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Murine cytomegalovirus retinitis during retrovirus-induced immunodeficiency (MAIDS) in mice: interleukin-2 immunotherapy correlates with increased intraocular levels of perforin mRNA

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Abstract

Mice with a retrovirus-induced immunosuppression (MAIDS) are susceptible to experimental murine cytomegalovirus (MCMV) retinitis, but can be rendered resistant to retinitis by systemic interleukin-2 (IL-2) immunotherapy. Experiments were performed to explore the mechanism by which IL-2 treatment during MAIDS might restore resistance to MCMV retinitis. Whereas 80% of untreated MAIDS mice were susceptible to MCMV retinitis, none (0%) of IL-2-treated MAIDS mice developed necrotizing retinitis. In comparison, 100% of both untreated and IL-2-treated perforin knockout mice (PKO mice) were susceptible to MCMV retinitis, and severity of retinitis and amounts of infectious intraocular MCMV in IL-2-treated PKO mice were equivalent to that in untreated PKO mice. A competitive quantitative RT-PCR assay was used to measure the levels of perforin mRNA within MCMV-infected eyes of immunologically normal mice, untreated MAIDS mice, and IL-2-treated MAIDS mice. Although the level of perforin mRNA within MCMV-infected eyes of untreated MAIDS mice susceptible to retinitis was significantly reduced when compared to the high level found within MCMV-infected eyes of normal mice resistant to retinitis, systemic treatment of MAIDS mice with IL-2 increased perforin mRNA within MCMV-infected eyes to levels found in normal mice. The ability of IL-2 treatment to increase intraocular levels of perforin mRNA diminished with the progression of MAIDS. Our findings support the hypothesis that systemic IL-2 immunotherapy during MAIDS provides protection against MCMV retinitis by upregulation of perforin-mediated cytotoxicity used by cytotoxic lymphocytes to kill virus-infected cells.

Keywords: Cytomegalovirus retinitis; AIDS; MAIDS; Interleukin-2; Cytokine immunotherapy; Perforin cytotoxicity

1. Introduction

Before the availability of highly active antiretroviral therapy (HAART), human cytomegalovirus (HCMV) retinitis caused vision loss and blindness in ∼40% of patients with AIDS at some time during the course of their disease (Bloom and Palenstine, 1988; Fay et al., 1988; Jabs et al., 1989). Although the HAART era has resulted in a significant decrease in the number of new cases of AIDS-related HCMV retinitis (Jabs and Bartlett, 1997; Palella et al., 1998), there has now been a halt to this decline, and the incidence of HCMV retinitis in HIV-1-infected patients remains at 25–30% of its peak incidence (Jabs and Bartlett, 1997; Jacobson et al., 2000). Thus, HCMV retinitis continues to be a significant ophthalmologic complication in patients with HIV-1 disease (Cunningham and Margolis, 1998).

Although management of HCMV retinitis in patients with HIV-1 disease continues to rely on traditional antiviral chemotherapy using such drugs as ganciclovir and foscarnet (Holland et al., 1996), issues of drug toxicity and drug resistance associated with these antivirals remain important clinical concerns. Our research has therefore been oriented toward the investigation of various forms of immune-based therapies as potential adjuvants to traditional antiviral chemotherapy for optimal management of AIDS-related HCMV retinitis in the HAART era. These studies have used an experimental model of murine cytomegalovirus

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(MCMV) retinitis in C57BL/6 mice with MAIDS (Dix et al., 1994), a retrovirus-induced immunodeficiency syndrome of mice that shares many immunopathologic features with AIDS in humans (Jolicoeur, 1991; Morse et al., 1992). Early studies suggested that antibody alone does not appear to affect retinal disease onset or progression since passive transfer of either hyperimmune serum or neutralizing monoclonal antibody against MCMV failed to reduce the frequency or severity of MCMV retinitis during MAIDS (Dix et al., 1997a). However, later studies revealed that systemic cytokine immunotherapy with interleukin-2 (IL-2), an important immunoregulatory Th1 cytokine with potent effects on T cells, B cells, and NK cells (Smith, 1988), restores resistance to MCMV retinitis during MAIDS (Dix et al., 1997b), a resistance observed in immunologically normal C57BL/6 mice following subretinal MCMV inoculation (Dix et al., 1994).

