Immune CD8+ T Cells Prevent Reactivation of Toxoplasma gondii Infection in the Immunocompromised Host

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Toxoplasma gondii remains a serious cause of morbidity and mortality in individuals that are immunosuppressed, patients with AIDS in particular. The cellular immune response, especially by gamma interferon (IFN-γ)-producing CD8+ T cells, is an essential component of protective immunity against the parasite. In the present study the role of CD8+ T cells during the reactivation of Toxoplasma infection in an immunocompromised murine model was evaluated. Chronically infected mice were challenged with LP-BM5 virus, and the kinetics of CD8+ T-cell function was studied. At 10 weeks after viral infection, mice showed obvious signs of systemic illness and began to die. At this stage, CD8+ T cells were unresponsive to antigenic stimulation and unable to kill Toxoplasma-infected targets. IFN-γ production by the CD8+ T cells from dual-infected animals reached background levels, and a dramatic fall in the frequency of precursor cytotoxic T lymphocytes was observed. Histopathological analysis of the tissues demonstrated signs of disseminated toxoplasmosis as a result of reactivation of infection. However, treatment of the dual-infected animals with immune CD8+ T cells at 5 weeks post-LP-BM5 challenge prevented the reactivation of toxoplasmosis, and mice continued to live. Our study for the first time demonstrates a therapeutic role for CD8+ T cells against an opportunistic infection in an immunocompromised state.

Toxoplasmosis is an opportunistic infection that induces a strong cellular immune response in the normal host. Cell-mediated immunity is essential for host resistance (31) against this parasite. Infection in immunocompetent individuals results in asymptomatic chronic infection maintained by dormant tissue cysts. In AIDS and other immunocompromised situations, the infection is reactivated, resulting in severe morbidity and mortality in the infected host (11). Although Toxoplasma spp. can infect all cells and organ systems, the predominant manifestation of toxoplasma infection in patients with AIDS is encephalitis (4). Toxoplasmic encephalitis in AIDS patients results in multiple brain lesions, suggesting that reactivation of infection is multifocal within the brain (21).

Loss of CD4+ T cells from progressive human immunodeficiency virus (HIV) infection correlates with reactivation of T. gondii infection in these patients (5). However, the underlying mechanism that results in the activation of disease in these immunocompromised subjects is not clear. Studies with mice have shown that gamma interferon (IFN-γ), a cytokine produced by both CD4+ and CD8+ T cells, is critical for protection during both acute and chronic infection (33, 34). Our laboratory and others have shown that the CD8+ T-cell population is more important for long-term survival of infection (6, 17, 35).

Infection of C57BL/6 mice with the LP-BM5 mixture of helper and etiologic defective murine leukemia virus (MuLV) leads to the development of murine AIDS (MAIDS), an inevitably fatal syndrome characterized by splenomegaly, lymphadenopathy, hypergammaglobulinemia, and a progressive loss of B- and T-cell responses to antigens and mitogens (3, 26). Cytotoxic-T-lymphocyte (CTL) responses against alloantigens were reduced at 8 to 9 weeks after viral infection (25). The loss of T-cell responsiveness in vitro correlates with increased susceptibility to a variety of infections (8, 14). While MAIDS is not a perfect model of HIV infection, the patterns of immunodeficiency induced in both syndromes are strikingly similar. The similarities include early selective defects in CD4+ T-cell function and impaired CD8+ T-cell response late in the course of the disease.

Previous studies by Gazzinelli et al. have shown that CD8+ T cells are important for resistance to reactivation of toxoplasmosis in mice infected by LP-BM5 MuLV virus (10). Variation in the susceptibility of mice to LP-BM5 infection has been reported to be dependent in part on CD8+ T cells (22). For example, prior antibody depletion of CD8+ T cells in resistant A/J mice infected with LP-BM5 MuLV resulted in symptoms similar to those seen in the susceptible C57BL/6 strain. In the present study, we have analyzed the role of CD8+ T cells during the course of reactivation of T. gondii infection in mice infected with LP-BM5 MuLV. The reactivation of disease coincided with the loss of CD8+ T-cell function and could be prevented by adoptive immunotherapy with CD8+ T cells from toxoplasma-vaccinated mice.

