Treatment with Soluble Interleukin-15Rα Exacerbates Intracellular Parasitic Infection by Blocking the Development of Memory CD8$^+$ T Cell Response

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Abstract

Interferon (IFN)-γ–producing CD8$^+$ T cells are important for the successful resolution of the obligate intracellular parasite *Toxoplasma gondii* by preventing the reactivation or controlling a repeat infection. Previous reports from our laboratory have shown that exogenous interleukin (IL)-15 treatment augments the CD8$^+$ T cell response against the parasite. However, the role of endogenous IL-15 in the proliferation of activated/memory CD8$^+$ T cells during toxoplasma or any other infection is unknown. In this study, we treated *T. gondii* immune mice with soluble IL-15 receptor α (sIL-15Rα) to block the host endogenous IL-15. The treatment markedly reduced the ability of the immune animals to control a lethal infection. CD8$^+$ T cell activities in the sIL-15Rα–administered mice were severely reduced as determined by IFN-γ release and target cell lysis assays. The loss of CD8$^+$ T cell immunity due to sIL-15Rα treatment was further demonstrated by adoptive transfer experiments. Naive recipients transferred with CD44^{hi} activated/memory CD8$^+$ T cells and treated with sIL-15Rα failed to resist a lethal *T. gondii* infection. Moreover, sIL-15Rα treatment of the recipients blocked the ability of donor CD44^{hi} activated/memory CD8$^+$ T cells to replicate in response to *T. gondii* challenge. To our knowledge, this is the first demonstration of the important role of host IL-15 in the development of antigen-specific memory CD8$^+$ T cells against an intracellular infection.

Key words: IL-15 • *Toxoplasma gondii* • IFN-γ • cytotoxic T cells • adoptive transfer

Introduction

The generation of an effective cellular immune response is key to the survival against intracellular pathogens (1) and therefore critical for vaccination strategies for the protection against initial and repeated infections. A prime example is infection with *Toxoplasma gondii*, an intracellular protozoan parasite against which T cell immunity plays a pivotal role for long-term host survival (2). Both CD4$^+$ and CD8$^+$ T cells have been reported to be important for protection against the infection (3). However, CD8$^+$ T cells are known to be the primary effector cells with CD4$^+$ T cells providing the necessary help (4, 5). Immune CD8$^+$ T cells from mice and humans secrete IFN-γ and exhibit in vitro cytotoxicity against infected target cells (6–9). Neutralization of either IFN-γ or CD8$^+$ T cells reversed the protective immunity against the parasite (4, 9, 10).

Studies from our laboratory have shown that exogenous treatment with IL-15 augmented the CD8$^+$ T cell response of mice against *T. gondii* infection (11). In a subsequent study, using a vaccine strain of *T. gondii*, we demonstrated that IL-15 treatment prolonged the duration of CD8$^+$ T cell immunity against *T. gondii* (12). The role of IL-15 in the proliferation and maintenance of long-term CD8$^+$ T cell response has been emphasized by the recent observations with IL-15 knockout mice. Mice lacking IL-15 or its receptor IL-15Rα are unable to generate a full memory CD8$^+$ T cell response (13, 14). Furthermore, IL-15 transgenic mice exhibited accelerated long-term CD8$^+$ T cell response by selectively propagating memory CD8$^+$ T cells (15, 16). However, the role of endogenous IL-15 in the induction and maintenance of memory CD8$^+$ T cells during a natural infection is unknown. In this study, we evaluated

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the effect of treatment with soluble IL-15Rα (sIL-15Rα)* on the ability of mice infected with *T. gondii* to survive a lethal secondary challenge.

IL-15 signals through a trimeric receptor complex that consists of a unique high affinity α chain, the IL-2R β chain, and the common γ chain (17–19). We have previously cloned and expressed a soluble fragment of IL-15Rα, which neutralizes IL-15 activity in vitro and in vivo. After a short period of administration, this protein profoundly suppressed the induction of collagen-induced arthritis in DBA/1 mice (20) and markedly prolonged the survival of allogenic heart grafts (21). Here we report that mice treated with sIL-15Rα developed a significantly more severe *T. gondii* infection. More importantly, sIL-15Rα exacerbated the disease by blocking the proliferation of antigen-specific memory $CD8^+$ cells crucial to the protective immunity against toxoplasmosis. These results clearly show that endogenous IL-15 plays a critical role in host defense against intracellular infection via the maintenance of specific memory $CD8^+$ T cells.