Attempts to define the mechanism by which IL-2 immunotherapy restores resistance to MCMV retinitis during MAIDS focused initially on quantification of natural killer (NK) cells and CD8+ T cells that infiltrate the eye in response to MCMV infection of the retina. MCMV-infected eyes of IL-2-treated mice with MAIDS were found to exhibit a 10-fold increase in the number of infiltrating CD8+ T cells when compared to MCMV-infected eyes of untreated mice with MAIDS (Dix and Cousins, 2003). Surprisingly, however, IL-2 treatment during MAIDS had no appreciable effect on the number of NK cells that infiltrate MCMV-infected eyes. Subsequent experiments exploring the mechanisms by which NK cells and cytotoxic CD8+ T cells kill virus-infected cells (Kagi et al., 1994; Lowin et al., 1994, 1995; Smyth and Trapani, 1998) provided evidence that susceptibility to MCMV retinitis during MAIDS correlates with a deficiency in the perforin-mediated cytotoxic pathway as measured by decreased amounts of perforin mRNA within splenic T-lymphocytes and MCMV-infected eyes of mice with MAIDS (Dix et al., 2003). The series of experiments described herein was therefore designed to test the hypothesis that systemic IL-2 immunotherapy during MAIDS correlates with increased levels of perforin mRNA and consequently upregulation of the perforin cytotoxic pathway within MCMV-infected eyes that serves to restore resistance to MCMV retinitis. Results demonstrate that MCMV-infected eyes of mice with MAIDS treated systemically with IL-2 have significantly increased levels of perforin mRNA when compared to MCMV-infected eyes of untreated mice with MAIDS. Moreover, the level of perforin mRNA detected within MCMV-infected eyes of IL-2-treated mice with MAIDS is equivalent to that found in immunologically normal mice that are naturally resistant to MCMV retinitis. We conclude that systemic IL-2 immunotherapy during MAIDS restores resistance to MCMV retinitis by upregulation of the perforin cytotoxic pathway that is used by NK cells and cytotoxic CD8+ T cells for clearance of infectious virus from retinal tissues and protection against necrotizing retinitis.

2. Materials and methods

2.1. Mice

Adult (8–10 weeks of age) C57BL/6 knockout mice with a null mutation in the perforin gene (perforin −/− mice; PKO mice) (Kagi et al., 1994; Lowin et al., 1994; Walsh et al., 1994) were obtained from the Trangenic Mouse Facility, University of Miami School of Medicine, Miami, FL. Adult C57BL/6 euthymic mice (Taconic Farms, Germantown, New York) were used for induction of MAIDS and also served as immunologically normal control mice in all retinitis experiments. Mice were allowed unrestricted access to food and water and maintained in alternating 12-h light/dark cycles. All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Viruses

Stocks of MCMV were prepared in mouse salivary glands of adult BALB/c mice (Taconic Farms, Germantown, New York) as described previously (Dix et al., 1994). Mice were infected intraperitoneally with 1×10^2 to 1×10^3 plaque-forming units (PFU) of the Smith strain of MCMV contained within a 0.2 ml volume. Fourteen days later, the salivary glands were removed aseptically, homogenized (10%, w/v) in Dulbecco's modified Eagle's tissue culture medium (DMEM) containing 10% fetal bovine serum, clarified by centrifugation, and 0.5 ml aliquots of the supernatants were stored in liquid N_2. Virus stocks were titered on monolayers of mouse embryo fibroblasts (MEF) grown in DMEM. A fresh aliquot of MCMV stock was thawed and used for a single experiment.

Stocks of the murine retrovirus mixture (LP-BM5–MuLV) were prepared using SC-1/MuLV–LP-BM5 cells obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Approximately 1×10^6 of SC-1/MuLV–LP-BM5 cells were mixed with approximately 1×10^6 of uninfected SC-1 cells and maintained for 6 days in DMEM. The cells were then scraped into the medium, pelleted by centrifugation, resuspended in phosphate-buffered saline (PBS), and frozen at −70 °C. Prior to use, the suspension was thawed and clarified by centrifugation to remove cellular debris.

2.3. Interleukin-2

Polyethylene glycol-modified human recombinant IL-2 (PEG-IL-2) (4×10^6 IU/mg) was a kind gift from Chiron Corporation, Emeryville, CA, USA. A new vial of PEG-IL-2 was reconstituted in sterile distilled H_2O immediately before use and administered as a single intramuscular injection (thigh muscle of the left or right leg) at a dose of ~23.5 ug (1 mg/kg per mouse) 2 days prior to subretinal MCMV challenge as done in a previous study (Dix et al.,

1997b). Protection against MCMV retinitis has been shown to occur when PEG-IL-2 is administered systemically at 3, 2, or 1 day prior to subretinal MCMV challenge (Dix et al., 1997b). The biologic activity of this PEG-IL-2 preparation was confirmed using an IL-2-dependent T cell line that undergoes apoptotic cell death in the absence of IL-2 (Deng and Podack, 1993) (data not shown).

