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Review

Cytomegalovirus reactivation in critically ill immunocompetent hosts: A decade of progress and remaining challenges

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ABSTRACT

Human cytomegalovirus (HCMV) is an undisputed pathogen in humans with severe immune compromise, which has historically been thought to carry little consequence in immunocompetent hosts. During the past decade, however, accumulating data suggest that significant numbers of immunocompetent humans reactivate HCMV during critical illness, and that these reactivation episodes are associated with worsened outcomes. Because most people are infected with this ubiquitous virus by adulthood, confirming pathogenicity has now become a clinical priority. In this article, we will review the incidence and implications of reactivation, the relevant immune responses and reactivation triggers relevant to the immunocompetent host. We will summarize the progress made during the past ten years, outline the work ongoing in this field, and identify the major gaps remaining in our emerging understanding of this phenomenon.

Contents

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1. Introduction

Millions of immunocompetent people suffer critical illness each year (Halpern and Pastores, 2010). Since our interest in cytomegalovirus (CMV) in this population began in the nineties (Cook et al., 1998), it has become increasingly clear that many of these individuals experience CMV reactivation during their critical illness. This finding has now been reproduced independently by eight different groups (Chiche et al., 2009; Chilet et al., 2010; Heininger et al., 2001; Jaber et al., 2005; Kutza et al., 1998; Limaye et al., 2008; von Muller et al., 2006; Ziemann et al., 2008). More importantly, these clinical data have shown that CMV reactivation during critical illness is associated with increased morbidity and mortality. In this review, we will discuss the incidence, causes, and potential consequences of CMV reactivation in non-immunosuppressed critically ill hosts. We will also highlight contemporary challenges facing researchers and clinicians in this field. Because the terms non-immunosuppressed and immunocompetent are both used frequently by different authors, we will use these terms interchangeably to distinguish patients as not receiving canonical immune suppression and not having immune compromise from HIV/AIDS. We do this understanding that critical illness can induce transient immune compromise.

2. Primary infection and latency

Cytomegaloviruses for all species are ubiquitous and have classic beta-herpes virus characteristics. Following host control of primary lytic infection, CMV establishes life-long infection, becoming dormant in multiple end organs, a state also referred to as latency. Previous infection is most often confirmed by the presence of CMV-specific IgG responses. Roughly 50–70% of school aged adolescents in the US are human CMV (HCMV) seropositive (Stadler et al., 2010; Stanberry et al., 2004; Staras et al., 2006), and this percentage increases to >80% with age (Musiani et al., 1988). Thus significant numbers of immunocompetent patients harbor latent virus, making them "at risk" for reactivation during critical illness.

3. Reactivation from latency

Defining viral reactivation must begin with the definition of latency. Although a full discussion of latency is beyond the scope of this review, most authors use some variation of an operational definition that requires the presence of viral DNA in tissues without transcription or translation of lytic or "late" gene RNAs to protein and thereby the absence of lytic virus (for review see (Reeves and Sinclair, 2008)). Thus from a purist point of view, viral reactivation can be defined as recovery of infectious virus following some period of viral latency. Importantly we have confirmed that recovery of lytic virus is possible from immunocompetent patients during critical illness (Cook et al., 2003). Nonetheless isolation of lytic virus appears to be less sensitive for detecting CMV reactivation in immunocompetent patients than molecular methods (Kalil and Florescu, 2009) just as it is in immunosuppressed patients (Weinberg et al., 2000). This insensitivity is complicated by the fact that immunocompetent patients manifest mostly non-specific signs and symptoms during primary infection or reactivation episodes, making these events frequently "occult" (Adler, 2008; Cook et al., 1998). In addition, immunocompetent patients have narrower windows of diagnostic opportunity given their ability to ultimately control reactivation episodes (Chilet et al., 2010; Limaye et al., 2008; von Muller et al., 2007).