Materials and Methods

Mice, Parasites, and Challenge. 5–6-wk-old female C57BL/6 and congenic Thy1.1 mice were obtained from The Jackson Laboratory. They were maintained in a pathogen-free environment in the Animal Research Facility at Louisiana State University Medical Center (New Orleans, LA). Mice were challenged perorally with cysts of 76K strain of *T. gondii* (provided by D. Bout, UFR Sciences Pharma Centiques, Tours, France). This strain is maintained by continuous oral passage of cysts. For primary infection, a dose of 10–15 cysts was used. Unless otherwise stated, the animals were infected orally with 100 cysts for secondary challenge.

sIL-15Rα Treatment. sIL-15Rα (T1) and its control mutant protein (M4; ref 20) were prepared as previously described (22). T1 span the entire extracellular domain of the murine IL-15R α chain, whereas M4 was constructed by a single site-directed mutation replacing the third cysteine of the "Sushi domain" of the α chain with aspartic acid (22). The recombinant 6-histidine–tagged proteins were expressed in *Escherischia coli* (XL-1 Blue; Stratagene) after isopropyl b-D-thioglactoside (Stratagene) induction and purified by a nickel-agarose purification system (QIAGEN) according to the manufacturer's recommendations. Purified proteins were analyzed by SDS-PAGE. The purity was >97% for all recombinant proteins. LPS was not detected by the Limulus amebocyte test (<0.01 ng/mg, E-toxate; Sigma-Aldrich). 1 d before secondary challenge, 4 wk after the primary infection, infected animals were injected intraperitoneally with T1 (40 mg/mouse). The treatment continued daily for a 10-d period. The control mice were treated with an equivalent amount of M4. In previous experiments, we failed to detect any anti–sIL-15Rα in mice treated under this regimen (unpublished data).

Quantitation of Parasite Burden. Gut, spleen, liver, and lung tissues from *T. gondii*–infected animals were collected on day 7 and 14 after secondary infection. DNA was extracted from tissues using the Qiamp tissue kit (QIAGEN), and 400 ng of each sample were analyzed by quantitative PCR. Amplification of parasite DNA was performed using primers specific for a 35-fold repetitive sequence of the toxoplasma B1 gene, 5′-GGAACTG-CATCCGTTCATGAG-3′ and 5′-TCTTTAAAGCTTCGTG-GTC-3′, which is found in all known parasite strains (23). A 134-bp competitive internal standard containing the same primer template sequences as the 194-bp B1 PCR fragment was also synthesized (24). Amplification of this 194-bp segment of the B1 gene and the 134-bp segment of the internal standard was performed using a 50-ml reaction mixture containing 1.25 U of Amplitaq DNA polymerase, 1× buffer (PerkinElmer), 0.2 mM each of dGTP, dATP, dTTP, and dCTP, and 0.4 mM each B1 primer. For each reaction, a known amount of DNA from the tissues was amplified with varying amounts of the internal standard. The levels of parasite load were estimated by comparison to the internal controls. To determine the parasite load in infected tissues, PCR was performed under the same conditions using a known number of parasites. The level of internal control was calculated per parasite (24).

Histopathological Analysis. Tissues from sIL-15Rα–treated and control animals were fixed in 10% buffered formalin and paraffin processed. 5-μm histological sections were stained with hematoxylin and eosin and photographed on an Olympus Van Ox microscope with Kodak Elite 100 film. The resulting images were digitized with a Polaroid Sprint scanner and processed using Adobe Photoshop™ software.

IFN-γ Production. Intracellular cytokine staining was used to determine IFN-γ production by $CD4^+$ and $CD8^+$ T cells at the single cell level as previously described (25). Spleen cells from day 7– and 14–infected mice were isolated and resuspended in RPMI 1640 containing 10% FCS. The cells (10^6 cells/well) were cultured in 96-well plates and stimulated with PMA (10 ng/ml; Sigma-Aldrich), ionomycin (500 ng/ml; Sigma-Aldrich), and monensin (2 μM, GolgiStop; BD PharMingen). Cultures were incubated for 4 h at 37°C in 5% CO_2 in a humidified incubator. Cells were then washed with PBS containing 1% FCS and stained with anti-CD8 or anti-CD4 antibody conjugated with fluorescein (BD PharMingen) for 30 min at 4°C. Intracellular staining was performed using a Cytofix/Cytoperm kit (BD PharMingen) according to the manufacturer's recommendations. In brief, after cell surface staining, cells were washed and then treated with formaldehyde and saponin to fix and permeabilize them. Intracellular staining was then performed using anti–IFN-γ or an irrelevant isotype-matched control antibody conjugated with phycoerythrin (BD PharMingen). Samples were resuspended in PBS containing 2% formaldehyde, acquired on a FACScan™ flow cytometer, and analyzed using CELLQuest™ software (Becton Dickinson).

