Immune Transfer Protects Severely Immunosuppressed Mice from Murine Cytomegalovirus Retinitis and Reduces the Viral Load in Ocular Tissue

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Cytomegalovirus (CMV) retinitis is a sight-threatening disease that affects immunosuppressed people and is prevalent in people with AIDS. The purpose of this study was to evaluate murine CMV (MCMV) retinitis in a replenishing model with adoptive immune transfer into severely immunosuppressed animals. Adult BALB/c mice, immunosuppressed with cyclophosphamide, were infected subretinally with $5 \times 10^7$ plaque-forming units of MCMV. Four to six hours later, $3-4 \times 10^7$ donor cells were transferred by intravenous infusion. Eight days after the transfer, the eyes that had received donor cells were studied histologically, titered for infectious virus, and analyzed with polymerase chain reaction (PCR). Adoptive transfer of total MCMV-immune lymph node (LN) cells or enriched LN lymphocytes specifically and significantly protected immunosuppressed mice from retinitis even after the initiation of infection. The transfer resulted in a reduced viral load, as measured by both plaque assay and PCR. This replenishment model will be useful for determining the specific immune parameters of protection from CMV retinitis.

Cytoamegalovirus (CMV) is a herpesvirus that generally causes inapparent infections in healthy individuals. However, in situations of immunosuppression such as organ transplantation or infection with AIDS, a primary infection or reactivation of latent CMV can result in significant disease. Within a susceptible host, CMV can infect and cause disease in several organ systems, including the eye (for reviews, see Britt and Alford [1], Hirsch [2], and Plachter et al. [3]).

CMV retinitis, a sight-threatening result of ocular infection, is a focal, progressive, necrotic infection of the retina. It is the most common infectious ophthalmic complication in people with AIDS [4-6], affecting up to 40% of patients with AIDS not receiving highly active antiretroviral therapy, and is the leading cause of blindness in this population [7, 8].

The histological features of CMV retinitis are well defined, but the species-specific host restrictions have limited the patho-genetic studies of human CMV (HCMV), including ocular infection. Fortunately, murine CMV (MCMV) has been shown to be biologically similar in many respects to HCMV infection and pathogenesis [9]. Recent advances in a murine model of ocular infection that uses a subretinal inoculation of MCMV by supraciliary injection have provided a system histopathologically similar to HCMV retinitis [10, 11]. Immunocompetent mice given a supraciliary inoculation of MCMV ($10^4$ plaque-forming units [pfu]) develop a high incidence (>60%) of focal, necrotizing retinitis [12], and the susceptibility increases if the mice are immunosuppressed before infection by either corticosteroid treatment or selective depletion of lymphocyte subsets [12, 13]. In the immunosuppressed animals, a low dose ($10^2$ pfu) of virus is sufficient to induce necrotizing retinitis at a frequency >80%.

Immunological control of CMV is critical for controlling disease progression, and various murine models have implicated CD8+ T cells and natural killer (NK) cells as important components for mediating protection [14-16]. T lymphocytes have also been implicated in regulating CMV infection of ocular tissue [12], and recent studies by Lu et al. [17] have shown that adoptive transfer of immune cells provides protection from the development of retinitis in mice depleted of T lymphocyte subsets. However, immune responses are often compartmentalized in immune-privileged sites such as the nervous system and the eye [18, 19], and the plasticity of the immune system often includes redundant and compensatory responses. This is exemplified by recent findings that NK cells are important contributors in preventing MCMV retinitis in both immunocompetent mice and mice depleted of T lymphocytes [20]. Therefore, the evaluation of T cell–subset depletion, with other cell populations remaining intact in recipient animals, limits the pa-
rameters of adoptive immunotherapy that can be examined, because T cells apparently are not the exclusive population that confers protection.

