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Interleukin-10 and Pathogenesis of Murine Ocular Toxoplasmosis

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To understand the role of interleukin-10 (IL-10) in ocular toxoplasmosis, we compared C57BL/6 (B6) and BALB/c background mice lacking a functional IL-10 gene (IL-10^{-/-}) and B6 transgenic mice expressing IL-10 under the control of the IL-2 promoter. Increased cellular infiltration and necrosis were observed in the eye tissue of IL-10^{-/-} mice of both the B6 and BALB/c backgrounds with associated changes in the levels of cytokines in serum. In contrast, there was no evidence of necrosis in the eye tissue from IL-10 transgenic mice following parasite exposure. Our results demonstrate that IL-10 is important in the regulation of inflammation during acute ocular toxoplasmosis.

Interleukin-10 (IL-10) is a cytokine with important anti-inflammatory and immunosuppressive properties (7), among which are pleiotropic effects on cells of the immune system, commonly associated with inhibition of cell-mediated immune responses (1, 15). Because of these inhibitory effects, it has been proposed that IL-10 may downregulate protective immune responses to several intracellular pathogens, such as *Leishmania* sp. (6), *Trypanosoma cruzi* (18), and *Toxoplasma gondii* (11). IL-10 has been shown to play an important role in the downregulation of gamma interferon (IFN- γ) production in C57BL/6 (B6) mice following peroral infection with *T. gondii* strain ME49, and IL-10^{-/-} B6 mice develop enhanced small intestine pathology characterized by increased cellular infiltration and intense necrosis (20). The role of IL-10 as a regulatory cytokine in ocular toxoplasmosis has not been reported.

B6 background IL-10^{-/-} mice and age (7 to 9 weeks old)- and sex-matched wild-type (WT) B6 and BALB/c mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). A breeding pair of BALB/c background IL-10^{-/-} mice was kindly provided by Donna Rennick (Immunex, Seattle, Wash.). Mice of the B6 background transgenic (Tg) for murine IL-10 under control of the promoter of the gene for IL-2 were bred from a homozygous Tg breeding pair obtained from Mitchell Kronenberg (La Jolla Institute of Allergy and Infection, La Jolla, Calif.). Mice were immunized by intraperitoneal injection of 10⁵ strain ts-4 tachyzoites and challenged by ocular inoculation of 100 strain RH tachyzoites 40 days postimmunization; naive mice were primarily infected with 100 strain RH tachyzoites by ocular inoculation at that time. For eye inoculation, mice were infected and ocular pathology was scored as previously described (10). The Wilcoxon rank sum test was

used for statistical evaluation of ocular pathological changes. *P* values of less than 0.05 were considered statistically significant.

At 11 days post-ocular infection, severe inflammation and necrosis were observed in the eye tissue of WT B6 mice (Fig. 1A), while moderate inflammation and necrosis were observed in the eye tissue of WT BALB/c mice (Fig. 1C). In contrast, enhanced necrosis was seen in the eye tissue of IL-10^{-/-} mice of the B6 and BALB/c backgrounds (Fig. 1E and G); however, few inflammatory foci and no evidence of necrosis were found in the eye tissue of IL-10 Tg B6 mice (Fig. 1I). At the same time point, significantly increased inflammatory scores were observed in IL-10^{-/-} B6 mice (3.65 \pm 0.3 for IL-10^{-/-} B6 mice versus 2.67 \pm 0.5 for WT B6 mice; *P* = 0.034) and IL-10^{-/-} BALB/c mice (3.50 \pm 0.5 for IL-10^{-/-} BALB/c mice versus 1.75 \pm 0.7 for WT BALB/c mice; *P* = 0.029), and significantly decreased inflammatory scores were observed in IL-10 Tg B6 mice (0.56 \pm 0.4 for IL-10 Tg B6 mice versus 2.67 \pm 0.5 for WT B6 mice; *P* = 0.0034). To better understand the effect of IL-10 on ocular pathogenesis, the histopathology of eye tissue at earlier time points was observed after infection with 4 \times 10⁴ RH tachyzoites. At 4 days postinfection, obvious inflammatory cells and tachyzoite proliferation were observed in the eye tissue of WT B6 mice (Fig. 2A). In contrast, intense infiltration of inflammatory cells (mostly neutrophil cells) was observed in the eye tissue of IL-10^{-/-} B6 mice (Fig. 2C); however, marked tachyzoite proliferation but few inflammatory cells were observed in the eye tissue of IL-10 Tg B6 mice (Fig. 2E). At 6 days postinfection, tachyzoite proliferation associated with tissue destruction was observed in the eye tissue of WT B6 mice (Fig. 2B). In contrast, tachyzoite proliferation associated with intense necrosis was observed in the eye tissue of IL-10^{-/-} B6 mice (Fig. 2D); however, marked tachyzoite proliferation with no evidence of inflammation or necrosis was observed in the eye tissue of IL-10 Tg B6 mice (Fig. 2F).

