Reconstitution of CD8 T cells is essential for the prevention of multiple-organ cytomegalovirus histopathology after bone marrow transplantation

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Cytomegalovirus (CMV) infection in the period of temporary immunodeficiency after haematopoietic treatment and bone marrow transplantation (BMT) is associated with a risk of graft failure and multiple-organ CMV disease. The efficacy of immune system reconstitution is decisive for the prevention of CMV pathogenesis after BMT. Previous data in murine model systems have documented a redundancy in the immune effector mechanisms controlling CMV. CD8 T cells proved to be relevant but not irreplaceable as antiviral effectors. Specifically, in a state of long-term in vivo depletion of the CD8 T-cell subset, CD4 T cells were educed to become deputy effectors controlling CMV by a mechanism involving antiviral cytokines. It is of medical importance to know whether one can trust in this ‘flexible defence’ in all clinical settings. It is demonstrated here that reconstitution of CD8 T cells is crucial for the prevention of fatal multiple-organ CMV disease under the specific conditions of BMT.

Patients undergoing bone marrow transplantation (BMT) associated with temporary immunodeficiency are at risk of fatal cytomegalovirus (CMV) disease with multiple organ involvement (for reviews see Winston et al., 1990; Einsele et al., 1998). Whether or not primary or recurrent CMV infections develop into disease appears to be related to the efficacy of haematopoietic and immune system reconstitution. Specifically, early reappearance of CD8 T cells is of positive prognostic value for recovery (Reusser et al., 1991). Accordingly, restoration of immunity to CMV by adoptive cytoreimmunotherapy with antiviral CD8 T-cell lines is in clinical trials (Riddell et al., 1992; Walter et al., 1995). In BMT performed across minor or class I major histocompatibility disparities, graft vs. host disease (GvHD) is a further risk factor (Einsele et al., 1998). In a major histocompatibility complex (MHC) class II-identical but MHC class I-disparate setting, selective depletion of class I-restricted CD8 T cells might be discussed as a regime for GvHD prophylaxis. However, if CD8 T cells were the only effectors...

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Fig. 1. (A) Lethality of CMV infection after selective T-cell subset depletion. Syngeneic BMT was performed on day 0 with BALB/c mice as BM cell donors and recipients. Recipients were infected with 1 × 10^5 p.f.u. of purified murine CMV ca. 2 h after BMT. In vivo depletion of T-cell subsets was performed twice, namely on days 7 and 14, by intravenous administration of 1 mg of the respective antibody. Kaplan–Meier plots show the survival rates for 20 recipients per experimental group as a function of time. (B) Efficacy and specificity of the T-cell subset depletions. Pulmonary infiltrate leukocytes were isolated on day 21 and analysed by three-colour cytofluorometry for the expression of TCR α/β (phycoerythrin fluorescence, FL-2), CD4 (RED613 fluorescence, FL-3) and CD8 (FITC fluorescence, FL-1). Gates were set on lymphocytes and on positive FL-2 in order to restrict the analysis to α/β T cells. Dot plots show the log FL-3 (ordinate) versus log FL-1 (abscissa) intensities for 20000 α/β T cells. The percentages of CD4 and CD8 T cells are given in the respective quadrants.

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Fig. 2. Enhancement of CMV replication in tissues by selective depletion of CD8 T cells. Virus replication on day 21 after BMT (see Fig. 1) was monitored by *in situ* DNA hybridization specific for the murine CMV genome. The label is provided by new fuchsin, yielding a brilliant red colour. The counterstaining was done with haematoxylin. Bars represent 25 µm throughout. The overview photographs document a selected tissue area of ca. 0.3 mm². For tissues with even distribution of the infected cells, numbers in the lower left corners of the photographs indicate the number of infected cells counted for a representative area of 10 mm² compiled from five BMT recipients per experimental group. Arrows in panels A3–C3 point to sites of interest resolved to greater detail in corresponding panels A3*–C3**. (Panels A) Sections of liver parenchyma, demonstrating inflammatory foci sequestering infected hepatocytes. Note the pronounced intranuclear inclusion bodies containing the viral DNA in the late stage of the virus replicative cycle. (Panels B) Sections of repopulated spleen tissue. The infection is localized in the stroma rather than within follicles. (Panels C) Brain tissue including the third ventricle in coronal section. Note the infection of choroid plexus epithelial cells (C3*) and of ependymal cells (C3**).
Fig. 3. Multiple-organ CMV infection and histopathology. Pairs of photographs compare virus replication in tissues after specific depletion of the CD4 T-cell subset (left) and the CD8 T-cell subset (right). The viral genome was detected as in Fig. 2 by in situ DNA hybridization. Bars represent 25 µm throughout. The photographs document a selected tissue area of 0.04 mm².

