REVIEW

Clinical relevance of cytomegalovirus infection in patients with disorders of the immune system

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ABSTRACT

Cytomegalovirus (CMV) infection is one of the most important infectious complications of solid-organ transplantation, and is also responsible for serious, life-threatening diseases in patients infected with human immunodeficiency virus (HIV). Tremendous progress has been made with respect to prevention and treatment of CMV disease in such patients. The use of anti-CMV drugs and the immune reconstitution achieved by use of anti-retroviral drugs has reduced the incidence of CMV disease dramatically. Nevertheless, problems of clinical relevance remain (e.g., drug toxicity, drug–drug interactions, antiviral resistance) and new problems have emerged. Intragenic recombination among different CMV strains has been identified as a possible source of novel CMV strains in patients with advanced HIV infection. Development of a protective CMV vaccine remains elusive, perhaps, in part, because of strain-specific variation in immunodominant epitopes. Late-onset CMV disease, which occurs several months or years after transplantation, has been recognised as a clinically relevant complication in transplant recipients. The most effective strategy for the prevention of CMV disease in transplant recipients (i.e., prophylaxis or pre-emptive therapy) remains a matter of debate. A link between CMV infection and Guillain–Barré syndrome, a neurological disease characterised by flaccid paralysis, has been substantiated, but the efficacy of antiviral therapy in such patients remains to be determined. This review summarises the current status of CMV disease in immunocompromised patients, and discusses some of the emerging issues of clinical relevance with regard to CMV infection in patients with disorders of the immune system.

Keywords Clinical complications, cytomegalovirus, Guillain–Barré syndrome, immunocompromised patients, review, transplant recipients

CYTOMEGALOVIRUS INFECTION

Cytomegalovirus (CMV) is a member of the β-herpes-virus group and is characterised by its strict species specificity, long life-cycle, and life-time persistence within the host [1]. CMV is transmitted via saliva, sexual contact, placental transfer, breast-feeding, blood transfusion and solid-organ or haematopoietic stem-cell transplantation. After entry, the virus disseminates within the host; this is probably facilitated by leukocytes. Productive infection resolves sponta-neously in the normal host, after which CMV establishes life-long latency or persistence within the infected individual. In the developed world, acquisition of CMV occurs progressively from an early age, with an overall seroprevalence of *c.* 60% [2]. Thus, a large proportion of the adult population remains susceptible to primary infection in developed countries. In contrast, homosexual men, poor socio-economic groups and residents of developing countries have seroprevalence rates of >90% [3,4].

Reactivation from latency is indicated by viraemia or shedding of CMV in various body fluids, including saliva, urine, tears, semen, cervicovaginal fluid and breast milk. Periods of virus shedding in urine become less common with increasing age of the infected individual, although reactivation from latency is observed in

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13% of healthy adults [5]. The factors leading to reactivation from latency are not completely understood. In particular, production of the stress hormones cortisol, adrenocorticotrophic hormone, epinephrine and norepinephrine has been implicated in the reactivation and shedding of CMV in urine [6]. In healthy individuals, productive CMV infection is usually asymptomatic, whereas immunocompromised patients may develop CMV disease.

CMV INFECTION AND DISEASE IN IMMUNOCOMPROMISED PATIENTS

CMV infection is one of the most important infectious complications of solid-organ transplantation [7], and is responsible for serious, life-threatening diseases in patients infected with human immunodeficiency virus (HIV) [8]. CMV infection, defined as a significant rise in the titre of CMV-specific antibodies, occurs in 44–85% of kidney, heart and liver transplant recipients, commonly within the first 3 months post-transplantation, when immunosuppression is most intense [9,10]. Transplant patients with no pre-existing CMV-specific immunity, i.e., CMV-seronegative recipients (R$^-$) of an organ from a CMV-seropositive donor (D$^+$), and HIV-infected patients with a CD4 cell count <100/μL, are at highest risk for CMV disease [11–13].

