Preemptive CD8 T-Cell Immunotherapy of Acute Cytomegalovirus Infection Prevents Lethal Disease, Limits the Burden of Latent Viral Genomes, and Reduces the Risk of Virus Recurrence

HANS-PETER STEFFENS, SABINE KURZ, RAFAELA HOLTAPPELS, AND MATTHIAS J. REDDEHASE*

Institute for Virology, Johannes Gutenberg-University, 55101 Mainz, Germany

Received 2 July 1997/Accepted 25 November 1997

In the immunocompromised host, primary cytomegalovirus (CMV) infection is resolved by the immune response without causing overt disease. The viral genome, however, is not cleared but is maintained in a latent state that entails a risk of virus recurrence and consequent organ disease. By using murine CMV as a model, we have shown previously that multiple organs harbor latent CMV and that reactivation occurs with an incidence that is determined by the viral DNA load in the respective organ (M. J. Reddehase, M. Balthesen, M. Rapp, S. Jonjic, I. Pavic, and U. H. Koszinowski. J. Exp. Med. 179:185–193, 1994). This predicts that a therapeutic intervention capable of limiting the load of latent viral genome should also reduce the risk of virus recurrence. Here we demonstrate the benefits and the limits of a preemptive CD8 T-cell immunotherapy of CMV infection in the immunocompromised bone marrow transplantation recipient. Antiviral CD8 T cells prevented CMV disease and accelerated the resolution of productive infection. The therapy also resulted in a lower load of latent CMV DNA in organs and consequently reduced the incidence of recurrence. The data thus provide a further supporting argument for clinical trials of preemptive cytoimmunotherapy of human CMV disease with CD8 T cells. However, CD8 T cells failed to clear the viral DNA. The therapy-susceptible portion of the DNA load differed between organs and was highest in the lungs. The existence of an invariant, therapy-resistant load suggests a role for immune system evasion mechanisms in the establishment of CMV latency.

Recurrence of productive infection by reactivation of latent viral genome in the immunocompromised host is a feature common to the members of the herpesvirus family (39; reviewed in reference 38). Specifically, in the case of human cytomegalovirus (CMV), the human herpesvirus type 5, primary as well as recurrent infection during the temporal immunodeficiency early after bone marrow (BM) transplantation (BMT) entails a risk of graft failure and severe organ manifestations of CMV disease (8, 44). Early findings by Quinnan et al. (24) have suggested a correlation between efficient reconstitution of the cellular immune response and the control of post-BMT CMV infection, and more recent clinical data have attributed this control to the reconstituted CD8 T cells (35). Accordingly, restoration of antiviral immunity in the critical phase before the reconstitution by BMT becomes effective should diminish the risk of CMV disease. Experimental re- search with the model of murine CMV infection has positively demonstrated the antiviral and protective efficacy of adoptively transferred acutely sensitized (31, 34) or memory (28) CD8 T cells recovered from immune donors as well as of short-term transferred CD8 T-cell lines propagated in culture (32). These studies have been pivotal for clinical trials of a preemptive CD8 T-cell immunotherapy of post-BMT human CMV infection in patients (37, 43).

Infection of the BMT recipient can accidentally result from the transmission of infectious virus, however, productive infection is more commonly initiated by reactivation of latent CMV in either the transplant or the recipient’s own organs or, occasion-
MATERIALS AND METHODS

BMT and concurrent CMV infection. BMT was performed as a syngeneic BMT with female BALB/c (H-2b) mice used at the age of 8 weeks as BM donors and recipients. Hematopoietic conditioning of the recipients was performed by total-body irradiation (300 rads) or a single dose of 6.5 Gy from a 137Cs source (DSB5; Buchler, Braunschweig, Germany). This irradiation is equivalent to a 50% lethal dose determined at day 30. Donor femoral and tibial BM cells (BMC) were obtained as described previously (22), and the indicated doses of BMC were injected intravenously (iv) into the tail vein of the recipients at ca. 6 h after the irradiation. Murine BM is practically devoid of mature T cells, and elimination of CD8 T cells by immunomagnetic sorting proved to have no influence on the results. Data, infected with 10^6 PFU of purified murine CMV, were obtained from 5 BALB/c, C57BL/6J, and C3H mice, purchased in 1981, was performed simultaneously in the left hind footpad at ca. 2 h after BMT. According to a recent reevaluation of viral infectivity assays, a dose of 10^6 PFU of purified murine CMV is equivalent to 5 × 10^7 viral genomes and 1 × 10^8 infectious units as measured by a reverse transcriptase (RT)-PCR-based focus expansion assay (17).

