Review

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Cytomegalovirus infection in burns: a review

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Sepsis is responsible for significant morbidity and mortality in patients suffering from severe burn injuries. Burn patients are known to be immunocompromised, and it is generally accepted that the immunosuppressed patient may experience human cytomegalovirus (HCMV) infection and disease. Review of the very limited available literature identifies a seroconversion rate of between 18 and 22 % for burn patients who were seronegative for HCMV prior to suffering their burn injury. Furthermore, approximately 50 % of HCMV antibody-positive patients may reactivate. Blood products and allografted skin have clinically been identified as possible sources of HCMV transmission in burn patients. Experience in the treatment of infection or disease in burn patients is very scarce and limited to immunoglobulin therapy. Animal experiments have demonstrated that murine cytomegalovirus (MCMV)-seropositive skin grafts are able to infect immunodeficient mice as well as burned mice. Murine studies have also demonstrated that infection with MCMV enhances susceptibility to secondary bacterial infection and increases mortality in these animals. Burned mice challenged with MCMV have a significantly higher level of bacterial translocation to mesenteric lymph nodes than either control thermally injured mice without MCMV inoculation or non-burned mice injected with MCMV alone. In summary, it remains controversial whether HCMV infection *per se* alters outcome for the majority of burn patients. Subgroups of severely burned, seronegative patients may benefit from blood products and skin from seronegative donors. Antiviral strategies are not yet evaluated for the burn patient. Further investigations utilizing modern diagnostic techniques seem necessary.

General considerations

Clinically significant viral infections are increasingly being recognized in a variety of patients who are immunocompromised. The immunosuppressed patient may experience cytomegalovirus (CMV) infection, and this disease state carries with it considerable morbidity and mortality, ranging from febrile illness to extensive dissemination with organ manifestations such as pneumonia, encephalitis, hepatitis and colitis (Ljungman *et al.*, 2002). Included in this broad category of immunocompromised patients are allograft recipients of solid organs or bone marrow and stem-cell transplants (Shelby & Shanley, 1987; Mazzulli *et al.*, 1993), and trauma patients (Heininger *et al.*, 2001). Similarly, burn patients are known to be severely immunocompromised (O'Mahony *et al.*, 1985; for review, see Munster, 1996). Currently, there is general agreement that the functional capacity of T cells in these patients to perform their normal physiologic responses is impaired (Zedler *et al.*, 1999). Burn patients are particularly susceptible to infection and sepsis (Shires, 1991) as a result of their inherent immunosuppression as well as the loss of the cutaneous barrier and the use of invasive catheters, endotracheal tubes and other devices. Pneumonia, wound infection and sepsis

are responsible for greater than 50 % of the mortality of patients suffering severe burn injuries (Huang *et al.*, 1992). Routine treatment of severely thermally injured patients includes allogeneic skin application for temporary wound coverage of excised burn wounds. Allogeneic skin procurement follows the guidelines of the American Association of Tissue Banks and involves either cryopreservation or glycerol preservation (Vloemans *et al.*, 2002). Previous reports on trauma patients have correlated septic episodes with CMV infection (Heininger *et al.*, 2001), yet data linking morbidity to CMV infection in burns remains limited. A recent case of a patient with a 60 % total body surface area burn, septic episodes and concomitant blood CMV DNA levels of about 10 000 copies per millilitre of plasma (Hamprecht *et al.*, 2005), and a survey of CMV infection at German burn centres (Pfau *et al.*, 2004), prompted us to review the pertinent literature. This review summarizes the available experimental and clinical data on CMV infection as it relates to burns.