We observed remarkable differences in ocular histopathology among infected IL-10^{-/-}, IL-10 Tg, and control mice, and eye lesions were surprisingly severe in IL-10^{-/-} mice of both the B6 and BALB/c backgrounds. Observation of the serial histopathologic changes in the eye tissue of B6 background mice following infection indicates that the pathology of the

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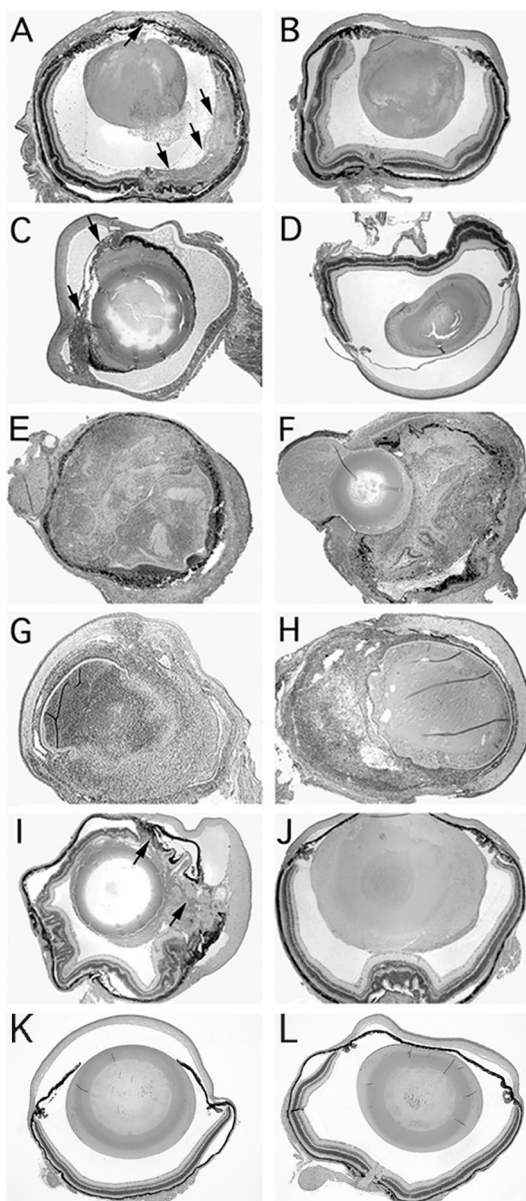


FIG. 1. Pathology of mouse eyes at 11 days post-ocular infection or challenge with 100 tachyzoites of *T. gondii* strain RH. Shown are the eyes of an infected (A) and challenged (B) WT B6 mouse, an infected (C) and challenged (D) WT BALB/c mouse, an infected (E) and challenged (F) IL-10^{-/-} B6 mouse, an infected (G) and challenged (H) IL-10^{-/-} BALB/c mouse, an infected (I) and challenged (J) IL-10 Tg B6 mouse, a normal B6 mouse (K), and a normal IL-10^{-/-} B6 mouse (L). Six mice were used in each group. This experiment is representative of two performed. Arrows indicate necrosis or inflammation. Original magnification, $\times 4$. Hematoxylin and eosin staining was used.