Generation of polyclonal antiviral CD8 T cells for immunotherapy. The generation of a polyclonal short-term T-cell line, its in vivo antiviral activity, its in vitro cytolytic T-lymphocyte (CTL) activity, and the molecular antiviral CTL specificities have been the subject of previous reports (29, 30, 32; reviewed in reference 16). In brief, immunocompetent BALB/c mice were sensitized by s.c. injection in the left hind footpad and lymphocytes were recovered on day 8 from the draining popliteal lymph node (LN) and propagated in culture at a density of 5 × 10^5 cells per 0.2 ml-round microtiter plate well in the presence of 10 U of recombinant interleukin-2 per culture. Importantly, to avoid antigen-specific selection in vitro, the plasmid LN lymphocytes were not restimulated with feeder cells and antigen. After 7 days of cultivation, CD4 T cells and, alternatively, CD8 T cells were depleted of CD4 or CD8 by using two rounds of treatment with anti-CD4 monoclonal antibody (MAb), clone GK-1.5, and complement and with anti-CD8 MAb, clone 19/178, and complement, respectively (32). The purity of the T-cell subsets was monitored by three-color cytofluorometric analysis of the expression of T-cell receptor α-β (clone H57-597; Pharmingen, San Diego, Calif.), CD4 (clone H129.19; GIBCO BRL, Eggenstein, Germany), and CD8 (clone 53-6.7; Becton Dickinson, San Jose, Calif.), and CD4 (clone H129.19; GIBCO BRL, Eggenstein, Germany), and CD8 (clone 53-6.7; Becton Dickinson, San Jose, Calif.), respectively.

Assays of viral infectivity and verification of latent infection. (i) Acute infection. Virus titers in organs after BMT and infection were measured from organ homogenates by a plaque assay on permissive mouse embryo fibroblasts (MEF) by the previously described technique of centrifugal enhancement of infectivity (17). Titers are expressed as PFU* to indicate the enhancement and are given as 10^7 infectious particles as measured by a reverse transcriptase (RT)-PCR-based focus expansion assay (17).

(ii) Latent infection. Criteria for the definition of CMV latency in organs were discussed in detail recently (2, 17). Absence of infectivity, e.g., in the lungs, was verified by testing the homogenate of the left lung and the postcaval lobe in total-body irradiated mice with a single dose of 6.5 Gy. Viral infectivity was measured on day 14 in organ homogenates by the RT-PCR-based focus expansion assay (17).

