Interleukin 17 Receptor Signaling Is Deleterious during Toxoplasma gondii Infection in Susceptible BL6 Mice

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Th17 cells are involved in host defense against several pathogens. Using interleukin (IL) 17RA−deficient mice, we demonstrated reduced ileitis with diminished neutrophil recruitment and inflammatory lesions in the ileum, in the regional lymph node, in the spleen, and in the liver at day 7 and prolonged survival after Toxoplasma gondii infection. In addition, IL-17A antibody neutralization reduced inflammation and enhanced survival in BL6 mice. Diminished inflammation is associated with augmented interferon (IFN) γ serum levels and enhanced production of IL-10 and IFN−γ in cultured splenocytes upon antigen restimulation. Finally, cyst load and inflammation in the brain at 40 days are greater in surviving BL6 mice than in IL-17RA−deficient mice. In conclusion, oral T. gondii infection increases IL-17 expression and contributes to the inflammatory response, and IL-17 neutralization has a partial protective effect against fatal T. gondii−associated inflammation.

Toxoplasma gondii is an opportunistic parasite with a worldwide distribution that triggers an innate immune response characterized by a rapid recruitment of neutrophils to the site of infection, followed by a strong Th1 protective response associated with the production of proinflammatory cytokines, including interleukin (IL) 12 and tumor necrosis factor (TNF) α. Neutrophils are critical and complementary to dendritic cells and macrophages, and the latter 2 populations are known to synthesize IL-12 soon after infection [1]. Bliss et al [2] showed that neutrophil depletion at the time of T. gondii infection reduced host resistance and was associated with the development of lesions in multiple organs; an impaired ability to produce early interferon (IFN) γ, TNF-α, and IL-12; and an increased parasite burden. Parasite-triggered chemokines from neutrophils have chemotactic activity on immature bone marrow–derived dendritic cells and promote Th1 differentiation [1]. Thus, neutrophils appear to have an important role in the early response to T. gondii infection.

IL-17A is the major cytokine for neutrophil recruitment after infection and is a potent proinflammatory mediator, but the role of the related IL-17F is less well defined [3, 4]. The signalling receptor IL-17RA is ubiquitously expressed and recognizes both IL-17A and IL-17F. Murine IL-17 signals through a complex consisting of at least 2 IL-17R subunits, which are preassembled in the membrane [5]. Stimulation of IL-17R with IL-
17A induces the activation of various transcription factors, including NF-kB and AP-1 [6].

A recent report suggested that IL-17R signalling has a protective effect, because IL-17RA–deficient mice succumb more rapidly to T. gondii infection [7]. Here, we conducted a similar study in IL-17RA–deficient mice but included IL-17A neutralizing antibody in C57BL/6 (BL6) mice that were orally infected with 30 76K-strain cysts, focusing on fatal ileitis [8]. We show here that IL-17RA–deficient mice and BL6 mice that received IL-17A neutralizing antibody were more resistant to T. gondii infection; presented fewer inflammatory changes and less tissue damage in the ileum, liver, spleen, and brain; produced more Th1 cytokines and IL-10; and are partially protected, compared with BL6 mice, suggesting that IL-17A contributes to the pathology of T. gondii infection.

**MATERIALS AND METHODS**

**Materials.** All reagents for cell culture were obtained from Invitrogen and Sigma-Aldrich; enzyme-linked immunosorbent assay (ELISA) reagents and recombinant cytokines were from BD Pharmingen, unless otherwise stated.

**Mice.** IL-17RA–deficient mice (KO) on C57BL/6 background were obtained from Jacques Peschon [9]. C57BL/6 (BL6) control mice were purchased from Jackson Laboratories (Bar Harbor, Maine) or from Janvier, France. Adult males weighing 20–25 g and 10–12 weeks of age were used in this study. All mice were bred under specific-pathogen–free conditions at Centre National de la Recherche Scientifique (Orléans, France). The animal experiments complied with the French Government’s ethical and animal experiment regulations.

