy of DHPG in the treatment of CMV pneumonia can be established.

Although clearly needed, and despite the well-established efficacy of ganciclovir for other symptoms of the CMV syndrome, such studies are not likely to occur for "ethical" reasons. Side effects of ganciclovir are very well documented and, although mild and reversible in most cases, they can be severe. In a study of 314 immunocompromised patients, the most common events during treatment with DHPG included neutropenia (absolute neutrophil count < 1000 cells/μl) in 42% of all patients, thrombocytopenia (< 50000 platelets/μl) in 19% of the patients, rash (6%), nausea (6%), fever (6%), infusion site reaction, vomiting, diarrhea, anemia (4% each) and eosinophilia, confusion, seizures, and abnormal mentation (3% each) [24]. Although most of the side effects are reversible, neutropenia may, in rare cases, be irreversible. Since ganciclovir has no effect on latent virus, relapses may occur in heavily immunocompromised patients with CMV disease after an initial good response to DHPG treatment, especially in patients with AIDS or with a poor humoral response to CMV. Thus, in order to stabilize the disease, maintenance therapy is sometimes required (e.g., 5 mg/kg five times a week) [95].

In this respect it is of great concern that resistance to DHPG of certain CMV strains has recently been described in the laboratory [15] as well as in vivo. Erice and associates [52] described the development of resistance of a certain CMV strain during treatment of DHPG in an immunocompromised patient who was on prolonged treatment with this drug. In the studies of Biron and coworkers [15], cells infected with the drug-resistant CMV mutant failed to convert ganciclovir to its active triphosphorylated form, suggesting that reduced phosphorylation of the drug accounted for the resistance of this mutant. Therefore, in order to avoid the selection of resistant strains of CMV by the unlimited use of ganciclovir, it might be wise to reserve this treatment for patients with life-threatening disease and for patients with CMV retinitis [52].

Recommendations for people who work with immunosuppressed patients with severe cytomegalovirus disease

Since there is no evidence of an increased risk of infection with CMV among personnel working in dialysis or oncology units [121, 185], no special precautions are recommended for the care of immunosuppressed patients who are known or believed to be excreting CMV. Good personal hygiene, especially hand-washing should be practiced after contact with urine or respiratory tract secretions of immunosuppressed patients. Gowns, masks, gloves, or private rooms are not necessary, and the transfer of susceptible personnel (including pregnant women) to other units is not recommended.

Pregnant women working with severely immunosuppressed patients should be informed of the risk of acquiring CMV infection, its possible effects on the fetus, and hygienic practices to prevent infection [121].

Conclusions

During the last decade, an abundance of data have emerged highlighting new aspects of the basic properties of CMV, information about transmission of the virus, and its relation to the immune system. New data have become available concerning the pathophysiological processes leading to the protean manifestations of the CMV syndrome. New methods for rapid diagnosis of CMV infection have become available. The CMV antigenemia assay, as well as the "accelerated" isolation methods of the CMV and possibly the PCR test, are especially promising new tools for the clinician dealing with the care of transplanted patients. For prevention of CMV infections, prophylactic treatment with hyperimmune globulin or possibly with high-dose oral acyclovir is becoming more and more a real possibility. Finally, promising new regimens for treatment of severe CMV infections have become available. DHPG, in particular, seems to be heralding a new era in the management of patients with life-threatening CMV disease. However, since no drugs are currently available to eradicate latent virus, it is unlikely that the many problems of this intriguing virus in organ transplantation will be solved in the near future.

As long as we act like meddlesome parents in the inevitable marriage between CMV and the immune system, introducing new, more potent immunosuppressive drugs, this virus will continue to challenge us to find ways of modifying its impact on graft and patient survival in organ transplantation.

