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Definitions of Cytomegalovirus Infection and Disease in Transplant Recipients

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Cytomegalovirus (CMV) infection and disease are important causes of morbidity and mortality among transplant recipients. For the purpose of developing consistent reporting of CMV in clinical trials, definitions of CMV infection and disease were developed and published. This study seeks to update the definitions of CMV on the basis of recent developments in diagnostic techniques, as well as to add to these definitions the concept of indirect effects caused by CMV.

During the past decade, major advances have been achieved regarding the management of cytomegalovirus (CMV) infection and disease. These advances have been made possible through the development of new diagnostic techniques for the detection of the virus and through the performance of prospective clinical trials of antiviral agents. It was apparent early in the development of these advances that it would be of value if similar definitions of important concepts could be used in these studies to allow for comparison of results from different trials. Therefore, a first set of CMV definitions was developed and published as part of the proceedings of the 4th International CMV Conference in Paris in 1993 [1]. These definitions were updated at the 5th International CMV Conference in Stockholm in 1995 [2] and have since been used in many published studies.

However, since 1995, many new developments in diagnostic technologies have occurred, and new concepts, such as the indirect effects of CMV, have been recognized. Therefore, the aim of this report is to update and expand the published definitions of CMV, taking into account current knowledge. The definitions have been developed primarily for application to transplant recipients, but they can also be applied to other immunocompromised individuals. We recognize that these definitions are, in part, unsuitable for application to HIV-infected patients.

Received 25 September 2001; revised 10 December 2001; electronically published 11 March 2002.

Financial support: Swedish Cancer Society.

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Clinical Infectious Diseases 2002; 34:1094–7

1058-4838/2002/3408-0009$03.00

DIRECT EFFECTS

CMV Infection

"CMV infection" is defined as isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen. It is recommended that both the source of the specimens tested (e.g., plasma, serum, whole blood, peripheral blood leukocytes, CSF, urine, or tissue) and the diagnostic method used be described clearly.

CMV Detection in Blood

Several specific definitions for CMV detection in blood are recommended.

Viremia. "Viremia" is defined as the isolation of CMV by culture that involves the use of either standard or shell vial techniques.

Antigenemia. "Antigenemia" is defined as the detection of CMV pp65 in leukocytes.

DNAemia. "DNAemia" is defined as the detection of DNA in samples of plasma, whole blood, and isolated peripheral blood leukocytes or in buffy-coat specimens. There are several techniques available for the detection of DNAemia, including PCR-based techniques, hybrid capture, and branched-chain DNA analysis. The tests can be either qualitative or quantitative. For quantitative tests, the technique used for quantification should be specified. It is recommended that true quantitative,

rather than semiquantitative, techniques be used to measure the virus load.

RNAemia. "RNAemia" is defined as the detection of RNA (e.g., by nucleic acid sequence–based amplification or noncommercial reverse transcriptase–PCR) in samples of plasma, whole blood, or isolated peripheral blood leukocytes or in buffy-coat specimens.

Primary CMV Infection

"Primary CMV infection" is defined as the detection of CMV infection in an individual previously found to be CMV seronegative. The appearance of de novo specific antibodies in a seronegative patient may also be acceptable for the diagnosis of CMV, provided that passive transfer of antibodies via immunoglobulin or blood products can be excluded.

Recurrent Infection

"Recurrent infection" is defined as new detection of CMV infection in a patient who has had previously documented infection and who has not had virus detected for an interval of at least 4 weeks during active surveillance. Recurrent infection may result from reactivation of latent virus (endogenous) or reinfection (exogenous).

Reinfection. "Reinfection" is defined as detection of a CMV strain that is distinct from the strain that was the cause of the patient's original infection. For cases in which infection can be demonstrated on 2 different occasions, reinfection may be documented by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic. Reinfection is diagnosed if the 2 strains are distinct. Reinfection may also be inferred if the patient develops new immune responses to epitopes known to be polymorphic; however, interference from passive antibody must be excluded.

Reactivation. Reactivation is assumed if the 2 strains are found to be indistinguishable either by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic.

CMV End-Organ Disease

A general problem involves how to report copathogens together with CMV. Each pathogen's relative importance is frequently difficult to assess, and, therefore, it is important that the presence of copathogens be reported clearly.