MATERIALS AND METHODS

Mice. Female C57BL/6 mice, 5 to 6 weeks old, obtained from Jackson Laboratory at Bar Harbor, Maine, were used for the study.

Infection with virus. LP-BM5 MuLV (MAIDS) virus was prepared as previously described (19). G6 cells, originally provided to one of us by Janet Hartley and Herbert Moses as a cloned cell line from SC-1 cells infected with LP-BM5 virus mixture were cocultured with uninfected SC-1 cells. Mice were infected intraperitoneally with 100 μl of a virus stock that was quantitated by an XC plaque assay (29) to contain approximately 7 × 105 ecotropic PFU/ml.

Parasites and antigen preparation. Chronic infection was established by 15 orally administered cysts of Me49 strain of T. gondii. Two weeks after feeding cysts, infection was confirmed by determining the toxoplasma serum antibody titer. Infected animals were challenged with LP-BM5 virus as described above.

Toxoplasma lysate antigen (TLA) was prepared from tachyzoites of the PLK strain of T. gondii. The parasites were cultured in human fibroblasts and released by forced extrusion through a 27-gauge needle. Parasites were isolated from host...
cell debris by separation by using a Percoll gradient. Purified parasites were essentially free of any fibroblast contamination. The parasites were pulse sonicated eight times (18,000 Hz) at 10-s intervals at 4°C. The sonicate was centrifuged at 10,000 × g for 15 min to remove the antigen, and the pellet concentration was determined by a commercial assay (Bio-Rad Laboratories, Cambridge, Mass.). The antigen was aliquoted and stored at −20°C until further use.

CD8+ T-cell purification and proliferation. After the administration of general anesthesia, the spleens were removed and homogenized in a petri dish and contaminating erythrocytes were lysed in an erythrocyte lysis buffer (Sigma Chemical Co., St. Louis, Mo.). After two to three washes in Hank's balanced salt solution (Life Technologies Inc., Gaithersburg, Md.) with 3% fetal bovine serum (HyClone Laboratories, Logan, Utah), the CD8+ T cells were separated by microbeads (Miltenyl Biotec, Auburn, Calif.). The separation procedure was conducted as recommended by the manufacturer. The purity of separated cells was >95% as determined by fluorescence-activated cell sorter (FACS) analysis. The assay was performed by previously described standardized method (17).

Antigen-specific proliferation of purified CD8+ T cells was determined by thymidine incorporation assay. CD8+ T cells were suspended in RPMI 1640 (Life Technologies) and cultured in 96-well flat-bottom plates in a 200-μl volume at a concentration of 2 × 10^5 cells/well. The cells were stimulated with either 5 μg/ml of concanavalin A (ConA) or 15 μg of TLA per ml in the presence of 10^5 irradiated feeder cells. The feeder cells were obtained from syngeneic mice and were irradiated at 3,000 rads. After 72 h of incubation at 37°C in 5% CO2, [3H]thymidine (0.5 μCi/well; Amersham, Arlington Heights, Ill.) was added to the wells. Pulsed splenocytes were harvested on a glass filter by use of an automated multiple sample harvester and dried, and incorporation of radioactive thymidine was then determined by liquid scintillation according to a standard protocol (17).

The IFN-γ assay. The IFN-γ assay of purified CD8+ T cells from infected animals was performed at various time points after LP-BM5 MuLV infection. CD8+ T cells from the infected animals were isolated as described above and stimulated in vitro with TLA and irradiated feeder cells in a 24-well plate. After 72 h of incubation, the cultures were harvested. Supernatants were then collected, centrifuged, and stored at −70°C until further use. The supernatants were assayed for IFN-γ production by cytokine enzyme-linked immunosorbent assay (ELISA) (Genzyme, Cambridge, Mass.).