We used supraciliary inoculation of MCMV into BALB/c mice immunosuppressed with cyclophosphamide (CY) to establish a replenishment model of adoptive immunotherapy for control of MCMV retinitis. Unlike previous studies that used selective T cell–subset depletion [17, 21], inducing a general immunosuppression in recipient animals allows a more direct assessment of the immune parameters of protection, with less interference of host immunity. Our results show that the adoptive transfer of MCMV-specific immune lymph node (LN) cells or enriched LN lymphocytes to CY-immunosuppressed recipient mice conferred specific and significant protection from the development of CMV retinitis. Importantly, protection occurred even after the onset of ocular infection. In addition, analysis of ocular tissue by plaque assay and polymerase chain reaction (PCR) suggests that the protection observed might in part be because of the reduction of viral load within ocular tissue.

Materials and Methods

Virus and tissue culture. The Smith strain of MCMV was prepared from murine salivary gland homogenates as described elsewhere [22, 23]. Mouse embryo fibroblast cells used for viral titration and production of control DNA were prepared from 18–20-day gestation BALB/c mouse embryos (Harlan Sprague-Dawley, Indianapolis) and maintained as described elsewhere [24]. The KOS strain of herpes simplex virus (HSV) type 1 was prepared by infecting rabbit skin cells [24, 27]. The Smith strain of MCMV was prepared by inoculation of New Zealand White rabbits (Harlan Sprague-Dawley, Indianapolis) and maintained as described elsewhere [24]. The KOS strain of herpes simplex virus (HSV) type 1 was prepared by infecting rabbit skin cells [24, 27].

Immunosuppression and infection of mice. Adult (6- to 8-week-old) female BALB/c (H-2d) mice (Harlan Sprague-Dawley) were used for all experiments. Mice were immunosuppressed with CY administered intraperitoneally 7, 5, and 3 days before infection or adoptive transfer [26]. For preparation of MCMV-specific donor cells, immunocompetent mice were infected intravenously with 6 × 10^3 pfu MCMV. Mice were infected by intraplantar inoculation of 1 × 10^5 pfu of strain KOS for preparation of HSV-specific donor cells. For MCMV ocular inoculations, the right eye of each recipient mouse was injected with 5 × 10^2–7.5 × 10^2 pfu per eye via the supraciliary route as described elsewhere [10], with slight modifications. A fresh aliquot of virus was thawed and diluted to the appropriate concentration in MEM (Gibco BRL, Grand Island, NY) immediately before the inoculation for each experiment. The titer of the inocula was confirmed by plaque assay. Mock-infected mice received diluent only.

Preparation of donor cells and adoptive transfer. MCMV-specific donor LN cells were collected from BALB/c mice systemically infected with MCMV [23]. Donor cells for control transfers were obtained from mice mock infected with MEM only. Eight days after infection of donor mice, the superficial inguinal, popliteal, axillary, brachial, and cervical LN cells were removed and pooled, and a single-cell suspension was made by passing the cells through a wire mesh, as described elsewhere [26]. For accessory cell deletions and lymphocyte enrichment, total LN cells were loaded onto a 30-mL packed Sephadex G10 (Sigma, St. Louis) column equilibrated with RPMI 10% fetal calf serum (RPMI-10; Gibco). The cells were incubated on the column for 30 min and eluted with RPMI-10. For HSV-specific transfers, the popliteal LN from HSV-infected or mock-infected donor mice were harvested and pooled, and a single-cell suspension was made. Viability of all donor cells was determined by trypan blue exclusion, and the concentrations were adjusted to 1.5–2.0 × 10^6 viable cells/mL. Three to five hours after supraciliary inoculation, 3–4 × 10^7 MCMV-specific, HSV-specific, or control donor cells were infused via the tail vein into each recipient mouse.

Histological scoring. Eight days after transfer, the mice were killed and the infected eyes were enucleated. Eyes analyzed histologically were fixed in buffered formalin, embedded in paraffin, cut into thin sections at 8 µm, and stained with hematoxylin and eosin. Each eye was scored in a masked fashion for retinitis severity on a scale of 0–4, as detailed elsewhere [12]. The average retinitis score for each group of animals was determined and the significance calculated by the Kruskal-Wallis 1-way analysis of variance.