disease is initially due to parasite invasion and multiplication. In contrast, the process of eye tissue destruction in IL-10^{-/-} mice is strongly influenced early on by the immune response. Our data demonstrate that IL-10 can affect the outcome of ocular infection with *T. gondii* in genetically distinct strains of inbred mice. This suggests that IL-10 is an important mediator in controlling the inflammatory process in response to ocular

parasite infection. Expression of IL-10 under the control of the promoter of the gene for IL-2 caused a markedly different response to ocular infection with *T. gondii* in our study. Compared with WT mice, IL-10 Tg B6 mice had much less severe ocular pathology and no evidence of necrosis in their eye tissue following acute infection. Endogenous IL-10 synthesis plays an important role in vivo in downregulating monokine and IFN- γ responses to acute intracellular infection, thereby protecting the host against an excessive and lethal Th1 cytokine response and preventing host immunopathology (4, 15). In addition, IL-10 induces several key antiparasite effector mechanisms while simultaneously limiting the extent of tissue pathology, thereby allowing progression of infection to the chronic stage (9). King et al. (14) reported that IL-10 may be a commonly used protective cytokine induced by many helminth parasites. Our data show that supplementation of IL-10 through the addition of a transgene can control the hyperimmune reaction induced by ocular infection with *T. gondii*. This demonstrates that overexpression of IL-10 could downregulate immune-mediated inflammation and prevent immunopathogenesis of acute toxoplasmosis.

At 11 days after an ocular challenge, nearly normal histology was observed in the eye tissue of WT B6, WT BALB/c, and IL-10 Tg B6 mice (Fig. 1B, D, and J). In contrast, intense necrosis was observed in the eye tissue of IL-10^{-/-} B6 and IL-10^{-/-} BALB/c mice (Fig. 1F and H). At the same time point postchallenge, significantly increased inflammatory scores were observed in IL-10^{-/-} B6 mice (3.00 ± 0.5 for IL-10^{-/-} B6 mice versus 0.83 ± 0.3 for WT B6 mice; $P = 0.0030$) and IL-10^{-/-} BALB/c mice (3.25 ± 0.6 for IL-10^{-/-} BALB/c mice versus 0.25 ± 0.2 for WT BALB/c mice; $P = 0.0032$). We observed that when vaccinated with strain ts-4 of *T. gondii*, WT B6, WT BALB/c, and IL-10 Tg B6 mice all developed nearly complete resistance to a subsequent ocular challenge with highly virulent strain RH whereas vaccinated IL-10^{-/-} mice of both the B6 and BALB/c backgrounds developed intense necrosis in their eyes. CD8⁺ T cells have been demonstrated to be critical to the protective immunity induced by strain ts-4 in vivo (8). IL-10 has been known to be a key regulatory cytokine of immune-mediated inflammation (13) and a stimulating factor for CD8⁺ cytotoxic T lymphocytes (16). When mice were immunized with irradiated cercariae of *Schistosoma mansoni*, IL-10 secretion from the spleen cells of all vaccinated groups increased after a challenge (2). Our data demonstrated that mice without the gene for IL-10 could not prevent the immune-mediated pathology that occurs following a *T. gondii* challenge. Thus, IL-10 appears to be important in the development of vaccine-based immunity to ocular toxoplasmosis.

Blood from each mouse was obtained by cardiac puncture at 11 days postinfection or postchallenge, and the serum samples were quantitated with enzyme-linked immunosorbent assay kits for IFN- γ and tumor necrosis factor alpha (TNF- α ; Biosource, Camarillo, Calif.). As shown in Fig. 3, the production of IFN- γ and TNF- α in serum from WT B6, IL-10^{-/-} B6, WT BALB/c, and IL-10^{-/-} BALB/c mice was significantly increased at day 11 postinfection ($P < 0.01$). All of these mice, except IL-10 Tg B6 mice, had serious ocular necrosis. At 11 days postchallenge, levels of IFN- γ in the serum of these mice and levels of TNF- α in the serum of IL-10^{-/-} B6 and IL-10^{-/-}