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References

1. Ackermann JR, LeFor W, Weinstein S, Kahana L, Shires DL, Tardif G, Baxter J (1988) Four year experience with exclusive use of cytomegalovirus (CMV) Ab-negative donors for CMV-Ab-negative kidney recipients. Transplant Proc 20 [Suppl 1]: 469-471
2. Alford CA Jr, Britt WJ (1985) Cytomegalovirus. In: Fields BN (ed) Virology, Raven Press, New York, pp 629-660
3. Ayulo M, Aisner SC, Margolis K, Moravec C (1980) Cytomegalovirus-associated gastritis in a compromised host. JAMA 243: 1364
4. Balfour HH Jr, Welo PK, Sachs GW (1985) Cytomegalovirus vaccine trial in 400 renal transplant candidates. Transplant Proc 17: 81-83
5. Balfour HH Jr, Chace BA, Stapleton JT, Simmons RL, Fryd DS (1989) A randomized, placebo-controlled trial of oral acyclovir for the prevention of cytomegalovirus disease in recipients of renal allografts. N Engl J Med 320: 1381-1387
6. Beck S, Barrell BG (1988) Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I-antigens. Nature 331: 269-271
7. Betts RF (1982) Cytomegalovirus infection in transplant patients. Prog Med Virol 28: 44-64
8. Betts RF, Schmidt SG (1981) Cytolytic IgM antibody to cytomegalovirus in primary cytomegalovirus infection in humans. J Infect Dis 143: 821-826
9. Betts RF, Freeman RB, Douglas RG, Talley TE, Rundell B (1975) Transmission of cytomegalovirus infection with renal allografts. Kidney Int 8: 385-392
10. Betts RF, Freeman RB, Douglas RG Jr, Talley TE (1977) Clinical manifestation of renal allograft derived primary cytomegalovirus infection. Am J Dis Child 131: 759-763
11. Bia MJ, Andiman W, Gaudio K, Kliger A, Siegel N, Smith D, Flye W (1985) Effect of treatment with cyclosporine versus azathioprine on incidence and severity of cytomegalovirus infection posttransplantation. Transplantation 40: 610-614
12. Bij W van der, Dijk RB van, Son WJ van, Torensma R, Prenger KB, Prob JM, Tegzess AM, The TH (1988) Antigen test for early diagnosis of active cytomegalovirus infection in heart transplant recipients. J Heart Transplant 7: 106-110
13. Bij W van der, Torensma R, Son WJ van, Anema J, Schirm J, Tegzess AM, The TH (1988) Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leukocytes. J Med Virol 25: 179-188
14. Biron KK, Stanat SC, Sorrell JB, Fyfe JA, Keller PM, Lambe CU, Nelson DJ (1985) Metabolic activation of the nucleoside analog 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine in human diploid fibroblasts infected with human cytomegalovirus. Proc Natl Acad Sci USA 82: 2473-2477
15. Biron KK, Fyfe JA, Stanat SC, Leslie KK, Sorrell JB, Lambe CU, Coen DM (1986) A human cytomegalovirus mutant resistant to the nucleoside analog 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine (BW B 759 U) induces reduced levels of BW B 759 U triphosphate. Proc Natl Acad Sci USA 83: 8769-8773
16. Black FL, Woodall JP, Evans AS, Liebhaber H, Henle G (1970) Prevalence of antibody against viruses in the Tiriyo, an isolated Amazon tribe. Am J Epidemiol 91: 430-438
17. Boyce NW, Hayes K, Gee D, Holdsworth SR, Thomson NM, Scott D, Atkins RC (1988) Cytomegalovirus infection complicating renal transplantation and its relationship to acute transplant glomerulopathy. Transplantation 45: 706-709
18. Brand A, Gratama JW (1985) CMV and blood transfusions. In: Meer JWM van der, Versteeg J (eds) Cytomegalovirus. Proceedings of postacademic course "Boerhaave", Leyden, pp 65-74
19. Brando B, Civati G, Busnach G, Braggi ML, Belli LS, Seveso M, Brunati C, Minetti L (1985) Cytomegalovirus infection in cyclosporine A treated renal transplant recipients. Transplant Proc 17: 2683-2685
20. Braun WE, Nankervis G, Banowsky LH, Protiva D, Bückert E, McHenny MC (1976) A prospective study of cytomegalovirus infection in 75 renal allograft recipients. Proc Clin Dial Transplant Forum 6: 8-12
21. Broughton WL, Cupples HP, Parver LM (1978) Bilateral retinal detachment following cytomegalovirus retinitis. Arch Ophthalmol 96: 618-619
22. Bruning JH, Bruggeman CA, Boven CPA van, Breda Vriesman PJC van (1986) Passive transfer of cytomegalovirus by cardiac and renal organ transplants in a rat model. Transplantation 41: 695-698
23. Bruning JH, Bruggeman CA, Boven CPA van, Breda Vriesman PJC van (1989) Enhancement of reactivation of latent rat cytomegalovirus by combination of immunosuppression and administration of allogenic immunocompetent cells. Transplantation: 47: 742-744
24. Buhles WC Jr, Mastre BJ, Tinker AJ, Strand V, Koretz SH (1988) Ganciclovir treatment of life or sight-threatening cytomegalovirus infection. Experience in 314 immunocompromised patients. Rev Infect Dis 10 [Suppl 3]: 495-506
25. Cacoub P, Deray G, Baumelou A, Le Hoang P, Rozenbaum W, Gentilini M, Soubrie C, Rousselie F, Jacobs C (1988) Acute renal failure induced by foscarnet: 4 cases. Clin Nephrol 29: 315-318
26. Carney WP, Hirsch MS (1981) Mechanisms of immunosuppression in cytomegalovirus mononucleosis. J Infect Dis 144: 47-54
27. Chachoua A, Dieterich D, Krazinsky K, Greene J, Laubenstein L, Wernz J, Buhles W, Koretz S (1987) 9-(1,3-dihydroxy-2-propoxymethyl) guanine (ganciclovir) in the treatment of cytomegalovirus disease with the acquired immunodeficiency syndrome. Ann Intern Med 107: 133-137
28. Chalmer JE, Mackenzie JS, Stanley NF (1977) Resistance to murine cytomegalovirus linked to the major histocompatibility complex of the mouse. J Gen Virol 37: 107-114
29. Chatterjee SN, Jordan GN (1979) Prospective study of the prevalence and symptomatology of cytomegalovirus infection in renal transplant recipients. Transplantation 28: 457-460
30. Cheeseman SH, Rubin RH, Stewart JA, Tolkoff-Rubin NE, Cosimi AB, Cantell K, Gilbert J, Winkle S, Herrin JT, Black PH, Russell PS, Hirsch M (1979) Controlled clinical trial of prophylactic human leukocyte interferon in renal transplantation: effects on cytomegalovirus and herpes simplex infection. N Engl J Med 300: 1345-1349
31. Cheeseman SH, Stewart JA, Winkle S, Cosimi AB, Tolkoff-Rubin NE, Russell PS, Baker GP, Herrin J, Rubin RH (1979) Cytomegalovirus excretion 2-14 years after renal transplantation. Transplant Proc 11: 71-74
32. Ch'ien LT, Cannon NJ, Whitley RJ, Diethelm AG, Dismukes WE, Scott CW, Buchanan RA, Alford CA Jr (1974) Effect of adenine arabinoside on cytomegalovirus infections. J Infect Dis 130: 32-39
33. Chou S (1986) Acquisition of donor strains of cytomegalovirus by renal transplant recipients. N Engl J Med 314: 1418-1423
34. Chou S, Merigan TC (1983) Rapid detection and quantitation of human cytomegalovirus in urine through DNA hybridization. N Engl J Med 308: 921-925
35. Chou S, Norman DJ (1985) Effects of OKT3 antibody therapy on cytomegalovirus reactivation in renal transplant recipients. Transplant Proc 17: 2755-2756
36. Chung A, Chang W, Leung MK, Ofosu FA, Moore BPL

