Review

Cytopathology or immunopathology? The puzzle of cytomegalovirus pneumonitis revisited

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Summary:

Various hypotheses have been proposed to explain why cytomegalovirus pneumonitis (CMV-P) is frequent and severe in bone marrow transplant patients while remaining rare and mild in HIV infected patients. One hypothesis suggests that CMV-P is an immunopathological condition that is common in bone marrow transplantation (BMT) under the effects of an abnormally regenerating immune system that reacts against CMV infected lung tissue. Such a hypothesis implicates CD4 T lymphocytes as one of the critical cell populations involved in immunopathology and also suggests that this process would be aborted by CD4 T cell deficiency in HIV infection. However, studies correlating the onset of CMV-P with lymphocyte reconstitution following BMT have revealed that CD4 cells are present at very low frequencies in the blood during the early period after transplantation when most cases of CMV-P occur. Furthermore, studies directly investigating bronchoalveolar lavage cell types during episodes of CMV-P in BMT patients have also failed to demonstrate significant CD4 involvement and, instead, have emphasized a predominance of natural killer (NK) cells and CD8 cells. These findings serve as the basis for questioning the validity of a CD4-driven immunopathological model of CMV-P in BMT. On the other hand, a variety of experimental and clinical observations support the protective role of CMV-specific CD3⁺ CD8 T lymphocytes against CMV in both immunocompetent individuals and BMT patients. In a murine BMT model, adoptive transfer of syngeneic BM cells was associated with massive increases in lung CD8 cells which resulted in the resolution rather than the exacerbation of existing CMV-P. In the light of these findings a more plausible hypothesis for CMV-P in BMT is that during the early period after transplantation adequate protective CD8 responses are absent and an uncontrolled CMV proliferation is allowed to develop. Once a critical viral load is reached a cytokine ‘storm’ may be triggered in the lung tissue that aggravates direct CMV-associated cytopathic effects. Likely candidates for this process would include the release of tumour necrosis factor-alpha (TNF-α) from alveolar macrophages stimulated by interferon-gamma (IFN-γ) released from NK cells that are reconstituted early after BMT. Bone Marrow Transplantation (2000) 26, 591–597.

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It has been more than a decade since the publication of a hypothesis that suggested that CMV-P in transplant recipients had an immunopathological basis.¹ Such a hypothesis addressed the need for an explanation as to why CMV-P in organ transplants in general and bone marrow transplants in particular was so severe, whilst in HIV infected individuals it was mild. However, the immunopathological hypothesis for CMV-P in man has not been universally accepted. Indeed, an alternative hypothesis, referred to here as the cytopathological model, has also been put forward that seeks to explain the pathogenesis of CMV-P in BMT as a result of uncontrolled CMV proliferation in the absence of immunoprotective CD8 cells. This hypothesis has been proposed on the basis of both murine² and human³,⁴ studies. The cytopathological hypothesis does not deny that CMV may trigger an immune-mediated damage through stimulating cytokine release, but it doubts that this pathogenetic process is aggravated and/or triggered by CMV-specific CD4⁺ or CD8⁺ T lymphocytes.

This review paper will assess the merits of each hypothesis in the light of recent observations and attention will be given to a critical evaluation of the observations on cells in the bronchoalveolar lavage (BAL) taken from patients with CMV-P.

The immunopathological hypothesis

The influential paper by Grundy et al¹ derived evidence for their hypothesis from both murine studies and clinical observations. In an experimental murine graft-versus-host (GVH) system, infection with murine CMV (MCMV) augmented the GVH reaction and led to the development of a fatal pneumonitis not seen with either MCMV or GVH alone.⁵ Moreover, examination of the lungs of mice who
developed pneumonitis with GVH and MCMV revealed mainly T lymphocytes of donor origin.\textsuperscript{6} Further murine experiments supported the role of T lymphocytes in the development of CMV-P by showing that MCMV given to athymic nude mice proliferated extensively in the lung but did not give rise to pneumonitis until the terminal stages of infection. However, when T cell immunity was allowed to reconstitute prior to MCMV challenge pneumonitis rapidly developed.\textsuperscript{1}

In addition to these murine studies, Grundy also drew on data from clinical observations to support the immunopathological model. It was noted that although the antiviral drug ganciclovir reduced the CMV load in the lungs of BMT patients with established CMV-P by more than 99.99\%, nine out of 10 treated patients died from their pneumonitis.\textsuperscript{7} To find only a limited clinical benefit from antiviral treatment despite its apparent virological efficacy suggested to the authors that factors other than direct viral cytopathic process and through stimulating the local release of cytokines such as TNF-\alpha were involved in the pathogenesis of CMV-P. However, this finding could be explained by the onset of adult respiratory distress syndrome (ARDS) provoked by CMV-P\textsuperscript{8} that would not be expected to resolve with antiviral medication.

