Med Microbiol Immunol (2008) 197:75–82
DOI 10.1007/s00430-008-0087-0

REVIEW

Human cytomegalovirus infection and antiviral immunity in septic patients without canonical immunosuppression

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Received: 7 February 2008 / Published online: 8 March 2008

Abstract The human cytomegalovirus (HCMV) is a relevant pathogen in patients with immunosuppressive therapy; however, reactivation and subsequent recurrence occurs also in individuals without canonical immunosuppression as e.g., in patients with septic shock. Analyzing the impact of NK- and T-cell immunity on the natural course of HCMV infection in patients with septic shock, it became clear that the presence of HCMV reactive T-helper cells did not prevent the development of reactivation but, the control of active infection was achieved mostly by specific T-cells. NK-cells seemed to be dispensable for clearance of active infection in this patient group with long-lasting NK-cell anergy.

Keywords Cytomegalovirus · Sepsis · Immunity · Latency · Reactivation · Therapy · Disease

Introduction

The human cytomegalovirus (HCMV) is a well-known opportunistic pathogen of the β-Herpesvirus family with lifelong latency following primary infection. Latency is accomplished by intermittent HCMV reactivation and recurrence [1]. Individuals without immunosuppressive therapy generally pass active HCMV infections without major clinical signs and symptoms, both after primary infection and also following reactivation. In patients with canonical immunosuppression the development of active HCMV infection causes substantial disease as was repeatedly shown for patients with solid organ or hemopoietic stem cell transplantation, for patients with AIDS and also for patients with an immature immune system as e.g., premature infants or infants with congenital infection.

Interestingly, the clinical signs and symptoms of HCMV disease are different between the patient groups, depending on the underlying immunodeficiency. Without antiviral therapy most patients with active HCMV infection develop pneumonitis following hemopoietic stem cell transplantation, patients after solid organ transplantation are frequently diagnosed with failure of the transplanted organs, and patients with AIDS may suffer from retinitis or gastrointestinal ulcers. Postnatal infection of premature infants may cause sepsis-like disease, whereas congenital infection is frequently followed by generalized disease with multiple organ failure. The different symptoms between the patient groups could indicate that cytopathic infection (direct effects) and antiviral immunity (indirect effects) may both contribute to the signs and symptoms of HCMV disease depending on immunocompetence or immunosuppression of the host [2].

Since models for HCMV latency and reactivation changed during recent years the definitions of HCMV infection and disease are of great importance [3], especially with respect to the term reactivation which is often used for different situations. On the one hand, reactivation describes the molecular steps responsible for the transition from latent to permissive infection [4–6]. On the other hand, the term reactivation has been used in diagnostic laboratories for HCMV recurrence detected by using diagnostic assay

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(e.g., cell culture, pp65-antigenemia or PCR) [7]. Based on the definitions of HCMV infection and disease the results of patients with septic shock will be discussed (Table 1). We tried to make Table 1 as concise as possible including relevant medical and virological aspects. However, we admit that some points are of common consent, whereas others may stimulate controversial discussion because not proven in detail for humans.

It is often stated that immunosuppression is a cause for HCMV recurrence. However, in the last years we and others could show that recurrence of active HCMV infection could be detected in one-third of HCMV seropositive patients with sepsis despite the lack of canonical immunosuppression [8–11]. HCMV recurrence of patients with septic shock was associated with prolonged mechanical ventilation (median, 33 vs. 17 days) and a prolonged stay in the intensive care unit. Strict host restriction of HCMV and the lack of suitable cell culture models raise a number of questions which can be answered only in humans [12]. By analyzing antiviral immune functions of patients with septic shock we are now able for the first time to evaluate the natural course of HCMV reactivation, recurrence, and viral clearance in a patient group without canonical immunosuppression [13].

Primary HCMV infection

Primary HCMV infection is defined as the first viral contact followed by local and systemic HCMV replication. The primary infection causes seroconversion, generation of specific HCMV reactive B- and T-cells and also of specific memory cells. Primary infections of immunocompetent hosts are usually controlled by the host immune system without symptoms of disease [12].