(1984) Assessment of methods for cytomegalovirus antibody testing of blood donation. Vox Sang 47: 343-347
37. Clarke J, Craig RM, Saffro R, Murphy P, Yokoo H (1979) Cytomegalovirus granulomatous hepatitis. Am J Med 66: 264-269
38. Cohen EB, Komorowski RA, Kauffman HM, Adams M (1985) Unexpectedly high incidence of cytomegalovirus infection in apparent peptic ulcers in renal transplant patients. Surgery 97: 606-612
39. Collaborative DHPG Treatment Study Group (1986) Treatment of serious cytomegalovirus infections with 9(1,3-dihydroxy-2-ropoxymethyl)guanine in patients with AIDS and other immunodeficiencies. N Engl J Med 314: 801-805
40. Condie RM, O'Reilly RJ (1984) Prevention of cytomegalovirus infection by prophylaxis with an intravenous, hyperimmune, native unmodified cytomegalovirus globulin. Randomized trial in bone-marrow transplant recipients. Am J Med 76: 134-141
41. Cooper NR (1988) Complement-dependent neutralization of viruses. In: Rother K, Till GO (eds) The complement system, Springer-Verlag, Berlin Heidelberg New York London Paris Tokyo, pp 342-348
42. Crawford SW, Bowden RA, Hackman RC, Gleaves CA, Meyers JD, Clark JG (1988) Rapid detection of cytomegalovirus pulmonary infection by broncho-alveolar lavage and centrifugation culture. Ann Intern Med 108: 180-185
43. Crumpacker C, Marlow S, Zhang JL, Abrams S, Watkins P (1988) Treatment of cytomegalovirus pneumonia. Rev Infect Dis 10 [Suppl 3]: 538-546
44. Daniele RP (1981) Immune-complex injury of the lung. Am Rev Respir Dis 124: 738-755
45. Diosi P, Arcan P, Plavosin L (1974) Genetic control of resistance to mouse cytomegalovirus infection. Arch Geschwulstforsch 44: 23-27
46. Dorfman LJ (1973) Cytomegalovirus encephalitis in adults. Neurology 23: 136-144
47. Dorp W van, Jonges E, Bruggeman CA, Daha MR, Es LA van, Woude FJ van der (1989) Cytomegalovirus infection directly induces MHC class I but not class II expression on endothelial cells. Transplantation (in press)
48. Dowling JN, Saslow AR, Armstrong JA, Ho M (1976) Cytomegalovirus infection in patients receiving immunosuppressive therapy for rheumatologic disorders. J Infect Dis 133: 399-408
49. Dowling JN, Wu BC, Armstrong JA, Ho M (1977) Enhancement of murine cytomegalovirus infection during graft-versus-host-reaction. J Infect Dis 135: 990-994
50. Drew WL (1988) Diagnosis of cytomegalovirus infection. Rev Infect Dis 10 [Suppl 3]: 468-476
51. Erice A, Jordan MC, Chace BA, Fletcher C, Chinnock BJ, Balfour HH (1987) Ganciclovir treatment of cytomegalovirus-disease in transplant recipients and other immunocompromised hosts. JAMA 257: 3082-3087
52. Erice A, Chou S, Biron KK, Stanat SC, Balfour HH, Jordan MC (1989) Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. N Engl J Med 320: 289-293
53. Es A van, Baldwin WM, Oljans PJ, Tanke HJ, Ploem JS, Es LA van (1984) Expression of HLA-DR on T lymphocytes following renal transplantation, and association with graft rejection episodes and cytomegalovirus infection. Transplantation 37: 65-69
54. Felsenstein D, D'Amico DJ, Hirsch MS, Neumeyer BS, Cedenberg DH, Miranda P de, Schooley RT (1985) Treatment of cytomegalovirus retinitis with 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl] guanine. Ann Intern Med 103: 377-380
55. Foucar E, Mukai KM, Foucar K, Sutherland DER, Buren CT van (1981) Colon ulceration in lethal cytomegalovirus infection. Am J Clin Pathol 76: 788-801
56. Franzin G, Muolo A, Griminelli T (1981) Cytomegalovirus inclusions in the gastro-duodenal mucosa of patients after renal transplantation. Gut 22: 698-701
57. Fryd DS, Peterson PK, Ferguson RM, Simmons RL, Balfour HH, Najarian JS (1980) Cytomegalovirus as a risk factor in renal transplantation. Transplantation 30: 436-439
58. Funjinami RS, Nelson JA, Walker L, Oldstone MBA (1988) Sequence homology and immunologic cross-reactivity of human cytomegalovirus with HLA-DRβ chain: a means for graft rejection and immunosuppression. J Virol 62: 100-105
59. Gaston JSH, Waer M (1985) Virus-specific MHC-restricted T lymphocytes may initiate allograft rejection. Immunol Today 8: 237-239
60. Gleaves CA, Smith TF, Shuster EA, Pearson GR (1985) Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. J Clin Microbiol 21: 217-221
61. Glenn J (1981) Cytomegalovirus infection following renal transplantation. Rev Infect Dis 3: 1151-1178
62. Gnann JW Jr, Ahlnen J, Svalander C, Olding L, Oldstone MBA, Nelson J (1988) Inflammatory cells in transplanted kidneys are infected by human cytomegalovirus. Am J Pathol 132: 239-248
63. Gold E, Nankervis GA (1982) Cytomegalovirus. In: Evan AS (ed) Viral infections of humans, epidemiology and control, 2nd edn. Plenum Press, New York London, pp 167-186
64. Greene MI, Fields BN (1988) Host response to viruses. In: Samter M, Talmage DW, Frank M, Austen KF, Claman HN (eds) Immunological diseases, vol 1, 4th edn. Little, Brown and Co, Boston Toronto, pp 899-921
65. Griffiths PD, Stirk PR, Ganczakowski M, Panjwani DD, Ball HG, Blacklock HA, Prentice HG (1984) Rapid diagnosis of cytomegalovirus infection in immunocompromised patients by detection of early antigen fluorescent foci. Lancet II: 1242-1244
66. Grundy JE, Mackenzie JS, Stanley NF (1981) Influence of H2 and non-H2-genes on resistance to murine-cytomegalovirus infection. Infect Immun 32: 277-286
67. Grundy JE, McKeating JA, Ward PJ, Sanderson AR, Griffiths PD (1987) β2-microglobulin enhances the infectivity of cytomegalovirus, and when bound to the virus enables class I HLA molecules to be used as a virus receptor. J Gen Virol 68: 793-803
68. Grundy JE, Shanley JD, Griffiths PD (1987) Is cytomegalovirus interstitial pneumonitis in transplant recipients an immunopathological condition? Lancet II: 996-999
69. Grundy JE, Super M, Lui S, Sweny P, Griffiths PD (1987) The source of cytomegalovirus infection in seropositive renal allograft recipients is frequently the donor kidney. Transplant Proc 19: 2126-2128
70. Grundy JE, Ayles HM, McKeating JA, Butcher RG, Griffiths PD, Poulter LW (1988) Enhancement of class I HLA antigen expression by cytomegalovirus infection: role in amplification of the virus. J Med Virol 25: 483-495
71. Grundy JE, Lui SF, Super M, Berry NJ, Sweny P, Fernando ON, Moorhead J, Griffiths PD (1988) Symptomatic cytomegalovirus infection in seropositive recipients: reinfection with donor virus rather than reactivation of recipient virus. Lancet II: 132-135
72. Hackman RC, Myerson D, Meyers JD, Shulman HM, Sale GE, Goldstein LC, Rastetter M, Flournoy N, Thomas ED (1985) Rapid diagnosis of cytomegaloviral pneumonia by tissue immunofluorescence with a murine monoclonal antibody. J Infect Dis 151: 325-329