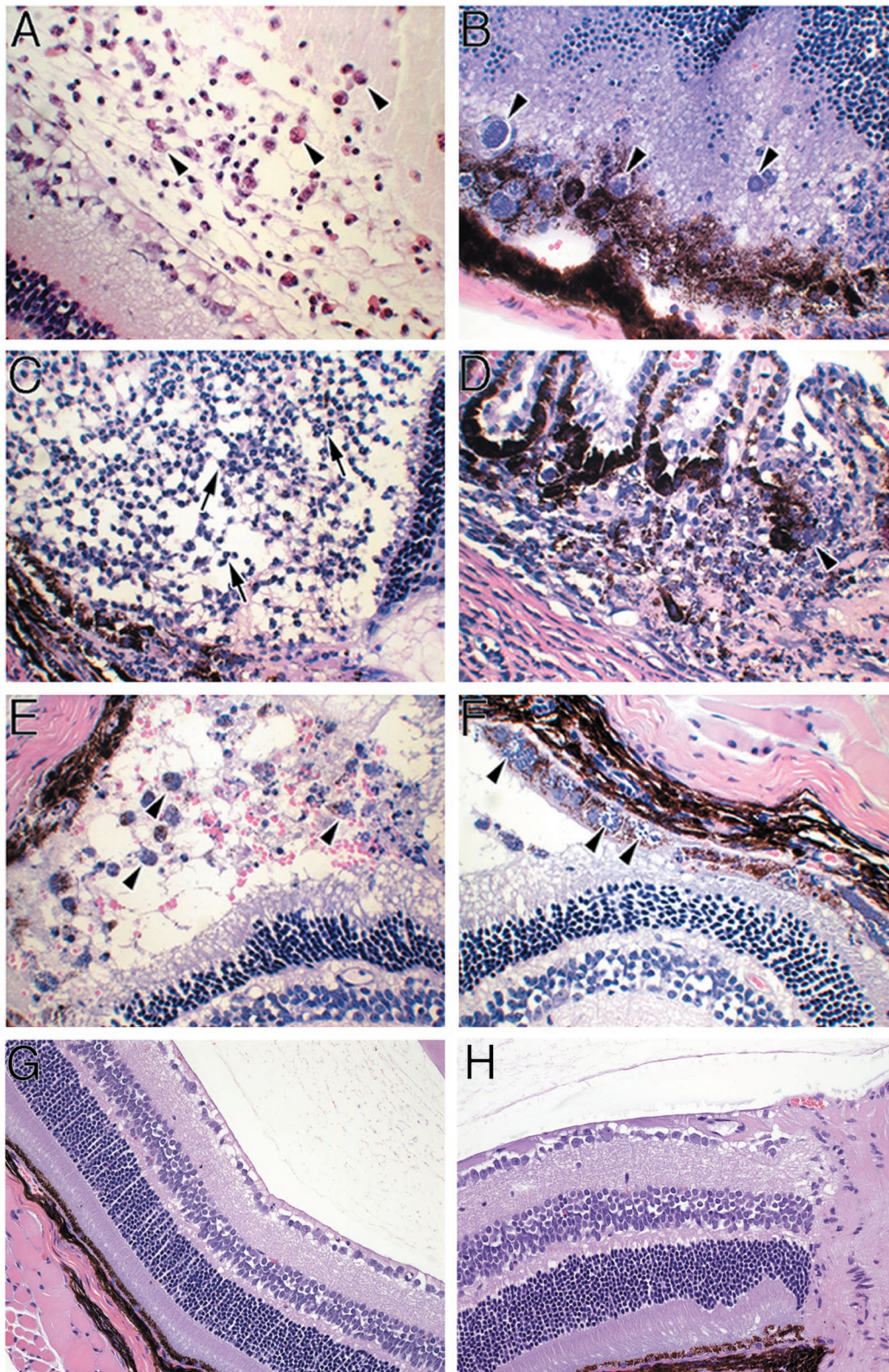


FIG. 2. Early histopathology of eye tissue from B6 background mutants and WT mice infected intracamerally with 4×10^4 tachyzoites of *T. gondii* strain RH. Shown are tissue samples from a WT mouse at 4 (A) and 6 (B) days postinfection, an IL-10^{-/-} mouse at 4 (C) and 6 (D) days postinfection, an IL-10 Tg mouse at 4 (E) and 6 (F) days postinfection, a normal B6 mouse (G), and a normal IL-10^{-/-} B6 mouse (H). There were six mice in each group. This experiment is representative of two performed. Arrowheads indicate *T. gondii* tachyzoites; arrows indicate inflammatory cells. Original magnification, $\times 40$. Hematoxylin and eosin staining was used.

