CHAPTER 11

OVERCOMING THE PROBLEM OF CYTOMEGALOVIRUS INFECTION AFTER ORGAN TRANSPLANTATION: CALLING FOR HERACLES?

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ABSTRACT

Although diagnosis of Cytomegalovirus (CMV) infections and treatment of CMV disease with effective antiviral drugs have become much easier, the persistent problem of CMV infection after solid-organ transplantation still requires solid knowledge of the pathophysiology of its clinical manifestations in order to minimize the impact of CMV infections in the future. The complex symptomatology of CMV infection after solid-organ transplantation is reviewed as well as some of the new theories attempting to explain the myriad of symptoms, seen after transplantation.

11.1 INTRODUCTION

Although a range of reliable and accurate diagnostic tests as well as effective antivirals is available, CMV infections remain a substantial clinical problem in modern organ transplantation, even now at the close of this century. Compared to 10-15 years ago, a substantial part of clinical problems caused by CMV have been solved. For instance CMV-related deaths are now relatively rare in solid-organ transplantation, but new problems have emerged. Aside from the warning signs of resistance of CMV to antivirals due to increasing (prophylactic?) use of those drugs in modern intensive immunosuppressive protocols, various complications have appeared in the aftermath of transplantation, some of which point to CMV as the culprit. For instance, CMV may be involved in the process of chronic transplant dysfunction ('chronic rejection') as reviewed by Lautenschlager [1] while others [2, 3] suspect a role for CMV in case of accelerated coronary atherosclerosis found in patients with CMV infection after heart transplantation. The necessity to solve old problems while at the same time new nuisances emerge reminds us somewhat of the Greek legend of the monster Hydra. According to this legend Hydra was a monster with nine heads (the number varies), the center one being immortal. The monster's haunt was the marshes of Lerna near Argos. The destruction of Hydra was one of the 12 labors of Heracles, which he accomplished with the assistance of his nephew Iolaus. The problem with this monster was that if one of his heads was cut off, two grew in its place. Therefore, Heracles decided to burn out the roots with firebrands and at last severed the immortal head from the body. So, to overcome new problems attributed to CMV after solving old ones, we probably need a solution such as that used by Heracles in the legend. However, as we do not appear to be on the verge of evolving a method of killing the virus in its latent state ('the immortal head') without causing lethal damage to its host, CMV infections will remain a problem in clinical solid-organ transplantation.

11.2 RISK FACTORS FOR CMV DISEASE

As far as symptomatology of CMV infection is concerned it is known that a substantial part of patients with an active CMV infection is asymptomatic. Risk factors for CMV disease are well recognized and include the following: transplanting a CMV-seronegative patient with a CMV-seropositive donor organ (leading to a primary infection) and the recipient's net state of immunosuppression, determined by

the characteristics of the immunosuppressive protocol (type, dose, duration, timing) and various host factors (co-morbidity, age, uremia, neutropenia, infections with other immunomodulating viruses) [4]. Bruning et al. [5] suggested an additional important role of allostimulation by the graft in the process of reactivation of CMV in a rat model.

As far as the type of immunosuppression is concerned, especially antilymphocyte antibodies and monoclonal antibody preparations such as OKT3 are well known to cause a high incidence of CMV disease when used for either induction or anti-rejection therapy. While the immunosuppressive potency of this class of drugs is usually suggested as the explanation for this high incidence of infection, other mechanisms might be involved. Recently a novel mechanism of reactivation has been proposed: the proinflammatory cytokine TNF-□ has been suspected to play a role in the reactivation of CMV by stimulation of the CMV major immediate early enhancer/promoter [6, 7, 8]. Since the use of OKT3 is well known for its abundant cytokine release (i.e. TNF-□) this might explain the high incidence of active CMV infection during OKT3 treatment. Finally, viral load might be an important factor. Several authors stress the importance of a high viral load in relation to the risk of clinical relevant CMV disease [4, 9-13].