73. Hall BM, Bishop GA, Duggin GG, Horvatti JS, Philips J, Tiller PJ (1984) Increased expression of HLA-DR antigens on renal tubular cells in renal transplants: relevance to the rejection response. Lancet II: 247-251
74. Hamilton JD (1982) Cytomegalovirus and immunity. In: Melnick JL (ed) Monographs in virology, vol 12. Karger, Basel New York
75. Harbison MA, De Girolami PC, Jenkins RL, Hammer SM (1988) Ganciclovir therapy of severe cytomegalovirus infection in solid-organ transplant recipients. Transplantation 46: 82-88
76. Hart IK, Kennedy PGE (1988) Guillain-Barré syndrome associated with cytomegalovirus infection. Q J Med [New Ser 67] 253: 425-430
77. Hecht DW, Snydman DR, Crumpacker CS, Barbara GW, Heinze-Lacey B (1988) Ganciclovir for treatment of renal transplant associated primary cytomegalovirus pneumonia. J Infect Dis 157: 187-188
78. Heieren MH, Woude FJ van der, Balfour HH (1988) Cytomegalovirus replicates efficiency in human kidney mesangial cells. Proc Natl Acad Sci USA 85: 1642-1646
79. Herrera GA, Alexander RW, Cooley CF, Luke RG, Kelly DR, Curtis JJ, Gockerman JP (1986) Cytomegalovirus glomerulopathy: a controversial lesion. Kidney Int 29: 725-733
80. Hirsch M, Schooley RT, Cosimi AB, Russell PS, Delmonico FL, Tolkoff-Rubin NE, Herrin JT, Cantell K, Farrell M, Rota TR, Rubin RH (1983) Effects of interferon-alpha on cytomegalovirus reactivation syndromes in renal transplant recipients. N Engl J Med 308: 1489-1493
81. Ho M (1982) Cytomegalovirus, biology and infection. Plenum Press, New York London, pp 3-213
82. Ho M, Suwansirikul S, Dowling JN (1975) The transplanted kidney as a source of cytomegalovirus infection. N Engl J Med 293: 1109-1112
83. Howard RJ, Najarian JS (1974) Cytomegalovirus-induced immunosuppression. Clin Exp Immunol 18: 109-118
84. Janssen HP, Loon AM van, Meddens HJM, Eickman-Josten ECM, Hoitsma AJ, Witte TJM de, Quint WGV (1988) Immunological detection of cytomegalovirus early antigen on monolayers inoculated with urine specimens by centrifugation and cultured for 6 days as alternative to conventional virus isolation. J Clin Microbiol 26: 1313-1315
85. Jesionek A, Kiolemenoglou B (1904) Ueber einem Befund von protozöen-artigen Gebilden in den Organen einen hereditairluetischen Fötus. Münch Med Wochenschr 51: 1905-1907
86. Jiwa NM, Gemert GW van, Raap AK, Rijke FM van de, Mulder A, Lens PF, Salimans MMM, Zwaan FE, Dorp W van, Ploeg M van der (1989) Rapid detection of human cytomegalovirus DNA in peripheral blood leukocytes of viraemic transplant recipients by the polymerase chain reaction. Transplantation 48: 72-76
87. Klintmalm G, Lönnqvist B, Öberg B, Gahrton G, Lernestedt G, Lundgren G, Ringdén O, Robert K, Wahren B, Groth C (1985) Intravenous foscarnet for the treatment of severe cytomegalovirus infection in allograft recipients. Scand J Infect Dis 17: 157-163
88. Kovarik J, Mayer G, Pohanka E, Schwartz M, Traindl O, Graf H, Smolen J (1988) Adverse effect of low-dose prophylactic human recombinant leukocyte interferon-alpha treatment in renal transplant recipients. Transplantation 45: 402-405
89. Kramer P, Kate FWJ ten, Bijnen AB, Jeekel J, Weimar W (1984) Recombinant leukocyte interferon A induces steroid resistant acute vascular rejection episodes in renal transplant recipients. Lancet I: 989-990
90. Krech UH, Jung M, Jung F (1971) Cytomegalovirus in man. Karger, Basel, pp 1-105
91. Lang DJ (1984) International forum on transfusion-transmitted CMV infections. Vox Sang 46: 387-414
92. Lang DJ, Ebert PA, Rodgers BM, Bogess HP, Rixse RS (1977) Reduction of postperfusion cytomegalovirus infections following the use of leucocyte depleted blood. Transfusion 17: 391-395
93. Laskin OL, Stahl-Bayliss CM, Kalman CM, Rosecan LR (1987) Use of ganciclovir to treat serious cytomegalovirus infections with AIDS. J Infect Dis 155: 323-327
94. Lehot JJ, Page Y, Xuan BB, Tardy JC, Chomel JJ, Girbert R, Bosshard S (1983) Severe acute pneumonia associated with cytomegalovirus. Infection 11: 175-176
95. Levin MJ (1988) Antiviral therapy of herpes virus infection in renal transplant recipients. Transplant Proc 20 [Suppl 8]: 19-23
96. Lewis RM, Johnson PC, Golden D, Buren CT van, Kerman RH, Kahan BD (1988) The adverse impact of cytomegalovirus infection on clinical outcome in cyclosporin-prednisolone treated renal allograft recipients. Transplantation 45: 353-359
97. Linnemann CC Jr, Dunn CR, First MR, Alvira M, Schiff GM (1978) Late onset of fatal cytomegalovirus infection after renal transplantation: primary or reactivation infection? Arch Intern Med 138: 1247-1250
98. Lopez C, Simmons RL, Mauer SM, Najarian JS, Good RA (1974) Association of renal allograft rejection with virus infections. Am J Med 56: 280-289
99. Löwenstein C (1907) Ueber protozoen-artigen Gebilde in den Organen von Kindern. Zentralbl Allg Pathol 18: 513-518
100. Luby JP, Shasby DM (1972) A sex-difference in the prevalence of antibodies to cytomegalovirus. JAMA 222: 1290-1291
101. Marker SC, Howard RJ, Groth KE, Mastri AR, Simmons RL, Balfour HH (1980) A trial of vidarabine for cytomegalovirus in renal transplant patients. Arch Intern Med 140: 1441-1444
102. Marker SC, Howard RJ, Simmons RL, Kalis JM, Connely DP, Najarian JS, Balfour HH Jr (1981) Cytomegalovirus infection: a quantitative prospective study of three hundred twenty consecutive renal transplants. Surgery 89: 660-671
103. Martin WJ, Smith TF (1986) Rapid detection of cytomegalovirus in bronchoalveolar lavage specimens by a monoclonal antibody method. J Clin Microbiol 23: 1006-1008
104. McKeating JA, Stagno S, Stirk PR, Griffiths PD (1985) Detection of cytomegalovirus in urine samples by enzyme-linked-immunosorbent assay. J Med Virol 16: 367-373
105. Merritt JC, Callender CO (1978) Adult cytomegalic inclusion retinitis. Ann Ophthalmol 10: 1059-1063
106. Metselaar HJ, Ploeg RJ, Loon AM van, Weiland HT, Rothbard PH, Paul LC, Brand A, Schaafsma R, Hendriks GFJ, Jeekel J, Weimar W (1988) Prevention of CMV infection by screening for CMV antibodies in renal allograft recipients and their blood and kidney donors. Scand J Infect Dis 20: 135-139
107. Meyers JD, McGuffin RW, Bryson YJ, Cantell K, Thomas ED (1982) Treatment of cytomegalovirus-pneumonia after marrow transplant with combined vidarabine and human leukocyte interferon. J Infect Dis 146: 80-84
108. Meyers JD, Reed EC, Shepp DH, Thornquist M, Danliker PS, Vicary CA, Flournoy N, Kirk LE, Kersey JH, Thomas ED, Balfour HH Jr (1988) Acyclovir for prevention of cytomegalovirus infection and disease after allogenic marrow transplantation. N Engl J Med 318: 70-75
109. Middeldorp JM (1985) Diagnostic and immunological aspects of the antibody response to human cytomegalovirus infection. Thesis, University of Groningen, Groningen