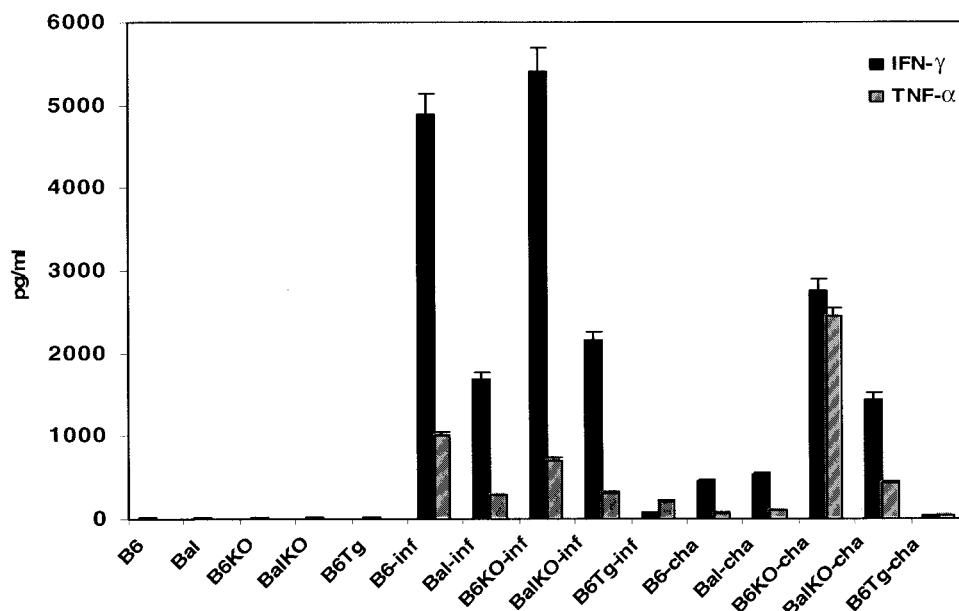


FIG. 3. Levels of IFN- γ and TNF- α in serum. Mutant and WT mice were infected or challenged intracamerally with 100 tachyzoites of *T. gondii* strain RH, and serum from each mouse was collected at 11 days postinoculation. Data are means \pm standard deviations from a single experiment with four mice in each group. Comparable results were obtained in three experiments.

BALB/c mice were significantly increased ($P < 0.01$), and serious necrosis was observed in the eye tissue of IL-10 $^{-/-}$ B6 and IL-10 $^{-/-}$ BALB/c mice; this was not the case in IL-10 Tg B6 mice. IFN- γ is critical for the prevention of tachyzoite proliferation and death in animals infected with *T. gondii* (19); at the same time, IFN- γ mediates the development of necrosis in the small intestines of IL-10 $^{-/-}$ mice following peroral infection with *T. gondii* (20). It has also been demonstrated that TNF- α plays a protective role during the acute phase of infection (5, 12) and is known to be a major mediator of tissue damage, as well as possibly implicated in the immunopathology of *T. gondii* infection (3, 4). Upon oral infection with *T. gondii*, TNF $^{-/-}$ mice fail to control intracerebral *T. gondii* and succumb to acute necrotizing *Toxoplasma* encephalitis (17). We observed that the ocular pathogenesis induced by *T. gondii* correlates with increased levels of IFN- γ and TNF- α in serum and that vaccine-induced protection from ocular toxoplasmosis correlates with increased levels of IFN- γ in serum. Our data reveal that the lack of endogenous IL-10 worsens the ocular pathology in infected IL-10 $^{-/-}$ mice and makes the challenged IL-10 $^{-/-}$ mice fail to prevent the immune-mediated pathology associated with increased levels of IFN- γ and TNF- α in serum. Overexpression of IL-10 could downregulate the production of IFN- γ and TNF- α , thereby controlling ocular inflammatory reactions. IL-10 is required to prevent the overproduction of IL-12, TNF- α , and IFN- γ during infection with ME49 (4). Both IFN- γ induction and its regulation by IL-10 are key elements in host resistance to this parasite (20). It has been reported that during acute infection with ME49, lack of IL-10 is associated with high levels of IL-12 and IFN- γ (4, 15). Surprisingly, in our study, the levels of IFN- γ and TNF- α were not significantly different between WT and IL-10 $^{-/-}$ mice following infection. Suzuki et al. (20) reported that significantly greater amounts of IFN- γ mRNA are detected in the lamina