In vivo tracking of donor cells. MCMV-specific donor LN cells were labeled with PKH67 red fluorescent cell linker dye (Sigma) according to the manufacturer’s instructions. The concentration of labeled viable cells was adjusted to 1.5–2 × 10^6 cells/mL, and 200 µL was transferred to each immunosuppressed recipient mouse as described above. Eight days after transfer, the eyes, spleens, and LNs of the recipient mice were harvested and single-cell suspensions made from each tissue. Quantitation of donor cells in each organ was determined by fluorescence microscopy and by flow cytometry. Quantitation by fluorescence microscopy was performed for each recipient organ by counting in triplicate samples the number of fluorescent cells in 1 × 10^6 cells that were cytopsulpt onto glass slides. Duplicate samples were analyzed with flow cytometry on a Coulter eXcel flow cytometer, made available through the Baylor College of Medicine Center for AIDS Research Flow Cytometry Core Facility.

Plaque assay. Eight days after transfer, inoculated eyes from recipient animals were enucleated, snap frozen in liquid nitrogen, and stored at −70°C. The tissue was thawed on ice and homogenized in 1 mL MEM–10% fetal calf serum (MEM-10). The clarified homogenate was titered on monolayers of mouse embryonic fibroblast cells in a 24-well plate as described elsewhere [23]. Following adsorption, the cells were overlaid with MEM-10 containing 0.18% Sea Kem agarose (FMC Bioproducts, Rockland, ME). The cells were incubated for 3 days, the overlay was removed, and the cells were stained as described elsewhere [23, 27, 28]. Plaques were counted and the viral titer (expressed as pfu per eye) was determined as described elsewhere [23].

PCR analysis. Total DNA was extracted from individual eyes in 1 mL Trizol Reagent (Gibco) and purified as described elsewhere [23, 29]. The primers and conditions for the PCR amplification of the MCMV immediate early–1 (ie-1) gene and the mouse adenine phosphoribosyltransferase (APRT) gene in a 50-µL reaction volume were identical to those described elsewhere, except no radiolabeled nucleotides were included [23]. The PCR conditions were designed to amplify the ie-1 and APRT DNAs in their respective linear ranges, which allows for semiquantitation of the amounts of DNA.
Figure 1. Effect of generalized immunosuppression on circulating white blood cells (WBCs) in BALB/c mice. Mice were given cyclophosphamide or were mock treated on days 0, 3, and 5. The WBC counts were kinetically monitored. Each time point represents the mean ± SD of 4–6 animals.

Figure 2. Infectious murine cytomegalovirus (MCMV) titers in eyes of immunocompetent and immunosuppressed mice. Eyes of immunosuppressed and immunocompetent mice were kinetically plaque titered for MCMV. Each time point represents the mean ± SD plaque-forming units (pfu) per eye of 3 animals.

Results

Induction of CMV retinitis. Methylprednisolone-induced immunosuppression has been shown elsewhere to increase the susceptibility of mice to MCMV retinitis [13]; however, the relatively long half-life of corticosteroids and their potential effects on transferred cells makes this approach of immunosuppression undesirable for adoptive transfer and replenishment studies. Therefore, we evaluated the biological effect of CY-induced immunosuppression on the kinetics of MCMV ocular infection and on induction of retinitis following supraciliary inoculation. The CY-induced immunosuppression of recipient animals was monitored kinetically by white blood cell (WBC) counts of peripheral blood (figure 1) by use of standard procedures from blood collected from retro-orbital sinuses [26]. The immunosuppression steadily reduced the number of WBC from an average of $8.2 \times 10^3$ WBC/mm$^3$ of blood (±892) to below the lower limit of detection (<40) by day 6 after treatment. The WBC counts remained below detectable limits for the 2 weeks analyzed, which correlated with the duration of the subsequent adoptive transfer experiments. No significant changes were observed in the WBC counts of mock-treated animals.