Additional clinical information observed in patients following BMT was also cited as evidence in support of the immunopathological model. It was noted that GVH was often associated with CMV-P, while CMV-P was rare amongst patients receiving syngeneic BMT where GVH is not expected to occur. Lastly, studies in HIV infected patients revealed that CMV-P is rare and generally not severe in this group. It has been suggested that the progressive depletion of CD4 cells attributed to HIV infection may protect against immune-mediated lung damage.\textsuperscript{1}

Thus, the immunopathological hypothesis as proposed by Grundy et al has placed an emphasis on the hypothetical adverse effects of CD3\textsuperscript{+} T cell responses during the pathogenesis of CMV-P and implicated the CD4 T cell populations in this pathology.

The cytopathological hypothesis

This hypothesis emphasizes that antigen-specific memory CD8\textsuperscript{+} T cells are crucial for protection against CMV-P. Thus the pathogenesis of CMV-P can be explained by uncontrolled CMV replication in the lung due to the absence of adequate numbers of immunoprotective CD8 cells during the early stages of bone marrow reconstitution. It is suggested here that once critical CMV viral loads are reached, lung damage may be caused both by a direct viral cytopathic process and through stimulating the local release of cytokines such as TNF-\alpha. The importance of blood viral load in determining the onset of CMV disease has been demonstrated in BMT patients.\textsuperscript{9}

Evidence for the cytopathological hypothesis has been provided by murine studies and also from clinical observations in BMT patients. In a carefully controlled murine experimental syngeneic BMT system, it has been shown that following haematoablation by \(-\)irradiation, the MCMV infected mice developed fatal multiple organ CMV disease. This lethal disease was, however, prevented by giving adequate doses of syngeneic BM cells.\textsuperscript{10} Examination of the lungs in these BM recipients revealed increases in alveolar macrophages as well as the infiltration of the lung parenchyma by CD8\textsuperscript{+} T cells. When the numbers of these CD8 cells in the lung were quantified, they were found to be 30 times higher than in the lungs after BMT without infection, and 120 times higher than seen in normal lungs.\textsuperscript{11} Importantly, this cellular CD8 infiltration was associated with the resolution of pneumonitis.

The protective role of CD8 cells against MCMV has been further investigated in a murine model where it was shown that depletion of CD8 cells with monoclonal antibodies after BMT in mice infected with MCMV resulted in expanded foci of CMV infected cells in the lungs and resulted in lethal infection. However, depletion of CD4 cells resulted in scattered, singly infected cells and was not associated with significant lethality.\textsuperscript{12}

Studies of BMT patients have also stressed the importance of CD8 cells in protection against CMV disease. In HLA-matched CMV-seropositive human donors, cytotoxicity against CMV infected autologous fibroblasts was mediated by CD8 cells.\textsuperscript{4} In this study, CMV-specific cytotoxic T lymphocytes were detected in only 10 out of 20 patients at 3 months post BMT. None of these 10 patients developed CMV-P, whereas six out of the 10 patients with undetectable CMV-specific CTL responses died from CMV-P. The same group has subsequently demonstrated that infusions of CMV-specific CD8 cells were well tolerated and protected BMT patients at risk from developing CMV disease.\textsuperscript{13} It is interesting to note that CMV-specific CD8 cells have not yet been given to patients with already established CMV – partly due to the fear that this treatment might exacerbate the pneumonitis through an immunopathological mechanism. However, there are sporadic published cases in which established CMV-P, refractory to antiviral treatment, responded to a leukocyte infusion from a CMV seropositive donor in one case,\textsuperscript{14} and to an autologous, expanded T cell infusion in another.\textsuperscript{15} In neither of these cases was there a significant deterioration of the pneumonitis.

