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REVIEW

Impact of cytomegalovirus load on host response to sepsis

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Abstract
There is a decades old association between cytomegalovirus reactivation and sepsis in immune-competent hosts. Much has been learned about this relationship, which has been described as bidirectional, meaning that the virus incites and is incited by the host's inflammatory response. More recent work has suggested that chronic viral infection leaves the host with exaggerated immunity to bacterial infections. In this review, the relationship between CMV and host responses to sepsis are reviewed, with particular attention to the impact that tissue viral load contributes to this phenomenon.

Keywords Cytomegalovirus reactivation · Sepsis · Viral load

Introduction

The inter-relationship between cytomegalovirus (CMV) and sepsis has become more complex as our understanding of it has grown. We have moved beyond the basic biology of how sepsis can trigger reactivation to what implications such reactivation events have for the immune-competent host. Despite our progress, there are still numerous observations that cannot be explained. The underpinning premise of this review is that nothing about CMV in humans will make sense until we understand the influence of host tissue viral load.

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CMV reactivation in immune competent hosts

Cytomegalovirus has been known for many years to reactivate in immune competent hosts during times of stress and immune compromise. There have now been 27 studies of CMV reactivation in previously immune-competent patients suffering critical illness [1–27]. When analyzing all comers, about 25% of patients who are at risk [identified by serum immunoglobulin-G (IgG)] have detectable reactivation (Fig. 1a). The significant variability in reported incidence can be mostly explained by variations in methodology or timing of testing. When limited only to patients with sepsis, the incidence of reactivation seems to be ~ 30% (Fig. 1b).

Although CMV remains the best studied of the herpesviruses, recent studies have cast a broader net, showing that multiple other herpes family viruses also reactivate during critical illness. Walton et al. showed that EBV, HSV, and HHV-6 reactivation also commonly reactivate during sepsis, and that close to 50% have reactivation of > 1 herpesvirus [11]. A more recent study showed similar results, also showing that varicella zoster reactivates infrequently (< 1%) [28]. Given the biologic similarities between the herpesviruses, as well as the need for immune control to maintain their latency, it is perhaps not surprising that so many of them reactivate during critical illness.

There are associations with poor outcomes in immune-competent patients with CMV reactivation during critical illness. The mortality risk associated with CMV reactivation is roughly twofold [29, 30], and there are now data suggesting

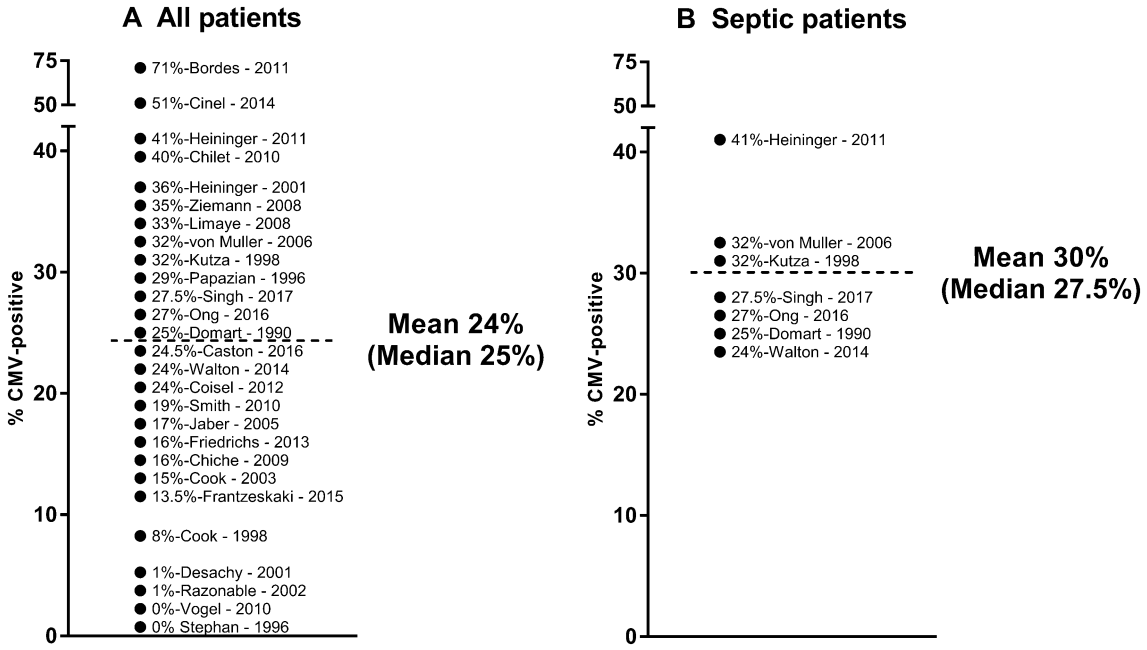

Fig. 1 Reported incidence of cytomegalovirus reactivation in immune-competent hosts. **a** Reported reactivation rates in studies of immune-competent hosts from both septic and non-septic hosts. **b** Reported reactivation rates for immune-competent patients with sepsis