2.4. Induction of MAIDS

MAIDS was induced in normal C57BL/6 mice by intraperitoneal injection of 1.0 ml of LP-BM5–MuLV preparation (Mosier et al., 1985). The inoculum contained approximately 5×10^3 to 5×10^4 infectious murine retroviruses. Mice with MAIDS of 8, 9, and 10 weeks duration were used in these studies. These animals consistently displayed visual clinical signs and symptoms (e.g. persistent generalized lymphadenopathy) consistent with MAIDS as described previously (Dix et al., 1994).

2.5. Experimental mouse model of MCMV retinitis

AIDS-related HCMV retinitis is thought to originate from virus that invades the retina from the blood during systemic infection (Holland et al., 1996). Intravenous or intraperitoneal injection of immunosuppressed mice with MCMV to produce systemic MCMV infection fails to cause retinal disease, although virus infects the choroid, retinal pigment epithelium (RPE), and ciliary body (but not the neurosensory retina) of the eye (Gao et al., 1995; Dix, 1998). Consequently, several laboratories (Atherton et al., 1991; Dix et al., 1994; Gao et al., 1995; Kercher and Mitchell, 2000) routinely induce experimental MCMV retinitis in mice by supraciliary (subretinal) MCMV injection, a procedure that induces reproducible retinal disease in mice with histopathologic features similar to those found in AIDS-related HCMV retinitis (Atherton et al., 1991). These include full-thickness retinal necrosis, retinal cells and RPE with prominent virus-induced nuclear inclusions, foci of cytomegalic cells (cytomegalocytes), hemorrhage, and abrupt transition zones between areas of involved and uninvolved retina (Dix et al., 1994). All mice in the present study were anesthesized and injected subretinally with approximately 1×10^4 PFU of MCMV as described previously (Dix et al., 1994).

2.6. Histopathologic analysis of eyes and evaluation of retinitis

At 10 days after subretinal MCMV injection, eyes were carefully removed from euthanized animals, fixed in 10% buffered formalin, paraffin-embedded, sectioned, stained with hematoxylin and eosin (H&E), and examined by light microscopy. Since necrotizing retinitis can remain regionally localized within the retina, at least six histopathologic sections representing different regions of the retina were examined. The histopathologic sections from MCMV-infected eyes were scored using a modification of two types of scoring systems described previously (Dix et al., 1994) to quantify the frequency and severity of MCMV retinitis in mice. Firstly, the sections were scored for the presence (i.e. score of 2 or more on at least two sections) or absence of necrotizing retinitis to determine the frequency of retinitis within each animal group. Secondly, each section was evaluated semiquantitatively for posterior segment inflammation. All six sections of each eye were scored, and the scores for all sections were summed (with a maximum score of 24). The median score and the percentage of the maximum score were calculated for each group. The frequency data were analyzed for significance by the chi-squared test. The severity data for posterior segment inflammation were analyzed for significance by the two-tailed Mann–Whitney *U*-test because the scoring system provided categorical rather than continuous measurements.

2.7. Measurement of mouse perforin mRNA

A competitive quantitative RT-PCR assay developed by our laboratory (Dix et al., 2003) was used to measure mouse perforin mRNA levels in pooled eyes collected from groups of normal mice, untreated MAIDS mice, and IL-2-treated MAIDS mice at 3 days after uniocular subretinal MCMV inoculation. Following extraction of total RNA (~20 ug per eye), reverse transcription and quantitative competitive RT-PCR for perforin mRNA content was performed using primers specific for mouse perforin (Gene Bank Accession Number M23182): 5′-CACAGTAGAGTGTCCGATGTA-3′ (upstream primer), 5′-CTTGGTTCCCGAAGAGCAGAT-3′ (downstream primer), and 5′-CTTGGTTCCCGAAGAG-CAGATAAAGAGGTGGCGATTTTGTGCT-3′ (composite primer). Conditions for PCR were 94 °C for 30 s, 53.4 °C for 30 s, and 72 °C for 2 min for a total of 40 cycles. The pattern of cDNA products (442 bp for authentic perforin and 380 bp for the competitive template) was visualized on ethidium–bromide-stained agarose gels. Sensitivity of the competitive RT-PCR assay was ~1 fg perforin mRNA per 1000 ng total RNA.