Bulk cytotoxic assay. A CTL assay was performed according to a standard procedure in our laboratory (17). Briefly, mouse peritoneal macrophages were obtained by lavage 2 days after intraperitoneal inoculation with 1 ml of thyoglycolate. The macrophages were washed three times in phosphate-buffered saline (PBS) and dispensed at a concentration of 3 × 10^5 cells/well in U-bottom tissue culture plates in medium. Macrophages were incubated overnight, and the next morning were labeled with 51Cr (0.5 μCi/well; New England Nuclear Research Products, Boston, Mass.) for 3 h at 37°C. After several washes in PBS (or until the supernatant contained <500 cpm), the macrophages were infected with freshly isolated cell culture-grown tachyzoites of the PLK strain and cultured in 96-well flat-bottomed plates in a 200-μl volume at a concentration of 2 × 10^5 parasites/well and incubated in phosphate-buffered saline (PBS) and dispensed at a concentration of 3 × 10^5 cells/well in U-bottom tissue culture plates in medium. Macrophages were incubated overnight, and the next morning, spontaneous lysis caused by overnight parasitic infection was measured, and wells exhibiting 50–50% or less destruction of the supernatants were excluded form the experiment. Macrophages were washed three times in PBS and incubated with purified CD8+ T cells at various effector/target (E/T) ratios in a final volume of 200 μl of culture medium. CD8+ T cells from T. gondii or dual-infected animals were purified by use of a magnetic procedure as described above. The purity was determined by flow cytometry. After the addition of T cells, the microtiter plates were centrifuged at 200 × g for 3 min and incubated at 37°C for 3 h. Supernatant samples of 100 μl were removed and assayed for released counts per minute (cpm) by scintillation counting. The percent lysis was calculated as follows: (mean cpm of test sample − mean cpm of spontaneous release)/(mean cpm of maximal release − mean cpm of spontaneous release) × 100.

pCTL assay. The cytolytic activity of the CD8+ T cells was quantitated by determining the precursor CTL (pCTL) frequency of the infected mice by using the limiting dilution assays. CD8+ T cells from the splenocytes of infected animals were isolated by magnetic separation with a resulting purity of 95% as determined by fluorescence-activated cell sorter (FACS) analysis. The assay was performed by previously described standardized method (17).

RESULTS

CD8+ T cells from dual-infected animals show decreased antigen-specific proliferation. A proliferation assay was performed to study the kinetics of antigen-specific CD8+ T-cell response in the T. gondii-infected animals after LP-BM5 MuLV infection. As expected, CD8+ T cells from the mice infected with T. gondii alone showed a significant (P = 0.005) proliferation in response to stimulation with TLA compared to control unstimulated cultures (Fig. 1A). On the contrary, CD8+ T cells from toxoplasma-infected mice, which were challenged 5 weeks earlier with LP-BM5 MuLV virus, exhibited significantly reduced antigen-specific proliferative response (P = 0.001). Although CD8+ T cells from the mice carrying dual infection or LP-BM5 MuLV virus alone proliferated in response to ConA, the level of stimulation was lower than for the uninfected animals or those infected with T. gondii alone. At 8 weeks after viral infection CD8+ T cells from the dual-infected mice continued to show significantly lower (P = 0.001) proliferation in response to antigen stimulation (Fig. 1B). At this time point the downregulation of mitogenic response of CD8+ T cells from virally infected animals was further enhanced. After 10 weeks after viral infection, CD8+ T cells from dual-infected animals completely fail to respond to antigenic stimulation (Fig. 1C). The ConA response of the cells from both dual-infected animals and the mice treated with virus alone at this time is completely absent.

Effect of LP-BM5 infection on the IFN-γ production by memory CD8+ T cells. Cytokine production upon antigen restimulation is an important characteristic of memory T cells (36). IFN-γ is known to be critical for protection against both acute and chronic toxoplasmosis infections (33). Immune CD8+ T cells from both dual-infected animals completely fail to produce IFN-γ in response to T. gondii infection (6, 18, 35). The effect of LP-BM5 virus on the IFN-γ production of immune CD8+ T cell was evaluated. Purified CD8+ T cells from the T. gondii-infected mice challenged 5 weeks earlier with LP-BM5 virus produced almost threefoldless IFN-γ compared to the control animals infected with T. gondii alone (Table 1). The IFN-γ production of these cells was further compromised at 10 weeks after viral infection, and nearly background levels of cytokine were released. CD8+ T cells from nonviral controls continued to produce high cytokine titers in response to antigenic stimulation at this time point. IFN-γ levels could not be detected in the wells containing feeder cells alone (data not shown).