Pneumonia. "CMV pneumonia" is defined by the presence of signs and/or symptoms of pulmonary disease combined with the detection of CMV in bronchoalveolar lavage fluid or lung tissue samples. Detection of CMV should be performed by virus isolation, histopathologic testing, immunohistochemical analysis, or in situ hybridization. Detection of CMV by PCR alone may be too sensitive for the diagnosis of CMV pneumonia and is therefore insufficient for this purpose. The presence of fungal copathogens, such as *Aspergillus* species, together with radiologic signs typical of *Aspergillus* pneumonia (e.g., a halo sign or a crescent sign) indicates fungal pneumonia rather than CMV pneumonia.

Gastrointestinal disease. "CMV gastrointestinal disease" is defined by identification of a combination of clinical symptoms from the upper or lower gastrointestinal tract, findings of macroscopic mucosal lesions on endoscopy, and demonstration of CMV infection (by culture, histopathologic testing, immunohistochemical analysis, or in situ hybridization) in a gastrointestinal tract biopsy specimen. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV gastrointestinal disease. Patients with CMV disease that involves the intestinal tract usually have mucosal abnormalities that can be seen by the endoscopist, but the appearance of some of these lesions is subtle. The spectrum of endoscopic lesions is variable and ranges from patchy erythema, exudates, and microerosions to diffusely edematous mucosa, to multiple mucosal erosions, to deep ulcers and pseudotumors. The diagnostic yield for CMV is higher when mucosal abnormalities are targeted for study. If CMV is detected in normal mucosa near a lesion consistent with those typical of CMV infection, this can be accepted as CMV gastrointestinal disease.

Hepatitis. "CMV hepatitis" is defined by findings of elevated bilirubin and/or enzyme levels during liver function testing, absence of any other documented cause of hepatitis, and detection of CMV infection (by culture, psychopathologic testing, immunohistochemical analysis, or in situ hybridization) in a liver biopsy specimen. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV hepatitis because it can imply the presence of transient viremia. Documentation of CMV (i.e., by immunohistochemical analysis) within the liver tissue is needed. Other pathogens, such as hepatitis C virus, may be present without excluding the diagnosis of CMV hepatitis.

CNS disease. "CNS disease" is defined by the identification of CNS symptoms together with the detection of CMV in CSF samples, by culture or PCR, or in brain biopsy specimens, by culture, histopathologic testing, immunohistochemical analysis, or in situ hybridization.

Retinitis. Lesions typical of CMV retinitis must be confirmed by an ophthalmologist.

Nephritis. "CMV nephritis" can be defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of histologic features of CMV infection in a kidney biopsy specimen obtained from a patient with renal dysfunction. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV nephritis. Furthermore, detection of CMV in the urine of a patient with kidney dysfunction does not fulfill the definition of CMV nephritis.

Cystitis. "CMV cystitis" is defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a bladder biopsy specimen obtained from a patient with cystitis. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV cystitis. Furthermore, detection of CMV in urine combined with identification of symptoms does not fulfill the definition of CMV cystitis.

Myocarditis. "CMV myocarditis" is defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a heart biopsy specimen obtained from a patient with myocarditis. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV myocarditis.

Pancreatitis. The definition of CMV pancreatitis requires the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a pancreatic biopsy specimen obtained from a patient with pancreatitis. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV pancreatitis.

Other disease categories. CMV can also cause disease in other organs, and the definitions of these additional disease categories include the presence of compatible symptoms and signs and documentation of CMV by biopsy (detection of CMV by PCR alone is insufficient), with other relevant causes excluded.

CMV syndrome. The term "CMV syndrome" should be avoided. Although it is recognized that CMV can cause the combination of fever and bone marrow suppression that is usually used to define the disease entity, the same symptoms can have several other different causes in stem cell transplant recipients, including such viral infections as human herpesvirus 6 (HHV-6), possibly human herpesvirus 7, and adenovirus. Antiviral drugs might have some effect against these viruses, making interpretation of causality difficult. Thus, if the term "CMV syndrome" is to be used, it must be used only after testing has been done for HHV-6, at the very least.

In solid-organ transplant recipients, CMV syndrome is better defined. At present, the minimum requirements for its definition are the documented presence of fever (temperature, >38°C) for at least 2 days within a 4-day period, the presence of neutropenia or thrombocytopenia, and the detection of CMV in blood. It is important that cases of CMV syndrome be differentiated from cases of end-organ disease when studies are reported.