3. Results

3.1. Comparison of susceptibility of PKO mice and mice with MAIDS to MCMV retinitis following systemic IL-2 immunotherapy

PKO mice harbor a null mutation in the perforin gene. These knockout mice are therefore deficient in the perforin-mediated pathway of cytotoxicity, but otherwise possess normal immune responses including cytotoxic lymphocytes with functional Fas/FasL cytotoxicity (Kagi et al., 1994; Lowin et al., 1994; Walsh et al., 1994; Clark et al., 1995). Previous work has shown that PKO mice and mice with MAIDS share equivalent susceptibilities to MCMV

Table 1
Frequency and severity of MCMV necrotizing retinitis at 10 days postinfection

	Frequency of necrotizing retinitis (%) (retinitis/total)	Median posterior segment score (range) % maximum
Mice with MAIDS[a]		
Untreated	80 (8/10)	14.0 (2.0–20) 56
IL-2-treated	0 (0/9)[b]	3.0 (1.5–3.5) 11[c]
PKO mice		
Untreated	100 (5/5)	16.5 (12.5–18.5) 68
IL-2-treated	100 (5/5)	18.5 (12.0–18.5) 70

[a] MAIDS of 8 weeks duration.
[b] Significantly different from untreated mice with MAIDS group, $P < 0.001$.
[c] Significantly different from untreated mice with MAIDS group, $P < 0.05$.

retinitis (Dix et al., 2003). If IL-2 immunotherapy confers protection against MCMV retinitis during MAIDS exclusively by upregulation of the classic perforin cytotoxic pathway and does not involve other immune mechanisms of immune clearance, then systemic IL-2 immunotherapy of PKO mice should have no effect on their susceptibility to MCMV retinitis. To test this hypothesis, groups of PKO mice and MAIDS mice were treated intramuscularly with PEG-IL-2 2 days prior to subretinal inoculation of MCMV; control PKO mice and MAIDS mice received distilled H_2O intramuscularly 2 days prior to subretinal MCMV inoculation. In agreement with previous studies (Dix et al., 2003), histopathologic analysis of MCMV-infected eyes collected from untreated PKO mice and MAIDS mice at 10 days after subretinal MCMV inoculation revealed a necrotizing retinitis in 100% (5 of 5) and 80% (8 of 10) of the animals, respectively (Table 1). Previous results (Dix et al., 1997b) also predicted that none (0%) of eyes recovered from PEG-IL-2-treated mice with MAIDS showed histopathologic features of classic MCMV retinitis (Table 1). Instead, sections of eyes from these animals showed either normal retinal architecture or retinal folds (Fig. 1A) similar to that observed in sections of eyes from normal mice (Dix et al., 1994). In sharp contract, however, 100% (5 of 5) of PEG-IL-2-treated PKO mice exhibited classic MCMV retinitis (Table 1) with full-thickness retinal necrosis and prominent cytomegalic cells (Fig. 1B). In addition, the severity of retinal disease in these animals was equivalent to that observed in untreated MCMV-infected PKO mice (Table 1). Finally, intraocular MCMV titers per eye as determined by standard plaque assay (Dix et al., 1994) were equivalent in the MCMV-infected eyes of untreated and PEG-IL-2-treated PKO mice (4.3 log 10 and 4.5 log 10, respectively) and at levels found in MCMV-infected eyes of untreated C57BL/6 mice with MAIDS that develop retinitis (4.2 log 10). In comparison, MCMV titers per eye of MCMV-infected eyes of untreated normal C57BL/6 mice or PEG-IL-2-treated mice with MAIDS that are resistant to