Compared with immunosuppressed animals, in which a low dose of MCMV is sufficient to induce a high frequency and severity of retinitis, immunocompetent animals require a high dose of MCMV to induce a similar frequency and severity of retinitis [13]. Therefore, immunocompetent mice and mice immunosuppressed with CY were infected by supraciliary inoculation with $1 \times 10^4$ and $5 \times 10^2$ pfu of MCMV, respectively. Eyes from the 2 groups were enucleated and titered for infectious MCMV over 3 weeks, to determine the infection kinetics. As expected, immunosuppression with CY increased the peak titer of infectious virus in the ocular tissue (~10-fold) and prolonged the clearance of the acute infection by >1 week (figure 2).

To evaluate the ability of MCMV to induce retinitis under these immunosuppressive conditions, eyes from the immunocompetent and immunosuppressed animals infected with MCMV by supraciliary inoculation were enucleated 8 days after infection and histologically scored for retinitis in a masked fashion. Similar to findings reported when methylprednisolone was used as the immunosuppressive agent, most (>80%) retinas from the CY-immunosuppressed mice infected with MCMV exhibited some degree of retinopathy, with the majority (60%) having severe focal necrotic retinitis (data not shown).
consistent with reports elsewhere in which researchers used suprachoroidal inoculation [13], the retinas from control animals receiving diluent as an inoculum were histologically normal except for slight retinal folding in some sections.

**In vivo tracking of donor cells.** For donor immune cells to potentially confer protection to recipient animals, the transferred cells must persist long enough to provide the needed immune function. For in vivo tracking of the transferred cells in our system, the donor cells from both mock- and MCMV-infected mice were prepared, labeled with fluorescent dye PKH26, and transferred into immunosuppressed recipient mice. The PKH26 dye efficiently labeled the donor cells, and, 8 days after transfer, adoptively transferred cells were present in both the recipient spleens and LNs (figure 3). Flow cytometric analysis showed that ~3%–4% of spleen cells (figure 3D) and 13% of the LN cells (figure 3F) in the recipient animals were derived from donors. Background fluorescence in splenic (figure 3C) or LN (figure 3E) samples was minimal. The flow cytometry results correlated well with the number of fluorescent cells observed with fluorescent microscopy of cytospun samples (data not shown). There were also fluorescent cells observed in the eyes of the recipient mice analyzed by fluorescent microscopy (data not shown), indicating dissemination or homing to this site. However, the background fluorescence was relatively high in ocular samples, making quantitation difficult.

**Transferred MCMV-specific LN cells protect recipient mice from retinitis.** The effect of adoptively transferred LN cells on the retinitis in immunosuppressed recipient mice infected with MCMV was determined and compared with mock-infected recipient mice 8 days after transfer. To determine whether the lymphocytes present in the transferred LN cells were responsible for or contributed to the protection observed in our studies, LN lymphocytes were enriched by the removal of accessory cells from the LN cell population with Sephadex G-10 columns [31]. The efficiency of enrichment was evaluated by differential counts of pre- and postcolumn samples and was

![Figure 3](https://academic.oup.com/jid/article-abstract/182/3/652/867049 by guest on 14 October 2018)

*Figure 3.* Flow cytometric analysis of adoptive transfer in recipient spleens and lymph nodes (LNs). Donor LN cells were mock labeled (A) or labeled with fluorescent dye PKH26 (B) before transfer. Eight days after transfer, the spleens (C and D) and LNs (E and F) were analyzed by flow cytometry. Each transfer was performed in duplicate, and representative examples are shown for individual animals.
found to be >90% for the depletion of accessory cells. In addition, the cells eluted from the columns were analyzed in a T cell functional assay and compared with precolumn samples. The results from these assays were consistent with accessory cell depletion because the precolumn cells were readily activated by treatment with the mitogen concanavalin A, but the eluted cells were unable to be stimulated even though they remained viable on the basis of examination by trypan blue exclusion staining (data not shown).