Precursor Cytotoxic T Lymphocyte (pCTL) Frequency Analysis. $CD8^+$ cytotoxic T cells were quantified by pCTL frequency analysis using limiting dilution assays (26). $CD8^+$ T cells from infected mice were purified by magnetic separation using microbeads coated with anti-CD8 antibody (Miltenyi Biotech). Purified $CD8^+$ T cells (>95% pure) were cultured by limiting dilution in 96-well round-bottom plates in RPMI 1640 medium (Life Technologies) containing appropriate growth factors, including 15 U/ml of recombinant IL-2 (R&D Systems), irradiated tachyzoites of the RH strain, and feeder cells. The dilutions ranged from 10,000 to 50,000 purified $CD8^+$ T cells/well. Control wells contained only irradiated parasites and feeder cells. After 1 wk, the cells were harvested and incubated with ^{51}Cr-labeled parasite-infected or -uninfected macrophages. The macrophages were collected and labeled as previously described (26). In brief, mouse peritoneal macrophages were obtained by a lavage 2 d after intraperitoneal inoculation with 1 ml thioglycollate. The cells were washed three times in PBS and dispensed at a con-

*Abbreviations used in this paper: FCM, flow cytometry buffer; pCTL, precursor cytotoxic T lymphocyte; sIL-15Rα, soluble IL-15 receptor α.

centration of 2×10^4 cells/well in 96-well U-bottom tissue culture plates. After overnight incubation, they were radiolabeled with ^{51}Cr (0.5 μCi/well; New England Nuclear Research Products) for 3 h at 37°C. After several washes in PBS, macrophages were infected with 10^4 freshly obtained RH parasites. The next morning, spontaneous lysis caused by overnight parasite infection was measured and wells exhibiting >250 cpm in the supernatant were excluded from the assay. Macrophages were washed in PBS and incubated with cultured $CD8^+$ T cells. The amount of radioisotope released was measured after a 4-h incubation. The wells were considered positive for lytic activity if the total counts per minute released were >3× SD over the control wells (mean counts per minute released by the target cells incubated with feeder cells and irradiated parasites alone). The pCTL frequency was calculated according to a standard formula (27).

Monoclonal Antibodies. Directly conjugated mAbs recognizing the following murine determinants were obtained from BD PharMingen: CD8-FITC (53-6.7, rat IgG_{2a}), CD90.1-PE (Thy1.1, OX-7, murine IgG_1), and CD44-PE–labeled (clone IM7) and isotype controls (A95-1–FITC, rat IgG_{2b}-FITC, and A112-2–PE, murine IgG_1). All mAbs were titrated and used at saturating concentrations.

Adoptive Transfer of Activated/Memory $CD8^+$ T Cells. C57BL/6 mice were infected with 10–15 cysts of *T. gondii*. The animals were killed 2 wk after infection and spleen cell suspensions were prepared. Red blood cells were lysed with a lysis buffer (ACK lysing buffer; Sigma-Aldrich). Cells were then centrifuged, washed twice with RPMI 1640, and counted on a hemocytometer using trypan blue exclusion to assess viability. $CD8^+$ T cells were purified by magnetic purification as previously described. Purified $CD8^+$ T cells were stained with PE-labeled anti-CD44 and the cells were separated into $CD44^{hi}$ and $CD44^{lo}$ population by flow cytometry. Before the transfer, the purity of the transferred cells was assayed by FACS® analysis. All preparations were fixed with 1% methanol-free formalin (Polysciences, Inc.), kept at 4°C, and analyzed within 7 d using a FACStar™ Plus (Becton Dickinson). For sorting experiments, $CD8^+$ T cells were stained on ice-cold sodium azide–free FCM (flow cytometry buffer) and analyzed immediately after labeling without fixation. $CD44^{hi}$ $CD8^+$ T cells (99.9% pure) were injected intravenously into naive syngeneic mice (10^6 cells/mouse). The recipients were then divided into two groups injected with either T1 or M4. The treatment, consisting of daily intraperitoneal injections of 40 μg/mouse for 10 d, began 14 d after cell transfer. The recipients were challenged with 100 cysts of 76K strain of *T. gondii* 24 h after the termination of treatment.