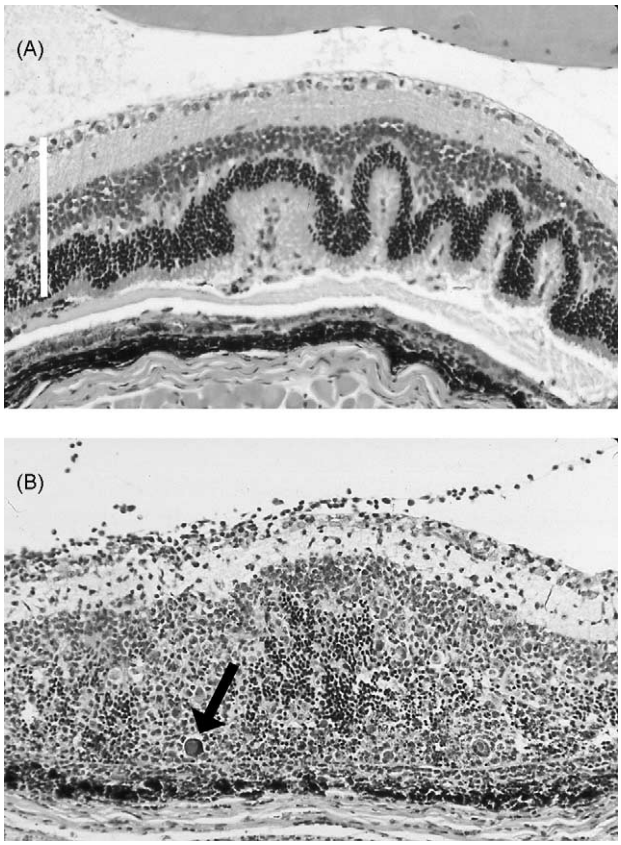

Fig. 1. (A) Photomicrograph of a section of retina of a PEG-IL-2-treated C57BL/6 mouse with MAIDS of 8 weeks duration at 10 days after subretinal inoculation with MCMV. Retinal necrosis is absent, but retinal folding is present as observed in MCMV-inoculated eyes of normal C57BL/6 mice (not shown). The neurosensory retina is indicated by the white bar. (B) Photomicrograph of a section of retina of a PEG-IL-2-treated PKO mouse at 10 days after subretinal inoculation with MCMV. There is full-thickness retinal necrosis that contains cytomegalic cells (arrow) as observed in MCMV-inoculated eyes of untreated PKO mice and untreated mice with MAIDS (not shown). Hematoxylin and eosin; original magnification, 200×.

retinitis were ~2.5 log 10. Thus, systemic IL-2 immunotherapy of mice deficient in the perforin cytotoxic pathway resulted in no detectable protection against MCMV retinitis as measured by frequency of retinal disease, severity of retinal disease, and amounts of infectious intraocular MCMV.

3.2. Effect of IL-2 immunotherapy during MAIDS on perforin mRNA levels in MCMV-infected eyes

We have previously provided evidence that the perforin cytotoxic pathway is diminished during MAIDS as suggested by a significant decrease in perforin mRNA levels within both splenic T-lymphocytes and MCMV-infected eyes of mice with MAIDS when compared with those of normal mice (Dix et al., 2003). This observation could explain the equivalent susceptibilities of MAIDS mice and PKO mice to MCMV retinitis. Since IL-2 immunotherapy during MAIDS restores resistance to MCMV retinitis, we

Table 2
Perforin mRNA levels in MCMV-infected eyes[a]

	Perforin mRNA (fg perforin mRNA/ 1000 ng total RNA)	MCMV retinitis
Normal mice	100	Resistant
Untreated MAIDS mice[b]	<1	Susceptible
IL-2-treated MAIDS mice[b]	100	Resistant

[a] Eyes pooled from six animals per group at 10 days after subretinal MCMV inoculation.
[b] MAIDS of 8 weeks duration.

hypothesized that IL-2-induced resistance during MAIDS would correlate with increased levels of perforin mRNA within MCMV-infected eyes destined to be resistant to retinitis. This hypothesis was tested by use of a competitive RT-PCR assay to measure perforin mRNA levels within MCMV-infected eyes of normal mice and untreated mice with MAIDS, and compare these perforin mRNA levels with those of MCMV-infected eyes of IL-2-treated mice with MAIDS. MCMV-infected eyes from groups of normal mice, untreated MAIDS mice, and IL-2-treated MAIDS mice (six animals per group) were collected at 3 days following subretinal MCMV inoculation, pooled, and subjected to quantitative RT-PCR assay for perforin mRNA expression. Results are shown in Table 2. In agreement with previous findings (Dix et al., 2003), MCMV-infected eyes from normal mice destined to be resistant to MCMV retinitis demonstrated 100 fg of detectable perforin mRNA (Fig. 2), whereas MCMV-infected eyes from untreated mice with MAIDS destined to be susceptible to MCMV retinitis demonstrated <1 fg of detectable perforin mRNA (Fig. 2). In comparison, intramuscular administration of PEG-IL-2 to mice with MAIDS 2 days prior to subretinal MCMV inoculation resulted in an increased level (100 fg) of perforin mRNA within MCMV-infected eyes destined to be resistant to retinitis (Table 2). Moreover, the level of perforin mRNA within MCMV-infected eyes of IL-2-treated mice with MAIDS was equivalent to that found within MCMV-infected eyes of normal mice, a level that correlated with resistance to retinitis.