11.3 SYMPTOMATOLOGY

Although in the era of rapid diagnostic tests for CMV and effective antivirals one might expect that the effect of CMV would fade away, CMV infections still have a substantial impact on graft and patient survival in solid-organ transplantation [4, 9, 14-22]. Of all patients who develop clinical manifestations of CMV infection more than 90% do so within 1□6 months after transplantation, and 60% of the febrile episodes during this period are due to CMV infections [4, 9]. Because of more potent immunosuppression used nowadays (with induction schemes including monoclonal and polyclonal anti-T-cell antibodies), the timetable of infectious complications after transplantation tends to 'shift to the left' [4], resulting in earlier appearance of clinically significant CMV infections. When patients are symptomatic, symptoms may vary greatly. Most of the patients have a so-called 'self-limiting syndrome', consisting of fever (often spiking), arthralgia, leukopenia and/or thrombocytopenia, and abnormal liver enzymes [4, 9].

With tapering of the immunosuppressive therapy, the great majority of patients recover completely from the syndrome. Aside from this self-limiting syndrome, CMV may cause a myriad of symptoms in the grafted patient. Gastrointestinal symptoms during CMV infections are numerous. They include gastrointestinal ulcers that may bleed or perforate (i.e. gastric and colonic ulcers) [4, 9, 23-28]. CMV associated vasculitis might be the common pathogenetic mechanism of this manifestation of the CMV syndrome in the immunocompromised patient [29]. Other gastrointestinal symptoms comprise pancreatitis [4, 9], granulomatous hepatitis [30] and pneumatosis intestinalis [4, 9]. The latter condition is of particular interest because the cysts may perforate, causing a sterile pneumoperitoneum. As a consequence, free air may be present under the diaphragm on chest X-ray; the awareness of the association of this condition with an active CMV infection may avoid unnecessary surgical intervention [31, 32]. Gastrointestinal symptoms may be present without other major symptoms of infection, and CMV may be present in the gastroduodenal tract without symptoms.

Franzin et al. [33] found evidence of CMV inclusion bodies in biopsies collected from the gastroduodenal mucosa of patients with a renal allograft in 9 out of 20 cases. The presence of these CMV inclusions was unrelated to viremia-induced or gastrointestinal symptoms at the time of endoscopy [33]. Several other symptoms have been attributed to CMV: lymphadenopathy, hepatosplenomegaly, pericarditis, myocarditis, encephalitis, retinitis, and skin ulcerations associated with vasculitis [4, 9, 34, 35]. CMV-induced Guillain-Barré syndrome is a well-known phenomenon [36] and is seen relatively frequently in AIDS patients but is a rarely encountered sequel of an active CMV infection in transplanted patient. In AIDS patients direct infection of the nerves has been suggested to play a role in the pathophysiology of the syndrome, as well as autoimmune phenomena elicited by the virus [37, 38]. The reasons why the incidence of this condition differs so greatly between the AIDS- and the transplanted population are unknown, but one may speculate about the significance of some overt differences that exist between the two groups. For instance, although AIDS patients are also immunosuppressed, the nature of this suppression differs considerably, which might reflect differences in sequelae leading to a possible autoimmune process elicited by the virus. Another item that might be important in order to explain the dissimilarity is the duration of viremia. In contrast to AIDS patients, in whom prolonged CMV viremia is the rule, duration of viremia is usually short in transplanted patients, being mostly confined to the period of maximum immunosuppression, i.e. shortly after initiation of anti-rejection therapy. One might speculate about the importance of prolonged viremia as a prerequisite for the development of autoimmune phenomena. If the latter is true this might be an important

clue in the pathophysiology of a patient characterized by an unusually prolonged period of CMV viremia, very recently described by De Maar et al. [38]. This patient presented with a chronic inflammatory demyelinating polyneuropathy after renal transplantation during recurrent CMV viremia. It is important, however, to stress that great caution should be exercised in designating CMV as the culprit in case of a given symptom: a single positive laboratory test may be not enough to consider the symptoms present as induced by CMV. Other possible causes must be excluded and laboratory signs for CMV infection have to be judged in concert with other signs of CMV infection.