110. Middeldorp JM, Jongsma J, Haar A ter, Schirm J, The TH (1984) Detection of immunoglobulin M and G antibodies against cytomegalovirus early and late antigen by enzyme-linked immunosorbent assay. J Clin Microbiol 20: 763-771
111. Minars N, Silverman JF, Escobar MR, Martinez AJ (1977) Fatal cytomegalic inclusion disease. Arch Dermatol 113: 1569-1571
112. Mintz L, Miner RC, Yeager AS (1980) Anticomplement immunofluorescence test that used isolated fibroblast nuclei for detection of antibodies to human cytomegalovirus. J Clin Microbiol 12: 562-566
113. Mitchell CD, Burns W, Santos G, Saral G, Wittek A, Rook A, Manischewitz J, Quinnan G (1986) Improved immmune-responses to cytomegalovirus infections in cyclosporine A versus cytoxan treated bone marrow transplant recipients. Clin Res 34: 677
114. Murray HW, Knox DL, Green WR, Susel RM (1977) Cytomegalovirus retinitis in adults. Am J Med 63: 574-584
115. Myerson D, Hackman RC, Meyers JD (1984) Diagnosis of cytomegaloviral pneumonia by in situ hybridization. J Infect Dis 150: 272-277
116. Najarian JS, Fryd DS, Strand M, Canafox DM, Ascher NL, Payne WD, Simmons RL, Sutherland DER (1985) A single institution, randomized, prospective trial of cyclosporine versus azathioprine - antilymphocyte-globulin for immunosuppression in renal allografts. Ann Surg 201: 142-157
117. Napel CHH ten, Houthoff HJ, The TH (1984) Cytomegalovirus hepatitis in normal and immune-compromised hosts. Liver 4: 184-194
118. Neiman PE, Reeves W, Ray G, Flournoy N, Lerner KG, Sale GE, Thomas ED (1977) A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogenic bone marrow grafts. J Infect Dis 136: 754-767
119. Oh CS, Stratta RJ, Fox BC, Sollinger HW, Belzer FO, Maki D (1988) Increased infections associated with the use of OKT3 for the treatment of steroid-resistant rejection in renal transplantation. Transplantation 45: 68-73
120. Olding LB, Jensen FC, Oldstone MBA (1975) Pathogenesis of cytomegalovirus infection. I. Activation of virus from bone marrow-derived lymphocytes by in vitro allogenic reaction. J Exp Med 141: 561-572
121. Onorato IM, Morens DM, Martone WJ, Stansfield SK (1985) Epidemiology of cytomegalovirus infections: recommendations for prevention and control. Rev Infect Dis 7: 479-497
122. Ozawa T, Stewart JA (1979) Immune-complex glomerulonephritis associated with cytomegalovirus infection. Am J Clin Pathol 72: 103-107
123. Pass RF, Whitley RJ, Diethelm AG, Whelchel JD, Reynolds DW, Alford CA (1980) Cytomegalovirus infection in patients with renal transplants: potentiation by anti-thymocyte-globulin and incompatible graft. J Infect Dis 142: 9-17
124. Pass RF, Griffiths PD, August AM (1983) Antibody response to cytomegalovirus after renal transplantation: comparison of patients with primary and recurrent infections. J Infect Dis 147: 40-46
125. Paya CV, Hermans PE, Smith TF, Rakela J, Wiesner RH, Krom RAF, Torres VE, Sterioff S, Wilkowske CJ (1988) Efficacy of ganciclovir in liver and kidney transplant recipients with severe cytomegalovirus infection. Transplantation 46: 229-234
126. Peterson PK, Balfour HH, Marker SC, Fryd DS, Howard RJ, Simmons RL (1980) Cytomegalovirus-disease in renal allograft recipients: a prospective study of the clinical features, risk factors and impact on renal transplantation. Medicine 59: 283-300
127. Peterson PK, Balfour HH, Fryd S, Ferguson R, Kronenberg R, Simmons RL (1983) Risk factors in the development of cytomegalovirus-related pneumonia in renal transplant recipients. J Infect Dis 148: 1121
128. Phipps PH, Grégoire L, Rossier E, Perry E (1983) Comparison of five methods of antibodies to human cytomegalovirus. J Clin Microbiol 18: 1296-1300
129. Plotkin SA, Farquhar J, Hornberger E (1976) Clinical trials of immunization with the Town 125 strain of human cytomegalovirus. J Infect Dis 134: 470-475
130. Plotkin SA, Starr SE, Brian CK (1982) In vitro and in vivo responses of cytomegalovirus to acyclovir. Am J Med 73: 257-261
131. Plotkin SA, Friedman HM, Fleisher GR, Dafoe DC, Grossmann RA, Smiley ML, Starr SE, Wlodaver C, Friedman AD, Barker CF (1984) Town-vaccine induced prevention of cytomegalovirus disease after renal transplantation. Lancet I: 528-530
132. Plotkin SA, Drew WL, Felsenstein D, Hirsch MS (1985) Sensitivity of clinical isolates of human cytomegalovirus to 9-(1,3-dihydroxy-2-proxymethyl)guanine. J Infect Dis 152: 833-834
133. Quinnan GV Jr, Kirmani N, Rook AH, Manischewitz JF, Jackson L, Moreschi G, Santos GW, Saral R, Burns WH (1982) Cytotoxic T cells in cytomegalovirus infection: HLA restricted T-lymphocyte and non T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus in bone-marrow-transplant recipients. N Engl J Med 307: 6-13
134. Ramsey PG, Fife KH, Hackman C, Meyers JD, Corey L (1982) Herpes simplex virus pneumonia. Ann Intern Med 97: 813-820
135. Ravin CE, Walker-Smith G, Ahern MJ, McLoud T, Putman C, Milchgrub S (1977) Cytomegaloviral infection presenting as a solitary pulmonary nodule. Chest 71: 220-222
136. Reed EC, Danliker PS, Meyers JD (1986) Treatment of cytomegalovirus pneumonia with 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine and high dose corticosteroids. Ann Intern Med 105: 214-215
137. Richardson WP, Colvin RB, Cheeseman SH, Tolkoff-Rubin NE, Herrin JT, Cosimi AB, Collins AB, Hirsch MS, McCluskey RT, Russell PS, Rubin RH (1981) Glomerulopathy associated with cytomegalovirus viremia in renal allografts. N Engl J Med 305: 57-63
138. Ringdén O, Lönnqvist B, Paulin T, Ahlmen J, Klintmalm G, Wahren B, Lernestedt JO (1986) Pharmacokinetics, safety and preliminary clinical experiences using foscarnet in the treatment of cytomegalovirus infections in bone marrow and renal transplant recipients. J Antimicrob Chemother 17: 373-387
139. Roenhorst HW, Beelen JM, Middeldorp JM, Schirm J, Tegzess AM, The TH (1985) Maintenance of CMV latency and host immune responses of long term renal allograft survivors. Clin Exp Immunol 61: 72-79
140. Roenhorst HW, Tegzess AM, Beelen JM, Middeldorp JM, The TH (1985) HLA-DRw6 as a risk factor for active cytomegalovirus but not for herpes simplex virus infection after renal transplantation. Br Med J 291: 619-622
141. Rook AH (1988) Interactions of cytomegalovirus with the human immune system. Rev Infect Dis 10 [Suppl 3]: 460-467
142. Rook AH, Quinnan GV Jr, Frederik W Jr, Manischewitz JF, Kirmani N, Dantzler T, Lee BB, Currier CB Jr (1984) Importance of cytotoxic lymphocytes during cytomegalovirus-infection of renal transplant recipients. Am J Med 76: 385-392
143. Rosen P, Armstrong D, Rice N (1973) Gastrointestinal cytomegalovirus infection. Arch Intern Med 132: 274-276
144. Rubin RH (1988) Infection in the renal and liver transplant patient in the compromised host. In: Rubin RH, Young LS