Experience from lung transplant patients, who have a high risk of developing CMV-P, has demonstrated the importance of CD4\textsuperscript{+} T lymphocytes in protection against CMV disease. One group has shown that lung transplant patients who failed to generate CMV-specific CD4\textsuperscript{+} T cell responses for a prolonged period of time post transplant all had recurrent CMV disease.\textsuperscript{16} The mechanism of CD4\textsuperscript{+} lymphocyte protection in the lung transplant model may be to augment CMV-specific cytotoxic effector CD8\textsuperscript{+} T lymphocytes. Indeed, the limited duration of protection against CMV noted in BMT patients given infusions of CMV-specific CD8\textsuperscript{+} lymphocytes\textsuperscript{13} may be due to the lack of CD4\textsuperscript{+} cell help.

Cell reconstitution in bone marrow transplantation

Following BMT, there is a characteristic period within 7–10 weeks post transplant when CMV-P tends to occur.\textsuperscript{17–19} Knowledge of both the rate of CD3\textsuperscript{+} T and NK cell reconstitution in blood and also the selective migration of these
cell types to the lung following BMT are important factors for an understanding of the mechanisms of CMV-P, since these events determine which cells are available to participate in protective or destructive immune responses during the critical period when CMV-P occurs.

The rate of cell reconstitution following BMT is dependent on a number of factors such as the age of the recipient,20 the histocompatibility between donor and recipient,21 the dose of transplanted CD34+ cells22 and whether the bone marrow is manipulated to deplete T lymphocytes in order to reduce the risk of GVHD.23,24 Several studies examining the phenotypic reconstitution following BMT have concurred with the findings of an early recovery of natural killer (NK) cells, a slower recovery of CD8 cells, and, in adults, a particularly protracted recovery of functional CD4 cells.21,25–27 Importantly, the reconstituted CD4 cells were functionally impaired as measured by proliferative responses to both mitogens and specific antigens.21,27 and they were also found to have defects in their production of interleukin-2.28 In this latter study, reconstituted CD8 cells were also found to be deficient in their production of interferon-γ.

The effects of T cell depletion on the rate of cell regeneration have been investigated in a small study of 23 BMT patients who underwent either full T cell depletion (TCD) with Campath-1, partial TCD or were given unmanipulated grafts.23 In this study, full TCD was defined as a greater than two log reduction of CD3+ cells and partial TCD was defined as a reduction of one to two log of CD3+ cells. There was a significant delay in CD4 cell recovery in the blood in those undergoing partial or full TCD, whereas those who were given unmanipulated grafts had CD4 numbers that reached the lower limit of the normal range at 6 months. These results have confirmed previous studies using T cell-specific depletion with the combination of CD6 and CD8 monoclonal antibodies.25 In both of these studies, CD8 recovery was more rapid and vigorous in the unmanipulated grafts, whilst those with full TCD had CD8 numbers that just reached the lower limit of the normal range after 6 months. Another study comparing T cell depletion using T cell-specific depletion with the combination of CD6 and CD8 monoclonal antibodies revealed that there were significant differences in T cell numbers between the two groups only during the first 3 months after transplantation.29

One prediction of the cytopathological hypothesis would be that episodes of CMV-P should be more common following TCD BMT when compared to unmanipulated grafts. This has been supported by a small study of 29 patients that showed that CMV infection occurred both more frequently and earlier in the TCD group as opposed to those receiving unmanipulated BMT.30 These investigators noted that there was a blood lymphocytosis composed of NK cells and/or CD8 cells associated with the episodes of CMV infection.

An important observation from BMT patients has been the increase of CMV-P in those with GVHD. It was noted that CMV-P was absent in a study of 100 cases of syngeneic BMT from identical twins,31 where GVHD is not expected to occur. In contrast, BMT patients with chronic GVHD were found to have significantly higher rates of infectious complications, including CMV-P, than those without chronic GVHD.32 The increased incidence of CMV-P in BMT patients with GVHD has been attributed to an immunopathological mechanism,1 whereby donor marrow T lymphocytes are thought to react both against host tissue and the CMV-infected lung. However, an alternative explanation is that the immunosuppressive effects both of GVHD and the therapy given to control it, result in profound delays to antigen-(CMV)-specific T cell reconstitution. Thus, GVHD results in particularly prolonged immunosuppression and may account for the cases of CMV-P that occur later than seen in BMT patients undergoing an uncomplicated course without GVHD.33,34

A similar view can be applied to understanding why the incidence of CMV-P in autologous BMT is much lower than in allogeneic BMT. In autologous bone marrow transplantation CMV-specific CD8 and CD4 responses are restored in a large proportion of CMV seropositive recipients by 3 months post transplantation.35 This can be compared with the allografted transplants, where CD4 and CD8 T cell reconstitution is slower and consequently the time available for CMV replication is longer.