CMV pneumonia after solid-organ transplantation is the manifestation that distinguishes serious illness from the more benign disease, and the condition is associated with high mortality, especially when assisted ventilation is required [4, 9, 39]. However, although still feared in the bone marrow transplantation, with the emergence of rapid diagnostic tests and the availability of effective prophylactic schemes as well as effective antivirals to treat CMV infection in an early stage, the incidence of CMV pneumonia has dramatically dropped.

Renal involvement during CMV infection remains controversial as far as pathogenesis is concerned. It has been suggested that CMV could trigger the immune mechanism of acute rejection either via i.e. increased MHC class II expression in the graft or via mimicry, since CMV was shown to have sequence homology and immunologic cross-reactivity with the HLA-DR β-chain [40, 41]. More recently Reinke et al. [42] described a series of patients with asymptomatic CMV infections which they linked with late acute rejection.

Alternatively, in 1981, Richardson et. al. [43] described a distinctive pattern of glomerular injury in renal allografts that they associated with CMV viremia without relation with allograft rejection. The pathological features consisted of diffuse endothelial hypertrophy and necrosis, accompanied by an accumulation of fine fibrillar webs of periodic-acid-Schiff (PAS)-positive material and mononuclear cells that resulted in obliteration of the glomerular capillaries. Fibrin, IgM as well as C3 were found by immununofluorescence. No viral particles were detectable by electron microscopy of immunofluorescenc using monoclonal antibodies directed to CMV early and late antigen [43].

The existence of this condition has been disputed by Herrera et al. [44] and Boyce et al. [45] who consider it to be a form of (vascular type or 'transplant glomerulopathy') rejection.

Aside from the directly attributable syndromes there are important indirect effects of CMV in the compromised host. CMV has important immunomodulating effects [4, 9] that contribute significantly to 'the net immunosuppressive' state of

a given patient, making him or her prone to superinfections with i.e. *Aspergillus* species and *Pneumocystis carinii* [4, 9, 46].

In a recent multicenter study by the Boston Center for Liver Transplantation a multivariate analysis showed that patients with CMV disease had significant more invasive fungal disease and bacteremia [47].

11.4 PATHOFYSIOLOGY OF CMV INFECTION

How CMV causes symptomatology and organ dysfunction in the recipient is still rather enigmatic and the subject of much debate and research. The question is whether symptomatology is caused by the cytopathic effect of the virus itself or is brought about by the (innate as well as specific) immune response elicited by the virus or virus infected cells (i.e. endothelial cells). On the one hand a clear relation exists between the viral load and the likelihood and the severity of CMV disease [4, 9-12], but on the other hand it has been known for years that notably the number of antigen-positive leukocytes (pp65 antigenemia), which has been shown to correlate well with viral load [48], is not always a reflection of the virtual severity of the clinical situation. The question remains unanswered whether this discrepancy is caused by a high viral load with a less virulent virus or a more mitigated immune response to the same virus. For instance there exists a lot of data indicating that virus-induced pneumonitis is not only the result of uncontrolled virus replication in the immunocompromised host, who is unable to control viral replication, but is rather the result of a T-cell-mediated immune response induced by viral antigens [49]. So, the protean manifestations of CMV seen after solid organ transplantation suggest that unrevealing of the pathophysiology will most likely reveal a very complex and multifaceted mechanism. Is CMV-induced endothelial cell damage ('vasculitis') (with or without secondary immune response directed to the infected endothelial cells) the key factor? The key role of the endothelial cell in the pathophysiology is in agreement with the work of Persoons et al. [50] using a rat model. They found that in this infection model multiple-organ involvement was associated with disseminated vascular pathology. CMV-infected endothelial cells might secondarily induce adhesion of leukocytes to the damaged endothelium [51], trigger the coagulation system [52] and induce cytokines like IL6 [53] which might contribute to the pathophysiology of CMV-induced multi organ disease. CMV also infects endothelial cells of transplanted patients; these cells may even detach from the vessel wall and subsequently be released in the circulation [54]. In the mono-