CD8+ T cells from dual-infected animals show reduced cytolytic response against T. gondii-infected targets. One of the important characteristics of CD8+ T cells is their ability to exhibit cytolytic response against antigen-specific target (1). Induction of CD8+ CTLs against T. gondii infection has been demonstrated earlier (12, 16, 32). Secondary in vitro CTL response was assessed to determine the effect of LP-BM5 virus on CD8+ T-cell function. At 5 weeks postinfection no difference in the cytolytic response between the CD8+ T cells from dual-infected animals and the mice infected with T. gondii alone was observed. CD8+ T cells from both of these animals showed almost 40 to 45% lysis of infected targets at 40:1 E/T ratio (Fig. 2A). At 10 weeks after LP-BM5 MuLV infection,
CD8+ T cells from dual-infected animals were unable to kill the toxoplasma-infected targets. The percent lysis at all E/T ratios were equal to the background levels (Fig. 2B). However, CD8+ T cells from mice infected with *T. gondii* alone continued to show 40% lysis of the infected targets at a 40:1 E/T ratio.

LP-BM5 MuLV infection downregulates the pCTL frequency of anti-CD8+ T cells from *T. gondii*-infected mice. In order to better ascertain the effect of LP-BM5 MuLV infection on the CTL function of CD8+ T cells, pCTL frequency analyses were performed. At 7 weeks after *T. gondii* infection, the pCTL frequency of the infected animals was 1/3,297 cells (Fig. 3, A1) compared to 1/9,982 cells (Fig. 3, A2) in the group of animals challenged with LP-BM5 virus 5 weeks earlier. These values are within the range of variability (7), suggesting that early LP-BM5 MuLV infection does not significantly affect the cytotoxic function of CD8+ T cells. However, when the assay was performed 10 weeks after viral infection, the pCTL frequency of the dual-infected animals was severely diminished (1/146,537 cells) (Fig. 3, B2) compared to mice infected with *T. gondii* alone (1/2,240 cells) (Fig. 3, B1).

Adoptive transfer of immune CD8+ T cells prevents reactivation of toxoplasmosis in immunocompromised animals. Treatment of naive animals with toxoplasma-immune CD8+ T cells results in almost complete protection against acute *T. gondii* infection (15). The role of CD8+ T cells during chronic toxoplasmosis, however, is not well studied. Experiments were performed to determine whether CD8+ T cells from toxoplasma-immunized animals can prevent the reactivation of infection in the LP-BM5 immunocompromised mice chronically infected with *T. gondii*. Mice carrying dual infection were treated with 10^7 purified immune CD8+ T cells at 5 to 8 weeks after LP-BM5 infection. None of the animals treated with immune CD8+ T cells 5 weeks after viral infection died for up to 16 weeks after viral challenge (Fig. 4). These mice did not develop any signs of sickness (ruffled fur, sluggishness, etc.). In comparison, 75% of the control animals treated with naive CD8+ T cells were dead by this time. Treatment of the dual-infected animals with 10^7 purified immune CD8+ T cells at 10 weeks after LP-BM5 infection was equally effective as treatment at 5 weeks after viral infection. Mice treated with immune CD8+ T cells at 10 weeks after LP-BM5 infection were cured of toxoplasmosis and did not develop any signs of sickness.

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**TABLE 1. IFN-γ levels of CD8+ T cells from *T. gondii*-infected mice challenged with LP-BM5 virus**

<table>
<thead>
<tr>
<th>Group</th>
<th>5 wks after LP-BM5 infection</th>
<th>10 wks after LP-BM5 infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected</td>
<td><em>T. gondii</em></td>
</tr>
<tr>
<td>Unstimulated</td>
<td>ND</td>
<td>20 ± 5.8</td>
</tr>
<tr>
<td>Stimulated</td>
<td>10 ± 0.5</td>
<td>2,587 ± 360</td>
</tr>
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*CD8+ T cells from *T. gondii*-infected animals carrying LP-BM5 virus were isolated at 5 or 10 weeks after viral challenge. A total of 10^6 purified CD8+ T cells (>95% pure) were cultured in 24-well plates in the presence of 15 μg of TLA per ml and 5 × 10^5 irradiated feeder cells. After 72 h of incubation, the cultures were collected, centrifuged, and stored at −70°C. The supernatants were assayed for IFN-γ by ELISA. ND, not detected.*
infected animals with immune CD4$^+$ T cells had no effect on the mortality of these animals (data not shown). Immune CD8$^+$ T cells failed to protect the dual-infected animals when the transfer was performed 8 weeks after LP-BM5 infection (data not shown). Like the mice treated with nonimmune CD8$^+$ T cells, 70 to 80% of these animals succumbed to infection by 12 weeks after LP-BM5 challenge.