Termination of primary HCMV infection

Priming of the previously immune-naive individuals is required to develop an adaptive antiviral immunity responsible for termination of active infection [14]. The stimulation of B- and T-cells after HCMV infection is followed by positive selection and subsequent differentiation (somatic hypermutations). Thereby, HCMV reactive antibodies synthesized in the course of primary HCMV infection develop an increasing neutralizing capacity for cell-free virus after the first months following seroconversion; however, the clinical experience shows that postnatally the protective role in immunosuppressed individuals is limited in vivo, although some reports have shown significant benefit in vivo and in the mouse model [15–18]. Termination of primary infection is mostly achieved by cytotoxic killing of permissively infected cells by adaptive cellular immunity (cytotoxic T-cells) [19] but also NK-cells may act against HCMV infected cells predominantly in the early phase of primary infections and also under conditions of impaired T-cell function as e.g., after T-cell depleted stem cell transplantation [20]. NK-cells display cytotoxic activity against HCMV infected fibroblasts in vitro [21] and the general importance of HCMV/NK-cell interaction is also emphasized by the immune escape strategies against NK-cells detectable in various CMV homologues [12]. A number of non-permissive but long-living cells can establish a latent HCMV infection, which cannot be cleared by the host immune system. It has been assumed that virus variants, the amount of the infecting viral inoculum, the route of infection and host factors such as age may influence signs and symptoms of infection but also the genomic viral load of latently infected cells in host organs [22].

Latency

During latency infectious viral particles cannot be detected by using standard diagnostic assays in seropositive individuals [23]. Immune evasion from innate and adaptive immunity is assumed to have an impact on HCMV genomic load in latently infected organs, thereby predisposing for reactivation and subsequent recurrence. The association between genomic load and recurrence was demonstrated in the mouse model [22, 24]. Also in humans a preliminary postmortem analysis of autopsy material indicates that the genomic load was heterogeneous in various organs and various individuals (unpublished data), which could influence the individual risk of HCMV reactivation.

Reactivation

In the mouse model it became clear that the initial steps of MCMV reactivation detected by expression IE transcripts occur also without canonical immunosuppression following allogeneic but not following syngenic transplantations [25]. MCMV reactivation with subsequent recurrence was stimulated also by polymicrobial infection with sepsis-like disease (caecal ligation and puncture, CLP) [26]. Pro-inflammatory cytokines stimulated by lipopolysaccharide (LPS) application may be responsible for MCMV reactivation; in other experiments TNF-α could activate the MCMV IE promoter and after intraperitoneal application also the additional checkpoints of viral latency could be overcome [6, 27].

Also for the human infection it becomes clear that HCMV reactivation develops independent of immunosuppression. In patients with renal transplantation we could

Table 1 Definitions in HCMV infection and HCMV disease

HCMV-host interaction	Definitions	Viral activity	Immune system	Variables/comments
Primary infection	Active infection after first virus-host contact	Active local and systemic infection	Generation of HCMV specific B- and T-cells (seroconversion)	Virus variants (virulence) Amount of viral inoculum Route of infection Host factors (e.g., genetic polymorphisms, age)
Termination of active infection	Clearance of active infection	Elimination of cells with permissive infection	Termination of viral replication by HCMV specific T-cells	May also activate CMV disease by indirect effects
		Persistence of latently infected cells	Assistance by antibodies and NK-cells	
Latency	Viral persistence without detectable viral replication	Latency associated gene expression	Lifelong production of specific antibodies (HCMV seropositive)	Amount of latent genomic viral load in the tissues may correlate with the risk of subsequent reactivation
		Inhibition of unrestricted gene expression at various "checkpoints" of viral replication	HCMV specific memory B- and T-cells	
Reactivation (at the cellular level)	Transition from latent to productive infection	Expression of CMV IE, E and L genes leading to virus replication	Specific immunity does not protect from reactivation	Inflammatory cytokines may stimulate reactivation
			Proliferation of specific memory T- and B-cells (may be detected as an indirect sign for reactivation)	Antiviral control or systemic infection dependent on host immunity
Recurrence	Re-detection of infectious virus following local reactivation	Viral replication in various organs and cell types Cytopathic effects by lytic infection	Viral dissemination following reactivation is dependent on host immunity	Viral load is correlated with the probability of end-organ-disease (direct effects)
Disease caused by direct effects (end-organ-disease, EOD)	Detection of histological signs of HCMV disease in biopsies	High amount of viral replication detected by viremia, antigenemia or DNAemia	Mostly in patients with canonical immunosuppression	Preemptive antiviral therapy and prophylaxis are effective
Disease caused by indirect effects	No clear-cut definition (detection of HCMV in affected organs is not obligate)	Indirect effects are not correlated with viral load (replication).	Inflammation and antiviral immunity is triggered by HCMV reactivation	More chronic disease Patients with immune imbalance or canonical immunosuppression Prevention is presumably achieved by prophylaxis but less effective by preemptive therapy