Thus, the evidence from bone marrow transplantation can be interpreted as being supportive of the cytopathological hypothesis that in the early post-transplant period CMV-P is due to viral proliferation in the absence of adequate CD8+ T cell control.

**BAL studies during CMV-P in bone marrow transplant patients**

The pathogenesis of CMV-P can perhaps best be understood by investigating the events that occur in the lung during CMV-P. Among the few papers published on this subject, Milburn et al36 reported on 10 episodes of pneumonitis associated with CMV in recipients of T cell-depleted BMT and compared these with nine episodes of pneumonitis due to other infectious and non-infectious causes. The results revealed that there was an increase in BAL lymphocytes in all patients with pneumonitis of any aetiology compared with controls, and interestingly, the BAL lymphocyte counts of those with CMV-P were less than those with pneumonitis due to causes other than CMV. In CMV-P, increased proportions of non-T, non-B (probably NK) cells were seen. In this particular study a bona fide T cell marker such as CD3 was not used, and the CD2+ cells seen among BAL lymphocytes could have been NK or T cells because both of these cell types express the sheep erythrocyte CD2 receptor.37 Another early study of BAL cell types during episodes of CMV-P also revealed an increase of lymphocytes with the morphological characteristics of NK cells in patients who died of CMV-P.38 Both of these papers failed to comment on the relationship between the timing of the bronchoscopies after BMT and the BAL cell phenotypes.

Further support for the role of NK cells in CMV-P has been provided by a study that investigated the cytotoxic activity of cells from the BAL of patients with and without CMV-P. Cytotoxic activity in patients with CMV-P, as measured by lysis of CMV infected target cells, was mediated in part through lymphocytes with the characteristics of NK cells, since there was no dependence on MHC.
class I restriction of the target cells. In addition, it was shown that depletion of NK cells abrogated this cytotoxic activity.39

The bulk of the published BAL data emphasizes a predominance of NK cells and CD8 T cells in the early risk period for CMV-P reflecting the cell phenotypes present in the blood during the same period. Nevertheless, there have been some exceptions. One group examined cryopreserved lung tissue in 12 BMT patients, 11 of whom died of interstitial pneumonitis, including seven who had CMV-P. The patients all had TCD BMT and the median time to onset of interstitial pneumonitis was 71 days after transplantation. The investigators found an absence of CD56+ (NK) cells, a large number of CD40+ B cells and CD68+ macrophages and twice as many CD4+ cells than CD8+ cells. These histopathological observations appear to be at variance with other studies investigating blood and BAL samples in cell suspensions. It remains to be determined whether in lung tissues CD4-positive staining of macrophages contributes to the CD4 counts. It is known that monocytes and macrophages are CD4+.41

In order to demonstrate the typical findings of cell phenotypes present in the lung and blood during the early post-transplant risk period for CMV-P, we have provided an illustrative example from a BMT patient (Figure 1). The patient received an unrelated, single locus mismatched donor marrow that was T cell depleted with Campath-1, 10 weeks prior to the bronchoscopy. A BAL was performed for an unexplained fever, although the patient had no respiratory symptoms. No respiratory pathogen was subsequently isolated. Flow cytometric analysis revealed the overwhelming dominance of CD56+ CD3– NK cells in the lavage (Figure 1a, b) and also the blood (Figure 1c, d) at this time.

Cytomegalovirus pneumonitis in HIV infection

Post-mortem studies have revealed that in patients who have died from AIDS for a variety of different reasons CMV virus was widely distributed in many tissues, of which the lung was one of the most frequently infected organs.42,43 Despite these findings, pneumonitis is a rare clinical feature of CMV disease in this population when compared with other CMV-related clinical complications such as retinitis, gastroenteritis and encephalomyelitis. Moreover, even in the rare cases, the decisive contribution of CMV to clinically significant pneumonitis in HIV infection has been questioned. In one study of HIV infected patients, CMV was detected as the single infectious agent only in six out of 166 (3.6%) of respiratory episodes requiring a diagnostic BAL.44 No specific anti-CMV therapy was given to these patients and all were alive and well 2 months later. In the same study, the mortality of patients in whom only Pneumocystis carinii was detected was similar to those who had both CMV and Pneumocystis carinii – implying that the severity of pneumonitis was determined solely by the pneumocystis pneumonia.