Animal experiments

Two murine animal studies utilizing a murine CMV (MCMV) strain (Shelby & Shanley, 1987; Kobayashi *et al.*, 1999) examined the role of skin-allograft-associated CMV

transmission by temporary skin grafting. In the study by Shelby & Stanley (1987), it was shown that transplanted donor-skin isografts from MCMV-infected animals were able to infect non-burned mice with a transmission rate between 50 and 60 %. In their report, these authors raised the question of the cellular source of the virus in the transplanted skin tissue, but were unable to definitively answer this question. Interestingly, *in vitro* experiments (Kobayashi *et al.*, 1999) with skin from CMV-latent mice failed to show replicating virus in culture by plaque methods in C127-I cells (Smee *et al.*, 1989); however, MCMV DNA and mRNA were retrieved from 70 % of these murine skin samples. In the study of Kobayashi *et al.* (1999), allogeneic skin from MCMV-infected mice was transplanted onto both burned and non-burned animals. The authors could not identify MCMV growth in the salivary gland in the non-burned and grafted mice, but a significant number of MCMV copies was detected in burned and grafted animals.

Several murine studies demonstrate that infection with CMV enhances susceptibility to secondary bacterial infection. In a series of experiments, Overall and his group (Hamilton *et al.*, 1976; Hamilton & Overall, 1978; Bale *et al.*, 1982) demonstrated that MCMV-infected mice were highly susceptible to infection by intestinal challenge with *Escherichia coli*, intravenous challenge with *Pseudomonas aeruginosa*, or combined infection with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. They concluded that MCMV infection decreases the inflammatory response by a diminished leukocyte migratory response, and as a result inhibits host resistance to bacterial infections. Mortality was significantly enhanced in the MCMV-infected animals compared to those animals infected with either the bacterial or the fungal pathogen alone.

The translocation of bacteria from the gastrointestinal tract to mesenteric lymph nodes and systemically is considered to be a possible source of infection in burns and other types of major stress (Tancrede & Andreumont, 1985). In burned mice challenged with MCMV, a significantly higher level of bacterial translocation to mesenteric lymph nodes was found compared to that of either control thermally injured mice without MCMV inoculation or non-burned mice injected with MCMV alone (Erickson *et al.*, 1990). In a subsequent experiment by Erickson *et al.* (1991), the authors reported a significant delay in the resolution of positive mesenteric lymph node cultures in thermally injured animals infected with MCMV compared to thermal insult or MCMV infection alone.

A recent mouse study evaluated the pathophysiology of the higher susceptibility to MCMV infection after thermal injury (Kobayashi *et al.*, 2001). These authors examined the role of the burn-associated T-cell response after burn injury. In their experiments, they found that 85 % of thermally injured BALB/c mice intraperitoneally inoculated with 2.5×10^5 p.f.u. MCMV died within 9 days. Unburned mice with a comparable inoculum had a significantly reduced mortality rate of 20 %. In subsequent experiments,

the same authors showed that normal splenic T cells from BALB/c mice could improve resistance to MCMV infection in immunodeficient mice and subsequently reduce mortality, while splenic T cells from thermally injured BALB/c mice were not able to improve resistance to MCMV infection.

Clinical experience

Reports concerning human cytomegalovirus (HCMV) infection in burn patients can be divided into two major categories of research papers and some case reports. The first group of research papers studies the epidemiology of CMV infection among patients with burn injuries. The second group of research papers addresses the question of HCMV transmission by transplanted allogeneic skin.

The first report of HCMV in a thermally injured patient was published in 1970 (Nash *et al.*, 1970). Later, Seeman & Konigova (1976) reviewed a series of 74 patients who suffered greater than 20 % total body surface area third degree burns, and who required skin grafting, blood transfusion and antibiotic regimens. Complement-fixing techniques revealed 27 % of the patients to be HCMV negative and 73 % to be HCMV positive. At least three of the deaths were assigned to pneumonia, and were felt to have resulted from a disseminated form of HCMV infection. In a follow-up paper, Seeman *et al.* (1980) reported a mortality rate attributable to systemic HCMV infection of 4.6 %.