To confirm that the observed mortality in these animals was due to the reactivation of T. gondii infection, histopathological analysis of the tissues was performed at 10 weeks after LP-BM5 infection. This is the time point when complete loss of CD8$^+$ T-cell function in the dual-infected animals was observed. Sections of various organs of the mice treated with naïve CD8$^+$ T cells revealed extensive T. gondii replication and necrosis. Areas of interstitial lymphocytic infiltration in the lung contained patches of necrosis in which abundant Toxoplasma tachyzoites could be identified (Fig. 5A). Within these areas of necrosis there were scattered lymphocytes, mononuclear cells, and polymorphonuclear granulocytes (PMN). The follicular architecture of the spleen was effaced, with loss of the normal organization replaced by a lymphocytic infiltrate in which numerous large phagocytic cells with engulfed pycnotic mononuclear cells were seen (Fig. 5D). Areas of necrosis and acute inflammatory infiltrate were seen, including many intracellular and extracellular T. gondii tachyzoites. Examination of LP-BM5 MuLV-infected mice at 8, 10, and 12 weeks after infection showed that the splenic effacement is seen in the absence of infection with T. gondii (data not shown). There was moderate fatty metamorphosis of the cytoplasm, with scattered small nodules of necrosis throughout the liver parenchyma (Fig. 5G). These nodules had few lymphocytes, scattered PMNs, and abundant T. gondii tachyzoites. There were scattered foci of necrosis in the white matter of the brain, with T. gondii tachyzoites evident (Fig. 5J), and the meninges and penetrating vessels had perivascular lymphocytic infiltrates.

Sections from mice at 5 weeks after transfer of immune CD8$^+$ T cells showed intense lymphocytic and mononuclear cell infiltrates in the lung with scattered plasma cells and PMNs, but there was no evidence of necrosis or T. gondii replication (Fig. 5B). Follicular architecture was preserved in the spleen, with scattered large phagocytes similar to those seen in the control mice receiving naïve CD8$^+$ T cells (Fig. 5E). Replicating T. gondii organisms were not evident. Hepatocytes had finely granular cytoplasm, and there were scattered small nodules of lymphocytic inflammatory cells throughout the parenchyma of the liver (Fig. 5H). The brain contained...
However, immunity during chronic infection has been re-
ported to be largely dependent on CD8\(^+\) T cells, with IFN-\(\gamma\) playing a central role in the protection (6, 35). Like HIV 

infection in humans, mice with MAIDS have been reported to 

exhibit a loss of CD4\(^+\) T-cell function with the passage of time 
(25, 28). This ultimately results in defective CD8\(^+\) T-cell re-

sponse in these animals. Thus, the mechanism of reactivation of 
T. gondii infection in the dual-infected animals is due to lack of 

CD4\(^+\) T-cell help to the effector CD8\(^+\) T-cell population.

Previous studies have shown that resistance to lethal reactiva-

tion of toxoplasmosis in animals with MAIDS is dependent on 

CD8\(^+\) cells and IFN-\(\gamma\) (10). These findings are strengthened 

by the reports that reactivation of toxoplasmosis during HIV 

infection occurs in individuals with full-blown AIDS, when 

CD8\(^+\) T-cell immunity is compromised (30).

In the present study, CD8\(^+\) T cells start to show a loss of 

toxoplasma-specific proliferation starting at 5 weeks after 

MAIDS infection. By 10 weeks after viral challenge, the im-

mune CD8\(^+\) T cells completely failed to respond to antigenic 

or mitogenic stimulation. The cells were unable to lyse infected 

targets, and IFN-\(\gamma\) production in response to antigenic stimu-

lation was barely detectable. Animals at this time point look 

very sick and started to die as a result of the reactivation of 

toxoplasma infection. However, if the dual-infected animals 

were treated with immune CD8\(^+\) T cells at 5 weeks after 

infection, the reactivation of toxoplasmosis was prevented and 

there was no dissemination of infection. These animals showed 

no sign of sickness and continued to live for up to at least 16 

weeks after viral challenge. Histopathological analysis of the 

tissues of these animals at 10 weeks after onset of MAIDS 

showed no evidence of reactivation of chronic toxoplasmosis. 