CMV disease manifests in the vast majority of transplant recipients as a viral syndrome that includes fever, malaise, myalgia or headache. End-organ disease affects 10–30% of patients with CMV disease [14,15], most commonly involving the transplanted organ. Consecutive dissemination to and involvement of other organs, e.g., the central nervous system, eye and the urogenital or gastrointestinal tracts (Fig. 1), are observed frequently [16]. In HIV-infected patients, retinitis is the single most common manifestation of CMV disease, accounting for 85% of all cases [8,12]. In developing countries, CMV retinitis is still the most frequent cause of visual loss in HIV-infected patients [17].

Paradoxically, the reconstitution of the immune response in HIV-infected patients can also have detrimental effects. Following the introduction of highly active anti-retroviral treatment (HAART), an unusually high incidence of vitritis, an inflammation of the eye chamber, has been noted. This phenomenon of inflammatory complications of
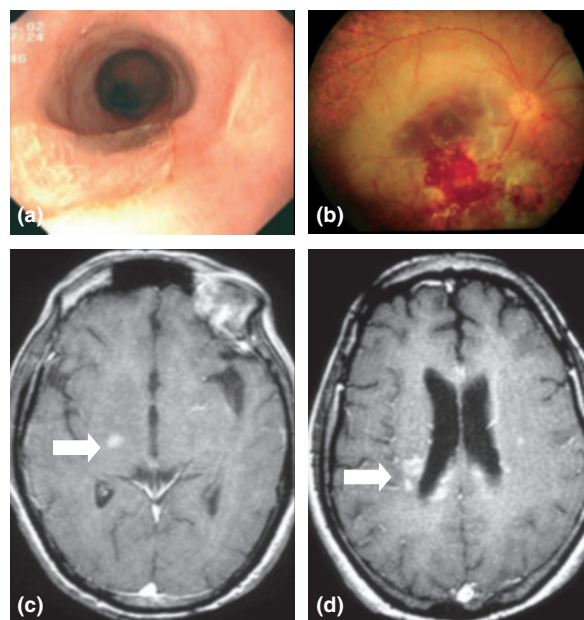

Fig. 1. Examples of cytomegalovirus (CMV) disease. (a) CMV oesophagitis presenting as a large, shallow ulcer with a punched-out border. CMV was demonstrated immunohistochemically from a representative biopsy of the ulcerated area (image kindly provided by M. Häfner, Medical University Vienna, Austria). (b) CMV retinitis in a patient with AIDS appears as an arcuate zone of retinitis with extensive haemorrhages and optic disk swelling (image kindly provided by Dr Dejaco-Ruhswurm, Medical University Vienna, Austria). (c, d) CMV ventriculoencephalitis. Multiple small, peri-ventricular lesions of the brain detected following brain magnetic resonance imaging (arrows) (image kindly provided by M. Thurnher, Medical University Vienna, Austria).

immune reconstitution has collectively been termed 'immune reconstitution inflammatory syndrome', and is thought to represent the response of the regenerating immune system to persisting antigens, such as CMV [18–20].

In addition to causing symptomatic disease, CMV employs multiple mechanisms to evade the host's innate and specific immunity [21]. The clinical significance of these in-vitro findings has been demonstrated in multiple clinical studies. In transplant recipients, CMV disease, as well as CMV infection, were significant independent predictors of acute rejection of transplanted organs [11,22] and reduced long-term graft function [23,24]. Probable mechanisms are CMV-induced up-regulation of vascular and intercellular adhesion molecules, and endothelial cell damage caused by alloreactive T-cells [25,26]. Transplant rejection rates were reduced

significantly by the use of antiviral prophylaxis against CMV [27].

New-onset diabetes mellitus has been linked to CMV infection in a cohort of kidney transplant recipients [28]. In the case of active CMV infection, the net level of therapeutic immunosuppression is augmented in transplant patients, and the susceptibility to a variety of other opportunistic infections is increased in transplant recipients and HIV-infected patients [7,29]. Finally, CMV disease has been associated with accelerated cardiac allograft vasculopathy in heart transplant recipients [30]. Among HIV-infected patients, those who are CMV-seropositive progress 2.5-fold more rapidly to AIDS and death than those who are CMV-seronegative [31–33]. In the case of primary CMV infection, even HIV-infected patients with a relatively high CD4 cell count ($>100/mm^3$) are at significantly increased risk for progression to AIDS [34].