Micrographs of representative results from adoptively transferred LN cells are shown in figure 4. Mock-infected recipient mice, whether they received donor cells from either MCMV-infected mice (figure 4A) or mock-infected mice (data not shown), had retinas histologically indistinguishable from the retinas of mock-infected animals not receiving any adoptively transferred cells. Recipient mice that received a supraciliary inoculation with MCMV and an adoptive transfer of control cells (figure 4B) demonstrated a retinal histopathology varying in severity from mild retinal folding to severe full-thickness necrotic retinitis, with several areas containing cytomegalic inclusion bodies (figure 4C). Compared with normal retinal tissue, the retinitis in the infected animals ranged from focal to fulminant, depending on the particular animal evaluated. The histology in these eyes contrasted sharply with that of the eyes of mice receiving a transfer of MCMV-specific LN cells (figure 4D). The LN cells from MCMV-infected donor mice conferred general protection from ocular histopathology and specific protection from retinal damage. In the animals receiving MCMV-specific donor cells, a moderate to mild retinal folding of the outer nuclear layer was seen, similar to that of mock-infected recipient animals. These eyes had inflammation that was only mild to not observable.

To quantitate the level of protection, histological results from all experimental groups were evaluated and scored in a masked fashion with the specific retinitis scoring system criteria. Scores <2 were considered negative for retinitis; a score of 2 was con-

Figure 4. Histological evaluation of adoptive transfer for protection of recipient mice from murine cytomegalovirus (MCMV) retinitis. Eight days after transfer, eyes were embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin. A, Section of retina from a mock-infected recipient mouse that received an immune transfer. Original magnification, ×200. B, Section of retina from an MCMV-infected recipient mouse that received a control transfer. Original magnification, ×100. C, Magnification of a region of mouse retina in B showing severe, full-thickness necrotic retinitis and several cytomegalic inclusions (arrowheads). Original magnification, ×200. D, Section of retina from an MCMV-infected mouse that received an MCMV-specific immune cell transfer. Original magnification, ×100. Scale bar, 100 mm.
considered mild retinitis; and scores of 3–4 were considered positive for moderate to severe retinitis, respectively. The average histological scores are summarized in table 1. Immunosuppressed animals infected with MCMV by supraciliary inoculation that received a transfer from mock-infected donor mice developed moderate to severe retinitis (average, 3.1). Those that received either full LN or lymphocyte-enriched cell suspensions from MCMV-infected donor mice were dramatically protected from the development of retinitis (average, 1.4 and 1.9, respectively). This observation was statistically significant (P < .001), compared with results from animals receiving a control transfer of cells from mock-infected donor mice. This protection was also CMV specific because cells transferred from HSV-infected donor mice did not protect the recipient mice. The average retinitis score of recipient animals that received HSV-specific donor cells was 2.3, which was significantly higher from mice given a full LN MCMV-specific transfer (P = .03) but not significantly different from the average score for animals that received control cell transfers from mock-infected donors (P = .17). Retinitis was not observed in mock-infected recipient animals, whether they received a transfer of cells from the infected donors (immune transfer) or mock-infected donors (control transfer), showing that the adoptive transfer alone caused no detectable ocular histopathology.

In addition to affecting the severity of retinal involvement, the adoptive transfer of MCMV-specific donor cells also impacted the incidence of retinitis (table 2). Most of the animals that received a transfer of cells from mock-infected or HSV-infected donor mice developed moderate to severe retinitis by 8 days after infection (82% and 62%, respectively). In contrast, the percentage of animals that developed moderate to severe retinitis was significantly reduced if the transferred full LN (14%) or lymphocyte-enriched LN cells (27%) were from MCMV-infected donor mice (P < .001).