Antibody titres in the serum of burned children have been analysed in both a retrospective study and a prospective study (Linnemann & MacMillan, 1981). In the latter, HCMV infection was diagnosed by a complement-fixing technique, and viral recovery was obtained from both throat swabs and urine. In the retrospective study, a significant increase in HCMV titres was identified in 22 % of the patients. In the prospective study, HCMV infection was diagnosed by virus isolation in 29 % of the patients, and serologically in 25 % of the patients, with an overall infection rate of 33 %. The prospective study directly correlated HCMV infection with more severe burns, more skin grafts and subsequent higher numbers of blood transfusions. The authors suggested that the suppression of cell-mediated immunity by the burn injury was a possible source of reactivation. In the prospective study, at least, the presumed source of primary HCMV infection seemed to be blood transfusions, as discussed earlier by Adler (1983). Both Linnemann & MacMillan (1981) and Deepe *et al.* (1982) observed a rise in HCMV antibodies during periods of unexplained fever and lymphocytosis.

In two similar surveys of burned patients (Kealey *et al.*, 1987; Bale *et al.*, 1990), it was shown that 22.5 and 18.6 %, respectively, of seronegative patients seroconverted. Among patients who were HCMV antibody positive, 56 and 52 %, respectively, reactivated, as assessed by a rise in titre of at least fourfold. However, differentiating passively acquired HCMV antibodies from production by an immunologic response was not addressed. Reactivation was associated

with the severity of the burn injury and the number of transfusions, as well as length of the hospital stay (Kealey *et al.*, 1987). For seronegative patients, seroconversion significantly correlated with the number of units of blood and blood products transfused, as well as the length of stay. No correlation could be established with relationship to total body surface area burn (Kealey *et al.*, 1987). Kealey and his group attributed seroconversion to transfusion and transplantation of seropositive blood products and allograft skin, respectively. They stated that HCMV infection did not appear to contribute to the development of morbidity or mortality in their patient population. In contrast, Kagan *et al.* (1985) demonstrated a higher incidence of septic episodes in HCMV-infected patients, as shown by serology. An association between the number of blood transfusions and the emergence of HCMV infections was identified; however, no correlation was found with respect to mortality.

Kealey *et al.* (1996) studied the incidence of HCMV transmission by skin allografts, and found that 22.7% of seronegative patients seroconverted after allograft transplantation. Unfortunately, the preservation methodology for the allografts was not stated. Significantly different viral potentials have been reported for fresh, cryopreserved and glycerol-preserved allograft temporary wound coverage (Van Baare *et al.*, 1994).

Destruction of the skin is another hallmark of burn injuries, and timely healing is essential in reducing morbidity and mortality. An interesting case report of three patients presented by Swanson & Feldman (1987) analysed HCMV infection and skin involvement. A shared clinical course of generalized non-healing wounds prompted microscopic evaluations, which revealed enlarged endothelial and periendothelial cells containing prominent intranuclear and intracytoplasmic inclusions diagnostic for HCMV. The authors concluded that HCMV infection of the skin may be more common than suggested by the few available reports. They did however note that it remains unclear in which way HCMV infection of the skin will systemically affect the individual patient.

Very recently, we published a detailed case analysis of a severely burned patient found to have HCMV infection in blood and lung (Hamprecht *et al.*, 2005). We could demonstrate that virus replication in blood was productive, with an HCMV DNA load in plasma of up to 10 000 copies per millilitre of plasma. Paralleling established observations of critically ill patients with sepsis (Heininger *et al.*, 2001), we investigated tracheal secretions in this patient for HCMV. HCMV DNA and HCMV late mRNA pp67 were detected in both haematologic and pulmonary-derived samples (bronchioalveolar lavage). Interestingly, it was not possible to demonstrate the presence of virus in blood in the form of pp65 antigenaemia. The course of the viral load in plasma gave evidence for a self-limiting virus replication in blood. Collectively, the information gained from the serologic follow-up of this severely burned patient underscores the very real potential to underestimate and inadequately

detect HCMV in burn patients. This patient received immediately on admission blood products of unknown initial serostatus. Thus it was impossible to rule out whether this patient suffered from viral reactivation or primary infection.