(iii) Recurrent infection. Recurrence was induced in lethally infected mice by γ-irradiation with a single dose of 6.5 Gy. Viral infectivity was measured on day 14 in organ homogenates by the RT-PCR-based focus expansion assay (17). In brief, mice organs or, specifically, the five lobes of the perfused lungs were homogenized separately, and aliquots equating 1/18 of the whole lungs were used to infect MEF under the influence of a 1,000-g centrifugal force. After 72 h of focus formation in the indicator cultures, poly(A)* RNA was isolated and a 100-ng aliquot was subjected to an RT-PCR specific for exon 4 of the viral 1g1 gene (2, 17). In addition to a negative result in this assay, chronic infection was confirmed by testing the homogenate of the left lung and the postcaval lobe in total-body irradiated mice with a single dose of 6.5 Gy. Viral infectivity was measured on day 14 in organ homogenates by the RT-PCR-based focus expansion assay (17). In brief, mice organs or, specifically, the five lobes of the perfused lungs were homogenized separately, and aliquots equating 1/18 of the whole lungs were used to infect MEF under the influence of a 1,000-g centrifugal force. After 72 h of focus formation in the indicator cultures, poly(A)* RNA was isolated and a 100-ng aliquot was subjected to an RT-PCR specific for exon 4 of the viral 1g1 gene. Amplification products (a 15-μl aliquot thereof) were analyzed by agarose gel electrophoresis (2% [wt/vol] agarose), Southern blotting, and hybridization with a γ-32P-end-labeled oligonucleotide probe (25) that was complementary to the gB-PCR, oligonucleotides 5'-84250–84269 and 5'-84269–84287 served as the forward and reverse primers, respectively, and oligonucleotide 5'-84450–84469 served as the probe (26; GenBank accession no. M15068 [complete genome]). Plasmids pE111 (21) and genomic murine CMV DNA derived from purified virions (17) were supplemented with organ DNA from uninfected mice and were titrated as standards. PCR products were performed in an MWG Biotech Omnigene thermocycler (Hybaid Ltd., Teddington, England) in either 0.5-ml safe-lock reaction tubes (Eppendorf, Hamburg, Germany) or conically walled polycarbonate 96-well (0.17 ml) microplates (Omniplate 96; Hybaid Ltd.). The reactions were performed in a volume of 30 μl containing 15 mM Tris-HCl (pH 8.4), 60 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, 20% (vol/vol) glycerol, 1 mM each deoxynucleoside triphosphate, 25 pmol of each primer, and 1.5 U Taq DNA polymerase (Eurobio, Raunheim, Germany). Amplification was performed in 30 cycles with denaturation at 95°C for 120 s in the first cycle and at 96°C for 30 s in the remaining cycles, primer annealing at 58°C for 60 s, and primer extension at 72°C for 60 s that was extended to 300 s in the last cycle. For monitoring the dynamic content of test samples, a 0.72-bp fragment of the single copy gene encoding tumor necrosis factor alpha TNF-α (40, 42) was amplified with PCR with oligonucleotides 5'-n5859–5880 and 5'-n6560–6540 as forward and reverse primers, respectively, and with oligonucleotide 5'-n6159–6183 serving as the probe (GenBank accession no. M38296). PCR buffers and conditions were as described above, except that the primer-annealing temperature of 58°C. Forcification of microplates was performed (26). Amplification products (20 μl) were vacuum blotted onto nylon membrane by using the Minifold dot blot manifold device (Schleicher & Schuell, Keene, N.H.) and hybridized accordingly. The radioactivity per dot was measured with a digital phosphorimaging system (Fuji film imaging system BAS 2000; Fuji, Tokyo, Japan) and is expressed as phosphostimulated luminescence units. Data analysis was performed with TINA 2.10 software (Raytest, Straubenhardt, Germany).

RESULTS

Endogenously reconstituted and exogenously restored antiviral CD8 T cells both protect against lethal CMV disease after BMT. After hematopoietic treatment of 8-week-old BALB/c mice with 6 Gy of γ-irradiation, murine CMV infection prevents endogenous BM reconstitution and causes lethal multiorgan disease combined with BM aplasia, referred to as CMV aplastic anemia (19, 22) (Fig. 1, top). Reconstitution of the BM by syngeneic BMC with 10^7 BMC is not sufficient to prevent lethal CMV disease but results in a slight delay of mortality (Fig. 1, top), which is due to an immediate but only temporary cessation of granulocytopenia and thrombocytopenia (not shown). Under these conditions, restoration of immunity by adoptive transfer of antiviral CD8 effector T cells is protective whereas adoptive transfer of sensitized CD4 T cells has no effect on the course of disease (Fig. 1, top). The antigen specificity of the antiviral CD8 T cells had been the subject of previous reports (for a review, see reference 16). Since the CD8 T cells were derived from primed lymph node cells without in vitro restimulation by viral antigens, they are thought to closely represent the authentic specificity repertoire. The polyclonal population comprises CTL clones specific for infected cells in all stages of the viral replicative cycle (29), with a high proportion of IE-phase-specific CTL (30).