**Parasites and T. gondii extract.** Tachyzoites of the RH strain of T. gondii were harvested from infected monolayers of human foreskin fibroblasts Hs 27 (ATCC CRL-1634) and were used as the source of T. gondii extract antigen (TE) [10]. Cysts of the 76K strain of T. gondii were obtained from brains of CBA/J mice that had been orally infected with 80 cysts 1 month earlier.

**Infection of IL-17RA KO mice and IL-17A antibody neutralization.** BL6 and IL-17RA–deficient mice were orally infected with 30 cysts of the 76K strain, obtained as described above. Furthermore, infected BL6 mice received a single anti–IL-17A antibody or isotype-control antibody (R&D Systems) administered intravenously at a dosage of 100 μg per mouse just before infection and at day 4 after infection. The mice were analyzed at day 7 for neutrophil recruitment in the ileum and morphological alterations of various organs.

**RNA extraction and polymerase chain reaction (PCR) in ileum.** Ileum from control infected BL6 mice was isolated, and RNA was extracted. Total RNA were isolated from <100 mg of intestinal tissue previously snap-frozen in liquid nitrogen. We performed RNA extraction in 2 steps to obtain a better quality sample. RNA was first extracted with RNA TRIzol reagent (Sigma) and, in the second step, was purified using a commercial kit (RNaseasy; Qiagen) following the manufacturer’s instructions. The purified total RNA were used to generate first-strand complementary DNA (cDNA) by reverse transcription using 2 μg of total RNA, M-MuLV Reverse Transcriptase (MP Biomedicals), and a random hexamer. Semi-quantitative PCR were realized using 100 ng of cDNA, 20 μM of each forward and reverse primer, 20 μM of deoxyribonucleotide triphosphate (MP Biomedicals), and Taq polymerase 1U (Amersham). HPRT expression was used to normalize the relative expression levels of IL-17A and IL-17F. The primers used in this study were the following: IL-17A forward, 5'-CGC AAA CAT GAG TCC AGG GAG AGC-3'; IL-17A reverse, 5'-TCA GGG TCT TCA TTG CGG TGG AG-3'; IL-17F forward, 5'-CCC GTG AAA CAG CCA TGG TCA-3'; IL-17F reverse, 5'-TGA CCC TG GCA TT GAT GCA G-3'; HPRT forward, 5'-CTG GTG AAA AGG ACC TCT CG-3'; and HPRT reverse, 5'-TGA AGT ACT CAT TAT AGT CAA GGG CAT-3'. Amplifications were performed as follows: IL-17A and IL-17F, 35 cycles with an annealing temperature of 58°C; HPRT, 35 cycles with an annealing temperature of 60°C.

**Antigen-induced cytokine production in culture.** Spleens and mesenteric lymph nodes (MLNs) were harvested 7 days after infection and were pressed through a stainless steel mesh. Single-cell suspensions were obtained by filtration through nylon mesh to remove tissue debris. Spleen erythrocytes were lysed by hypotonic shock, and spleen and MLN cells were resuspended in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 5% fetal calf serum, HEPES (25 mM), L-glutamine (2 mM), sodium pyruvate (1 mM), β-mercaptoethanol (5 × 10⁻⁵ M), and penicillin (5 U/mL) in combination with streptomycin (50 μg/mL). Spleen and MLN cells were cultured in 24-well plates at 10⁶ cells per well in 1 mL of culture medium, alone or containing TE (10 μg/mL). The plates were incubated for 4 days in 5% CO₂ at 37°C. Cell culture supernatants were harvested between 24 h and 4 days and kept at −20°C until cytokine assays could be performed.

**Cytokine and nitric oxide concentration measurement.** Spleen and MLN cell supernatants were tested for IL-2, IL-4, IL-10, IL-12 p70, IFN-γ, MCP-1, TNF-α, and nitric oxide activities. Cytokine productions were evaluated using commercial ELISA kits according to the manufacturer’s instructions. Concentrations were determined by reference to standard curves constructed with known amounts of mouse recombinant IL-2, IL-4, IL-10, IL-12, IFN-γ, MCP-1, TNF-α, and nitric oxide activities. The sensitivity limits for the assays were 3.1 pg/mL for IL-2, 7.8 pg/mL for IL-4, 15.6 pg/mL for MCP-1 and TNF-α, 31.3 pg/mL for IL-10 and IFN-γ, and 62.5 pg/mL for IL-12.