DECLINING INCIDENCE OF CMV DISEASE IN IMMUNOCOMPROMISED PATIENTS

Tremendous progress has been made in the prevention and therapy of CMV disease among transplant recipients and HIV-infected patients. Apart from improvements in immunosuppressive therapy, the control of CMV infection following solid-organ transplantation has been considered to be the most significant advance in organ transplantation to have taken place in the past 20 years [35]. CMV disease has become a rare complication of organ transplantation, and the effects that are associated indirectly with CMV disease, namely rejection and opportunistic infection, have been reduced dramatically [36–38]. A meta-analysis of clinical trials evaluating the use of ganciclovir revealed a reduction in the development of CMV disease of 72–80% compared with that of controls [39].

CMV infection was one of the most important opportunistic infections in HIV-infected patients before the introduction of HAART. Approximately 40% of HIV-infected patients with advanced disease suffered from one of several manifestations of CMV disease during their life [40–43]. Although prophylaxis and treatment of other AIDS-related opportunistic infections had improved the overall prognosis and extended the life-expectancy of HIV-infected patients

significantly, the lifetime risk of CMV disease had increased concomitantly shortly before the introduction of HAART [44]. In 1995–1996, the concept of combining at least three anti-retroviral drugs of different classes (i.e., HAART) for the treatment of HIV-infected patients was introduced. Since then, HAART has increased life-expectancy and quality of life dramatically, with persistent suppression of HIV viraemia. Overall HIV-related mortality decreased from 29.4/100 patient-years in 1994 to 8.8/100 patient-years in 1997 [45]. In parallel with persistent immune reconstitution, decreased CMV replication and a reduced incidence of CMV disease in HIV-infected patients were observed [45–49]. Accordingly, the incidence of CMV retinitis, which is the most common CMV disease among HIV patients, decreased from 17.1/100 patient-years to 5.6/100 patient-years [45,50].

CURRENT ISSUES IN THE PREVENTION AND MANAGEMENT OF CMV DISEASE

Considering the dramatic drop in the incidence of CMV disease among transplant recipients and HIV-infected patients, is it still necessary to be concerned about this infection? Multiple factors concerning the prevention and treatment of CMV disease in immunocompromised patients have remained highly relevant, including the toxicity of antiviral drugs, antiviral resistance, drug–drug interactions, patient adherence to treatment regimens, availability of antiviral drugs in resource-poor settings, and economic costs associated with the use of antiviral drugs [17,51–59]. Moreover, selected groups of patients are still at risk for CMV disease. For example, a considerable number of HIV-infected patients have a CD4 cell count below the critical threshold of $100/\mu L$, for the simple reason that an increase in a CD4 cell count to $>100/\mu L$ usually requires a period of >6 months after initiation of HAART. In particular, patients are still at high risk of developing CMV disease during the initial months of anti-retroviral treatment and before full reconstitution of cellular immunity [49,60–62]. In addition, CD4 cell counts remain $<100/\mu L$ in 10–20% of patients treated with HAART [63], and a substantial number of HIV-infected patients at risk for CMV disease do not receive HAART because of non-compliance or intolerance to prescribed regimens

[64]. Additional challenges in the prevention and treatment of CMV disease have emerged more recently and may gain further in significance. The remainder of this review will focus on some of these emerging challenges.

GENETIC DIVERSITY OF CMV STRAINS AND EMERGENCE OF NOVEL CMV GENOTYPES

The CMV genome is highly conserved and exhibits low genetic variation. Clinical and laboratory reference CMV strains (Towne and AD169) are closely related at a genomic level, as indicated by an overall DNA sequence homology of 90–95% [65,66]. Electrophoretic patterns of virion or infected-cell proteins from different strains are similar, to the extent that differentiation among strains is impossible using these tools [67,68]. Nevertheless, the more sensitive tools of restriction site polymorphism analysis [69] and sequence analysis by PCR have allowed the differentiation of distinct CMV genotypes by analysis of more variable regions of the CMV genome [70,71]. A large number of CMV strains are apparently in circulation worldwide, and these have different geographical frequency distributions [72]. Based on an analysis of nine genomic sites, it has become apparent that, in theory, a virtually infinite number of CMV strains may exist [72].