Numbers in the lower left corners give the number of infected cells counted in representative areas of 10 mm² (panels A–D, G and H) or of 2 mm² (E and F), compiled from five BMT recipients per experimental group. (A1, A2) Lung tissue with infected cells in proximity to bronchioles (b). (B1, B2) Gastrointestinal infection, represented by infection of the lamina propria in the villi of small intestine, shown in transverse section. Note that infected cells are also found in the colon and the gastric mucosa (not depicted). (C1, C2) Infected cells in the mucous acini of the submandibular gland. The inset shows infected acinar epithelial cells resolved to greater detail. Cytoplasmic spots of staining (arrows) represent the virion DNA of numerous monocapsid virions that are packaged in huge vacuoles for secretion. (D1, D2) Myocardial infection. Cardiac muscle cells and endothelial cells are the main target cells in the heart muscle. (E1, E2) Infection of the adrenal cortex. Shown are foci of infection localized in the zona glomerulosa (zag) underneath the capsule as well as in the zona fasciculata (zf). (F1, F2) Infection of the adrenal medulla. Panel F1 includes the junction between adrenal medulla (m) and the X-zone (xz) of the adrenal cortex. (G1, G2) Selective infection of the glomeruli in the kidney cortex and absence of infection in the convoluted cortical tubules. (H1, H2) Absence of infection in the tubular system of the kidney medulla. Opposed arrows mark tubules in longitudinal section.
controlling CMV, such a treatment would entail a risk of promoting the progression of subclinical CMV infection to severe organ disease.

Murine models have been instrumental in studying specific aspects of CMV pathogenesis. Depending on the genetic background, the immune control of murine CMV infection in the immunocompetent host is dominated by MHC-unrestricted natural killer (NK) cells or by MHC-restricted CD8 T cells (Lathbury et al., 1996). Murine CMV infection of the genetically susceptible BALB/c mouse inbred strain (MHC haplotype H-2d) so far represents the model closest to human CMV pathogenesis. Specifically, in this model, CD8 T cells appeared to be the principal antiviral effectors (Reddehase et al., 1985; for a review see Koszinowski et al., 1993). Upon adoptive transfer for pre-emptive cytimmunotherapy of murine CMV disease in the immunocompromised host, antiviral CD8 T cells prevented lethality as well as histopathology by controlling virus replication (Reddehase et al., 1987a, b; Dobonici et al., 1998). Furthermore, they limited the establishment of latent infection (Steffens et al., 1998a). In these immunotherapy protocols, CD4 T cells derived from infected donors did not confer antiviral protection, and the protective CD8 T cells did not depend on a helper function of CD4 T cells. Thus, CD8 T cells proved to be dominant and autarkic in the control of murine CMV. This straight view was challenged by the finding that BALB/c mice which were long-term depleted of the CD8 subset controlled murine CMV infection perfectly well (Jonjic et al., 1990). Along the same line of evidence, β-2m<sup>−/−</sup> mutants on C57BL/6 (H-2<sup>b</sup>) genetic background cleared the productive infection, even though they were lacking functional MHC class I molecules and, associated with this defect, were also lacking CD8 T cells (Polić et al., 1996). Thus, irrespective of the genetic background, the knockout of CD8 T cells was compensated. Notably, in both models, this compensation was not performed by NK cells but required CD4 T cells and was mediated by the induction of cytokines, specifically of interferon-γ and tumour necrosis factor-α (Pavić et al., 1993; Lučin et al., 1994). Apparently, in the absence of the CD8 T-cell subset, the immune system gets reorganized and can then cope with CMV infection by an alternative mechanism of defence.