The lower sensitivity of culture for detecting HCMV reactivation in humans has led to the development of newer and more sensitive methods, which are all byproducts of advances in monitoring immunosuppressed patients. Historically, elevations in anti-CMV IgG titers were used to diagnose reactivation in latently infected hosts (Nagington, 1971), but antibody titer fluctuations lacked specificity leading to abandonment of this method. Although recovery of lytic virus appears to correlate better with symptomatic CMV reactivation, its lower sensitivity has led to its disuse (Hebart and Einsele, 1998). Currently, molecular methods that quantitate CMV DNAemia or antigenemia are considered by most to be the most sensitive and, therefore, the most widely used for immunosuppressed patients (Weinberg et al., 2000), and these also appear to be the most sensitive for immunocompetent patients (Kalil and Florescu, 2009). Increasing sensitivity in detecting CMV reactivation has come at a price, requiring distinction between "CMV disease" and "viral shedding" in immunosuppressed patients, because not all positive patients with reactivation show disease manifestations. We suspect that this phenomenon might also be true for immunocompetent patients, and that some reactivation episodes could be trivial while others are not. Indeed, the scant data available to date suggest that higher viral loads during reactivation are associated with worse outcomes (Limaye et al., 2008), and this topic will require keen attention in future clinical trials.

4. Reactivation incidence

Trying to pin down the actual incidence of CMV reactivation during critical illness has been confounded by several factors. One is the monitoring methodology chosen as previously discussed. When only CMV IgG positive patients are analyzed, reactivation incidence is observed in 22–42% (Kalil and Florescu, 2009). Timing of monitoring also influences detection because reactivation does not occur immediately. As shown by recent studies, reactivation typically occurs between 1 and 3 weeks after critical illness begins (Chilet et al., 2010; Cook et al., 2003; Limaye et al., 2008). Thus if testing is done too early, the incidence of reactivation is grossly underestimated, as highlighted by the two studies monitoring for reactivation within 4 days of admission (Desachy et al., 2001; Razonable et al., 2002). The etiology of one's critical illness also appears to influence reactivation rates, with burn and trauma patients possibly at higher risk than cardiac or medical ICU patients (Limaye et al., 2008). Finally, a recent study that evaluated bronchoalveolar lavage fluid suggested even higher rates of reactivation (42%) than those seen from peripheral blood (Chilet et al., 2010), suggesting that the site of testing can influence detection. Taken together, if one excludes studies with very early monitoring and monitors only those with latent CMV, a reasonable estimate of reactivation appears to be one in three non-immunosuppressed critically ill patients.

5. Reactivation implications

Despite the incontrovertible evidence that HCMV reactivates in non-immunosuppressed patients during critical illness, the question remains over the clinical consequence. It is relevant to note that this is the same conundrum that faced transplant surgeons almost 40 years ago (Lopez et al., 1974). During the subsequent decades, HCMV reactivation has become recognized as a pathogen in those without fully functional immune systems (Gaytant et al., 2002; Gor et al., 1998; Simmons et al., 1977; Steininger, 2007). Intensivists are now facing the same question in patients who were immunocompetent before they became critically ill? Is reactivated HCMV a pathogen in these patients, or an innocent bystander identifying those with transient immune suppression or immunological insult?

The preponderance of recent clinical data supports the hypothesis that HCMV is a pathogen during critical illness. Studies to date have demonstrated consistent morbidity in these patients,

including increased durations of mechanical ventilation (Chiche et al., 2009; Cook et al., 2003, 1998; Curtsinger et al., 1989; Heininger et al., 2001; Jaber et al., 2005; Kutza et al., 1998; Limaye et al., 2008; von Muller et al., 2006; Ziemann et al., 2008) and even acute respiratory distress syndrome (ARDS) (Papazian et al., 2007, 1998). Consistent with increased duration of mechanical ventilation, there are also associated increases in ICU lengths of stay (Chiche et al., 2009; Chilet et al., 2010; Cook et al., 2003; Curtsinger et al., 1989; Domart et al., 1990; Heininger et al., 2001; Jaber et al., 2005; von Muller et al., 2006; Ziemann et al., 2008). There is striking consistency between studies, despite a variety of etiologies for critical illness and diagnostic methods to identify viral reactivation. We have been particularly intrigued by the association between HCMV reactivation and increased durations of mechanical ventilation and ARDS because lungs are a primary site of latent virus and a consistent site of reactivation (Cook et al., 2003, 1998; Heininger et al., 2001; Jaber et al., 2005; Kutza et al., 1998; Limaye et al., 2008; Toorkey and Carrigan, 1989; von Muller et al., 2006).