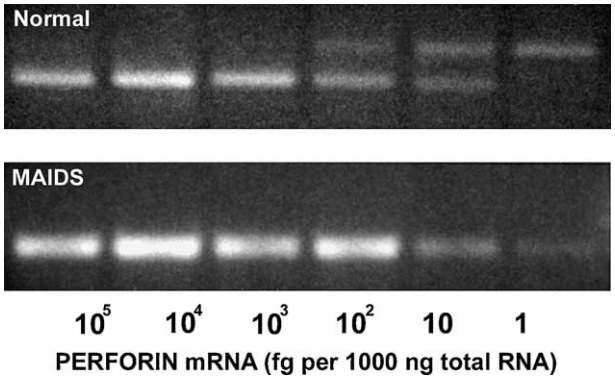

Fig. 2. Ethidium–bromide-stained agarose gel of competitive RT-PCR assay for mouse perforin mRNA in pooled eyes collected at 3 days after subretinal MCMV inoculation. Top, MCMV-inoculated eyes from normal C57BL/6 mice showing equivalence at ~100 fg of perforin mRNA per 1000 ng of total RNA. Bottom, MCMV-inoculated eyes from mice with MAIDS of 8 weeks duration showing lack of detection of authentic perforin mRNA (authentic perforin mRNA = 442 bp; competitive template = 380 bp).

3.3. *Effect of IL-2 immunotherapy on perforin mRNA levels within MCMV-infected eyes during the progression of MAIDS*

The IL-2 immunotherapy experiments described above as well as cytokine immunotherapy experiments performed in a previous study (Dix et al., 1997b) used mice with MAIDS of 8 weeks duration (MAIDS-8), a time after retrovirus infection when CD8+ T cells exhibit abnormal cytotoxic responses (Hartley et al., 1989). However, since NK cell activity is preserved until 8 weeks, but decreases rapidly thereafter, another series of experiments was performed to determine if systemic IL-2 immunotherapy would increase intraocular perforin mRNA levels within MCMV-infected eyes of mice with MAIDS at either 9 weeks (MAIDS-9) or 10 weeks (MAIDS-10) after LP-BM5–MuLV infection. Previous work in our laboratory has shown the frequency of MCMV retinitis in MAIDS-8 animals to be 80–100%, whereas 100% of MAIDS-9 and MAIDS-10 animals develop retinitis (Dix et al., 1994, 1997a,b; Dix and Cousins, 2003). Thus, 2 days after intramuscular administration of either PEG-IL-2 or H_2O, groups of MAIDS-8, MAIDS-9, and MAIDS-10 animals were infected with MCMV by subretinal inoculation (six animals per group). MCMV-infected eyes from normal mice served as controls (six animals per group). MCMV-infected eyes from each animal group were collected at 3 days after subretinal inoculation, pooled, and subjected to competitive RT-PCR assay for quantitation of perforin mRNA levels. Results are shown in Table 3. In agreement with earlier results (Table 2), MCMV-infected eyes of normal mice resistant to retinitis showed intraocular perforin mRNA at a level of 100 fg, while MCMV-infected eyes of MAIDS-8, MAIDS-9, or MAIDS-10 mice susceptible to retinitis showed a dramatic decrease in the level of

Table 3
Perforin mRNA levels in MCMV-infected eyes at different times during MAIDS[a]

	Perforin mRNA (fg perforin mRNA/1000 ng total RNA)			
	Normal mice	MAIDS-8[b] mice	MAIDS-9[b] mice	MAIDS-10[b] mice
Untreated	100	<1	5	<1
IL-2-treated	Not done	100	50	10

[a] Eyes pooled from six animals per group at 10 days after subretinal MCMV inoculation.
[b] MAIDS-8, MAIDS-9, and MAIDS-10 indicates mice with MAIDS of 8, 9, and 10 weeks duration, respectively.

intraocular perforin mRNA that ranged from <1 to 5 fg. However, whereas MCMV-infected eyes of IL-2-treated MAIDS-8 animals showed perforin mRNA at a level of 100 fg, perforin mRNA levels in MCMV-infected eyes of MAIDS-9 and MAIDS-10 animals were found to be 50 and 10 fg, respectively. Thus, the ability of IL-2 treatment to restore intraocular perforin mRNA levels to those found in MCMV-infected eyes of normal mice diminished remarkably during the progression of MAIDS. This might explain the inability of systemic IL-2 immunotherapy to restore resistance to MCMV retinitis in mice with MAIDS of 12 weeks duration (unpublished data).