(eds) Clinical approach to infection in the compromised host, 2nd edn. Plenum Press, New York London, pp 569-583
145. Rubin RH, Colvin RB (1986) Cytomegalovirus infection in renal transplantation: clinical importance and control. In: Williams GM, Burdick JF, Solez K (eds) Kidney transplant rejection. Diagnosis and Treatment. Dekker, New York Basel, pp 283-304
146. Rubin RH, Cosimi AB, Tolkoff-Rubin NE, Russell PS, Hirsch MS (1977) Infectious disease syndromes attributable to cytomegalovirus and their significance among renal transplant recipients. Transplantation 24: 458-464
147. Rubin RH, Russell PS, Levin M, Cohen C (1979) Summary of a workshop on cytomegalovirus infection during organ transplantation. J Infect Dis 139: 728-734
148. Rubin RH, Cosimi AB, Hirsch MS, Herrin JT, Russell PS, Tolkoff-Rubin NE (1981) Effects of antithymocyte globulin on cytomegalovirus infection in normal transplant recipients. Transplantation 31: 143-145
149. Rubin RH, Tolkoff-Rubin NE, Oliver D, Rota TR, Hamilton J, Betts RF, Pass RF, Hillis W, Szmuness W, Farell ML, Hirsch MS (1985) Multicenter seroepidemiologic study of the impact of cytomegalovirus infection on renal transplantation. Transplantation 40: 243-249
150. Rubin RH, Wilson EJ, Barrett LV, Medearis DN (1986) The protective effects of hyperimmune anti-murine cytomegalovirus antiserum against lethal viral challenge: the case for passive-active immunization. Clin Immunol Immunopathol 39: 151-158
151. Schirm J, Timmerije W, Bij W van der, The TH, Wilterdink JB, Tegzess AM, Son WJ van, Schröder FP (1987) Rapid detection of infectious cytomegalovirus in blood with the aid of monoclonal antibodies. J Med Virol 23: 31-40
152. Schooley RT, Hirsch MS, Colvin RB, Cosimi B, Tolkoff-Rubin NE, McCluskey RT, Burton RC, Russell PS, Herrin JT, Delmonico FL, Giorgi JV, Henle W, Rubin RH (1983) Association of herpes-infections with T-lymphocyte-subset alterations, glomerulopathy, and opportunistic infections after renal transplantation. N Engl J Med 308: 307-313
153. Schuster V, Matz B, Wiegand H, Traub B, Kampa D, Neumann-Haefelin D (1986) Detection of human cytomegalovirus in urine by DNA-DNA and RNA-DNA hybridization. J Infect Dis 154: 309-314
154. Shanley JD, Pesanti EL, Nugent KM (1982) The pathogenesis of pneumonitis due to murine cytomegalovirus. J Infect Dis 146: 388-396
155. Shepp DH, Danliker PS, Miranda P de, Burnette TC, Cederberg DM, Kirk E, Meyers JD (1985) Activity of 9-[2-hydroxy-1-(hydroxymethyl)ethyxomethyl]guanine in the treatment of cytomegalovirus pneumonia. Ann Intern Med 103: 368-373
156. Shorr RI, Longo WL, Oberley TD, Bozdeck MJ, Walker DL (1987) Cytomegalovirus-associated tubulointerstitial nephritis in an allogenic bone-marrow-transplant recipient. Ann Intern Med 107: 351-352
157. Simmons RL, Weil R, Tallent MB, Kjellstrand CM, Najarian JS (1970) Do mild infections trigger the rejection of renal allografts? Transplant Proc 3: 419-423
158. Simmons RL, Lopez C, Balfour HH Jr, Kalis J, Ratazzi LC, Najarian JS (1974) Cytomegalovirus: clinical, virological correlations in renal transplant recipients. Ann Surg 180: 623-634
159. Simmons RL, Matas AJ, Ratazzi LC, Balfour HH Jr, Howard RJ, Najarian JS (1977) Clinical characteristics of the lethal cytomegalovirus infection following renal transplantation. Surgery 82: 537-546
160. Smiley ML, Wlodavir CG, Grossmann RA, Barker CF, Perloff LJ, Tustin NB, Starr SE, Plotkin SA, Friedman HM (1985) The role of pretransplant immunity in protection from cytomegalovirus disease following renal transplantation. Transplantation 40: 157-161