In addition to morbidity, there are now data associating CMV reactivation during critical illness with mortality risk. Recent work from Ziemann's group has associated HCMV reactivation with increased risk of dying (Ziemann et al., 2008), and Limaye et al. have shown excellent correlation between increasing HCMV viral load during reactivation and worsened outcomes (Limaye et al., 2008). In addition, a recent meta-analysis has shown that immunocompetent patients with CMV reactivation during critical illness have a doubled risk of death (Kalil and Florescu, 2009). It is interesting that this CMV-associated risk is similar to that seen in HIV patients with HCMV DNAemia (2–4X more likely to die – independent of HIV load and CD4 counts and despite highly active antiretroviral therapy) (Wohl et al., 2005). Thus there are sufficient correlative clinical data to suggest that CMV reactivation in previously immunocompetent patients may indeed have clinical consequences during critical illness.

6. Animal models

Because of the purely correlative relationship between CMV reactivation and poor outcomes, and both ethical and practical limitations in human studies, we have turned to animal models to study mechanisms of reactivation and disease. Although there are several described models of CMV, perhaps the most popular is murine CMV (MCMV) (Gonczol et al., 1985). MCMV and HCMV share significant genetic and functional similarities, and MCMV is considered an excellent model for HCMV infection, latency and reactivation (Gonczol et al., 1985; Ho, 1982; Hummel and Abecassis, 2002; Rawlinson et al., 1996; Reddehase et al., 2002). Following acute primary infection of immunocompetent mice or humans, there is control of the acute infection but not eradication of virus. This leads to lifelong viral latency, and both MCMV and HCMV can be reactivated by any number of triggers (reviewed in (Hummel and Abecassis, 2002) and below). Interestingly, despite affording the ability to directly analyze tissues for viral transcription and lytic virus, these models are plagued with the same difficulties of reactivation detection as described in immunocompetent humans. Importantly, MCMV has the same proclivity as HCMV for several organs, including lungs (Balthesen et al., 1993), which makes it an ideal model to study the pulmonary effects of reactivation (Balthesen et al., 1993; Collins et al., 1993; Koffron et al., 1998; Kurz et al., 1997).

7. Immune responses to CMV

It is important to note that the immune responses to MCMV and HCMV also share many similarities. Both innate and adaptive immunities are important for viral control during acute and latent infections (Lemmermann et al., in press; Lenac et al., 2008; Polic et al., 1998; Pyzik et al., 2010; Rolle and Olweus, 2009; Wilkinson et al., 2008). HCMV and MCMV both induce very broad CD8 and CD4 T-memory responses (Babel et al., 2009; Holtappels et al., 2008, 2002; Kern et al., 2002, 2000; Munks et al., 2006a,b; Walton et al., 2008). Likewise, both viruses induce transient IgM followed by lifelong IgG antibody responses (Lawson et al., 1988). Although there is some dissimilarity between receptors and ligands, NK cells are important to control both viruses (Andrews et al., 2010; Babić et al., 2010; Jackson et al., in press; Lenac et al., 2008; Wilkinson et al., 2008). Finally, although not genetically identical, both viruses contain numerous immune evasion genes, several with functional similarity, that influence both innate and adaptive immunities (reviewed in (Jackson et al., in press; Lemmermann et al., in press)).

For CD8 T-cells, some hosts develop "inflationary" CMV-specific memory responses that grow to occupy very large percentages of the T-memory compartment (Gillespie et al., 2000; Holtappels et al., 2002; Karrer et al., 2003, 2004; Olsson et al., 2001; Ouyang et al., 2003; Reddehase et al., 1985; Sierro et al., 2005; Sylwester et al., 2005; Vescovini et al., 2007). The purpose of these inflated T-cell responses has vexed investigators since their first description (Reddehase and Koszinowski, 1984), but recent data suggest that these T-cells help to maintain latency (Simon et al., 2006). Curiously, unlike mice undergoing high titer infections, not all humans develop T-memory inflation (Gillespie et al., 2000; Sylwester et al., 2005; Vescovini et al., 2007). We have recently shown that the development of such inflationary memory may depend upon the conditions of the original infection (Thomas et al., 2010), and we are currently attempting to determine whether repeated reactivation episodes can cause MCMV-specific memory inflation as has been suggested to occur in humans.