Spleen Cell Preparation and BrdU Staining. Congenic C57BL/6 Thy1.1 mice were infected perorally with 10–15 cysts of *T. gondii*. The mice ($n = 5$) were killed and spleen cells were collected 2 wk after infection. $CD8^+$ T cells from the spleens were isolated and then separated into $CD44^{hi}$ and $CD44^{lo}$ populations as previously described. Purified $CD8^+$ $CD44^{hi}$ T cells (10^6) were injected intravenously into naive Thy1.2 mice. The recipients were challenged orally 10 d later with 80 cysts of *T. gondii*. Groups of mice were injected intraperitoneally with 40 μg/mouse of T1 or M4 1 d before challenge. The treatment continued daily for 7 d. The animals were given BrdU (0.8 mg/ml; Sigma-Aldrich) in the drinking water starting on the day of challenge. The proliferation of $Thy1.1^+$ cells in the splenic and hepatic populations was analyzed by flow cytometry. Hepatic lymphocytes were isolated as previously described (28). In brief, the livers were perfused with 10 ml cold PBS, excised minced, and passed through a meshed screen. The cells were washed in cold PBS, and one liver equivalent was suspended in 15 ml Percoll in the presence of 100 IU/ml heparin. The suspension was centrifuged for 10 min at 500 *g* and the pellets were pooled and resuspended in cold FCM and counted on hematocytometer. After washing in FCM, the spleen and liver cell preparations (2.5×10^5/well) were labeled with PE-labeled anti-Thy1.1 antibody in 96-well round-bottom polypropylene microtiter plates (Costar Corp.), incubated for 30 min on ice, and then washed twice with 200-μl changes of FCM buffer. After extracellular staining, the intracellular staining for BrdU was performed using a commercially available BrdU flow kit (BD PharMingen)

Statistical Analysis. Statistical analysis of the data was performed by Student's *t* test (29).

Results

sIL-15Rα Abrogated the Protection Against a Secondary Challenge with T. gondii. We have reported earlier that exogenous IL-15 treatment enhanced $CD8^+$ T cell response against *T. gondii* in the infected mice (11). However, the importance of endogenous IL-15 during the infection in a normal host is unclear. Therefore, we determined the role of endogenous host IL-15 in the protection of immune animals against a lethal toxoplasma infection. C57BL/6 mice were immunized orally with 10–15 cysts of 76K strain of *T. gondii* and challenged 4 wk later with a lethal dose of 100 cysts. 1 d before the challenge infection, the mice were injected intraperitoneally with 40 μg/mouse sIL-15Rα (T1) or the control mutant protein (M4). The treatment continued daily for 10 d. As shown in Fig. 1, although all of the mice treated with M4 recovered from the infection, 80% of the animals treated with T1 succumbed to *T. gondii* challenge infection.

To confirm that sIL-15Rα–treated mice had a reduced ability to clear *T. gondii* parasites, gut, spleen, liver, and lung tissues from these animals were analyzed for parasite load by quantitative PCR at day 7 and 14 after challenge. As shown in Fig. 2 A, mice treated with T1 had severalfold higher parasite numbers compared with M4-treated animals in all the tissues examined. By day 14 after infection, the parasite load in tissues from the T1-treated animals increased or stayed high, whereas the M4-administered control animals were able to clear the infection (Fig. 2 B).

Histopathological analysis of the infected mice was performed at day 14 after secondary challenge. The liver of the control mice showed modest fatty infiltration of hepato-

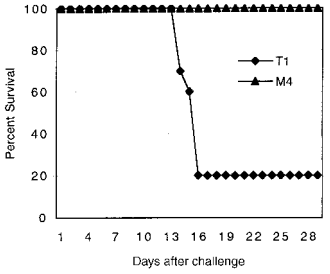

Figure 1. The effect of sIL-15Rα treatment on long-term protection against *T. gondii*. C57BL/6 mice were infected orally with 10–15 cysts of 76K strain of *T. gondii*. 4 wk later, the mice were challenged orally with 100 cysts of the same strain. The animals were injected intraperitoneally with 40 μg/mouse sIL-15Rα (T1) or the control protein (M4) starting 1 d before challenge and continuing daily for 10 d. Data are mean ± SEM ($n = 10$) and representative of two separate experiments.

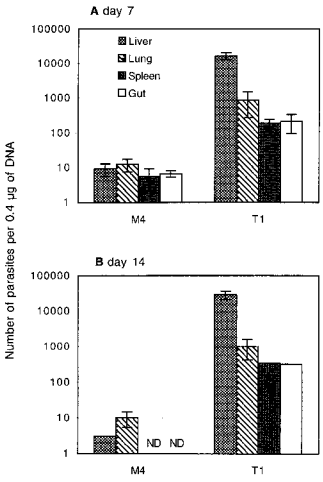

Figure 2. Levels of parasite DNA in the tissues of sIL-15Rα–treated mice. C57BL/6 mice were immunized and subsequently challenged with *T. gondii*. Animals were administered sIL-15Rα (T1) or the mutant protein (M4) as described in Fig. 1. (A) At day 7 and (B) 14 after challenge, the mice (three per group) were killed and tissues were analyzed for the level of parasite DNA by quantitative PCR. Data are mean ± SD and representative of two separate experiments. ND, not detected.