nuclear cell fraction of peripheral blood of a patient with a CMV infection after heart transplantation Grefte et al. [54] unexpectedly found distinctly large cells (35-45 μm diameter), reminiscent of the classical 'cytomegalic inclusion body cells'. In a subsequent study Grefte et al. [55] showed that those cells could be demonstrated in a substantial part of patients with an active CMV infection after solid-organ transplantation, especially in those with a high viral load in their blood. Immunostaining revealed those cells to be endothelial cells positive for HCMV antigens of all three stages of the viral replication cycle (with the typical nuclear and cytoplasmic localization of the distinct antigens) [54], while transmission electron microscopic studies showed that viral capsids, viral particles and dense bodies were present in the in the nucleus and cytoplasm, respectively, indicating that those cells represent a site of virus production [55].

Whether those cytomegalic endothelial cells play a role in the dissemination of the virus and/or organ dysfunction is far from settled, but it surely fits in with the data obtained from animal models [50-53] that suggest a key role for the CMV-infected endothelial cells in the pathophysiology of CMV infection. CMV infection may thus be looked on as a systemic disease with a multifaceted symptomatology. In this respect, it is of interest that subclinical organ involvement can be demonstrated in the majority of patients after organ transplantation even with an asymptomatic CMV infection [56]. A majority of patients with an active CMV infection after renal transplantation show subtle pulmonary dysfunction (decreased diffusion capacity for carbon monoxide) without clinical pulmonary symptoms [56]. This may point to subclinical pneumonitis due to either a direct cytopathic effect of CMV on pulmonary tissue, or to cellular immune response directed to e.g. infected pulmonary endothelial cells, with or without serum complement activation by the classical or alternate activation pathway [56, 57]. Another explanation might be that large cytomegalic endothelial cells 35-45 μm in diameter [54] plug into the lung capillaries, which have a diameter of only 5 μm. This was subsequently studied by De Maar et al. [58] using a method by which both components of diffusion of CO could be studied separately; the membrane factor Dm (i.e. affected by edema of the alveolar membrane) and the capillary ('blood') factor Vcap, which is most likely to be affected by plugging of those large cells into the capillary bed. We concluded from this study that, since both Dm and the Vcap decreased during active CMV infection, the decreased diffusion capacity for CO was not caused by plugging of the cytomegalic endothelial cells only [58]. Other factors might be involved: i.e. since they contain active replicating virus [54, 55], the cytomegalic endothelial cells may lead to spreading of the virus into the capillary bed which in turn together with complement activation and/or locally induced cytokine production [51, 53, 59], might

cause edema of the alveolar membrane and hence to the decreased Dm found in these patients [58]. Recently, we have also shown subclinical involvement of the gastro-intestinal tract [60]. Increased intestinal permeability indicating intestinal epithelial damage has been found in a substantial number of patients with an active CMV infection after renal transplantation [60]. Although this also points towards a more systemic nature of CMV infections, the exact reasons for those findings still have to be elucidate.

11.5 SUMMARY AND CONCLUSIONS

Even in modern transplantation, with the availability of rapid diagnostic tests and effective antiviral drugs, CMV infections remain the single most important infectious complication after solid-organ transplantation. In the last decade much has been learned about its pathophysiology, although it requires much more thought to comprehend the exact mechanism of the protean manifestations of CMV infection after organ transplantation. Since we continue to meddle in the inevitable marriage between CMV and host immunity, introducing new, non-selective immunosuppressive drugs, we probably need a 'Heracles-like' solution for solving the CMV problem in modern transplantation. Since it is very unlikely that anti-CMV drugs will become available in the near future to influence the latent state of this virus by nontoxic means, CMV will remain a serious problem, even at the dawn of the new millennium.

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