8. Reactivation triggers

Reactivation of latent HCMV can be triggered by a variety of stimuli. The most obvious cause of reactivation is immunosuppression and patients receiving immunosuppressive medications after transplantation or who have immune compromising diseases such as HIV are prone to CMV reactivation and disease (Steininger, 2007). HCMV reactivation in immunocompetent patients has been associated with stress (Toro and Ossa, 1996), inflammatory states such as sepsis (Cook et al., 1998; Heininger et al., 2001; Kalil et al., 2010; von Muller and Mertens, 2008), inflammation (Döcke et al., 2003, 1994; Fietze et al., 1994; Prosch et al., 1995; Stein et al., 1993), and even endogenous catecholamines (Prosch et al., 2000; Salem et al., 2006). To further characterize these associations, we have used the MCMV model to confirm that bacterial sepsis, toll-like receptor 4 signaling and inflammatory cytokines can all trigger reactivation of latent CMV in lungs of immunocompetent mice (Cook et al., 2002, 2006a). Although steroid use during critical illness has been associated with CMV reactivation in a few reports (Chiche et al., 2009; Cook et al., 2003; Jaber et al., 2005; Wiener-Well et al., 2006), we have been unable to confirm this using our animal model (Forster et al., 2009). Our most recent work suggests that transient contraction of MCMV-specific memory induced by heterologous bacterial antigens (such as LPS) during sepsis may actually trigger reactivation (Cook et al., manuscript in review). From our perspective most of the inflammatory "triggers" have at least transient immune suppressive consequences that could influence T-memory thereby facilitating reactivation. Host immunity in immunocompetent patients during CMV reactivation has been reported by only two investigators to date (Chilet et al., 2010; von Muller et al., 2007), with two

additional studies ongoing: one in burn patients (Cairns et al. NCT00467532) and the other in ICU patients (Papazian et al. NCT00699868). We consider this a very fertile area of investigation that should help to better define those most at risk for reactivation and poor outcomes.

9. Reactivation versus reinfection

Although it may be considered hair-splitting by some, it is possible that not all "reactivation" events identified in ICU patients are truly reactivation. It is clear that humans, mice, and non-human primates can all be reinfected with different CMV strains despite previous infection and pre-existing CMV-specific immune responses (Gorman et al., 2006; Hansen et al., 2010; Ross et al., 2010). This raises the possibility that critically ill humans are not reactivating latent virus, but are simply being reinfected during their illness. It is well accepted that CMV can be transmitted by blood transfusions (Adler et al., 1985; Cheung and Lang, 1977; Drew and Miner, 1982; Kantor et al., 1970; Lerner and Sampliner, 1977; Stevens et al., 1970), and we and others have observed a higher incidence of transfusions in patients with active CMV during critical illness (Chiche et al., 2009; Cook et al., 2003; Curtsinger et al., 1989; Jaber et al., 2005). One mechanism for this putative occurrence could be passenger leukocytes in banked blood transmitting infection to the recipient (Schrier et al., 1985). Alternatively, passenger leukocytes might provide allogeneic stimulation which can trigger CMV reactivation (Forster et al., 2009; Soderberg-Naucler et al., 1997). Leukoreduction has been shown to reduce risk of blood related CMV transmission (Eisenfeld et al., 1992; Hillyer et al., 1994), and possibly reactivation during critical illness (Stephan et al., 1996), but an influence on CMV detection during critical illness is not supported by meta-analysis (Kalil and Florescu, 2009). Moreover, in all the studies to date, the overwhelming majority of patients that have documented viral activity during critical illness were CMV-IgG positive to begin with. While it is possible that CMV IgG-positivity identifies those most susceptible to reinfection, the simpler explanation is that endogenous latent virus is reactivating. The fact that reactivation can be recapitulated in our murine model proves that reactivation is possible in immunocompetent hosts (Cook et al., 2002, 2006a). The dynamics of different HCMV strains within the same host have just recently been described by deep sequencing HCMV in transplant patients (Gorzer et al., 2010), and such studies in critically ill patients would help to answer this re-infection question. Thus, current evidence supports reactivation as the primary source of CMV activity during critical illness, though we acknowledge the possibility that some of these episodes may ultimately prove to represent reinfections.