cytes consistent with a mild inflammatory response, which suggests rapid clearance of parasites as is the case with immune animals (30). Multiple mixed lymphocytic inflammatory nodules of 50–100-μm diameter were found throughout the parenchyma and no intracellular *T. gondii* were detected (Fig. 3 A). In contrast, mice treated with sIL15Rα (T1) showed marked fatty infiltration of hepatocytes with numerous scattered inflammatory nodules throughout the hepatic parenchyma made up of lymphocytes and granulocytes (Fig. 3 B). The small bowel of the T1-treated mice showed severe necrosis and hemorrhage, whereas only patchy superficial necrosis was seen in that of the control mice (Fig. 3, C and D). Toxoplasma induced immunopathology by IFN-γ–producing $CD4^+$ T cells in the gut and liver tissues of naive animals during acute infection has been previously reported (31). However, due to the rapid clearance of parasites, the immune animals do not develop an inflammatory response (12). Our results demonstrate that sIL-15Rα (T1) treatment reduces the ability of the immunized mice to clear *T. gondii* infection. Uncontrolled parasite replication in these animals might have caused immunopathology similar to that seen in naive mice.

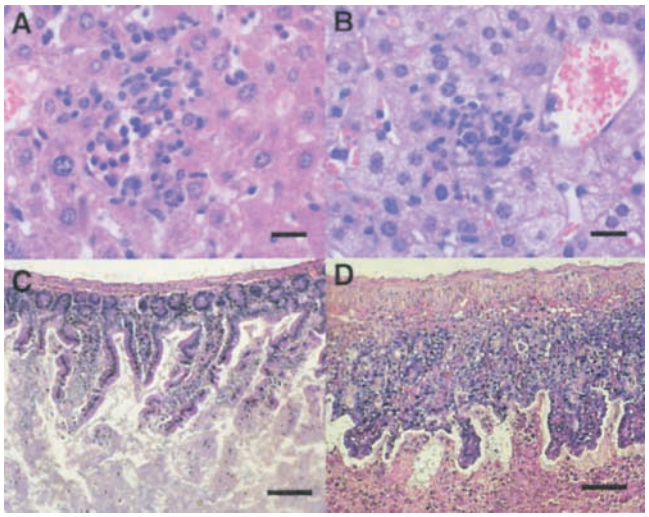

Figure 3. Histological analysis of tissue from mice infected with *T. gondii* and treated with sIL-15Rα (T1) or the mutant protein (M4). C57BL/6 mice were immunized with a low dose of *T. gondii* and challenged 4 wk later. The mice were treated with T1 or M4 as described in Fig. 1. 14 d after challenge, animals were killed and gut and liver tissue sections were stained with hematoxylin and eosin. (A) Liver of M4-treated mice. Bar, 100 μm. (B) Liver of T1-treated mice. Bar, 50 μm. (C) Gut of M4-treated mice. Bar, 100 μm. (D) Gut of T1-treated mice. Bar, 50 μm. Data are representative of three mice per group.

sIL-15Rα Treatment Reduced Memory $CD8^+$ T Cell Response. As IL-15 is considered important for $CD8^+$ T cell maintenance (32), we analyzed the effect of sIL-15Rα treatment on the *T. gondii*–specific $CD8^+$ T cell memory response. This was performed by first estimating the levels of $CD8^+$ cytotoxic T cells using a pCTL assay. Mice were immunized with *T. gondii* cysts and challenged 4 wk later with the same strain of parasite. sIL-15Rα (T1) treatment started 1 d before challenge and continued daily for 10 d as previously described. At day 7 and 14 after challenge, mice were killed and $CD8^+$ T cells were separated by magnetic isolation. Purified $CD8^+$ T cells were cultured by limiting dilution and pCTL assay was performed. As shown in Fig. 4, the treatment of immune mice with T1 led to a significant reduction ($P < 0.05$ on day 7 and $P < 0.005$ on day 14) in pCTL frequency compared with treatment with the control protein, M4.

In addition to their direct cytolytic activity on infected targets, memory $CD8^+$ T cells also secrete IFN-γ (33). The memory $CD8^+$ T cell profile in the sIL-15Rα–treated mice was further evaluated by estimating the number of IFN-γ–producing $CD8^+$ T cells. On day 7 and 14 after secondary challenge, the mice were killed and the $CD8^+$ and $CD4^+$ T cell populations were analyzed for IFN-γ production by intracellular staining. Treatment with T1 significantly reduced the number of IFN-γ–producing $CD8^+$ T cells in the *T. gondii*–infected animals ($P < 0.01$ on day 7 and $P < 0.001$ on day 14 after infection) compared with mice treated with M4 (Fig. 5, A and B). It is interesting to note that the number of IFN-γ^+ $CD4^+$ T cells was not affected by treatment with T1. These observations further confirm that IL-15Rα selectively blocks the development of memory $CD8^+$ T cells in *T. gondii*–infected mice.