show that pretransplant immunosuppression alone did not stimulate the development of HCMV recurrence, whereas inflammation (graft rejection with subsequent rejection therapy) was regularly associated with development of active infection as a consequence of reactivation [28]. Reactivation signals may stimulate the differentiation of latently infected cells into permissive cells with the potential of unrestricted viral replication [4]. The differentiation of bone marrow derived peripheral blood stem cells into dendritic cells stimulates the activity of viral promotors by chromatin re-modeling, which is required for lytic gene expression and generation of infectious progeny virus [29, 30].

The current knowledge of HCMV reactivation, recurrence, and termination of active infection is still limited due to the lack of cell culture or animal models. All animal models imply the use of animal CMV homologues. However, similarities between the murine CMV homologue (MCMV) and HCMV are restricted to a number of open reading frames (about 70 of 200 CMV genes) and nucleotide homologies are not generally found at the exact DNA sequence level [12]. Consequently, the biology of the animal CMV homologues and HCMV is different for a number of viral functions but interestingly, the different CMV homologues contain a number of evolutionarily distinct genes with very similar functions (evolutionary convergence). For example, the high prevalence of genes responsible for immune escape mechanisms in the CMV homologues argues that these strategies are of general importance for CMV replication in vivo [31]. Therefore, despite obvious differences at the DNA sequence level, the high biologic similarity of various viral–host pairs allowed us to get insight into some common checkpoints of viral–host interaction by using animal models (e.g., for latency, reactivation, and antiviral immunity). Cell culture models for HCMV latency and reactivation are not generally available to date although a number of studies suggest that alloreactive cytokines may stimulate HCMV reactivation of hemopoietic stem cell derived cells with latent infection [29, 32].

HCMV can be reactivated upon certain stimuli, such as extended cytokine secretion ("cytokine storm") which was shown for patients with [28] and without canonical immunosuppression [8, 33]. Both, hyper-inflammation (severe inflammatory response syndrome, SIRS) and anti-inflammation (compensatory anti-inflammatory response syndrome, CARS) of sepsis are associated with extended cytokine secretion, which seems to be responsible for HCMV reactivations in this patient group. We could show that HCMV recurrence was detected very similarly in groups with and without HCMV reactive Th1-cells, which means that CMV reactivation at the cellular level is not caused by immunosuppression. HCMV recurrence was followed by an increase of HCMV reactive Th1-cells which mostly failed to appear in the group without pp65-antigenemia [13] However, a subgroup of pp65-negative seropositive patients displayed a strong increase in HCMV reactive Th1-cells (Fig. 1) and we propose that reactivation without HCMV recurrence could have been the basis for stimulation and subsequent proliferation of HCMV reactive Th1-cells. We therefore assume that reactivation at the cellular level can be identified by increasing frequencies of HCMV reactive Th1-cells even when HCMV recurrence is not found. Intermittent HCMV reactivation without recurrence may regularly alert the host immune system of normal immunocompetent humans. Thereby, a sustained T-cell response is achieved with high frequencies of HCMV reactive T-cells [34].