10. The $64,000 question

As we have recently suggested, one remaining $64,000 question is whether reactivated CMV during critical illness is a pathogen or a bystander (Cook, 2009). We have shown that CMV reactivation in immunocompetent mice can cause lung injury, and more importantly that both reactivation and this lung injury can be prevented by antiviral therapy (Cook et al., 2006b). Our most recent data clearly show that waiting for reactivation to occur mitigates the benefits of antiviral treatment, and that the best treatment effects occur when those at risk receive early antiviral "prophylaxis" (Forster et al., 2010). Antiviral treatment in critically ill humans published to date has been limited to patients with demonstrated reactivation, and our animal data, therefore, might explain the lack of response to antivirals seen in this setting (Cook et al., 1998; Heininger et al., 2001; Jaber et al., 2005). Based upon this, we and others have suggested that to definitively answer this pathogen versus bystander question, a randomized prospective trial of antivirals in critically ill patients at risk is required (Cook, 2007; Griffiths, 2010; Limaye et al., 2008; Osawa and Singh, 2009). Fortunately, Boeckh et al. have recently been funded to begin such studies in critically ill humans with acute lung injury at risk for reactivation (NHLBI 1U01HL102547). Although their primary endpoint is not mortality, their work should be foundational to answering this most relevant question. For now, however, we continue to recommend against antiviral therapy in critically ill immunocompetent patients outside of a randomized clinical trial.

11. Barriers to progress

While we await results from the Boeckh clinical trial, there are at least four major barriers that need to be addressed to continue moving this field forward. First we must better understand how CMV injures immunocompetent hosts. Second, we need to identify those most at risk for reactivation so that we can target interventions. Third, new therapies must be developed that will allow treatment of critically ill patients without causing them harm. Finally, adequate resources need to be allocated to continue pursuit of these major gaps.

11.1. Barrier 1 – How does CMV injure immune competent hosts?

Whatever the mechanism of injury, it seems reasonable to assume that there will be significant differences between immunocompetent and immunosuppressed hosts. Unlike HIV or transplant patients with disease tempered or intentionally inhibited immune responses, immunocompetent patients bring their full complement of T- and B-cells to the onset of critical illness. Indeed, immunocompetent critically ill patients do not seem to be dying from overwhelming viremia (Chilet et al., 2010; Limaye et al., 2008; von Muller et al., 2007), but there are several other mechanisms by which CMV reactivation might cause them disease. First is direct cytopathology from reactivated lytic virus injuring organs that harbor virus (Barry et al., 2000). Second is an immunopathologic effect, in which damage to tissues is a collateral consequence of the immune response to virus (Bolovan-Fritts et al., 2007; Grundy et al., 1987; Soderberg-Naucler, 2006). Finally,

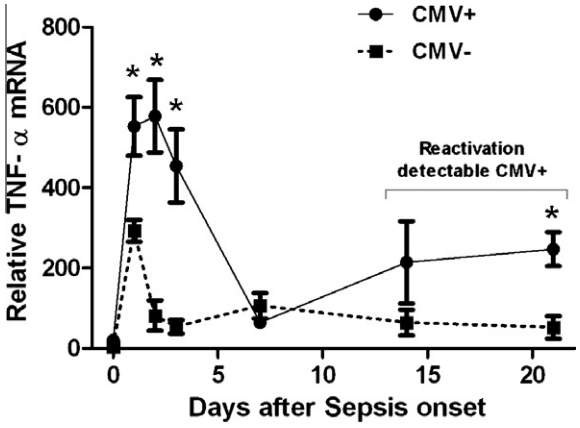

Fig. 1. Influence of previous cytomegalovirus infection on pulmonary TNF-α response to sepsis. BALB/c mice latently infected with 10^6 pfu Smith murine cytomegalovirus (CMV+) or naïve (CMV−) underwent sepsis by cecal ligation and puncture. Lung homogenates from cohorts (n = 3–5) were evaluated for TNF-α and β-actin mRNA 1, 2, 3, 7, 14 and 21 days after sepsis. TNF-α mRNA are expressed relative to β-actin and * indicates significant difference from CMV- mice (two-tailed Student's t-test $p < 0.05$). Figure produced from previously published data (Cook et al., 2006b).