**Transferred MCMV-specific LN cells reduces the viral load in ocular tissue.** One potential mechanism of the protection described above is by the transferred cells either directly or indirectly reducing the amount of virus in the ocular tissue and thereby diminishing retinal damage. To measure the level of infectious virus, recipient mice were infected with MCMV or were mock infected and given an adoptive transfer of immune or control donor cells. Although infectious virus was detectable 8 days after transfer in all MCMV-infected recipients (figure 5), there was a ~30-fold decrease in the amount of infectious virus in the eyes of animals that received an immune cell transfer, compared with those that received a control cell transfer (P = .11). Consistent with the histological findings, no virus was detected in mock-infected eyes of recipient animals. The difference in viral load within the ocular tissue was also measured molecularly by semiquantitative PCR 8 days after transfer. Representative results are shown in figure 6 and were consistent with the plaque assay results. Only eyes from recipient mice infected with MCMV had detectable viral DNA, whereas eyes from mock-infected mice were negative for viral DNA (figure 6A). There was a slight variation in the amount of total DNA recovered from individual samples as measured by APRT PCR (figure 6A); however, this variation was consistent between the different experimental groups. The plasmid pH1, which contains a region of the MCMV ie-1 gene cloned into pUC19 [23, 29, 30], was used as a positive control and a semiquantitation standard for the PCR studies. The dynamic range of linear amplification of pH1 was determined by densitometric analysis of serial diluted plasmid and found to be $10^6$ through $10^7$ copies (data not shown). The amount of viral DNA detected by PCR analysis was within the dynamic range of the PCR amplification regardless of whether the animal received a transfer of immune or control donor cells (figure 6B).

Densitometric analysis of PCR results was standardized to the total amount of DNA present for each sample on the basis of amplification of the murine APRT gene. The average amount of viral DNA present in the eyes of animals that received the adoptive transfer of MCMV-specific immune cells was lower than the amount of viral DNA present in the eyes of animals that received LN cells from mock-infected donor mice (figure 6B). There was a ~1.2-fold decrease in relative densitometric value, which corresponded to a 4–5-fold decrease in copy number of viral DNA. The difference between the infected eyes receiving an immune cell transfer (densitometric average, 152 ± 7.5) and a control transfer (densitometric average, 187 ± 12.2) was statistically significant (P = .01).

**Discussion**

We developed a replenishing model for adoptive immune transfer to test the hypothesis that MCMV-immune cells could protect CY-immunosuppressed mice from developing retinitis even after the initiation of ocular infection. We chose a replenishment approach in order to limit interference by recipient immunity. Our results demonstrate that adoptive transfer of...
Table 2. Incidence of animals developing murine cytomegalovirus (MCMV) retinitis.

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Retinitis severity</th>
<th>Total MCMV-immune LN cells</th>
<th>Lymphocyte enriched</th>
<th>Total herpes simplex virus-immune LN cells</th>
<th>Control</th>
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<tbody>
<tr>
<td>MCMV infected</td>
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<td>100</td>
<td>100</td>
<td>ND</td>
<td>100</td>
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<tr>
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<td>0</td>
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<td></td>
<td>Severe</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Mock infected</td>
<td>None</td>
<td>50</td>
<td>33</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>36</td>
<td>40</td>
<td>15</td>
<td>12</td>
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<tr>
<td></td>
<td>Severe</td>
<td>14</td>
<td>27</td>
<td>62</td>
<td>82</td>
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NOTE. LN, lymph node; ND, not done.

a Cyclophosphamide-immunosuppressed recipient mice were subretinally infected with MCMV or mock infected, then given the indicated cell transfer.

b Mean histological scores/severity of retinitis: none, 0±1; mild, 2; severe, 3±4.

c Percentage of animals within an experimental group with the indicated severity of retinal involvement (n > 13 animals per group).

MCMV-specific immune cells protected severely immunosuppressed recipient animals from retinitis. This protection was significant in animals that received either a full LN transfer or a lymphocyte-enriched transfer and was MCMV specific. Plaque titer and PCR analyses suggested that the effectiveness of the transfer might in part be due to a reduction of the amount of virus in the ocular tissues.