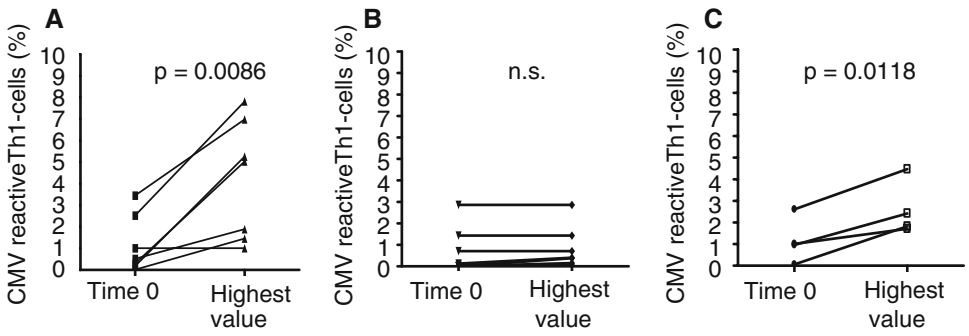

Fig. 1 HCMV reactive Th1-cells in patients with septic shock. The frequency of HCMV reactive Th1-cells at the beginning of septic shock (Time 0) and during the follow-up (highest value) was shown for different patient groups. **a** Patients with HCMV recurrence (pp65-antigen positive) showed an increase in HCMV reactive Th1-cells. **b** Patients without reactivation (pp65-antigen negative) showed stable and generally low frequencies of HCMV reactive Th1-cells. **c** Patients with presumed reactivation at the cellular level but without recurrence showed an increase in HCMV reactive Th1-cells despite negative pp65-antigenemia. Statistical analysis was performed by using paired *t*-test. N.s. means not significant

Active HCMV infection

Active HCMV infection following reactivation seems to be the consequence of a multistep process (Fig. 2): Inflammation stimulates HCMV reactivation at the cellular level which can be followed by HCMV recurrence with viral dissemination in the case of immune imbalance (e.g., sepsis) or canonical immunosuppression (e.g., transplantation). While reactivation at the cellular level occurred independent of immunosuppression, the development of subsequent recurrence is mainly influenced by the immunocompetence of the host. Therefore, the natural course of reactivation is different comparing different individuals and different clinical situations. Reactivation at the cellular level seems to be very common also in immunocompetent individuals and may occur without detection of active CMV infection by standard diagnostic assays due to the inhibition of viral spread already at the local level. Reactivation after T-cell depleted stem cell transplantation is followed by unrestricted viral replication and high viral load [7, 35, 36]. However, when HCMV reactive T-cells were detected following transplantations the development of HCMV recurrence was associated with a lower viral load and at least in part by viral clearance without antiviral therapy [37]. Consequently, recurrence of patients without canonical immunosuppression was characterized by low viral load [8] and also reactivation without recurrence might have been occurred (Fig. 1).

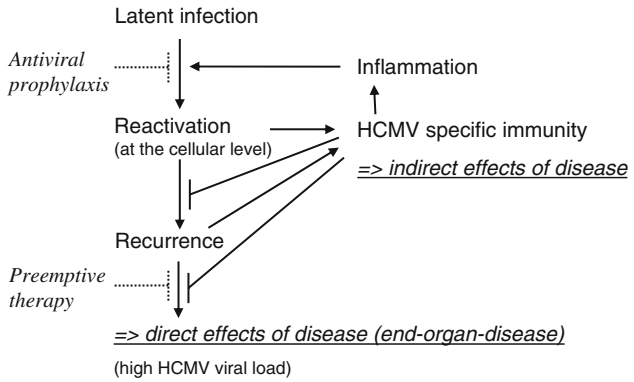

Fig. 2 Model of HCMV reactivation and disease HCMV reactivation is stimulated by inflammation (e.g., SIRS or alloreactive immune response). Recurrence with dissemination is influenced by CMV specific immunity. Unrestricted viral replication with high viral load, direct cytopathic effects and end-organ disease can develop in case of canonical immunosuppression. HCMV reactivation of patients with mild or without immunosuppression activates HCMV reactive T-cells, which is associated with an increase of HCMV reactive T-cells, a low viral load and clearance of active infection. Thereby, indirect effects of CMV infection associated with an antiviral inflammatory response can be stimulated. Intervention by antiviral therapy is available by prophylaxis or preemptive therapy acting at different points of the reactivation/recurrence cascade

Onset of active infection was detected at different times following transplantation or sepsis which means that the timing of reactivation was different in both groups. Reactivation with systemic infection was mainly detected 1 to 3 months following transplantations [38]; however, in patients with septic shock a systemic infection was detected already in the first 2 weeks following onset of septic shock (average day of onset, 7 days) [8]. However, the mechanisms responsible for delayed or accelerated reactivation are still unknown.