that worsened outcomes are proportional to degree of DNAemia during reactivation [11, 6]. Whether poor outcomes are a consequence of viral activity or simply an indicator of severity of illness is a subject of significant current interest [31]. There have been two randomized prospective clinical trials that show that reactivation can be prevented in this patient population, but both were underpowered to address the causality question [32, 33]. Because only 1 in 3 patients has reactivation, there is still significant interest in understanding who is most at risk for reactivation to avoid treatment of patients unlikely to reactivate.

Latency, sepsis and reactivation

Latency has recently come to be regarded as a functional state wherein multiple copies of viral genome that are mostly transcriptionally silent lie dormant in a host's cells. The occasional stochastic transcriptional activation that occurs is rapidly controlled by the host with intact immunity, but in immunocompromised hosts can progress to full replication, viremia and even disease. We have previously proposed that functional latency results from a balance between three factors: inflammation, epigenetic regulation and host immunity [34].

These three factors can be categorized broadly as either stimulatory (inflammation) or suppressive (epigenetics, host immunity) influences on virus activity. The major immediate early promotor (MIEP) is a highly promiscuous promoter with consensus sequences within its enhancer element that make it exquisitely sensitive to stimulation by inflammatory pathway signaling [35, 36]. Counterbalancing this promiscuity are two regulatory forces; first is the cells tendency to chromatinize CMV, functionally blocking MIEP transcription, with active immune surveillance "riding shotgun", controlling viral activity if the epigenetic blockade is overcome.

Given these forces, it is perhaps not surprising that there is a longstanding relationship between sepsis and virus reactivation. Sepsis is a prototypical inflammatory state that by itself can influence both epigenetic regulation and immune function [37, 38]. The connection between sepsis and CMV reactivation was first made almost three decades ago when it was recognized that immunecompetent patients with mediastinitis had high rates of CMV reactivation [10]. This was followed by corroborating work from others, confirming an association between sepsis and reactivation as well as showing how inflammatory mediators are stimulatory to the CMV-MIEP [39–44]. Combining a murine model of sepsis and latent CMV, we confirmed the association experimentally [45], and it has since been shown that individual inflammatory mediators are also capable of inducing transcriptional reactivation [46–49].

Before these inflammatory mediators can have their effect, the MIEP must be accessible, and the influence that epigenetic regulation has on initiating and maintaining latency has been underappreciated until recently. The first hints came from work showing that MIEPreporter constructs are silenced invivo by cellular mechanisms, and that

stimulation with lipopolysaccharide can transiently restore MIEP function and reporter expression [50]. Subsequent work from several investigators using different models has demonstrated that epigenetic regulation contributes significantly to intracellular control of MIEP transcriptional activity [51–55]. The role of inflammation in epigenetic regulation has been since confirmed using an allogeneic stimulation model, showing that inflammation can release epigenetic control allowing MIEP transcription. Sepsis can also impact acute and chronic epigenetic regulation, and although the subject is in sore need of study, it seems logical that sepsis similarly influences the epigenetic regulation of herpes viruses allowing transcriptional activity.

The final checkpoint for reactivation is active immune surveillance. This is most clear in patients with significant immunosuppression, in whom CMV reactivation frequently occurs. The importance of T-cells in active immune surveillance has been demonstrated using elegant viral mutation methodology [56, 57]. It is known that sepsis can induce profound immune compromise [58, 59], and using our murine model we have shown that this includes contraction of CMV-specific T-cells [60]. Sepsis and critical illness are also associated with contraction of B-cells and CMV-specific immunoglobulin-G [61, 22], and the importance of CMV-specific IgG in confining recurrent virus after reactivation from latency has been shown by Jonjic et al. [62] (reviewed in this issue of MMIM [63, 85]). Given that some hosts develop very large CMV-specific T-cell and antibody responses [64–67], and because these T-cell responses show signs suggesting constant stimulation [56, 68, 69], it seems reasonable to assume that this immune checkpoint is frequently challenged.

In summary, inflammation associated with sepsis likely first influences epigenetic regulation, allowing access to the chromatinized viral DNA. Once the virus, and in particular the MIEP is unwound to some degree, then the cellular signaling pathways from inflammatory mediator activation can potentiate transcriptional activity. Simultaneously, the septic response also triggers host immune compromise, opening a window of opportunity for the virus to fully reactivate. Once reactivation gains momentum, viremia can contribute further to septicemia, provoking further inflammation and providing positive feedback to the reactivation event [70].