A therapeutic approach to the treatment of burn patients with elevated HCMV antibody titres was reported in two separate papers by Munster and coworkers (Munster *et al.*, 1987; Moran *et al.*, 1988), in which intravenous infusions of commercially available human immunoglobulin preparations were administered. Although IgG therapy led to the development of high titres of CMV-specific antibodies, the data could not indicate whether this therapy favourably influenced the clinical course of HCMV infection. At the present time, a review of the literature can establish no specific recommendation for the therapeutic antiviral use of ganciclovir, foscarnet or cidofovir for the treatment of HCMV in burn patients.

Conclusions

A review of HCMV and burns shows that severely thermally injured patients who are seronegative are at risk of developing HCMV seroconversion, while CMV antibody-positive patients may reactivate. HCMV infection in burn patients can lead to a broad spectrum of infection and disease, ranging from mild febrile illness to extensive viral dissemination and organ manifestation. While the burn injury alone can cause immunosuppression, HCMV infection itself is immunosuppressive by impairing or evading cell-mediated immunity (Reddehase, 2002). Experimental evidence has demonstrated that a significant thermal injury in mice causes susceptibility to MCMV infection. MCMV infection can lead to an increased susceptibility to bacterial infection, and may lead to an increased and prolonged incidence of bacterial translocation, which might additionally predispose to an increase in bacterial infections. Septic episodes remain a major cause of death in burn patients. Attributing bacterial sepsis and mortality directly to HCMV infection in burn patients remains controversial, as the causal relationship between bacterial sepsis and HCMV disease has not been directly examined. Severely thermally injured patients are routinely exposed to several sources of HCMV: they receive numerous blood and blood-product transfusions, and their acutely excised wounds are often temporarily covered with allogeneic skin. In order to minimize transfusion-associated transmission of HCMV to seronegative patients, filtered or leukocyte-depleted blood products should be considered for this patient population (Roback, 2002). Transfusion of leukocyte-depleted packed red cell units is the current standard modality in the authors' country, as well as in others. Seronegative blood products are another possible option. Until recently, few efforts appear to have been taken to distinguish between HCMV infection and HCMV disease in burn patients. In the literature, it remains controversial whether HCMV infection *per se* alters clinical outcome for the majority of burn patients. Antiviral strategies as utilized in myeloablated

Fig. 1. Diagnostic algorithm for monitoring herpesviral infections (HCMV and/or HSV) of severe burn patients. Abbreviations: GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase.

patients are not yet established for the burn patient. Further investigations are necessary to establish the value of an antiviral therapy or immunomodulatory regimen with regard to CMV disease and outcome. This review of HCMV infection and burn patients shows that mainly sero-epidemiologic studies have been performed to confirm HCMV infection. However, blood-product transfusions may have also served as a source of passive antibody transmission. As in the transplant patient population, the currently available diagnostic methods have to be compared in order to assess their diagnostic value (Preiser *et al.*, 2001). The available repertoire of modern diagnostic methods could identify those patients with extended HCMV infection as well as monitor subsequent disease development.

Based on our experience of the diagnosis of herpes virus infections in critically ill patients with sepsis (Heininger *et al.*, 2001), we would like to suggest the following diagnostic algorithm for detecting HCMV and HSV-mediated infection in severely burned patients (Fig. 1). An initial ELISA test reveals patients at risk of primary or secondary or recurrent HCMV/herpes simplex virus (HSV) infections. Qualitative PCR of leukocytes and plasma then allows for the accurate detection of DNAemia. Quantitative DNA detection using in-house or standardized commercially available protocols is required only in cases of positive plasma viral DNAemia. Additionally, in the case of patients respirationally automatically, viral cultures from tracheal secretions may help to establish a potential pathogenic role for HCMV or HSV.

For the future, in cases where positive virus results are obtained, quantitative detection of HCMV in both blood and lung, and HSV in throat and lung, may be required to establish the clinical relevance of HCMV and HSV in burn patients, and further studies may also be needed to analyse the potential benefit of ganciclovir or aciclovir treatment in these patients.

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