In the BALB/c mouse model, which is a model that closely resembles human CMV pathogenesis, CD8+ T cells are the primary antiviral effectors and are critical for controlling CMV in several major organ systems (for review, see Koszinowski et al. [32]). Preemptive adoptive immunotherapy to severely immunosuppressed or immunodeficient recipient mice has been shown to be effective in controlling MCMV in several tissues, including lungs [15], salivary glands [33], and adrenal glands [34]. These results from mice correlate with findings from patients receiving preemptive CD8 immunotherapy for posttransplant CMV infection [35]. Experimental bone marrow transplantation models in mice have provided evidence that reconstitution of CD8+ T cells is essential for the prevention of lethal CMV disease and that these cells are responsible for resolving the acute infection [14, 33]. Similarly, in human bone marrow transplantation, the reconstitution of cellular immunity is critical for control of CMV disease resulting from both primary and recurrent infections [36, 37].

It is known, however, that immunological control of CMV disease is more complex than just CD8+ cytotoxic T lymphocyte activity. The specific mechanism of control might be organ dependent, because there is evidence that CD4+ cells are responsible for control in the salivary glands [38, 39]. It is also clear that NK cells play an important role in protection [40, 41]. In addition, there are reports of immunological compensation in mice that are either depleted of CD8+ cells or β-2m−/− for the long term, in which both mouse strains clear the productive infection in the absence of obvious cytotoxic T lymphocyte activity [38, 42].

In terms of ocular infection, the precise cellular factors critical for the protection from CMV retinitis are unclear but appear to be multifactorial. Recent evidence from adoptive transfer studies in selectively depleted recipient mice implicates both NK cells and CD8+ cytotoxic T lymphocytes [20, 21]. To evaluate immune parameters of protection in adoptive transfer studies, the method of recipient immunosuppression is important for the interpretation of results. Other studies of immunosuppression with subretinal inoculation of MCMV have utilized generalized immunosuppression by corticosteroid treatment or selective in vivo depletion of CD4+ and CD8+ T lymphocyte subsets [12, 13]. The disadvantages of these respective
Figure 6. Polymerase chain reaction (PCR) analysis of murine cytomegalovirus (MCMV) DNA in mouse eyes 8 days after transfer of peripheral lymph node donor cells. A. Immunosuppressed mice subretinally infected with MCMV (indicated as [+CMV]) or mock infected (indicated as [−CMV]) received cells from MCMV-infected donors (immune transfer) or mock-infected donors (control transfer). Eight days after transfer, eyes were analyzed by PCR specific for the MCMV immediate early–1 gene, which produces a 476-bp product. The plasmid pRE1 was used as a positive control, and water served as a negative control. PCR specific for the mouse adenine phosphoribosyltransferase (APRT) gene was used as an internal sample control. B. Densitometric analysis of the viral load in eyes of the different groups of mice from A. Each bar represents the mean ± SD of 4–5 animals per group.

treatments are long drug/antibody half-lives in the body, which could potentially affect transferred donor cells, and suppression of specific immune cell types, which limits the range of immune mechanisms evaluated. We chose the acylating agent CY because of its well-defined and rapid biological effect, short half-life of the drug in the body, and general immunosuppressive properties. Although possible interactions between donor and resident recipient cells cannot be excluded, the CY treatment does produce a broader spectrum of suppression than selective in vivo depletion by antibody treatment. Consistent with previous work by Atherton et al. [12] in which methylprednisolone was used to induce a general immunosuppressive condition, immunosuppression with CY increased the susceptibility of the mice, so that a low dose of virus was sufficient to induce retinitis in a majority of animals. These results support the use of CY for the induction of immunosuppression in this system and afford the benefits mentioned for a replenishing model.

A major question of adoptive immunotherapy in this and other systems is whether cells from donor mice can confer protection to an immunosuppressed recipient without inducing immunopathology. In AIDS patients, highly active antiretroviral therapy–associated immune recovery has led to improved survival and lower rates of opportunistic infections, but there are associated problems. Recent reports have described in some patients with preexisting CMV retinitis the development of intraocular inflammation termed immune recovery uveitis [43]. The dramatic reduction we observed in the severity of retinal damage after adoptive transfer demonstrates protection, but we did not see any indication of inflammation correlating to immune recovery uveitis. These findings suggest that no immunopathologic problems are associated with adoptive immune transfer, but it is important to note that our analyses were performed at only 8 days after transfer. Future studies should incorporate analyses at several weeks or months after transfer to evaluate the potential long-term consequences in this model.