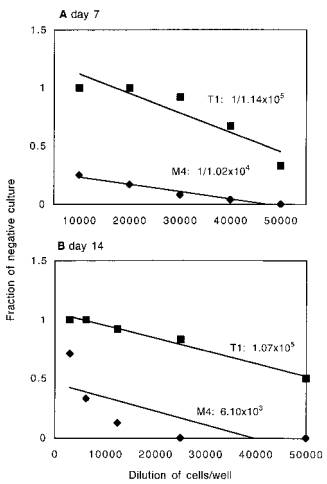

Figure 4. The effect of sIL-15Rα treatment on the memory $CD8^+$ T cell response against *T. gondii* infection. Toxoplasma-immunized C57BL/6 mice were challenged orally with 76K strain of the parasite. The animals were treated with sIL-15Rα (T1) or the mutant protein (M4) as described in Fig. 1. (A) $CD8^+$ T cell response of the immune animals was evaluated by pCTL analysis at day 7 and (B) 14 after challenge.

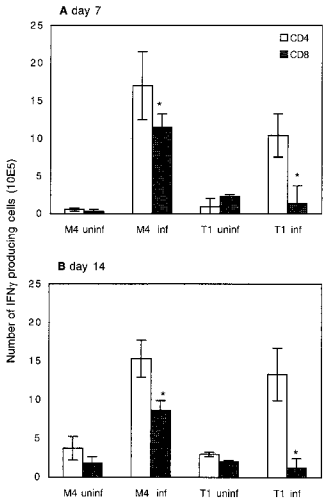

Figure 5. Analysis of IFN-γ–producing T cells from mice infected with *T. gondii* and treated with sIL-15Rα. C57BL/6 mice immunized and subsequently challenged with 76K strain were treated with sIL-15Rα (T1) or the control protein (M4) as described in Fig. 1. (A) Spleen cells were harvested on day 7 and (B) 14 after challenge infection, pooled ($n = 3$), and cultured in vitro with PMA, ionomycin, and monensin for 4 h. The cultured cells were then labeled for CD4 or CD8 before intracellular staining for IFN-γ. Data are presented as number (mean ± SD) of $CD4^+$ or $CD8^+$ T cells positive for IFN-γ and are pooled from two different experiments.

sIL-15Rα Abrogates the Protective Effect of Donor $CD8^+$ T Cells. Adoptive transfer of immune $CD8^+$ T cells protects naive recipient animals from a lethal *T. gondii* infection (9). Therefore, we determined whether the $CD8^+$ T cell immunity against *T. gondii* infection could be adoptively transferred to sIL-15Rα (T1)–treated animals. Activated/memory $CD8^+$ T cells from C57BL/6 mice infected 2 wk earlier with *T. gondii* were isolated by affinity purification. Purified $CD8^+$ $CD44^{hi}$ T cells (99% pure) were injected intravenously into naive C57BL/6 mice. 2 wk after cell transfer, the recipients were injected intraperitoneally with T1 or M4 daily for 10 d. 1 d after beginning the treatment, the animals were challenged with 100 cysts of *T. gondii*. Fig. 6 shows that adoptive transfer of activated/memory $CD8^+$ T cells failed to protect the recipients treated with T1, but provided normal protection to M4-treated mice.

To determine whether sIL-15Rα affects the proliferation of memory $CD8^+$ cells, we monitored the number of donor activated/memory $CD8^+$ T cells recovered after *T. gondii* challenge in the recipient animals. $CD44^{hi}$ $CD8^+$ T cells (10^6) isolated from *T. gondii*–immunized congenic Thy1.1 mice were injected into Thy1.2 mice. 2 wk later, the mice were injected daily for 8 d with T1 or M4. 1 d after the start of treatment, the mice were challenged with 80 cysts of *T. gondii* and given BrdU via drinking water for 7 d. On day 8 after challenge, the animals were killed and the proliferation of donor Thy1.1 cells in the spleen and liver was analyzed by determining the $BrdU^+$ donor $CD8^+$ T cell population. The number of donor $Thy1.1^+$ $CD8^+$ T cells recovered from the T1-treated mice was significantly lower in comparison to both spleens ($P < 0.01$) and livers ($P < 0.001$) of control M4-injected animals (Fig. 7). Therefore, these findings confirm that sIL-15Rα treatment blocked the proliferation of activated/memory $CD8^+$ T cells in response to challenge infection.