Immune consequences of CMV infection

In the course of our studies of sepsis and CMV, we made the observation that latently infected mice had more dramatic pulmonary inflammation and injury during sepsis than naïve mice [71]. Using our model we observed prolonged expression of inflammatory mediators TNF-α, KC, MIP-2 and IL-1β in lungs of latently infected mice during sepsis. This work suggests a biphasic inflammatory response, with an early exaggerated TNF-α response occurring immediately after sepsis begins, well before any viral activity can be detected, followed by a later TNF elevation caused by CMV reactivation. This suggested that previous CMV infections somehow condition hosts to a more pronounced antibacterial response. Shortly after our report, Barton et al. showed that previous infection with murine herpesvirus-68 or CMV can both confer protection against subsequent bacterial infections, suggesting that monocyte macrophage activation might be responsible [72]. Given that this exaggerated response can also be harmful to the lungs of the latently infected host [71], we coined the phrase CMV- associated lung injury (CMV-ALI) to describe this immunopathologic potential [73].

The idea that previous immune experience shapes subsequent immune responses is not new and must be first credited to Selin and Welsh who popularized the idea of "heterologous immunity" [74]. We made the point in a previous review that there has been significant discordance demonstrated between human and murine responses to sepsis or trauma [75], and that such differences might be explained because the murine systems are relatively "clean", while the human system is "dirtied" with numerous previous infections, vaccinations, etc. [76]. Indeed Masopust's group has recently shown that "dirty mice" actually show significant alterations in their immune responses that more closely imitate human responses ([77] and discussed in greater detail by Jergovic et al. in this issue [78]). Collectively, it seems that CMV exposure can alter host immunity in ways that influence subsequent immune responses to novel antigens.

Making sense of CMV biology with viral load

Despite everything that we know about CMV biology, there are still a number of gaps that remain. For example, why do only 30% of seropositive patients have reactivation during sepsis (Fig. 1b) while 100% of mice in our sepsis model develop transcriptional reactivation [45]? Likewise, why do some patients undergoing transplantation have CMV reactivation and others do not? More curious still is the observation that CMV drives huge adaptive memory responses in some hosts, while still others have nominal responses [65, 66]. Similarly inexplicable are a number of studies showing that some CMV seronegative hosts have detectable CMV DNA in their PBMC [79–81]. There are undoubtedly numerous contributing factors that influence the answers to these questions. These might include differences in genetics, severity of illness, types of bacterial infection, donor or recipient conditions, severity of immunosuppression and sensitivity differences between serology and DNA PCR among others.

One factor that has been largely ignored until recently is the role of underlying host viral load. It is important here to distinguish carefully our definition of viral load. In almost all recent publications, this term refers to viral DNA load in the circulation of infected hosts. Monitoring host viral activity by quantitating CMV DNA in the circulation of solid organ transplant recipients has in fact become the foundation upon which diagnosis and treatment can be monitored in such patients [82]. Nevertheless, how circulating viral loads relate to virus in specific tissues of the host remains largely unstudied. For the rest of this review, however, unless specifically mentioned our references to viral load are specific to tissue viral load (see also contribution by Reddehase and Lemmermann in this issue [83]).

It has been known for 25 years that the conditions of primary infection and the infectivity of the virus impact cytomegaloviral load in tissues and the subsequent host risk of reactivation ([84] see also contribution of Adler and Reddehase in this issue [85]). We were inadvertently reminded of this a decade later when for a period of time, due to an error in virus titration, mice in our model were infected with only 10^3 pfu of Smith-mCMV (instead of 10^5 or 10^6 pfu), and our sepsis-reactivation model stopped working (unpublished data). More recently the profound impact that the initial infection has on memory responses, and in particular the development of memory inflation has been confirmed [86–88]. Perhaps most important has been confirmation of the importance of these infection conditions upon host viral load [86, 87, 89]. It is now clear that high titer primary infections lead to higher tissue viral loads and larger memory responses, while low titer infections result in proportionately lower viral loads and immune responses.

Although this relationship has been well worked out in SPF inbred mice, there are currently few data on viral loads in naturally infected outbred hosts. Progress here has been limited by several factors, including not knowing the time of onset or viral inoculum of the primary infection and moreover by the need for tissues to analyze. Nonetheless it has been shown that there is significant variability in host viral load after natural infections in mice [90], humans [91] and most recently pigs [89]. Given available data from murine models, the simplest explanation for this variability in tissue viral load after natural infections is that hosts encounter different virus titers at the time of primary infection. It is equally probable that other factors contribute, such as viral fitness and the immune state of the host at the time of infection. Together, these factors likely combine to explain the differences in viral load as well as the widely variable CMV-specific immune responses that have been observed in humans [65, 66].