If the transfer was delayed to 8 weeks after LP-BM5 MuLV 

infection, the reactivation of toxoplasmosis could not be pre-

vented. Evidence of disseminated infection similar to the con-

trol animals treated with naive CD8\(^+\) T cells was revealed 

upon histopathological examination of the tissues. The intra-

cellular multiplication of T. gondii and necrosis of tissues was 

superimposed on the lymphoid infiltrates, a finding typical of 

LP-BM5 MuLV infection (38). Infection with LP-BM5 MuLV 

alone produces an appearance in the spleen similar to that 

seen in dual-infected mice, without the evidence of increased 

phagocytosis.

The inability of immune CD8\(^+\) T cells to protect the immu-

nocompromised animals at 8 weeks after onset of MAIDS may 

be due to the fact that the toxoplasma infection by this time has 

completely reactivated and the parasites have disseminated to 

various tissues. Immune CD8\(^+\) T cells are thus ineffective in 

controlling overwhelming toxoplasma infection. This view is 

supported by the observations of Gazzinelli et al., who re-

ported significantly decreased cyst numbers in the brains of 

dual-infected animals beginning 8 weeks after viral infection 

(10).

Although endogenous CD8\(^+\) T cells from the dual-infected 

animals are not severely compromised at 5 weeks after viral 

infection, these cells are unable to prevent the reactivation of 

toxoplasma in the host. This finding can be attributed to the 

reduced ability of these cells to proliferate and produce 

IFN-\(\gamma\) in response to T. gondii stimulation. In contrast, 

immune CD8\(^+\) T cells from the mice infected with T. gondii alone 

exhibit significantly higher proliferation and IFN-\(\gamma\) produc-

tion when stimulated with toxoplasma antigen. When trans-

ferred to the immunocompromised host the immune CD8\(^+\) T cells 

prevent the reactivation of infection in immunocompromised an-

imals, which results in their survival. The possibility that, like 

the endogenous CD8\(^+\) T-cell population, the donor CD8\(^+\) T 

cells ultimately may undergo immunosuppression cannot be 

ruled out. However, based on our current observations, these

rare foci of necrosis and inflammation, with T. gondii 
tachyzoites and evidence of cyst formation (Fig. 5K and L). 
The meninges and penetrating vessels showed evidence of lym-

phocytic infiltration. On the contrary, mice treated with im-

mune CD8\(^+\) T cells 8 weeks after LP-BM5 challenge showed 
extensive necrosis and T. gondii replication in the lung, spleen, 

and liver (Fig. 5C, F, and I), as well as in mesenteric fat and 

brain (data not shown), results similar to those seen in animals 
treated with naive CD8\(^+\) T cells.

DISCUSSION

Toxoplasmosis is a major problem in immunocompromised 
individuals, in particular in individuals with AIDS. Earlier 

studies by Lang et al. have shown that among the nonviral 

opportunistic infections, encephalitis due to T. gondii was the 

most frequent cause of mortality in the HIV-infected popula-

tion (20). In a majority of these cases toxoplastic encephalitis 

was due to recrudescence of chronic infection as a result of 

loss of cellular immune responses. We used a mouse model for 

retrovirus-induced immunodeficiency to study the reactiva-

tion of chronic T. gondii infection. Our findings suggest that 

reactivation of toxoplasmosis in the immunocompromised mice 

coincides with the loss of CD8\(^+\) T-cell function. The reactiva-

tion of the disease could be prevented if these animals are 
treated with immune CD8\(^+\) T cells from immunocompetent 

mice. These studies clearly demonstrate that immune CD8\(^+\) T 

cells are critical for the prevention of reactivation in mice 

chronically infected with T. gondii. A protective effect of adop-

tively transferred immune CD8\(^+\) T cells has been previously 

reported (15). However, the present findings for the first time 
demonstrate the role of these cells in preventing the reactiva-

tion of toxoplasma infection in an immunocompromised host.