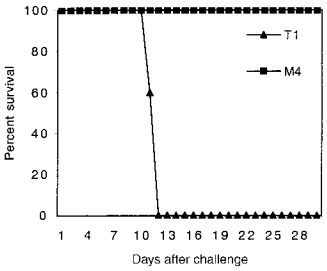

Figure 6. The effect of sIL-15Rα treatment on the antitoxoplasma protection of naive mice transferred with immune $CD8^+$ T cells. C57BL/6 mice were infected with 10–15 cysts of 76K strain of *T. gondii*. Mice were killed 2 wk later and spleen cells were pooled ($n = 5$). $CD8^+$ T cells were isolated by magnetic separation (>95% pure) and were stained with PE-labeled anti-CD44 and separated into $CD44^{hi}$ (activated/memory) and $CD44^{lo}$ naive population by flow cytometry. $CD8^+$ $CD44^{hi}$ T cells (10^6) were injected intravenously into naive syngeneic mice. Mice were challenged orally with 100 cysts 15 d after the cell transfer. The recipients were injected intraperitoneally daily for 10 d with T1 or M4 from the day before challenge infection. Data are pooled from two experiments ($n = 10$).

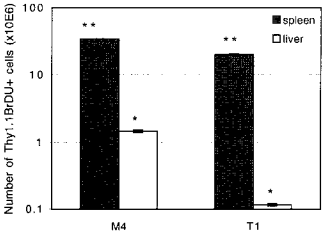

Figure 7. The effect of sIL-15R treatment on the proliferation of adoptively transferred memory $CD8^+$ T cells. Congenic Thy1.1 mice ($n = 5$) were infected orally with 10–15 cysts of *T. gondii* and $CD44^{hi}$ $CD8^+$ T cells were isolated on day 14 and transferred intravenously (10^6/mouse) to naive Thy1.2 animals. 2 wk after the transfer, the recipient animals ($n = 4$) were challenged with 80 cysts of *T. gondii* and treated with T1 or M4 as described in Fig. 6. The animals were given BrdU via drinking water (0.8 mg/ml) for 7 d. Recipients were killed on day 8 and spleen and liver were analyzed for $BrdU^+$ $Thy1.1^+$ T cells by flow cytometry. Data are mean ± SD ($n = 4$). *, $P < 0.01$; **, $P < 0.001$.

We then evaluated the effect of sIL-15Rα treatment on $CD8^+$ $CD44^{hi}$ T cell populations in naive and immune mice infected with a low dose of parasite but not recipients of a secondary *T. gondii* challenge. As expected, a relatively low number of $CD8^+$ $CD44^{hi}$ T cells was observed in the naive mice. This was not affected by the T1 treatment (Fig. 8). In contrast, the administration of T1 caused a significant decrease ($P < 0.002$) in the $CD8^+$ $CD44^{hi}$ population of the immune animals (Fig. 8). Therefore, these results demonstrate that treatment with sIL-15Rα inhibited the expansion of memory $CD8^+$ T cells and had little or no effect on resting cells.

Discussion

Immunologic memory is a hallmark of the immune system and its maintenance is necessary for the host to resist recurrent infections or the reactivation of chronic disease

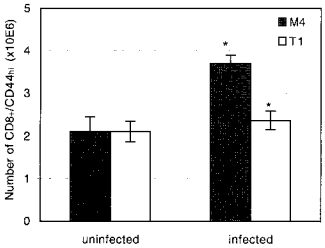

Figure 8. The effect of sIL-15Rα on activated/memory and resting memory $CD8^+$ T cells. C57BL/6 mice were either infected orally with 10–15 cysts or uninfected. 2 wk after the infection, mice ($n = 3$) were treated daily with T1 or M4 for 12 d as previously described. Mice were killed on day 13 and the spleen cells were analyzed for $CD8^+$ $CD44^{hi}$ population. sIL-15Rα markedly reduced the expansion of $CD8^+$ $CD44^{hi}$ T cells from infected mice but not uninfected mice. Results are mean ± SD. *, $P < 0.002$.

(34). An essential role for memory $CD8^+$ T cells in the long-term protection against several intracellular pathogens and tumors has been previously described (35, 36). For example, on recovery from acute infection with influenza, Sendai, or Lymphochoriomeningitis virus, mice develop lifelong $CD8^+$ T cell memory (33, 37, 38). Memory $CD8^+$ T cells have also been reported to be important for intracellular bacterial infections such as *Listeria monocytogenes* and *Salmonella* (39, 40). Similarly, lack of $CD8^+$ T cells compromises the host's ability to clear malarial infection (41). A crucial role of $CD8^+$ T cells in the protection against reactivating and recurrent *T. gondii* infection has been documented (3, 12). However, the factors responsible for the induction or maintenance of a robust memory $CD8^+$ T cell immunity against these infectious agents have not been extensively studied. Understanding the mechanism involved in the generation and maintenance of memory $CD8^+$ T cell response is crucial for the development of therapeutic agents against these pathogens.