With this context, we can begin to frame explanations to several of the gaps articulated above. For example, when we study immune-competent humans for reactivation, we are really studying a cohort with a range of viral loads. The murine experimental equivalent would require mice with a varying range of viral loads, and based on previous data [84] and our unpublished experience, we could expect that those with the highest tissue viral loads will reactivate, while those with the lowest tissue viral loads would not. Likewise, we have already shown that extremely low titer infections (1 pfu) can transmit virus to a host without inducing measurable CMV-specific immunity [92], thereby explaining how some "seronegative" patients have CMV-DNA in their PBMC. This framework thus leaves us wanting a methodology to predict tissue viral loads in a host without doing invasive biopsies.

Just as important as bridging these gaps, better understanding of viral loads should also allow us insight into how viral load impacts heterologous immunity and immunopathological changes associated with previous CMV infection. For example, will the immune protection against bacterial infection described by Barton et al. develop after low titer infections, or will it be limited only to those with the highest viral loads? Similarly, will the lung injurious response that we have described previously during bacterial sepsis in mice infected with high titer CMV also occur after low titer infection? It seems logical that any immunopathology associated with previous CMV infection should be viral load dependent just as memory inflation is, but this hypothesis remains to be tested.

Surrogates for tissue viral load

Unfortunately there has historically been no way to distinguish patients that have significant tissue viral loads from those with barely detectable viral burdens. In an attempt to predict who might be at greatest risk for CMV reactivation during critical illness, we recently evaluated whether semi-quantitative CMV-specific IgG could be useful [89]. Mice infected with varying titers of mCMV in SPF conditions show excellent correlation between IgG titer and tissue viral load, we reasoned that naturally infected hosts might show a similar correlation. This might allow identification by IgG titer of those with the highest reactivation risk. Collaborating with Limaye et al., we evaluated patient sera from a previously published trial [6], and showed that CMV-specific IgG levels are not predictive of reactivation risk [89]. This failure of prediction suggests possible divergence between serum IgG and tissue viral loads in naturally infected hosts. Because human paired tissue/blood specimens are not readily available, we instead studied pigs, first finding that there is a wide range of porcine CMV (pCMV) DNA in lung tissues of these naturally infected hosts. Interestingly, there was no correlation between pCMV IgG antibody and tissue DNA, suggesting that naturally infected outbred hosts

have influences on viral load, IgG titer or both that are not captured in our experimental model, such as superinfection [87] or reactivation [93]. This divergence fits with recently published human results showing that CMV-specific IgG increases gradually with age, while monocyte viral load follows another pattern [94].

Another possible clinical surrogate of tissue viral load is CMV-DNA in PBMC. Detection of hCMV DNA in PBMC is not a new idea, and has been evaluated by numerous previous investigators. Recent studies using very sensitive nested PCR shows that just over half of elderly CMV IgG+ individuals have detectable CMV DNA in their circulating monocytes, and interestingly those with CMV + PBMC had significantly higher CD8 T-cell responses [95] and higher immune activation indicated by neopterin levels [96]. An even more recent study using droplet digital PCR showed that roughly 30% of healthy people had hCMV DNA in their circulating CD14+ monocytes [94]. Perhaps it is just coincidence, but it is intriguing to wonder whether these are the same patients that might have reactivation when they become critically ill? The counterpoint to this hypothesis is that individuals seem to be intermittently positive over time, having detectable CMV-DNA in their PBMC at some times but not others [97]. Future studies of reactivation in human subjects may benefit from such monitoring to try to identify those most at risk of reactivation.

Another non-invasive surrogate for viral load might be virus shed in urine or saliva, and there have been a number of studies that have looked at these as potential portals into virus activity in children [98], adolescents [99] and women of childbearing age [100]. Although shedding has been equated in the past with reactivation in adults [101], it is unclear that these asymptomatic episodes relate in any way to the incidents observed during critical illness. To date there are no correlative studies of tissue viral load and viral shedding in humans, so it is unknown whether shedding in saliva or urine can be useful in predicting tissue viral load or risk of reactivation.

Conclusions

Altogether, current data suggest that naturally occurring CMV infections are not created equally. If we start with the reasonable assumption that there is variability in the amount of virus encountered during natural primary infections, then it stands to reason that each host leaves that first transaction with a different viral load in their tissues. This in turn could leave them more or less at risk for having reactivation, and perhaps more importantly imprint an immune phenotype that potentiates a larger or smaller inflammatory response. If we are to make sense of the variability of outcomes seen in human hosts, we must develop new approaches to determine tissue viral load in patients, and abandon the less meaningful binary identification of CMV-positive or -negative based on IgG results. Until we move past this historical binary to a richer and more precise definition of CMV that includes viral load, we will be left scratching our heads about how/why our CMV IgG-positive patients behave so differently.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

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