161. Smith MG (1954) Propagation of salivary gland virus of the mouse in tissue culture. Proc Soc Exp Biol Med 86: 435-440
162. Smith RD, Wehner RW (1980) Acute cytomegalovirus nephritis: an experimental model. Lab Invest 43: 278-286
163. Snydman DR (1988) Ganciclovir for cytomegalovirus-disease associated with renal transplants. Rev Infect Dis 10 [Suppl 3]: 554-562
164. Snydman DR, Werner BG, Heinze-Lacey B, Berardi VP, Tilney NL, Levey AS, Strom TB, Carpenter LB, Levey RH, Harmon WE, Zimmerman CE, Shapiro ME, Steinman T, LoGerfo F, Idelson B, Schröter PJ, Levin MJ, McIver J, Leszczynski J, Grady GF (1987) Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal transplant recipients. N Engl J Med 317: 1049-1054
165. Snydman DR, Werner BG, Tilney NL, Kirkman RL, Milford EL, Cho SI, Bush HL Jr, Levey AS, Strom TB, Carpenter CB, Berardi VP, Levey RH, Harmon WE, Zimmerman CE, Tenny A, Heinze-Lacey B, Shapiro ME, Steinman T, LoGerfo F, Idelson B, McIver J, Leszczynski J, Grady GF (1988) Further analysis of primary cytomegalovirus disease prevention in renal transplant recipients with a cytomegalovirus immunoglobulin: interim comparison of a randomized and an open-label trial. Transplant Proc 20 [Suppl 8]: 24-30
166. Sommadossi J, Bevan R, Ling T, Lee F, Mastre B, Chaplin MD, Nerenberg C, Koretz S, Buhles WC Jr (1988) Clinical pharmacokinetics of ganciclovir in patients with normal and impaired renal function. Rev Infect Dis 10 [Suppl 3]: 507-514
167. Son WJ van, Jagt EJ van der, Woude FJ van der, Slooff MJH, Meijer S, The TH, Tegzess AM, Slikke LB van der, Donker AJM (1984) Pneumatosis intestinalis in patients after cadaveric kidney transplantation: possible relationship with an active cytomegalovirus infection. Transplantation 38: 506-510
168. Son WJ van, Woude FJ van der, Hem GK van der, The TH, Ockhuizen T, Tegzess AM (1985) Complement activation associated with active cytomegalovirus infection in renal transplant patients, and its absence in transplant rejection episodes. Transplantation 39: 510-514
169. Son WJ van, Peset R, Duipmans JC, Mark TW van der, The TH, Tegzess AM (1987) Cytomegalovirus infection after renal transplantation: pulmonary dysfunction measured by decreased diffusing capacity for carbon monoxide (KCO) in patients with symptomatic and asymptomatic infection. Transplantation 44: 149-150
170. Son WJ van, Tegzess AM, The TH, Duipmans JC, Slooff MJH, Mark TW van der, Peset R (1987) Pulmonary dysfunction is common during a cytomegalovirus infection after renal transplantation, even in asymptomatic patients: possible relationship with complement activation. Am Rev Respir Dis 136: 580-585
171. Son WJ van, Bij W van der, Tegzess AM, Anema J, Giessen M van der, Hem GK van der, Marrink JM, The TH (1989) Complement activation during an active cytomegalovirus infection after renal transplantation: due to circulating immune complexes or alternative pathway activation? Clin Immunol Immunopathol 50: 109-121
172. Son WJ van, Bij W van der, The TH, Peset R, Mark TW van der, Tegzess AM (1989) Evidence for pulmonary dysfunction in all patients with symptomatic and asymptomatic cytomegalovirus (CMV) infection after renal transplantation. Transplant Proc 21: 2065-2068
173. Spector SA, Rua JA, Spector DH, McMillan R (1984) Detection of human cytomegalovirus in clinical specimens by DNA-DNA hybridization. J Infect Dis 150: 121-126
174. Stagno S, Volanakis J, Reynolds DW, Stroud R, Alford CA