Active HCMV infection of patients with septic shock was detected in the blood compartment and in one-third of the patients also in bronchial aspirates. The predisposition for a reactivation in the lung might be associated with high levels of latently infected cells in this organ as was demonstrated in the mouse model [22].

Termination of active infection following reactivation

Although, recurrence occurs in the presence of specific antibodies and memory T-cells very similar mechanisms may generally be responsible for termination of active infection, both after primary infection and recurrence. The clinical experience with HCMV recurrence following transplantations and also the animal models showed that anti CMV antibodies could neither stop active HCMV infection nor prevent HCMV recurrence or disease [1]. It was conclusively shown by adoptive transfer experiments that HCMV specific T-cells are highly effective against active HCMV infection [39, 40]. Adaptive cellular immunity is composed by CD8+ cytotoxic T-cells with effector cell activity and CD4+ T-helper cells with regulatory functions supporting long-term activity of CD8+ cytotoxic T-cells. HCMV, MCMV, and also other CMV homologues developed various strategies for down modulation of major histocompatibility antigen (MHC) class I [41]. However, the cells with low MHC class I expression are recognized and eliminated by natural killer cells (NK-cells) [42]. Despite cytotoxic activity of NK-cells against HCMV infected target cells in vitro, [21] the clear evidence for the NK-cell activity against HCMV is still missing in vivo. The evolution of viral inhibitors of NK-cell activity within human and animal CMV homologues strongly argues that NK-cells are important for virus-host interaction but the existence of viral inhibitors also implies that NK cells could work less effective. Cytotoxic NK-cell activity was shown in vitro after infection with laboratory strains but not with wild-type or endothelial cell adapted wild-type-like strains [21] which means that immune-escape strategies against NK-cells are highly effective. We demonstrated that cytotoxic NK-cell activity was profoundly suppressed in patients with septic shock despite the presence of CD16+/

CD56+ cells. Therefore, loss of NK-cell activity seems to be the result of NK-cell anergy and not of NK-cell depletion. This long-lasting NK-cell anergy could not be changed by testing purified NK-cells nor by addition of IL-2 in vitro [13, 43]. The mechanism why NK-cells lost the ability to become activated is not clear but cytokines of SIRS could inhibit NK-cell activity in vitro, which was shown recently for IL-6 [44]. Therefore, it might be proposed that NK-cell function is not essential for clearance of active HCMV infection, at least in patients with septic shock.

For monitoring of HCMV specific immunity we decided to analyze the frequency of HCMV reactive CD4+ Th1-cells. HCMV reactive Th1-cells can be detected in vitro after stimulation with HCMV antigens from HCMV infected cells [45]. Also following transplantations the analysis of HCMV reactive CD4+ T-cells was useful to predict whether patients were protected against HCMV disease despite active HCMV infection [37, 46]. Activation of HCMV reactive CD4+ T-cells is achieved by using the exogenous pathways of antigen presentation acting for all HLA-types. Therefore, analysis of HCMV reactive CD4+ T-cells could be applied also to a patient group with unknown HLA-types. CD8+ cytotoxic T-cells need the support of CD4+ T-helper cells and the activity of both T-cell subsets is closely associated [37].

During the follow-up the frequency of Th1-cells significantly increased in patients with septic shock following reactivation and recurrence [13]. The increase in CD4+ HCMV specific Th1-cells was presumably accompanied by increased activity of HCMV specific CD8+ cytotoxic T-cells as was shown before for patients after transplantation [39]. Since active infection of patients with septic shock was terminated without support from cytotoxic NK-cells it can be concluded that HCMV specific T-cells were sufficient to terminate active HCMV also in patients with septic shock.

To our surprise, also a more general reactivity of Th1-cells was detected in the group with active infection which was detected following superantigen stimulation (staphylococcal enterotoxine B, SEB) [47]. Both, the frequency of HCMV and SEB reactive Th1-cells remained generally low in the group without HCMV recurrence. To date it is not clear if increased frequencies of T-cells without HCMV specifity developed as a sign of polyclonal expansion of bystander T-cells or by a more general change from T-cell anergy to reactivity in patients with septic shock.