Variation in CMV genes translates into multiple strain-specific epitopes on different viral proteins [73]. Antibodies produced against one CMV strain generally react with other strains, but in the case of CMV infection with a different strain (re-infection), strain-specific immunity confers little cross-protection [74]. Although there is considerable interest in developing a CMV vaccine, and several candidates are in development, attempts have not succeeded to date. Single candidate proteins include glycoprotein B (gB) (with the aim of inducing neutralising antibody) and the pp65 tegument protein (with the aim of inducing a strong cytotoxic T-lymphocyte (CTL) response) [75]. An intact live virus vaccine approach is also being pursued, using recombinant virus produced from strain AD169 and the Toledo isolate, which is closer to clinical isolates in its tropism and genetic structure [76]. Results in rodent models have been very promising. In clinical trials using single protein as well as intact virus vaccines, neutralising antibodies and detectable CMV-specific cellular immunity could be induced [77–79]. However, to date, none of the vaccines evaluated in larger cohorts has provided reliable protection against CMV infection [80,81]. The variation in immunogenic epitopes among clinical CMV strains may explain some of the disappointing clinical results obtained with CMV vaccine candidates. Laboratory strains used for vaccine design may differ significantly from clinical strains, in terms of antigenic epitopes, and some clinical strains may be very difficult to culture.

Subtype classification of CMV strains has been based most frequently on the gB gene (UL55) [82]. Glycoprotein B plays an important role in cell-to-cell transmission [83], is a major functional region promoting virion entry, as well as cell-to-cell spread of infection and fusion [84,85], and is a major target of neutralising antibodies [86]; thus, it has been considered to be a prime candidate for a CMV vaccine. Four major gB subtypes, with different geographical frequency distributions, have been characterised to date [82,87]. However, co-infection with more than one CMV strain is not unusual, and primary CMV infection with several CMV strains simultaneously has been observed in immunocompetent individuals [88]. An analysis of CMV strain-specific antibodies has revealed that exposure to multiple CMV strains may occur in *c.* 20% of the healthy population [89].

Co-infection with different CMV strains is also of clinical relevance. Humar *et al.* [90] demonstrated that infections with mixed CMV gB-subtype strains in solid-organ transplantation may be associated with delayed clearance of the virus from blood during therapy. This finding is of special interest, as delayed virus clearance during receipt of antiviral therapy may also signify a higher risk of development of drug resistance. In another study, mixed gB-subtype infections were associated with increased graft rejection and disease progression [91].

HIV-infected patients are co-infected with different CMV strains even more frequently than the general population [92], and often with more than one CMV gB subtype [93]. Reactivation of CMV is observed most frequently in patients with advanced HIV infection and significant immunosuppression [94]. There is also evidence of intragenic recombination as a source of novel CMV strains in HIV-infected patients with severe immunodeficiency [95]. The majority of CMV gB strains detected in HIV-infected patients with

CMV encephalitis differed significantly from those found in immunocompetent patients [95]. Previous studies have suggested that homologous recombination contributes to the variability of the gB gene, both *in vitro* and *in vivo*, and cell culture experiments have demonstrated that co-infection with two CMV strains may give rise to viable recombinant CMV gB strains [96]. Co-infection by several CMV strains might provide the basis for CMV intragenic recombination and for the emergence of CMV variants with altered biological properties. The fact that CMV evades immunity by mutations in immunodominant domains may provide a significant biological advantage for CMV.