Interestingly, upon increasing the number of BMC in the BMT, survival times and the final survival rates improved, and, specifically, after reconstitution with 10^7 BMC, an additional adoptive transfer of CD8 T cells at the time of the BMT, as judged just by the criterion of survival (Fig. 1, middle and bottom). This raised the question whether protection by CD8 T-cell immunotherapy and protection by high-dose BMT operated by the same mechanism. By cytoluminometric analysis of the reconstituting immune system, it was found that T cells
reappear in the recipients in week 3 after BMT but remain absent in thymectomized, uninfected BMT recipients (data not shown). This indicated that all the T cells were derived from hematolymphopoietic reconstitution. The protective effect of high-dose BMT was completely abrogated by selective in vivo depletion of the reconstituting CD8 T cells, whereas the selective in vivo depletion of reconstituting CD4 T cells had no effect on the survival rate (Fig. 1, bottom). In conclusion, the reconstitution of CD8 T cells is decisive for the control of CMV infection after BMT, and a preemptive CD8 T-cell immunotherapy can substitute for inefficient endogenous reconstitution.

**CD8 T-cell immunotherapy accelerates the resolution of acute primary CMV infection.** After BMT performed with $10^7$ BMC, a beneficial effect of preemptive CD8 T-cell immunotherapy was no longer apparent with regard to survival (Fig. 1, bottom). More detailed information on the course of CMV infection in the surviving recipients is provided by measuring the extent of virus replication in organs (Fig. 2). Endogenous reconstitution of CD8 T cells requires up to 4 months to resolve virus replication in the major target organs affected by florid CMV infection, with a typical succession of rapid clearance of productive infection in the adrenal glands, delayed clearance in the lungs, and slow clearance in the salivary glands, which represent the preferred site of chronic CMV replication (Fig. 2, top). Therapy with increasing doses of CD8 T cells proved to be beneficial in that it significantly reduced the extent and duration of virus replication in vital organs, such as the lungs and the adrenal glands. The phase of chronic, asymptomatic CMV replication in the salivary glands was shortened by 2 months, which is of relevance with regard to the
risk of virus transmission after BMT. In conclusion, preemptive CD8 T-cell immunotherapy modulates the course of primary infection by increasing the efficacy of antiviral control in quantitative and in kinetic terms.

Verification of the establishment of CMV latency after resolution of acute infection. Latent infection implies that the viral genome is retained while infectious virus is absent. The discrimination between molecular latency and persistent infection below the detection limit of infectivity assays has been a long-debated problem. The recently described RT-PCR-based focus expansion assay (17) can detect infectivity with the utmost possible sensitivity. This assay was therefore used to verify the absence of infectivity in organs. It is important to note that at the time of analysis 12 months after BMT and infection, viral DNA was absent from blood. This finding excludes hematogenous dissemination of virus from an unknown remote site of persistent productive infection (detailed in reference 17; not shown here). Infectious virus was then no longer detectable in any organ tested, including the salivary glands, the spleen, and the adrenal glands (data not shown). Since the lungs represent a major organ site of CMV latency and recurrence (2, 17, 27), we focus here on the lungs for documenting the data for the group that did not receive immunotherapy (Fig. 3). For each individual mouse included in the latency analysis, the left lung and the postcaval lobe were used for the infectivity assay and the remaining three lobes were used to verify the presence of latent viral DNA. The total homogenate of the left lung and the postcaval lobe were distributed to nine indicator cultures for the RT-PCR-based focus expansion assay. Aliquot 1 was supplemented with 0.05 PFU of purified murine CMV as a positive control, whereas for each of the remaining cultures, 100 ng of poly(A)^+ RNA was subjected to ie1 exon 3/4-specific RT-PCR, leading to an amplification product of 188 bp. The autoradiograph was obtained after hybridization with a γ-32P-end-labeled oligonucleotide probe directed against the splice junction. For culture 9, the presence of RNA was verified by an RT-PCR specific for the HPRT housekeeping gene transcript. (Left) DNA isolated from the SL, ML, and IL was subjected to an IE1 IE111 that contains gene ie1 (14, 21) were titered to titer determination in parallel (Fig. 3, left). The specific internal probe hybridized exclusively to an amplification product of the correct size. The assay detected 10 copies of the control plasmid and revealed the presence of the sequence within a lower limit of 30 ng of the lung cell DNA. In conclusion, viral DNA was present in the lungs in the absence of infectivity.