CMV disease caused by direct and indirect effects

Only the minority of immunocompetent hosts develops HCMV disease following primary infection and reactivations. However, under conditions of immunological imbalance or canonical immunosuppression HCMV disease is caused by direct and indirect effects of infection or by combinations of these. By definition, direct effects of cytopathic HCMV infection cause HCMV end-organ-disease which can be identified by viral detection in biopsies of affected organs [3, 48]. Viral load, direct effects, and the probability of end-organ-disease are highly correlated. Direct effects of HCMV infection can be inhibited by preemptive antiviral therapy and also by prophylaxis as shown for patients with hemopoietic stem cell transplantation [7].

The indirect effects of HCMV infection are very heterogeneous and even less characteristic from the clinical point of view [2, 49]. The diagnosis of indirect effects is difficult to achieve because clear-cut criteria are missing. Indirect effects are not correlated with the viral load and may even occur in the absence of detectable virus. Consequently, reliable diagnostic assays for detection of indirect effects are not available. Indirect effects are caused mainly by triggering of antiviral immunity following HCMV reactivation. The indirect effects of HCMV infection can be inhibited by antiviral prophylaxis but presumably not as well by preemptive antiviral therapy because preemptive antiviral therapy is initiated when exposition to the potential triggering signals of the indirect effects was already present [50]. Indirect effects can be associated with graft rejection, accelerated coronary artery atherosclerosis after organ transplantations, predisposition for severe bacterial, and fungal infections [51, 52]. Active HCMV infection of patients with septic shock was associated with prolonged mechanical ventilation and ICU stay. Increased morbidity of patients with septic shock was conclusively found in all prospective studies despite low viral load and successful clearance of active infection within a few weeks [8–11]. This clinical observation argues that CMV associated disease of patients with septic shock is caused predominantly by indirect effects of infection. In the polymicrobial mouse model of MCMV reactivation TNF-α was identified as the key-cytokine for the reactivation and also for the pathology in the lungs [27]. Ganciclovir prophylaxis but not early antiviral therapy could inhibit MCMV reactivation and TNF-α production. Pathogenicity in the lungs could thus be attributed to indirect effects of MCMV infection [53] which could have occurred very similarly also in the patients with septic shock [54].

In addition to the definitions of HCMV infections and disease (Table 1) [3] we propose a schematic model of HCMV reactivation, recurrence, and disease (Fig. 2). Reactivation at the cellular level is induced by still unknown factors of inflammation. Thereby, cells with latent infection may become terminally differentiated which allows permissive viral replication with generation of progeny virus [4]. In the case of an immunocompetent host a reactivation at

the cellular level may be locally cleared [55]. The subsequent expansion of HCMV reactive T-cells is assumed to be an indirect sign of reactivation, which might be used in a diagnostic way to identify patients with reactivation but without recurrence (Fig. 1). Reactivation at the cellular level is identified in animal organs by analyzing IE and E transcripts; however, such an invasive procedure is not applicable for diagnostic purpose in humans, which means that in the clinical situation HCMV reactivation at the local level remains undetectable by the standard virologic assays. Quantitative viral load is correlated with the development of end-organ-disease caused by cytopathic effects following viral replication. However, HCMV associated disease may also occur due to indirect effects of infection which are caused mostly by antiviral immunity of patients with low viral load (e.g., patients with septic shock) [2, 49]. As outlined in this model, reactivation is stimulated independent from immunosuppression whereas the grade of viral dissemination (viral load) and the risk of end-organ-disease are influenced by the immune status of the host.

Conclusion and outlook

By investigating antiviral immunity and active HCMV infection of patients without canonical immunosuppression we confirmed that immunosuppression was not the cause for HCMV reactivation. However, recurrence, viral dissemination, and the development of HCMV disease are thoroughly controlled by antiviral immunity. Although the molecular mechanisms of HCMV reactivation are still under investigation, there is increasing evidence that pro-inflammatory cytokines stimulate HCMV reactivation at the cellular level. The “cytokine storm” of sepsis is likely to be relevant for the high prevalence of HCMV reactivation in this patient group. Prevention of HCMV associated morbidity of patients with sepsis might be possible by antiviral therapy, which remains still to be shown by an interventional study.

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