(1977) Immune-complexes in congenital and natal cytomegalovirus infections in man. J Clin Invest 60: 838-845
175. Stagno S, Pass RF, Reynolds DW, Alford CA (1981) Diagnosis of cytomegalovirus infections. In: Nahmias AJ, Dowdle WR, Schinazi RF (eds) The human herpes viruses, an interdisciplinary perspective. Elsevier, New York Oxford, pp 363-373
176. Stimler NP, Bloor CM, Hugli TE (1983) Immunopharmacology of complement anaphylatoxins in the lung. In: Newball HH (ed) Immunopharmacology of the lung. Dekker, New York, pp 401-434
177. Stirk PR, Griffiths PD (1987) Use of monoclonal antibodies for the diagnosis of cytomegalovirus infection by the detection of early antigen fluorescent foci (DEAFF) in cell culture. J Med Virol 21: 324-337
178. Stoffel M, Gianello P, Squifflet JP, Pirson Y, Alexandre GPJ (1988) Effects of 9-(2-hydroxy-1-[hydroxymethyl]-ethoxymethyl)guanine (DHPG) on cytomegalovirus pneumonitis after renal transplantation. Transplantation 46: 594-595
179. Suwansirikul S, Rao N, Dowling JN, Ho M (1977) Primary and secondary cytomegalovirus infection: clinical manifestation after renal transplantation. Arch Intern Med 137: 1026-1029
180. Tabak MA, Jordan SC, Ettenger RB, Sakai R, Dupuis MA, Fine RN (1985) Diagnosis of cytomegalovirus infection post-transplantation via fine needle aspiration biopsy. Transplant Proc 17: 153
181. Tamura T, Chiba S, Chiba Y, Nakao T (1980) Virus excretion and neutralizing antibody response in saliva in human cytomegalovirus infection. Infect Immun 29: 842-845
182. The TH, Klein G, Langenhuysen MMAC (1974) Antibody reactions to virus specific early antigens (EA) in patients with cytomegalovirus (CMV) infection. Clin Exp Immunol 16: 1-12
183. The TH, Bij W van der, Berg AP van den, Giessen M van der, Weits J, Sprenger HG, Son WJ van (1989) Cytomegalovirus-antigenemia. Rev Infect Dis (in press)
184. Till GO, Johnson KJ, Kunkel R, Ward PA (1982) Intravascular activation of complement and acute lung injury. J Clin Invest 69: 1126-1135
185. Tolkoff-Rubin NE, Rubin RH, Keller E, Baker GP, Stewart JA, Hirsch MS (1978) Cytomegalovirus infections in dialysis patients and personnel. Ann Intern Med 89: 625-628
186. Tsuji Nose Y, Inouye Komeri K, Yumamura M (1981) HLA-D/DR linked immune response to candida allergen: an in vivo and in vitro study in Japanese. Tokai J Exp Clin Med 6: 113-120
187. Volpi AV, Whitley RJ, Ceballos R, Stagno S (1983) Rapid diagnosis of pneumonia due to cytomegalovirus with specific monoclonal antibodies. J Infect Dis 147: 1119-1120
188. Wade JC, Hintz M, McGuffin RW, Springmeyer SC, Connor JD, Meyers JD (1982) Treatment of cytomegalovirus pneumonia with high dose acyclovir. Am J Med 73: 249-256
189. Waner JL, Weller TH, Kevy SV (1973) Patterns of cytomegaloviral complement-fixing antibody reactivity: a longitudinal study of blood donors. J Infect Dis 127: 538-543
190. Wehner RW, Smith RD (1983) Progressive cytomegalovirus glomerulonephritis: an experimental model. Am J Pathol 112: 313-325
191. Weir MR, Irwin BC, Maters AW, Genemans G, Shen SY, Charache P, Williams GM (1987) Incidence of cytomegalovirus disease in cyclosporine-treated renal transplant recipients based on donor/recipient pretransplant immunity. Transplantation 43: 187-192
192. Weir MR, Irwin BC, Maters AW, Genemans G, Shen SY, Charache P, Williams GM (1987) Morbid outcome of cytomegalovirus-negative transplant recipients receiving cytomegalovirus-positive kidneys. Transplant Proc 19: 2137-2141
193. Weir MR, Henry ML, Blackmore M, Smith J, First MR, Irwin B, Shen S, Genemans G, Alexandre JW, Corry RJ, Nghiem DH, Ferguson RM, Kittur D, Shield CF, Sommer BG, Williams GM (1988) Incidence and morbidity of cytomegalovirus disease associated with a seronegative recipient receiving seropositive donor-specific transfusion and living-related donor transplantation. Transplantation 45: 111-116
194. Weller TH (1970) Cytomegalovirus: the difficult years. J Infect Dis 122: 532-539
195. Weller TH, Hanshaw JB, Scott DE (1960) Serological differentiation of viruses responsible for cytomegalic inclusion disease. Virology 12: 130-132
196. Wertheim P, Buurman C, Geelen J, Noordaa J van der (1983) Transmission of cytomegalovirus by renal allograft demonstrated by restriction enzyme analysis. Lancet I: 980-981
197. Whitley RJ (1988) Ganciclovir - have we established clinical value in the treatment of cytomegalovirus infections? Ann Intern Med 108: 452-454
198. Willebrand E von, Pettersson E, Ahonen J, Häyry P (1986) CMV infection, class II antigen expression, and human kidney allograft rejection. Transplantation 42: 364-367
199. Williams PF, Wreghitt T, Joysey V, Evans DB, Calne RY (1988) Cytomegalovirus matching in renal transplantation. Lancet II: 569
200. Wink K, Schmitz H (1980) Cytomegalovirus myocarditis. Am Heart J 100: 667-672
201. Winston DJ, Ho WG, Lin CH, Bartoni K, Budinger MD, Gale RP, Champlin RE (1987) Intravenous immunoglobulin for prevention of cytomegalovirus infection and interstitial pneumonia after bone marrow transplantation. Ann Intern Med 106: 12-18
202. Winston DJ, Ho WG, Bartoni K, Holland GN, Mitsuyasu RT, Busuttil RW, Champlin RE (1988) Ganciclovir therapy for cytomegalovirus infection in recipients of bone-marrow transplants and other immunosuppressed patients. Rev Infect Dis 10 [Suppl 3]: 547-553
203. Wu BC, Dowling JN, Armstrong JA, Ho M (1975) Enhancement of mouse cytomegalovirus infection during host-versus-graft reaction. Science 190: 56-58