Preemptive CD8 T-cell immunotherapy limits the load of latent viral DNA in the lungs. We have shown in a previous report that the extent of virus dissemination in the acute phase of infection determines the load of latent viral genome in organs (27). Accordingly, the modulation of the acute infection by CD8 T cells (Fig. 2) should affect the viral DNA load measured many months later. We therefore quantitated the viral DNA in latently infected lungs 12 months after BMT, infection, and immunotherapy, by PCR specific for exon 4 of the ie1 gene. Since the radioactive internal probe hybridized to a single, specific band of 363 bp (Fig. 3), it was possible to do the quantitation in a dot blot system followed by phosphorimaging. It is worth emphasizing that there was no signal after PCR with DNA derived from the lungs of age-matched uninfected BMT recipients. Plasmid pIE111 added to this control DNA served as a positive standard for the PCR (Fig. 4, top). The amount of cellular DNA in all test groups was monitored by a PCR specific for the gene that encodes TNF-α (data not shown). In essence, the autoradiograph of the plate shows that the amount of latent viral DNA is smaller in the groups given CD8 T-cell therapy. The copy numbers of the viral sequence in lung cell DNA were then estimated from the linear portions of the log-log plots of dilution versus radioactivity (Fig. 4, bottom). Based on the fact that 6 µg of DNA represents the DNA content of 10^6 diploid mammalian cells, the latent viral DNA...
and hence may be incapable of reactivating to productive infection. To exclude this possibility, we have chosen a sequence located ca. 100 kbp away, namely, a 510-bp sequence within the gB gene, representing positions 84250 to 84759 within the *Hind*III D fragment (25, 26). The latent CMV DNA load in the salivary glands was then determined for the extreme cases, namely, the group given no therapy and the group given maximal therapy, by comparing IE1-specific and gB-specific amplification (Fig. 5). Purified virion DNA, in which both genes are present in a single copy, was chosen to provide an identical standard for both PCRs. The latent DNA contained both regions of the CMV genome with identical copy numbers, and hence may be incapable of reactivating to productive infection. To exclude this possibility, we have chosen a sequence located ca. 100 kbp away, namely, a 510-bp sequence within the gB gene, representing positions 84250 to 84759 within the *Hind*III D fragment (25, 26). The latent CMV DNA load in the salivary glands was then determined for the extreme cases, namely, the group given no therapy and the group given maximal therapy, by comparing IE1-specific and gB-specific amplification (Fig. 5). Purified virion DNA, in which both genes are present in a single copy, was chosen to provide an identical standard for both PCRs. The latent DNA contained both regions of the CMV genome with identical copy numbers, and there was no indication that the therapy-susceptible and the therapy-resistant fractions of the load would differ in this respect.