Is this now a licence to deplete CD8 T cells for GvHD prophylaxis in CMV-infected recipients of MHC class I-disparate BMT? Can we generally count on the flexibility of the immune system? The answer to these questions is of medical importance and of theoretical interest. It will tell us whether in the specific situation of immunological reconstitution after BMT the newly generated CD4 T cells can functionally replace the CD8 T-cell subset.

We approached the problem by experimental syngeneic BMT performed with BALB/c mice as bone marrow (BM) cell donors and haematoablated BALB/c mice as transplantation recipients. A concurrent infection, initiated on the day of BMT by intraplantar application of 10<sup>8</sup> p.f.u. of murine CMV strain Smith does not lead to CMV disease provided that the number of transplanted BM cells suffices for an effective repopulation of the recipient BM (Steffens et al., 1998a, b). Insufficient reconstitution, however, is associated with fatal CMV disease (Mutter et al., 1988), including a pathogenesis within the BM that is characterized by the infection of BM stroma cells and a deficiency in the expression of stroma cell-derived haematopoietins (Mayer et al., 1997; Dobonici et al., 1998; Steffens et al., 1998b). On the basis of this previous experience, BMT was herein performed with 1 × 10<sup>7</sup> BM cells and haematoablatative treatment of the recipients with 6 Gy of total-body <sup>137</sup>S as γ-irradiation in order to achieve a high rate of survival (Fig. 1A, •). Elimination <i>in vivo</i> of reconstituting CD4 T cells by intravenous infusions of anti-CD4 monoclonal antibody YTS 191.1 (Cobbold et al., 1984) on days 7 and 14 was not associated with significant lethality (Fig. 1A, □). This finding is not trivial, as it implies that CD4 T cells are dispensable for survival not only with respect to a direct effector function but also as helper cells for other immune effectors. Further, depletion of CD4 T cells is known to preclude the generation of antiviral antibody (Jonjic et al., 1989). Thus, antibodies are apparently not essential for prevention of fatal murine CMV disease after BMT. This conclusion is in accordance with the previous finding of effective resolution of primary murine CMV infection in B-cell-deficient μ-chain<sup>−/−</sup> mice (Jonjic et al., 1994). By contrast, <i>in vivo</i> elimination of reconstituting CD8 T cells with anti-CD8 monoclonal antibody YTS 169.4 (Cobbold et al., 1984) on days 7 and 14 resulted in absolute lethality (Fig. 1A, ○). It should be noted that CD8 depletion was not fatal after BMT in the absence of infection (not shown). The efficacy of the depletions was monitored by three-colour cytofluorometric analysis of T cells that were infiltrating the lungs, a major organ site of CMV pathogenesis and latency (Reddehase et al., 1985; Balthesen et al., 1993). Pulmonary interstitial leukocytes were isolated (Holt et al., 1985) and were labelled with fluorochromated antibodies specific for T-cell receptor (TCR) α/β, CD4 and CD8. Pulmonary T cells were analytically selected by gating on positive TCR α/β expression, and the subset composition is documented by dot plots of CD4 versus CD8 expression (Fig. 1B). The <i>in vivo</i> depletions proved to be effective and specific, eradicating CD4 and CD8 T cells, respectively. Most importantly, the treatment not only depleted lymphoid tissues but indeed prevented any recruitment of the respective T-cell subsets to an important organ site of CMV pathogenesis. In conclusion, survival was strictly linked to the presence of CD8 T cells.

So far, the data have suggested that the reconstituted CD8 T cells did perform their protective function by controlling virus replication. Hard evidence was provided by a detailed <i>in situ</i> analysis of CMV replication and histopathology after BMT (Figs 2 and 3). Productive murine CMV infection was monitored by <i>in situ</i> hybridization detecting the viral DNA that is found in infected cells accumulated in an intranuclear inclusion body during the late phase of the virus replicative
cycle. The staining was performed essentially as described in a recent report (Mayer et al., 1997), except that the sensitivity of detection was enhanced by replacing the previously used 1.5-kbp gB gene DNA probe with a mixture of digoxigenized plasmids containing HindIII fragments A, I, and K, representing 51.2 kbp of the murine CMV strain Smith genome (Ebeling et al., 1983; Rawlinson et al., 1996).