Consistent with previous reports implicating a role for lymphocytes in preventing or limiting MCMV retinitis [13, 17, 21], the LN-enriched lymphocytes tested in our system protected recipient mice from retinitis. However, protection by the transfer of full LN cell suspensions appeared to be slightly better than protection from the transfer of the lymphocyte-enriched suspensions. Although the difference was not statistically significant, the biological condition of the cells after column purification, the absolute number of effector cells transferred, a partial selective loss of B cells, or the loss of some required accessory cell function because of removal of cells during purification could have contributed.

There was a relatively large population of nonfluorescent cells in the spleen and in the LNs of recipient mice 8 days after transfer. We cannot exclude the possibility of residual immune cells remaining after CY treatment, but this result could partially be explained by transferred donor effector cells undergoing expansion after transfer. Cell division results in less dye per cell, and the fluorescence intensity decreases to background levels by 8 doublings [44].

The protective mechanism of the immune transfer is not clear, but the findings of the plaque assay and PCR suggest a limiting of viral replication or an altering of viral dissemination within the ocular tissues. Although the reduction in plaque titers in the immune transfer group was not statistically significant, the results were reproducible, and the variations between animals within an experimental group were consistent. The differences
in measurements of viral DNA were significant. In the infected recipients that received an immune cell transfer compared with recipients that received a control transfer, there was a ~30-fold decrease in mean infectious titer and a 4–5-fold decrease in viral DNA. The fact that these 2 methods rely on different end points might account for the apparent discrepancy between the assays, and the results suggest that the immune transfer had at least part of its effect on the virus after DNA replication. Regardless of the absolute numbers, results from both assays suggest that the adoptive transfer reduced the ocular viral load and indicate that carryover virus from the donor mice was not problematic for these studies.

It is important to note that the measurements by both plaque assay and PCR were on entire eyes, so obviously there might be larger effects on viral load specifically within particular tissues such as the retina. Also, the analyses were performed on day 8 after transfer—the optimal time for the development of retinitis. Evaluation at later times might show greater reductions in viral load.

In previous ocular studies, it is indicated that the transfer was performed before the inoculation of virus [17, 21]. Because CMV is ubiquitous in the human population, and because most cases of AIDS-related CMV retinitis are believed to result from reactivation of a latent virus that disseminates to the ocular tissue [45], it is important clinically that the protection we observed was after the initiation of ocular infection.

Similar to other models of CMV disease, analysis of our data supports the idea that activated lymphocytes are critical for the control of CMV retinitis, but the exact mechanism remains unknown. Although experimental studies to date support CD8+ T cells as the primary effectors, clinically, the development of CMV retinitis is strongly correlated with the decline in the CD4+ lymphocyte count in AIDS patients [6]. This raises an important question as to whether the control of CMV retinitis is strictly a CD4+ independent mechanism. Although the generalized immunosuppression with CY in mice does not directly mimic the selective, gradual CD4+ loss seen in AIDS patients, it does result in histopathology similar to that observed with AIDS-related CMV retinitis. An advantage of the replenishment model described here, in addition to its use in determining the immune effectors, is its potential to define the afferent arm of immunity. Cells that present antigen or provide a helper function in the induction of protective immunity can be identified by use of selective in vivo depletion of cell populations in the donor animals before infection with CMV. There are many factors, both viral and immunological, that contribute to the pathogenesis of CMV retinitis in AIDS patients, and effective long-term treatment strategies will ultimately depend on a more complete understanding of the host immune mechanisms. This model provides one system for evaluating the immune mechanisms that control CMV retinitis.

Acknowledgments

The authors wish to thank Drs. K. Wilhelmus and S. Wardwell and Ms. L. Ward for critical review of the manuscript and helpful discussions. We also wish to thank the Baylor College of Medicine Center for AIDS Research Flow Cytometry core facility and the Department of Ophthalmology histology, digital imaging, and molecular biology core facilities.

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