Organ-specific differences in the load of latent CMV DNA and in the increment of CD8 T-cell immunotherapy. The load of latent viral DNA was also determined for the adrenal glands (data not shown). All the results, expressed as copy number per 10^6 cells, are compiled in Fig. 6. In the group with no CD8 T-cell therapy, the highest load was determined for the lungs (5,000 copies), followed by the salivary glands (3,200 copies) and the adrenal glands (3,000 copies). As indicated by the slope of the graphs, the efficacy of CD8 T-cell immunotherapy differs between organs and, specifically, the establishment of latency in the salivary glands is less accessible to control by antiviral CD8 T cells. Notably, after therapy, the loads in the lungs and the adrenal glands and, by extrapolation of the graph, the load in the salivary glands did not fall below a constant value of ca. 1,000 copies per 10^6 cells. Apparently, this is not a problem of dosage but indicates a principal limit, that is, a fraction of latent DNA which resists CD8 T-cell therapy. Notably, after therapy, the loads in the lungs and the adrenal glands and, by extrapolation of the graph, the load in the salivary glands did not fall below a constant value of ca. 1,000 copies per 10^6 cells. Apparently, this is not a problem of dosage but indicates a principal limit, that is, a fraction of latent DNA which resists CD8 T-cell therapy.
consequent recrudescent disease. By comparing incidences of CMV recurrence in mice infected either as neonates or as adults, we previously documented a positive correlation between the latent CMV DNA load and the risk of recurrence (27). Accordingly, down-modulation of the load of latent CMV by CD8 T cells should reduce the incidence of recurrence. However, this postulate is not as clear as it might appear. So far, the data have revealed a therapy-susceptible fraction of the load, which was 4,000 copies per 10^6 cells in the lungs and 2,000 copies per 10^6 cells in salivary glands and adrenal glands, and an invariant therapy-resistant fraction of 1,000 copies per 10^6 cells in any of the three organs tested. At present, we do not know whether the viral DNA detected by PCR represents functional genomes or, if so, whether this then applies to both fractions of viral DNA load. In the extreme assumption, only the resistant fraction might contain latent genome capable of reactivation. In this case, CD8 T-cell immunotherapy would result in a reduced amount of retained viral DNA but without any beneficial consequence with respect to virus reactivation. To test this objection, we determined the incidence of recurrence after immunosuppressive treatment for the latently infected group given no therapy in comparison to the group that had received 10^7 CD8 T cells. The lungs were chosen for the readout, because this is the organ with the highest absolute load as well as the highest load difference between the groups. In the group given no therapy and with a consequent high load, recurrence was detected within only 14 days in all five mice tested and, with some variance in quantity, in all five lobes of the lungs. This indicated a high frequency of productive reactivation events after the ablation of immune control. In contrast, in the group given therapy and with consequently low load, recurrence occurred in only two out of five mice and only in a single lobe in each (Fig. 7). Even though the incidence of recurrence was low, the few positive cases demonstrate that the therapy-resistant latent viral DNA included functional viral genomes capable of productive reactivation.

In conclusion, CD8 T-cell immunotherapy of acute CMV infection can reduce the risk of virus recurrence from latency.

DISCUSSION

The importance of latent tissue reservoirs and of the viral genome load as a predictor for progression from an asymptomatic to an acute state of infection is a topic of increasing interest, in particular in AIDS research (3). For herpes simplex virus reactivation from latency, Roizman and Sears have proposed a decisive role for the copy number of the latent viral DNA (39). Experimental evidence was provided by our own work on murine CMV latency (27). Mice infected as neonates underwent a prolonged productive primary infection resulting in a high load of latent viral DNA in multiple organs. By contrast, mice infected as immunocompetent adults rapidly cleared the productive primary infection and retained only a low load of latent viral DNA. Most importantly, high and low load correlated with high and low risk of recurrent infection, respectively. The same principle was revealed by the organ-specific load. Organs with a high load, such as the lungs (2), proved to be favored sites of CMV recurrence (2, 27). In the present study, we asked whether the latent CMV DNA load could be manipulated by a therapeutic intervention for reducing the risk of recurrent CMV infection in an experimental setting of BMT.

Encouraged by the promising results with the murine model, a preemptive CD8 T-cell immunotherapy of CMV disease is in clinical trials (37, 43; reviewed in reference 36) and has so far proved to be successful in terms of reducing the incidence of CMV disease after BMT. The benefit of the therapy is difficult to verify for the individual patient, because it remains unknown whether the individual would have developed CMV disease in the absence of therapy. The uncertainty is because not all high-risk patients reactivate CMV after BMT and because even after diagnosed CMV infection, the incidence of CMV disease is only ca. 50%. This raises the question whether preemptive immunotherapy is medically indicated.

Our present work has recalled previous data by demonstrating that a preemptive CD8 T-cell immunotherapy prevents lethal CMV disease in recipients suffering from an inefficient hematolymphopoietic CD8 T-cell reconstitution. Sensitized CD4 T cells did not substitute for CD8 T cells. Likewise, in mice with efficient endogenous reconstitution, harmless CMV infection progressed to lethal CMV disease after selective in vivo depletion of reconstituting CD8 T cells, whereas depletion of reconstituting CD4 T cells did not interfere with the control of infection. This is an important finding, since it appears to contradict previous work on the efficient control of murine CMV infection in mice undergoing long-term depletion of either CD4 T cells (12) or CD8 T cells (13). Apparently, the mechanism by which CD4 T cells acquire antiviral function in
the long-term absence of the CD8 subset cannot develop in the short period during hematolymphopoietic reconstitution after BMT. Graft-versus-host disease prophylaxis by depletion of CD8 T cells is therefore not indicated during a diagnosed CMV infection. This conclusion is in line with clinical experience made after an in vivo-ex vivo T-cell depletion for allogeneic BMT (10).