propria lymphocytes from infected IL-10 $^{-/-}$ mice than in those from infected control mice; however, the IFN- γ -to- β -actin mRNA ratios do not differ in intraepithelial lymphocytes from infected IL-10 $^{-/-}$ mice and control mice (20). Meanwhile, Wille et al. (21) also observed that the levels of IFN- γ in serum show no significant difference between WT and IL-10 $^{-/-}$ mice of the BALB/c background at days 5 and 7 postinfection with strain RH of *T. gondii*. Our data suggest that IL-10 is not the sole regulator of IFN- γ production during acute ocular infection with *T. gondii*.

In this report, we describe an in vivo model with which to assess the role of IL-10 in the regulation of primary ocular infection with *T. gondii*, as well as protective immune responses induced in the eyes by *T. gondii*. It demonstrates that IL-10 is important in the pathogenesis of ocular toxoplasmosis and that IL-10 is required for the restriction of ocular immunopathology in both B6 and BALB/c mice following primary ocular infection and in the protective immune responses following a challenge with *T. gondii*.

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REFERENCES

1. de Waal Malefyt, R., H. Yssel, M.-G. Roncarolo, H. Spits, and J. E. de Vries. 1992. Interleukin-10. *Curr. Opin. Immunol.* 4:314-320.
2. el-Malky, B. S., M. Soliman, A. A. Mageid, H. E. el-Nemr, E. M. el-Nashar, and S. Hawas. 2001. IL-10 gene expression as a marker for detection of protective immunity levels with irradiated cercarial vaccine against experimental *Schistosoma mansoni* infection. *J. Egypt. Soc. Parasitol.* 31:745-760.
3. Freund, Y. R., G. Sgarlato, C. O. Jacob, Y. Suzuki, and J. S. Remington. 1992. Polymorphisms in the tumor necrosis factor alpha (TNF- α) gene correlate with murine resistance to development of toxoplasmic encephalitis