These results can be interpreted in favour of an immunopathological explanation for CMV pneumonitis in which CD4 responses weakened by HIV infection mitigate against immune-mediated lung damage and thus only rarely lead to clinically significant pneumonitis. Tentative evidence for some role of CD4+ T cells in CMV-P in HIV infection has been the demonstration of two cases of severe CMV-P in HIV infected individuals with CD4 counts of greater than 200/µl. These two patients required mechanical ventilation and were contrasted with nine other HIV infected individuals who all had CD4 counts below 100/µl and who developed only a mild respiratory illness with CMV being the only isolate on BAL.45 The implication of these two severe cases of CMV-P was that their immune responses might have been better preserved, thus contributing to immunopathology. Unfortunately, however, no comment might have been better preserved, absolute CD4 counts may not exclude impaired CMV-specific immune repertoires.

Despite the apparent non-pathogenicity of CMV in HIV-associated lung disease, in a few cases of HIV infected individuals with advanced immunodeficiency CMV was the only isolate on BAL and on post-mortem examination a pneumonic illness with CMV inclusion bodies was found in the lung.42,46

One of the potentially disturbing predictions of the immunopathological hypothesis was that the reconstitution of immune responses in HIV infection should lead to an increasing rather than decreasing incidence of significant CMV-P. Indeed, highly active anti-retroviral therapy (HAART) has been successfully introduced to recover immune reactivity to a variety of recall antigens and infectious agents,47,48 but there have been no reported cases of clinical pneumonitis developing after the introduction of
HAART as yet. This is somewhat surprising as HAART has been shown to occasionally lead to a significant clinical deterioration in patients with *Mycobacterium tuberculosis*,, and with *Mycobacterium avium intracellulare* infection, and with CMV retinitis. Thus, while the immunopathological hypothesis offers a tentative explanation for the rarity of CMV-P in the HIV infected population, it fails to explain why CMV-P does not develop following immune reconstitution with HAART.

**Conclusions**

These are three-fold. Firstly, evidence of the rate of reconstitution of different lymphocyte subsets following BMT, in conjunction with the knowledge that NK and CD8 cells predominate in the lungs of BMT patients with CMV-P, has led us to conclude that CD4+ lymphocytes are unlikely to be a significant factor in the pathogenesis of CMV-P in the early period following BMT. In addition, the fact that lymphocyte infusions in sporadic human cases with established CMV-P and CD8 infusions in murine CMV disease may be curative and do not appear to exacerbate the pneumonitis lends support to the cytopathological hypothesis presented here.

Secondly, there is now evidence that various lymphocyte populations show different homing to tissues – and that both NK-like cells in the early period following BMT and activated CD8+, CD45RO+ T cell populations of ‘memory’ type in HIV infection migrate to the lung. Consequently, only the focused analysis of lymphocyte populations in the BAL, confirmed by immunohistological studies of lung tissue in selected cases, will finally resolve the immunopathology of CMV-P.

The most important conclusion, however, is that the resolution of problems such as the mechanisms underlying CMV-P are now amenable to the powerful new techniques that have recently been introduced into immunology. Analysis of antigen-specific CD8 responses using HLA-tetrameric complexes have already provided useful information for the study of acute viral illnesses such as Epstein–Barr virus, and HIV infection. Nevertheless, there are as yet no published data using tetramer technology to study BAL fluid. Alternative methods of detecting antigen-specific cells such as the Elispot system, or flow cytometric techniques following activation with antigen can provide additional functional observations about intracellular cytokine production and thus phenotypic differentiation of antigen-specific cells.

The precise nature of the processes involved in the pathogenesis of CMV-P remain to be elucidated. TNF-α seems to be a likely candidate and human CMV infection has been shown to induce TNF-α synthesis. It may be that the initial stimulus derives from NK released IFN-γ which then stimulates TNF-α release from alveolar macrophages. Whatever the precise mechanisms involved, the major conclusion of this review is that CD4 and CD8 cells are unlikely to be the primary harmful component of the pathogenesis of CMV-P in BMT. We thus favour the cytopathological model as presented here, whilst recognising that an immunopathology due to cytokine release may be an important component of this hypothesis (Figure 2).

This review has important clinical implications, as it provides a rationale for the potential use of immunotherapy with CMV-specific CD8+ lymphocytes not just for prophylaxis against CMV disease, but also for the treatment of established episodes of CMV-P.
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