In BMT recipients in which hematolymphopoietic reconstitution of endogenous CD8 T cells sufficed for preventing lethal CMV disease, a preemptive supplementary CD8 T-cell immunotherapy was not without benefit. Specifically, the therapy accelerated the resolution of acute infection and limited the establishment of latency, as reflected by a lower load of latent CMV DNA in the organs. In consequence, the therapy also reduced the incidence of CMV recurrence. Demonstrating the correlation between the viral genome load and recurrence is not feasible for human CMV latency in patients, since this would require organ biopsies in virtually healthy individuals followed by experimental immunosuppression. Clearly, this question is a case for the murine model. The results documented here provide a supporting argument in favor of a preemptive CD8 T-cell immunotherapy in BMT patients who are at risk of a CMV infection.

Having shown the benefits of CD8 T-cell immunotherapy, the model also revealed the limits. The data indicate that CD8 T cells cannot prevent the establishment of CMV latency. At first glance, this conclusion is not at all new. Previous attempts to prevent CMV latency with CD8 T cells have also failed (1). However, it remained unknown whether the failure in clearing the viral DNA was a trivial problem of dosage or revealed a fundamental limit. This question has now been answered. In three quite different organs, namely, the lungs, the salivary glands, and the adrenal glands, the latent virus DNA load was susceptible to preemptive therapy, with the notable exception of a therapy-resistant fraction of ca. 1,000 genomes per 10^6 tissue cells. Specifically, for the lungs, 10^6 CD8 T cells reduced the load from 5,000 to 1,000 copies but a 10-fold increase in the number of CD8 T cells did not further improve the therapeutic effect. Likewise, in the adrenal glands, a load of 1,000 genomes from BM and blood (17). Hematopoietic progenitors and circulating leukocytes, including blood monocytes, are therefore not the carrier cells of the latent viral genome detected here, whereas resident histiocytes have still to be considered. Clearly, the search for the latently infected cell type(s) in CMV infection is far from its end.

Instead of proposing two sites of extraleukocytic CMV latency, one could argue that CD8 T cells might control the hematogenic spread of the virus from the plantar site of inoculation to the target organs rather than controlling the establishment of latency within the organs. This objection is not supported by the data. After therapy with a high dose of CD8 T cells, productive infection still disseminated to the salivary glands and lungs whereas the titer in the adrenal glands was below the level of detection. Nevertheless, the therapy-resistant load was the same for all three organs. Along the same line of argument, the therapy-resistant load was reached in the adrenal glands after therapy with only 10^5 CD8 T cells, although productive infection was then still detectable in the adrenal glands. Likewise, if we compare the salivary glands and lungs, the prolonged and high virus productivity in the salivary glands did not result in an accordingly high load. Since productive CMV infection is cytolytic for fully permissive cells in situ (19), the cells that account for the virus titer measured during acute infection are distinct from those that retain the latent viral genome. This explains the lack of correlation between virus productivity and the load of latent viral DNA (1, 27).

In conclusion, CMV latency still keeps secrets to be addressed in future research. The data presented here have re-
vealed the first evidence of two types of latency that differ with respect to immune system evasion properties in the early stage of their establishment. This finding is of theoretical and practical importance, since a significant portion of the latent virus load and consequent risk of recurrent infection can be prevented by antiviral CD8 T cells. The model of murine CMV infection thus provides a supportive argument for a preemptive CD8 T-cell immunotherapy of human CMV disease.

ACKNOWLEDGMENTS

This work was supported by a grant to M.J.R. by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF), Collaborative Research Project on CMV, individual project 01K1 9319/2, and by the Deutsche Forschungsgemeinschaft, project RE 712/3-2.

REFERENCES