CMV is known to make hosts susceptible to bacterial or fungal super-infection (Hamilton and Overall, 1978; Hamilton et al., 1976), and indeed there has been association shown between immunocompetent patients with CMV reactivation and bacterial infections (Chiche et al., 2009; Cook et al., 2003, 1998; Domart et al., 1990; Jaber et al., 2005). We therefore suspect that patients with CMV reactivation suffer these more insidious injuries to end organs, such as lungs, making them susceptible to poor outcomes. Our group has previously focused only on lungs, but many other organs are known to harbor latent virus (Collins et al., 1993) and might also be influenced by reactivation during critical illness.

One hypothesis that we are investigating is that latently infected immunocompetent hosts maintain anti-CMV defenses that carry pathologic potential during other illnesses. This concept was first proposed over 20 years ago, although in the context of immunosuppressed patients (Grundy et al., 1987). We have previously shown that CMV-latent mice are primed for an exaggerated pulmonary inflammatory response to sepsis, a phenomenon that we now term CMV-associated lung injury (CMV-ALI) (see Fig. 1, (Cook et al., 2006b)). Given the previously discussed innate and adaptive immune responses to CMV, it is possible that either or both could contribute to CMV-ALI. For example, CMV-specific inflationary CD8 T-effector memory (T_{EM}) cells are known to secrete TNF-α and IFN-γ in response to cognate antigen (Babel et al., 2009; Khan et al., 2007; Vescovini et al., 2007). We have recently shown that these same MCMV-specific CD8 T-cells are activated in MCMV-latent mice during sepsis (Forster et al., 2010). There is precedent for this hypothesis because T-cells in MCMV-latent mice activated non-specifically with anti-CD3 antibody cause a lethal "non-viral" pneumonitis (Tanaka et al., 1995, 1994). Similarly, T-memory has been shown to have immunopathologic potential in lungs following infection with other viruses, such as lymphochoriomeningitis virus and respiratory syncytial virus (Cannon et al., 1988; Chen et al., 2001). The clinical implication of these observations is significant when one considers that ganciclovir therapy does not prevent activation of these CMV-specific T-cells during sepsis (Forster et al., 2010). It is, therefore, possible that ganciclovir, which is effective for CMV-related disease in immunocompromised hosts, might not be as effective in immunocompetent hosts. Only by understanding CMV-related mechanisms of injury in immunocompetent hosts will we be able to develop the most effective therapies for these patients.

11.2. Barrier 2 – Identification of patients most at risk?

Although the Boeckh antiviral prophylaxis trial should begin to define the causative role for CMV reactivation and poor outcomes, a critical barrier to improving outcomes in these patients remains: how to identify those most at risk for CMV reactivation, as previously discussed, only ~1 of 3 latently infected critically ill patients will have CMV reactivation during their critical illness (Kalil and Florescu, 2009). Practically speaking this leaves two of three very sick patients in this clinical trial exposed to the risks of antiviral therapy – likely without benefit. It is, therefore, vital that methods be developed to allow better identification of those most "at-risk" for CMV in this patient population.

We and others have previously proposed that reactivation risk is a consequence of underlying latent viral load and the severity of illness (Cook, 2007). Nonetheless, despite exhaustive analyses including a recent meta-analysis of multiple available data sets it has been impossible to narrow the group at risk for reactivation beyond ~1/3, even when severity of illness is considered (Kalil and Florescu, 2009). Tissue viral load, however, has not been tested as a risk factor in humans, mainly because non-invasive methods to determine latent viral load in tissues do not exist. Interestingly, available animal data suggest that tissue latent viral load is a critical determinant of reactivation risk ((Reddehase et al., 1994) and our unpublished data). In addition, the magnitude of the host immune response to CMV seems directly proportional to the viral load during the original infection (Thomas et al., 2010), suggesting that host tissue viral load might be approximated by the immune response that it provokes. Confirmation of this hypothesis is ongoing in our laboratory.