CMV-associated graft failure. Several publications have suggested that CMV can induce graft failure after stem cell transplantation. It is difficult to define CMV-associated graft failure, because several other possible causes of graft failure exist, including graft rejection, relapse of hematologic disease, drug toxicity, and infection with other viruses (e.g., HHV-6, Epstein-Barr virus, and parvovirus). If the term "CMV-associated graft failure" is to be used, the minimum requirements for its definition are severe pancytopenia, bone marrow hypoplasia, detection of CMV (by culture) in bone marrow together with exclusion of rejection, relapse (determined by use of appropriate techniques), and HHV-6.

Future Perspectives

Several new diagnostic techniques are in development, the most important of which are techniques for assessment of virus load. These techniques could be used to define end-organ disease as well, but they cannot be introduced into a document on definitions until carefully performed prospective clinical trials have been performed to compare the results of virus load measurements in patients with CMV disease (according to current definitions) with those in patients without CMV disease.

INDIRECT EFFECTS

In addition to directly causing end-organ diseases, CMV is associated statistically with graft rejection, accelerated atherosclerosis, and fungal or bacterial superinfection, which collectively are known as the "indirect effects" of CMV [3]. CMV infection should have been documented earlier than the indirect effect assumed to be associated with CMV. The evidence for association of CMV with these conditions is based on epidemiologic findings that show an increased risk for indirect effects caused by CMV among patients already infected with CMV. Other evidence is based on findings of reduced incidence of indirect effects during trials of antiviral therapy. This evidence will be reviewed briefly, together with postulated mechanisms.

Acute Graft Rejection

Evidence from several cohort studies shows that CMV infection is associated with an increased risk of acute graft rejection. This has been shown for recipients of heart [4, 5], lung [6], kidney [7–9], and liver [8] transplants.

In a randomized, double-blind, placebo-controlled trial, valacyclovir significantly decreased biopsy-confirmed rejection in D^+R^- (CMV-seropositive donor/CMV-seronegative recipient) transplant recipients [10]. The Kaplan-Meier curves presented in the study by Lowance et al. [10] provide an estimate of the timing of the CMV-induced graft rejections prevented by prophylaxis.

Transplantation Atherosclerosis

After heart transplantation, CMV infection was associated with greater incidence and greater severity of coronary atherosclerosis and a higher rate of graft loss in CMV-seropositive heart

transplant recipients [4]. In a rat model, CMV infection accelerated cardiac allograft atherosclerosis [11]. This effect could be prevented by administration of prophylactic ganciclovir [12]. A post hoc analysis of a trial, which showed that prophylactic administration of ganciclovir after heart transplantation inhibited CMV disease, reported that this drug also reduced the incidence of atherosclerosis [13]. Because the risk of developing posttransplantation atherosclerosis is decreased by the use of calcium-channel blockers, patients were stratified according to their use of such drugs. In a comparison of the ganciclovir and placebo groups, a significant difference was seen in the incidence of atherosclerosis among patients who were not taking calcium-channel blockers, but no difference was apparent among patients who were taking calcium-channel blockers.

CMV infects and alters vascular smooth muscle cell growth through inhibition of the tumor suppressor *p53* [14]. Loss of *p53* activity may facilitate smooth muscle proliferation and, thus, increased intimal thickness. CMV gene *US 28* is a chemokine receptor that causes chemotaxis toward a site of inflammation when it is transfected into smooth muscle cells [15]. CMV infection can also induce intracellular reactive oxygen species in vascular smooth muscle cells and then can use them to facilitate its own gene expression and replication via activation of NF-κB [15]. CMV may exert a procoagulant effect by expressing glycoproteins at the surface of infected endothelial cells, thereby increasing the adherence of polymorphonuclear leukocytes [4, 16].

Secondary Infections

CMV seropositivity is a risk factor for invasive fungal infection in recipients of bone marrow transplants [17] and liver transplants [18]. For heart transplant recipients, administration of prophylactic ganciclovir can reduce the incidence of fungal infection [19]. A large randomized, double-blind, placebo-controlled trial that involved kidney transplant recipients showed that valacyclovir can significantly reduce the incidence of nonherpesvirus infections in the D^+R^- group [10]. Secondary infections might develop through different mechanisms; for example, CMV could disrupt mucosal surfaces, predisposing the patient to superinfection, or it could cause alterations in humoral and cell-mediated immunity.