LATE-ONSET CMV DISEASE IN SOLID-ORGAN TRANSPLANT RECIPIENTS

Antiviral prophylaxis, i.e., the treatment of all patients before the detection of CMV replication, is highly effective in preventing CMV disease in transplant recipients, particularly in R^-/D^+ patients, who are at highest risk for CMV disease [39]. However, prophylaxis is also associated with a considerable risk of increased toxicity and antiviral drug resistance following prolonged exposure to antiviral compounds. Late-onset CMV disease has been recognised more recently as a significant complication of antiviral prophylaxis. Patients are protected during the period of antiviral prophylaxis, but are at risk for CMV disease thereafter. In the late transplantation period, the level of immunosuppression is usually less intense, and the incidence of CMV disease is therefore clearly lower than during the early transplantation period (i.e., before and after 6 months post-transplantation) [97]. Nevertheless, late-onset CMV disease is observed increasingly, and is far from trivial. Among kidney and kidney–pancreas transplant recipients, late-onset CMV disease was documented in 47% of R^-/D^+ patients, 12% of R^+/D^+ patients, 7% of R^+/D^- patients, and 4% of R^-/D^- patients [98]. In a cohort of haematopoietic stem-cell transplant recipients, late-onset CMV disease occurred in 18% of patients, with a mortality rate of 46% in these patients [99]. In addition, late-onset CMV disease may have atypical manifestations, making the evaluation of symptomatic patients even more difficult [100,101].

An increasing body of evidence suggests that antiviral prophylaxis may even increase the risk of late-onset CMV disease. Delayed recovery of CMV-specific T-cell responses has been reported in association with the administration of antiviral drugs for prolonged periods [102]. Lack of reconstitution of CMV-specific cellular immunity in transplant recipients has been a significant factor in the subsequent development of CMV disease in haematopoietic stem-cell and solid-organ transplant recipients [102]. Delayed recovery of the virus-specific host response is not associated uniquely with the use of ganciclovir, and has also been observed following prolonged exposure to other nucleoside antiviral agents and with other herpes viruses [103]. Prolonged ganciclovir prophylaxis in solid-organ transplant recipients may interfere with the humoral immune response [104], which is required to limit viral dissemination throughout the host [105]. Immunoglobulin class-switching from IgM to IgG antibodies was impaired, and antibody maturation was inhibited in a subgroup of solid-organ transplant recipients who received ganciclovir prophylaxis [104].

As an alternative, the concept of pre-emptive therapy aims at suppression of viral replication before the occurrence of CMV disease. This is usually achieved by screening blood samples routinely for the presence of CMV viraemia, with the administration of a short course of antiviral treatment in the case of a positive laboratory result. Pre-emptive therapy is also highly effective in preventing CMV disease [39]. A meta-analysis of prospective, randomised trials involving solid-organ transplant recipients revealed that the overall reduction in risk of CMV-based organ disease was comparable with pre-emptive therapy (80%, 95% CI 53–90%) and prophylaxis (77%, 95% CI 65–85%) [39]. Nevertheless, the effects of the two strategies on the occurrence of late-onset CMV disease could not be evaluated, because of the limited data available [39]. Thus, as both strategies are highly effective in preventing CMV disease, it remains unclear whether an individual patient should receive prophylaxis or pre-emptive therapy. This question has sparked an intense discussion [35,106] that has also prompted an indirect comparison of meta-analyses of the two prevention strategies [107]. Prophylaxis and pre-emptive therapy were similarly effective in preventing CMV disease in general, but ineffective in preventing CMV disease occurring >90 days after

transplantation. However, comparison of the two strategies, with respect to late-onset disease, was based on only 22 and five cases, respectively [107].

Direct comparisons of antiviral prophylaxis against pre-emptive therapy have only involved small trials, designed primarily to show equivalence of the two strategies in preventing CMV disease [108,109]. Well-designed, direct-comparison trials are urgently required to provide definitive data and to clarify whether prophylaxis or pre-emptive therapy, or a combination of both, is most effective in preventing early- and/or late-onset CMV disease.