Recently, attention has been drawn to the role of cytokines in the maintenance of memory $CD8^+$ T cells (12, 42–44). Studies conducted by different laboratories suggest that IL-15, a cytokine closely related to IL-2, is crucial for the maintenance of $CD8^+$ memory T cells (13, 44). The specificity for IL-15 versus IL-2 is provided by the cytokine-specific α chain receptors that complete the IL-15$\alpha\beta\gamma$ and IL-2$\alpha\beta\gamma$ heterotrimeric high affinity receptor complexes and thereby allow differential responsiveness (19). Although IL-2 is produced primarily by $CD4^+$ T cells (45), IL-15 is secreted by multiple cell types, including both immune and nonimmune cells such as dendritic cells, macrophages, and placental cells (17, 46).

We previously reported that exogenous IL-15 treatment enhanced $CD8^+$ memory T cell response against *T. gondii* infection (11). Subsequently, an important role for IL-15 in the selective stimulation of $CD8^+$ T cells was demonstrated (47). Mice lacking IL-15 or IL-15Rα gene had markedly reduced $CD8^+$ memory T cell response (13, 14). Recent investigations from our laboratory have shown that optimal $CD8^+$ T cell immunity in the mice immunized with a vaccine (nonpersistent) strain of the parasite could not be maintained beyond a 9-mo period (12). The exogenous treatment with IL-15 restored the declining $CD8^+$ T cell protective response in these vaccinated animals. However, the role of IL-15 in the regulation of $CD8^+$ T cells directed against infection is unknown. Our current observations clearly demonstrate that the blockade of IL-15 by IL-15Rα in mice infected with a natural (persistent or cyst forming) strain of toxoplasma abrogates the host's ability to survive a challenge infection. This could be attributed to the down-regulation of memory $CD8^+$ T cell response in these animals. The treated animals exhibited poor $CD8^+$ T cell response manifested by decreased pCTL frequency and reduced IFN-γ production within this population. An important role of IL-15 in the maintenance of memory $CD8^+$ T cells is its ability to induce the proliferation of these cells upon a challenge infection. This was demonstrated by adoptive cell transfer experiments in which the transfer of protection against a lethal infection by activated/memory $CD8^+$ T cells into naive syngeneic recipients was blocked by sIL-15Rα treatment. Moreover, the donor $CD8^+$ T cells in the sIL-15Rα–treated recipient mice proliferated poorly in response to infection compared with controls. The sIL-15R treatment affected the activated/memory ($CD44^{hi}$) $CD8^+$ T cell population in the immune animals but not in resting $CD8^+$ T cells. These results clearly show that IL-15 is critical for the expansion of memory $CD8^+$ T cells both during primary and recurrent toxoplasma infection. The blockade of IL-15 activity inhibits the expansion of the memory $CD8^+$ T cell population during repeat infection, which leads to unchecked infection and a fatal outcome. It should also be noted that IL-15 also serves as a growth factor for NK (14) and $CD4^+$ T cells (48), although $CD8^+$ memory cells appear to be particularly sensitive to IL-15 activation (13, 44). Although our results do not exclude the effect of sIL-15Rα on NK and $CD4^+$ cells, the role of these cells in *T. gondii* infection appears to be secondary to $CD8^+$ T cells (3, 12).

The first event that takes place during an intracellular *T. gondii* infection, in which long-term protection is highly dependent on $CD8^+$ T cells, might be that during the early phase of infection there is a marked increase in activated $CD8^+$ T cells. After this initial expansion, as the infection is resolved or reaches chronicity (15–30 d after infection), a period of cell death ensues during which 90–95% of activated T cells undergo apoptosis (49). The next phase is characterized by a pool of memory $CD8^+$ T cells that are important for immune surveillance, protecting the host against recurrent *T. gondii* infections (12). IL-15 plays an important role in the generation of optimal memory $CD8^+$ T cells, as blockade of IL-15Rα causes a decrease in the $CD8^+$ $CD44^{hi}$ T cell population during the infection. When recurrent infection does take place, memory $CD8^+$ T cells proliferate vigorously causing a quick resolution of the infection. Thus, the rapid proliferation of memory $CD8^+$ T cells is highly dependent on IL-15, the absence or neutralization of which severely compromises the immunity against the pathogen. This is consistent with a recent report by Weninger et al. (50) that naive $CD8^+$ T cells stimulated with IL-15 developed into "central memory cells" homing avidly to lymphoid organs and mediated rapid recall responses.

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