- and with levels of TNF- α mRNA in infected brain tissue. *J. Exp. Med.* **175**:683–688.
4. **Gazzinelli, R. T., M. Wysocka, S. Hieny, T. Scharton-Kersten, A. Cheever, R. Kuhn, W. Muller, G. Trinchieri, and A. Sher.** 1996. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on CD4⁺ T cells and accompanied by overproduction of IL-12, IFN- γ , and TNF- α . *J. Immunol.* **157**:798–805.
 5. **Gazzinelli, R. T., S. Hieny, T. A. Wynn, S. Wolf, and A. Sher.** 1993. Interleukin-12 is required for the T-lymphocyte-independent induction of interferon-gamma by an intracellular parasite and induces resistance in T cell-deficient hosts. *Proc. Natl. Acad. Sci. USA* **90**:3442–3447.
 6. **Ghalib, H. W., M. R. Piuvezam, Y. A. W. Skeiky, M. Siddig, F. A. Hashim, A. M. El-Hassan, D. M. Russo, and S. G. Reed.** 1993. Interleukin-10 production correlates with pathology in human *Leishmania donovani* infections. *J. Clin. Investig.* **92**:324–329.
 7. **Groux, H., M. Bigler, J. E. de Vries, and M. G. Roncarolo.** 1998. Inhibitory and stimulatory effects of IL-10 on human CD8⁺ T cells. *J. Immunol.* **160**:3188–3193.
 8. **Hakim, F. T., R. T. Gazzinelli, E. Denkers, S. Hieny, G. M. Shearer, and A. Sher.** 1991. CD8⁺ T cells from mice vaccinated against *Toxoplasma gondii* are cytotoxic for parasite-infected or antigen-pulsed host cells. *J. Immunol.* **147**:2310–2316.
 9. **Hoffmann, K. F., S. L. James, A. W. Cheever, and T. A. Wynn.** 1999. Studies with double cytokine-deficient mice reveal that highly polarized Th1- and Th2-type cytokine and antibody responses contribute equally to vaccine-induced immunity to *Schistosoma mansoni*. *J. Immunol.* **163**:927–938.
 10. **Hu, M. S., J. D. Schwartzman, A. C. Lepage, I. A. Khan, and H. L. Kasper.** 1999. Experimental ocular toxoplasmosis induced in naive and preinfected mice by intracameral inoculation. *Ocul. Immunol. Inflamm.* **7**:17–26.
 11. **Hunter, C. A., J. S. Abrams, M. H. Beaman, and J. S. Remington.** 1993. Cytokine mRNA in the central nervous system of SCID mice infected with *Toxoplasma gondii*: importance of T-cell-independent regulation of resistance to *T. gondii*. *Infect. Immun.* **61**:4038–4044.
 12. **Johnson, L.** 1992. A protective role for endogenous tumor necrosis factor in *Toxoplasma gondii* infection. *Infect. Immun.* **60**:1979–1983.
 13. **Kaufman, H. L., J. B. Rao, K. R. Irvine, V. Bronte, S. A. Rosenberg, N. P. Restifo, and K. R. Irvine.** 1999. Interleukin-10 enhances the therapeutic effectiveness of a recombinant poxvirus-based vaccine in an experimental murine tumor model. *J. Immunother.* **22**:489–496.
 14. **King, C. L., A. Medhat, I. Malhotra, M. Nafeh, A. Helmy, J. Khandary, S. Ibrahim, M. El-Sherbiny, S. Zaky, R. J. Stupi, K. Brustoski, M. Shehata, and M. T. Shata.** 1996. Cytokine control of parasite-specific anergy in human urinary schistosomiasis. IL-10 modulates lymphocyte reactivity. *J. Immunol.* **156**:4715–4721.
 15. **Neyer, L. E., G. Grunig, M. Fort, J. S. Remington, D. Rennick, and C. A. Hunter.** 1997. Role of interleukin-10 in regulation of T-cell-dependent and T-cell-independent mechanisms of resistance to *Toxoplasma gondii*. *Infect. Immun.* **65**:1675–1682.
 16. **Santin, A. D., P. L. Hermonat, A. Ravaggi, S. Bellone, S. Pecorelli, J. J. Roman, G. P. Parham, and M. J. Cannon.** 2000. Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8⁺ cytotoxic lymphocytes. *J. Virol.* **74**:4729–4737.
 17. **Schluter, D., L. Y. Kwok, S. Lutjen, S. Soltek, S. Hoffmann, H. Korner, and M. Deckert.** 2003. Both lymphotoxin-alpha and TNF are crucial for control of *Toxoplasma gondii* in the central nervous system. *J. Immunol.* **170**:6172–6182.
 18. **Silva, J. S., P. J. Morrissey, K. H. Grabstein, K. M. Mohler, D. Anderson, and S. G. Reed.** 1992. Interleukin 10 and interferon gamma regulation of experimental *Trypanosoma cruzi* infection. *J. Exp. Med.* **175**:169–174.
 19. **Suzuki, Y., M. A. Orellana, R. D. Schreiber, and J. S. Remington.** 1988. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science* **240**:516–518.
 20. **Suzuki, Y., A. Sher, G. Yap, D. Park, L. E. Neyer, O. Liesenfeld, M. Ford, H. Kang, and E. Gufwoli.** 2000. IL-10 is required for prevention of necrosis in the small intestine and mortality in both genetically resistant BALB/c and susceptible C57BL/6 mice following peroral infection with *Toxoplasma gondii*. *J. Immunol.* **164**:5375–5382.
 21. **Wille, U., E. N. Villegas, B. Striepen, D. S. Roos, and C. A. Hunter.** 2001. Interleukin-10 does not contribute to the pathogenesis of a virulent strain of *Toxoplasma gondii*. *Parasite Immunol.* **23**:291–296.

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