One of the major complications of HIV infection is the 
progressive loss of CD4\(^+\) T cells (5). Both CD4\(^+\) and CD8\(^+\) T 
cells are known to play a role in resistance to T. gondii infection 

(9). However, immunity during chronic infection has been re-

FIG. 4. Adoptive transfer of immune CD8\(^+\) T cells prevents the reactivation of 
T. gondii infection in the LP-BM5-infected host. C57BL/6 mice infected with 
T. gondii were challenged with LP-BM5 virus as described above. Dual-infected 
animals were injected intravenously with 10\(^7\) immune CD8\(^+\) T cells at 5 weeks 
after LP-BM5 infection. The animals were monitored daily for morbidity or 
mortality. There were 8 animals per group, and the experiment was performed 
three times with similar findings. The data presented are representative of one exper-
iment. Symbols: ●, naive CD8; ■, immune CD8.
FIG. 5. Microphotographs of sections of lung (A to C), spleen (D to F), liver (G to I), and brain (J to L) of T. gondii-infected animals challenged with LP-BM5 virus. Magnifications: A to C, ×170; D, ×85; E, ×42.5; F, ×85; G to I, ×170; J and K, ×85; L, ×170. (A) Lung of LP-BM5 and toxoplasma-infected mouse with adoptively transferred naive CD8$^+$ T cells at 5 weeks after LP-BM5 infection showing necrosis of the lung parenchyma with identifiable tachyzoites. (B) Lung of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells at 5 weeks after LP-BM5 challenge showing lymphocytic infiltration of the interstitial space (no tachyzoites can be identified). (C) Lung of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells at 8 weeks after LP-BM5 infection showing extensive necrosis of lung tissue with rare tachyzoites. (D) Spleen of dual-infected mouse with adoptively transferred naive CD8$^+$ T cells showing effacement of follicular architecture and necrosis. (E) Spleen of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells showing preservation of follicular architecture. (F) Spleen of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells at 8 weeks after LP-BM5 infection showing necrosis and effacement of follicular architecture. (G) Liver of dual-infected mouse with adoptively transferred naive CD8$^+$ T cells showing extensive tachyzoite replication and necrosis. (H) Liver of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells at 5 weeks showing lymphocytic infiltrate within the parenchyma but no identifiable tachyzoites. (I) Liver of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells at 8 weeks showing extensive necrosis and tachyzoite multiplication. (J) Brain of dual-infected mouse with adoptively transferred naive CD8$^+$ T cells at 5 weeks showing a microscopic focus of inflammation and necrosis and perivascular lymphocytic infiltrates. (K) Brain of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells at 5 weeks showing extensive perivascular lymphocytic infiltration but no necrosis. (L) Brain of dual-infected mouse with adoptively transferred immune CD8$^+$ T cells at 5 weeks showing cyst without surrounding cellular reaction.
cells definitely play a protective role in the initial stages of reactivation of *T. gondii* infection.

Studies with LP-BM5 infection have shown that the infected animals show early loss of CD4+ T-cell effector functions, including help for the CD8+ T-cell response (26). The CD4+ help has been reported to be critical for certain infectious disease and tumor models, where protective immunity is dependent on CD8+ T cells (23, 24, 37), as is the case with *T. gondii* infection, where CD8+ T-cell immunity is required for resistance against the parasite (2, 15, 33). CD8+ T cells and IFNγ keep the infection under check by restricting it to a chronic or dormant state. However, a lack of adequate CD4+ T-cell help, probably as a result of LP-BM5 infection, is followed by a poor CD8+ T-cell response against the parasite. This results in the absence of sufficient host immune surveillance required for preventing the reactivation of infection. Treatment with CD8+ T cells from an immunocompetent host can make up for this loss, and the infection can be controlled.

Adoptive immunotherapy with activated CD8+ T cells has been suggested as a therapy for HIV infection (13, 40). In one of these studies the transfer of activated CD8+ T cells to AIDS patients resulted in stable CD4+ and CD8+ T-cell counts, lower levels of antigenemia, and minimal toxicity over the period of study (40). The treated individuals improved clinically and remained stable. The ability of adoptively transferred CD8+ T cells to protect naive mice against acute toxoplasmosis infection has been demonstrated (15). The role of immune CD8+ T cells in the reactivation model of *T. gondii* infection has not been thoroughly studied. Our studies for the first time demonstrate that immunotherapy with immune CD8+ T cells can prevent reactivation of toxoplasmosis in a retrovirus-induced immunocompromised state.

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**REFERENCES**


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