Fig. 2 shows the effect of T-cell subset depletions on virus replication at 3 weeks after BMT in liver (Fig. 2, top row), spleen (centre row) and brain (bottom row). In these organs, murine CMV replication was almost completely prevented by immune reconstitution after BMT if no T-cell depletion was performed (Fig. 2, first column). Depletion of the CD4 T-cell subset resulted in only a slight enhancement of virus replication in liver and spleen with only single cells positive in the documented tissue area (Fig. 2, second column). By contrast, depletion of the CD8 T-cell subset was associated with a dramatic increase in the number of infected cells, and, under these conditions, murine CMV disseminated also to the brain (Fig. 2, third column for overview and fourth column for details). It should be noted that simultaneous depletion of CD4 and CD8 T cells gave results indistinguishable from those seen with selective CD8 T-cell depletion (not shown). In the liver, the hepatocyte is the predominant target cell of murine CMV infection (Fig. 2, A3 and A3*). Infiltrating mononuclear cells sequester infected hepatocytes, resulting in inflammatory foci (panel A3* that are reminiscent of NK cell foci described recently in another model system of murine CMV hepatitis (Salazar-Mather et al., 1998), with the notable difference that the inflammation observed here in CD8 T-cell-depleted mice was apparently not protective. Infected cells in the spleen are likely to represent stromal cells (panel B3*). Virus replication in the brain is confined to the ventricles (panel C3). Infected cell types are the cerebrospinal fluid-producing choroid plexus epithelial cells (panel C3*) as well as the ependymal cells lining the ventricle cavity (panel C3**). Interestingly, the restricted distribution of this β-herpesvirus in the brain resembles that of a neuroattenuated variant of herpes simplex virus type 1 which also infects ependymocytes and destroys the ependymal lining (Kesari et al., 1998).

Fig. 3 compiles the results obtained after CD4 as well as after CD8 T-cell depletion for further sites relevant to CMV disease. The histological findings were alike in undepleted and in CD4 T-cell-depleted recipients, and therefore the results for the undepleted group are not separately presented. The lungs and the gastrointestinal system (Fig. 3 A, B) belong to a group of organs, including those already shown in Fig. 2, in which significant virus replication occurred only in the absence of CD8 T cells. Infected cell types in the lungs are interstitial fibroblasts, pneumocytes and cells of the capillary endothelium (Reddehase et al., 1985). Notably, only single infected cells were found scattered in the lungs of CD4 T-cell-depleted mice (Fig. 3, A1), whereas expansion to extended foci of infection occurred after depletion of CD8 T cells (panel A2).

In the stomach, small intestine (panel B) and large intestine, infection is localized in the lamina propria. By contrast, salivary glands (panel C), heart (panel D), adrenal gland cortex as well as medulla (panels E and F) and kidney cortex (panel G) are sites at which murine CMV replicates also in undepleted (not shown) and in CD4 T-cell-depleted mice. This result was not unexpected for salivary glands and kidney, which represent known sites of asymptomatic virus shedding also in the immunocompetent host. Notably, in the acinar epithelial cells of salivary glands, viral DNA is detectable also in cytoplasmic inclusions, which correspond to vacuoles filled with numerous mononodispersed virions shown previously by electron microscopy (Jonjić et al., 1989). In the kidney cortex, infection is confined to the glomeruli (panel G). Notably, tubules in the kidney medulla were not infected (panel H).

In summary, in the salivary glands and, unexpectedly, also in the adrenal medulla, virus replication was not significantly controlled by either T-cell subset early after BMT. By contrast, depletion of CD8 T cells led to enhanced virus replication in most relevant organs resulting in fatal multiple-organ disease, including hepatitis, splenitis, ventriculitis, pneumonitis, gastroenterocolitis, a moderate carditis, cortical adrenitis and glomerulitis.

In conclusion, in this setting of experimental BMT and murine CMV infection, CD4 T cells or other effectors apparently did not compensate for CD8 T cells in the control of virus replication and disease. The precise conditions for the development of alternative antiviral mechanisms in long-term CD8 T-cell-deficient mice have never been defined. Our data are therefore not in conflict with previous work, but they clearly tell us that the alternative antiviral defence mechanisms are not educated when CD8 T cells get depleted during haematopoietic reconstitution. We hence predict that CD8 T-cell depletion for GvHD prophylaxis will be a great risk in BMT patients who are positive for human CMV.

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References


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