4. Discussion

Our results suggest that the mechanism by which systemic IL-2 immunotherapy restores resistance to MCMV retinitis during MAIDS is through upregulation of the perforin-mediated pathway of cytotoxicity used by NK cells and cytotoxic CD8+ T cells to kill virus-infected cells (Kagi et al., 1994; Lowin et al., 1994, 1995; Podack, 1995; Smyth and Trapani, 1998). This conclusion is supported by two key observations. Firstly, PKO mice that are susceptible to retinitis following subretinal MCMV inoculation fail to show a reduction in the frequency or severity of MCMV retinal disease following IL-2 treatment. These knockout mice have a null mutation in the perforin gene (Kagi et al., 1994; Lowin et al., 1994), and therefore are deficient in perforin-mediated cytotoxicity. However, with the exception of this one genetic defect that impairs perforin-mediated cytotoxicity by NK cells and cytotoxic T-lymphocytes, PKO mice display relatively normal cellular immune responses (Walsh et al., 1994; Clark et al., 1995). If IL-2 immunotherapy targeted an immune response that is critical to resistance against MCMV retinitis other than the perforin cytotoxic pathway, IL-2-treated PKO mice should at least show a decrease in the severity of MCMV retinal disease. In fact, 100% of both untreated and IL-2-treated PKO mice developed necrotizing retinitis after subretinal MCMV inoculation, and the severity of retinitis as well as intraocular MCMV titers in these animal groups were equivalent.

One might argue that IL-2 treatment could target the Fas/FasL pathway, a receptor-mediated cytotoxicity pathway that is also used by NK cells and CD8+ T cells to kill virus-infected cells (Goldstein, 1995; Nagata and Goldstein, 1995; Russell and Ley, 2002; Smyth and Trapani, 1998). Like normal mice, however, mutant mice deficient in FasL (*gld* mice) (Nagata and Goldstein, 1995; Nagata and Suda, 1995), and therefore deficient in the Fas/FasL cytotoxic pathway, are resistant to MCMV retinitis (Dix et al., 2003). PKO mice also exhibit normal Fas/FasL-mediated cytotoxicity (Walsh et al., 1994; Clark et al., 1995). Thus, the Fas/FasL cytotoxic pathway apparently plays no significant role in protection against experimental MCMV retinitis.

Secondly, IL-2-induced protection against MCMV retinitis during MAIDS correlates with an increase in perforin mRNA levels within MCMV-infected eyes that is equivalent to that found in MCMV-infected eyes of immunologically normal mice who are naturally resistant to retinitis. We have shown previously that mice with MAIDS exhibit a significant decrease in perforin mRNA levels within splenic T-lymphocytes and individual MCMV-infected eyes when compared with those of normal mice (Dix et al., 2003), an observation that suggests that susceptibility to MCMV retinitis during MAIDS is due to a retrovirus-induced loss of the perforin cytotoxic pathway. The findings of the present investigation confirm and extend this observation. In agreement with our earlier observation, perforin mRNA was detected in MCMV-infected eyes of untreated mice with MAIDS susceptible to retinitis at a level that was significantly less than that found in MCMV-infected eyes of normal mice resistant to retinitis. Of potential therapeutic importance, however, is evidence unique to the present study that systemic administration of IL-2 during MAIDS dramatically increased the level of perforin mRNA within MCMV-infected eyes that would become resistant to retinitis. While important, the fact that MCMV-infected eyes of IL-2-treated mice with MAIDS achieved a high level of perforin mRNA identical to that observed in MCMV-infected eyes of immunologically normal mice is perhaps even more remarkable. Thus, there is a direct correlation between resistance to MCMV retinitis and a high level (100 fg) of perforin mRNA within MCMV-infected eyes. When intraocular perforin mRNA levels fall to levels of <1 fg, however, MCMV-infected eyes are susceptible to retinitis. The minimum level of intraocular perforin mRNA needed to sustain resistance to MCMV retinitis remains unknown at the present time.