The data presented in all studies cited in the Indirect Effects section strongly imply that the indirect effects of CMV in transplant recipients are real and important, and they also suggest that future trials of antiviral drugs should be designed to include large-enough study populations and well-defined end points so that these effects can be properly assessed.

References

1. Ljungman P, Griffiths P. Definitions of cytomegalovirus infection and disease. In: Michelson S, Plotkin S, eds. Multidisciplinary approach to understanding cytomegalovirus disease. Amsterdam: Excerpta Medica International Congress Series, **1993**:233–7.
2. Ljungman P, Plotkin S. CMV definitions and new syndromes. In: Ehrnst A, Ljungman P, eds. Proceedings from the 5th International Cytomegalovirus Conference (Stockholm). Scand J Infect Dis **1995**; 99(Suppl): 87–9.
3. Rubin RH. Impact of cytomegalovirus infection on organ transplant recipients. Rev Infect Dis **1990**; 12(Suppl 7):S754–66.
4. Grattan MT, Moreno-Cabral CE, Starnes VA, Oyer PE, Stinson EB, Shumway NE. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. JAMA **1989**; 261:3561–6.
5. Loebe M, Schuler S, Zais O, Warnecke H, Fleck E, Hetzer R. Role of cytomegalovirus infection in the development of coronary artery disease in the transplanted heart. J Heart Transplant **1990**; 9:707–11.
6. Bando K, Paradis IL, Similo S, et al. Obliterative bronchiolitis after lung and heart-lung transplantation: an analysis of risk factors and management. J Thorac Cardiovasc Surg **1995**; 110:4–13.
7. von Willebrand E, Pettersson E, Ahonen J, Hayry P. CMV infection, class II antigen expression, and human kidney allograft rejection. Transplantation **1986**; 42:364–7.
8. Fietze E, Prosch S, Reinke P, et al. Cytomegalovirus infection in transplant recipients: the role of tumor necrosis factor. Transplantation **1994**; 58:675–80.
9. Reinke P, Fietze E, Ode-Hakim S, et al. Late-acute renal allograft rejection and symptomless cytomegalovirus infection. Lancet **1994**; 344: 1737–8.
10. Lowance D, Neumayer HH, Legendre CM, et al. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group. N Engl J Med **1999**; 340:1462–70.
11. Lemstrom K, Koskinen P, Krogerus L, Daemen M, Bruggeman C, Hayry P. Cytomegalovirus antigen expression, endothelial cell proliferation, and intimal thickening in rat cardiac allografts after cytomegalovirus infection. Circulation **1995**; 92:2594–604.
12. Lemstrom K, Sihvola R, Bruggeman C, Hayry P, Koskinen P. Cytomegalovirus infection–enhanced cardiac allograft vasculopathy is abolished by DHPG prophylaxis in the rat. Circulation **1997**; 95:2614–6.
13. Valantine HA, Gao SZ, Menon SG, et al. Impact of prophylactic immediate posttransplant ganciclovir on development of transplant atherosclerosis: a post hoc analysis of a randomized, placebo-controlled study. Circulation **1999**; 100:61–6.
14. Speir E, Modali R, Huang ES, et al. Potential role of human cytomegalovirus and *p53* interaction in coronary restenosis. Science **1994**; 265:391–4.
15. Speir E. Cytomegalovirus gene regulation by reactive oxygen species: agents in atherosclerosis. Ann N Y Acad Sci **2000**; 899:363–74.
16. Span AH, Van Boven CP, Bruggeman CA. The effect of cytomegalovirus infection on the adherence of polymorphonuclear leucocytes to endothelial cells. Eur J Clin Invest **1989**; 19:542–8.
17. Tollemar J, Ringdén O, Boström L, Nilsson B, Sundberg B. Variables predicting deep fungal infection in bone marrow transplant recipients. Bone Marrow Transplant **1989**; 4:635–41.
18. George MJ, Snydman DR, Werner BG, et al. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Am J Med **1997**; 103:106–13.
19. Wagner JA, Ross H, Hunt S, et al. Prophylactic ganciclovir treatment reduces fungal as well as cytomegalovirus infections after heart transplantation. Transplantation **1995**; 60:1473–7.