CMV INFECTION IN PATIENTS WITH GUILLAIN–BARRÉ SYNDROME

Textbooks of clinical medicine describe several diseases of unknown aetiology, but which have features that suggest the possible role of virus infections in their pathogenesis, e.g., Wegener's granulomatosis or Guillain–Barré Syndrome (GBS). GBS has become the most frequent cause of acute flaccid paralysis in Western countries, following the near-elimination of poliomyelitis. The current annual incidence is estimated to be 0.75–2 cases/100 000 population [110]. Good progress has been made in elucidating relevant pathomechanisms and identifying effective therapeutic approaches, but understanding of the mechanisms causing GBS is still very limited. An increasing body of knowledge supports the concept that GBS is the result of an aberrant organ-specific immune response that may follow triggering events [111,112]. Infectious agents have been suggested as possible triggers of GBS, as some form of respiratory or gastrointestinal infection precedes nearly two-thirds of GBS cases [113].

Infection with CMV is the most common antecedent virus infection, as identified by the presence of IgM antibodies in 10–15% of patients at the onset of GBS [114–116]. However, antiviral therapy is currently not recommended in cases of GBS, since the disease is considered to be post-infectious. Recently, the presence of CMV DNA has been demonstrated in almost one-third of serum and cerebrospinal fluid samples from GBS patients who were positive for CMV-specific antibodies at the onset of the neurological disease [117]. Furthermore, the time of lumbar puncture was critical for the detection of CMV DNA, as the probability of the presence of CMV DNA decreased significantly with an increasing interval between the onset of GBS and the time of sample collection. This association suggests that even more patients may have been carrying CMV in cerebrospinal fluid at a very early stage of GBS. Active CMV infection supports the link between neurological disease and virus infection, but the clinical relevance of this finding has still to be elucidated.

Visser *et al.* [118] found that GBS patients with CMV-specific IgM antibodies had clinical profiles that differed from those without serological evidence of a recent CMV infection. CMV IgM-positive patients were younger, and had more severe sensory abnormalities, more frequent facial weakness, and a more frequent severe general weakness that required artificial ventilation, with a consequent delayed recovery. These findings were confirmed in a more recent study [119]. CMV-specific IgM antibodies are produced during primary infection, but also during reactivation and re-infection [112]. The mechanisms of pathogenesis and the immunological consequences are significantly different in these two instances. Following primary infection, CMV-specific antibodies increase to high titres rapidly; however, antibody avidity is initially low, and maturation requires >1 year [105,120]. Molecular mimicry, which has been proposed as a relevant mechanism in the pathogenesis of GBS [113], and cross-reactivity of CMV-specific antibodies with neuronal structures, would be expected to be more likely in primary CMV infection than during the course of virus reactivation because of the lower antibody specificity and antibody affinity soon after infection. However, detectable CMV-specific IgM antibodies in the presence of CMV-specific IgG antibodies in a serum sample may not prove primary infection, since IgM antibodies are also produced during reactivation and re-infection [112].

Primary CMV infection occurring within 6 months of the onset of GBS was found to be common in CMV-seropositive patients [119]. Low-avidity IgG antibodies, which indicate an immature immune response to a specific antigen following recent primary exposure, were found in 20% of CMV-seropositive GBS patients [119]. However, 4% of GBS patients were positive for CMV-specific IgM antibodies, but had IgG

antibodies of high avidity, indicative of a previous CMV infection. The presence of CMV-specific IgM antibodies or CMV viraemia, despite a previous CMV infection, might also be explained by re-infection with a different CMV genotype. It is well-known that CMV infection confers little cross-protection against infection with a different genotype [74]. Re-infection may be associated more frequently than reactivation with the presence of CMV viraemia or IgM antibodies in serum, but cannot be detected with currently available assays. Re-infection could also explain why primary CMV infection has not been associated with a more severe course of GBS.

CONCLUSIONS

Prevention and therapy of CMV disease remains a clinical challenge, despite the availability of highly effective antiviral and anti-retroviral drugs. Antiviral drug resistance, toxicity, the emergence of novel CMV genotypes with altered biological properties, the development of a protective CMV vaccine and late-onset CMV disease are all current topics of high clinical relevance. The possible link between GBS and CMV infection requires further investigation. The clinical significance of such a link, and the consequences with respect to patient management, remain to be elucidated, with particular reference as to whether GBS patients should receive antiviral therapy. Nevertheless, the tremendous progress during the past two decades in the prevention and treatment of CMV disease justifies optimism for the future.

ACKNOWLEDGEMENTS

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