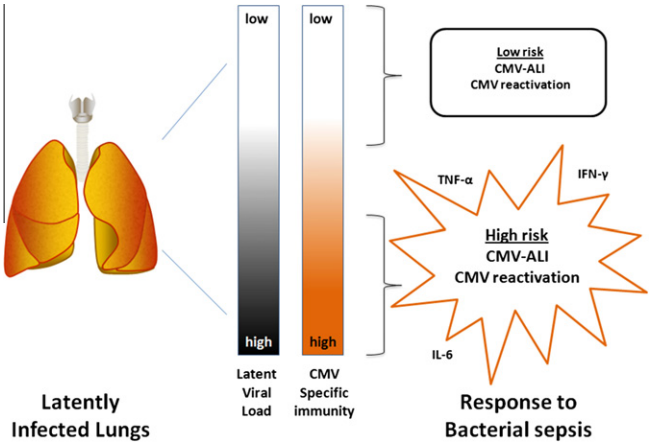

Fig. 2. Model for immunopathogenesis after cytomegalovirus infection. Following primary infection, cytomegalovirus (CMV) becomes latent in the lungs of the infected host. The conditions of the original infection determine lung latent viral load (Reddehase et al., 1994) as well as CMV-specific immunity, with high viral loads inducing "inflated" CMV-specific immunity (Thomas et al., 2010). During a bacterial septic challenge there is activation of CMV-specific immunity (Forster et al., 2010). In this model, hosts with heavy latent viral load and inflated immunity are prone to CMV reactivation and an exaggerated pulmonary inflammatory response (Cook et al., 2006b), leading to CMV-associated lung injury (CMV-ALI).

Independent of this correlation, recent literature suggests that CMV-related risk may come in another form in immunocompetent patients. The magnitude of the immune response to CMV has been recently associated with all cause mortality in the elderly (Roberts et al., 2010; Strandberg et al., 2009; Wang et al., 2010). In these population-based studies, patients with the highest quartile anti-CMV IgG antibody responses have worse all-cause mortality. Although it is somewhat counterintuitive, mice with the highest viral IgG titers are also at the highest risk for reactivation (Reddehase et al., 1994). While it is, therefore, tempting to speculate that CMV reactivation might be contributing to worsened outcomes in these high-titer patients, the relationship is likely to be much more complex. Elderly patients with chronic herpes virus infections are known to develop progressive immune senescence (Almanzar et al., 2005; Khan et al., 2004, 2002; Pawelec and Derhovanessian, in press), and the elevated antibody titers in some patients may simply be a consequence (or indicator) of their deteriorating cellular immunity.

Conversely, we have recently observed that anti-CMV IgG titers correlate very well with CMV-specific CD8 T-cells in mice (Cook et al., manuscript in preparation), so it is possible that patients with high CMV-specific IgG also have "inflated" CMV-specific T-memory. If these cells carry immunopathologic potential as previously discussed, then patients with memory inflation might prove to be those most predisposed to poor outcomes during critical illness (Fig. 2). We, therefore, suggest that CMV-related risk in immunocompetent hosts should be more broadly defined, combining both underlying latent viral load and corresponding reactivation risk together with underlying host anti-CMV immune responses. Although it may seem intuitive that the viral load and the immune response to it are inextricably intertwined, clear evidence linking either to disease in the critically ill has not been defined, and both

seem promising avenues to explore in order to identify those most likely to benefit from therapy.

11.3. Barrier 3 – New approaches to prevent reactivation

The third major barrier to progress is the paucity and toxicity of currently available antiviral drugs. Critically ill patients are by definition the sickest patients in our hospitals, and available antiviral therapies have borderline or unacceptable safety profiles, especially for the two of three patients that will not benefit from such therapy. The toxicities of Ganciclovir, Foscarnet and Cidofovir are well known (reviewed in (Biron, 2006; Prichard and Kern, in press)), with Ganciclovir being least toxic and currently the leading choice of these agents. Although Valganciclovir may have a somewhat better safety profile, its oral formulation precludes early use during critical illness when many patients have ileus and unreliable intestinal absorption. Moreover, four of the five FDA approved drugs to treat CMV (Ganciclovir, Valganciclovir, Foscarnet and Cidofovir) all target the DNA polymerase and emergence of drug resistant strains (harboring a range of mutations in either the UL97 kinase or UL54 polymerase genes) is a significant problem (Biron, 2006; Gilbert et al., 2002). The fifth approved drug, Fomivirsen, is an antisense oligonucleotide against the HCMV – immediate early-1 protein and is licensed only for intraocular use.