The results of the present investigation also indicate that the ability of systemic IL-2 immunotherapy to protect against MCMV retinitis during MAIDS diminishes as the retrovirus-induced immunodeficiency syndrome progresses. The natural history of MAIDS has been defined with some precision (Mosier et al., 1985; Morse et al., 1989; Hartley et al., 1989; Gazzinelli et al., 1992), and indicate complex changes over time in the phenotype and function of all components of the immune network. Within 1–3 weeks after infection with the murine retrovirus mixture, LP-BM5–MuLV, C57BL/6 mice develop a progressive immunodeficiency characterized by persistent generalized lymphadenopathy, early polyclonal B cell activation, and hypergammaglobulinemia. Dysfunction of cellular immunity commences at ~3 weeks after retrovirus infection as cytokine production shifts from a Th1 profile to a Th2 profile. By 6 weeks, major phenotypic changes occur in CD4+ T cells that are associated with prominent functional deficits including a decreased ability to provide help for induction of cytotoxic T-lymphocyte responses. The phenotype and function of CD8+ T cells also decline during the evolution of MAIDS, but at a rate different from that of CD4+ T cells. The ability

of CD8+ T cells to mediate cytotoxic T-lymphocyte responses to altered self or to alloantigens becomes abnormal at ~8 weeks after retrovirus infection, as does the ability of intact mice to reject MHC class I disparate skin grafts. The activity of splenic NK cells is preserved until ~9 weeks postinfection, however, but then decreases rapidly thereafter. Death due to complications of MAIDS usually occurs after 14 weeks of progressive immunodeficiency.

An analysis of MCMV-infected eyes in mice with MAIDS of 8, 9, and 10 weeks duration demonstrated exceedingly low or undetectable amounts of intraocular perforin mRNA, an expected finding since these animals are susceptible to MCMV retinitis. Also expected was our finding that systemic IL-2 treatment of mice with MAIDS of 8 weeks duration resulted in a striking increase in the amount of perforin mRNA within MCMV-infected eyes to an amount found within MCMV-infected eyes of normal mice resistant to retinitis. This relatively high (and protective) level of perforin mRNA within MCMV-infected eyes diminished rapidly, however, as mice progressed to MAIDS of 9 and 10 weeks duration. Thus, the ability of systemic IL-2 immunotherapy to restore the level of perforin mRNA within MCMV-infected eyes of MAIDS mice to that of MCMV-infected eyes of immunologically normal mice rapidly diminishes in a temporal window that extends from 8 to 10 weeks after LP-BM5–MuLV infection. This finding might explain our inability to protect against MCMV retinitis in mice with MAIDS of 12 weeks duration following IL-2 treatment. It is noteworthy that this temporal window corresponds to the time of rapid loss of NK cell function and somewhat later in time than loss of CD8+ T cell function (Jolicoeur, 1991; Morse et al., 1992).

Since induction of key immunoregulatory cytokines is impaired as a consequence of HIV-1 infection (Clerici and Shearer, 1993), AIDS patients might also benefit from therapy with exogenously administered cytokines, especially IL-2 that is a major product of Th1 helper T cells (Paul and Seder, 1994). Several clinical trials of both native and recombinant IL-2 have been performed in recent years to assess the ability of this immunoregulatory cytokine to restore immune function in HIV-1-infected patients (Kovacs et al., 1995, 1996, 2000, 2001; Davey et al., 1997, 1999; Connors et al., 1997; Chun et al., 1999; Weissman et al., 2000; Miller et al., 2001). Results of these clinical trials have been encouraging and show that intermittent courses of high-dose IL-2 coupled with potent antiretrovirus drug therapy produces substantial and sustained increases in CD4+ T cell number and function in patients with both early and late HIV-1 disease (Mitsuyasu, 2001). A progressive accelerated accumulation of circulating NK cells in addition to CD4+ T cells has also been observed in HIV-1-infected patients who receive daily self-administered low doses of IL-2 over weeks to months (Smith, 2001). Collectively, however, these clinical trials have focused on the immunologic consequences of IL-2 immunotherapy on HIV-1 replication, and have not addressed directly the impact of IL-2 immunotherapy on the onset or progression of AIDS-related opportunistic infections such as HCMV retinitis. Our studies therefore provide the first proof-of-principle that systemic IL-2 immunotherapy will indeed reduce the frequency of cytomegalovirus retinitis in a setting of pathogenic retrovirus-induced immunosuppression, and does so by upregulation of the perforin-mediated pathway of cytotoxicity used by NK cells and cytotoxic CD8+ T cells for clearance of infectious virus from retinal tissue and prevention of retinal disease.

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