Unfortunately, there appear to be few prospects in the pipeline, with only two of 10 new antivirals recently reported to have anti-CMV activity (Dropulic and Cohen, 2010). One of the most promising was Maribavir, which showed potential in phase II clinical trials (Winston et al., 2008), but has since failed to show clinical efficacy for CMV prevention in phase III trials (http://www.fiercebiotech.com/press-releases/viropharma-incorporated-vphm-reports-results-phase-3-clinical-trial-maribavir-bone-ma). CMX-001 and AIC246 are candidates in phase II trials for CMV reactivation prevention (NCT00942305 and NCT01063829, respectively), but as such are realistically years away from availability (Dropulic and Cohen, 2010).

While an exhaustive review of future antivirals with anti-CMV potential is beyond the scope of this review, it is clear that there is a significant gap between need and available treatments that hopefully will be spanned in the near future. For now we must attempt to strike a balance between efficacy and toxicity with Ganciclovir, and hopefully this will favor efficacy as it has in transplant patients and our murine model (Cook et al., 2006b; Forster et al., 2010). If toxicity becomes an issue, the need for novel agents will become even more pressing. In addition, as suggested before, understanding how CMV injures immunocompetent patients might allow development of completely novel therapeutics to mitigate CMV-related risk and improve outcome during critical illness.

11.4. Barrier 4 – Lack of funding

Perhaps the biggest consequence of lack of pharmaceuticals in the pipeline is the consequent lack of funding for investigations in this area. Despite years of lobbying the pharmaceutical industry by numerous groups, without patent protected agents in the pipeline there has been limited interest in funding clinical trials. This is despite the fact that the market potential for critically ill patients is orders of magnitude larger than the transplant or HIV/AIDS patient populations. Most surprising to us is that companies with monitoring technology have not been more enthusiastic to support such studies, given that CMV-monitoring might play an integral role in determining treatment end points. This situation underscores the need for public funding of research in this area. Thankfully Boeckh et al. have been persuasive enough to secure funding for the requisite first clinical trial, and we expect that this potential market may see a pause in development until their trial results become available.

12. Limitations

In addition to the barriers outlined above, there remain numerous other limitations in our understanding of herpes family viruses during critical illness. As previously acknowledged, it is possible that CMV reactivation is merely an indicator of immune compromise and illness severity and that the worsened outcomes are merely associations. For example do burn patients show the highest rates of reactivation because of the profound immune compromise that they suffer following their burn injury, with superimposed bacterial colonization/infection of their open burn wounds? Further, although we have chosen to focus on CMV in this review, humans are frequently infected with multiple herpes viruses during a lifetime, including most commonly herpes simplex (HSV) and Epstein–Barr (EBV) viruses. Similar to CMV, HSV reactivates in immunocompetent hosts during critical illness (Bruynseels et al., 2003; Camps et al., 2002; Cook et al., 2003, 1998; Cushing et al., 1993; Hayden et al., 1994; Kagan et al., 1985; Linssen et al., 2008; Luyt et al., 2007; Ong et al., 2004; Porteous et al., 1984; Schuller, 1994). Some of these HSV studies have shown associations with worse outcome similar to CMV (Linssen et al., 2008; Luyt et al., 2007; Ong et al., 2004), and pathogen versus bystander also remains very much a question for HSV (Simoons-Smit et al., 2006). EBV has not been well studied during critical illness, but has been suggested to reactivate during times of stress in immunocompetent hosts (Glaser et al., 1999, 1994, 1991). Human herpes virus-6 also appears to reactivate, but the data for consequences of such reactivations virus are far less complete (Razonable et al., 2002). Given the myriad combinations of just these four viruses, trying to understand individual contributions of each may become problematic; leaving carefully controlled animal models as an important adjunct to clinical studies.

13. Conclusion

It is clear that the majority of our immunocompetent population harbors latent CMV, and that reactivation occurs in ~1/3rd of them when they become critically ill. Considering that millions of patients become critically ill every year, the potential for work in this field to significantly contribute to patient outcome is enormous. The Boeckh prophylaxis trial using ganciclovir represents a major first step, but it is clear that there are still major gaps in our understanding of CMV in these patients. Given the limitations inherent to both human subjects and animal models, continued traverse between bench and bedside will be required to close these most important gaps. It is our considered opinion that narrowing or closing these gaps will be imperative to continue moving this new field forward.

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