The nitric oxide production was evaluated using the Griess
IL-17R Signalling in T. gondii Infection

T. gondii infection upregulates intestinal IL-17 expression, and survival is enhanced in IL-17RA deficient mice. Oral infection with T. gondii causes severe ileitis. To determine the functional implication of IL-17A and IL-17F in inflammation, wild-type BL6 and IL-17RA KO mice were infected with 30 cysts of T. gondii. We first asked whether IL-17 is expressed in the ileum. We found that IL-17A and IL-17F expression were upregulated in the inflamed ileum, compared with the control ileum, at day 7 after infection (Figure 1A).

To evaluate the potential relationship between the presence of IL-17 and the survival of mice with acute infection, clinical
signs, weight, and survival of mice that had been administered an oral infectious dose of 30 cysts were studied (Figure 1B and 1C). BL6 mice rapidly showed clinical symptoms of disease and lost body weight, and all mice were dead within 7 days, whereas 70% of the IL-17RA KO mice survived for 9 days, and 30% of them survived for the experimental period of 40 days. Moreover, 60% of mice that received anti-IL-17A antibody survived the acute phase of infection, whereas all isotype-treated mice were dead at day 7. Thus, the data suggest that endogenous IL-17 may have a detrimental role in infection, because IL-17RA KO mice display a partial protection against fatal *T. gondii* infection.

**Reduced acute intestinal inflammation in the absence of IL-17RA signalling.** The ileum at day 7 after infection was distended and the intestinal wall reddened in BL6 mice, whereas inflammation was minimal in IL-17RA KO mice (Figure 2A). Microscopic investigations also revealed a marked acute ileitis with erosions and ulcers of the intestinal epithelium in BL6 mice, which was largely absent in IL-17RA KO mice (Figure 2A). We then used neutralizing IL-17A antibody in BL6 mice.
and found reduced neutrophil recruitment in the ileum, as assessed by MPO activity (Figure 2B) with reduced severity of ileitis and inflammatory lesions comparable to IL-17RA deficient mice (Figure 2C). We determined IFN-γ and TNF-α levels in the ileum as well as the parasite burden of wild-type and IL-17RA KO mice. This showed no difference between the 2 groups 7 days after infection (data not shown). Therefore, IL-17A antibody neutralization prevents inflammation and mortality in T. gondii-infected BL6 mice, as observed in IL-17RA–deficient mice.

Reduced systemic inflammation in the absence of IL-17RA signalling. We next investigated the inflammatory response in other organs. The liver showed multiple focal lesions which clearly were more pronounced in BL6 mice than in IL-17RA KO mice (Figure 3A). Microscopic examinations from BL6 mice revealed focal inflammation, characterized by mononuclear cell and neutrophil recruitment, single-cell necrosis, and thrombotic lesions in the portal areas (Figure 3A, left panel). These changes were less pronounced in the liver of IL-17RA KO mice (Figure 3A, right panel). The spleens from BL6 mice were enlarged, granular, and half-sized, compared with the IL-17RA KO spleens (Figure 3B). Microscopically, the BL6 spleens showed congestion in the red pulp and lymphocyte depletion in the white pulp, whereas these changes were less prominent in the IL-17RA-deficient mice. The draining lymph nodes were increased in size, and the follicular structure was effaced by inflammation and necrosis; the inflammatory response was less severe in IL-17RA KO mice (Figure 3C). The data suggest that IL-17RA signalling augments inflammatory pathology in the intestine, draining lymph node, liver, and spleen and reduces survival in response to acute phase of T. gondii infection.

Augmented Th1 cytokine response in the absence of IL-17RA signalling. To explain the higher survival rate of IL-17RA deficient mice, we studied cytokines in serum samples, spleens, and MLNs during the acute phase of infection (Figure 4). Ten days after infection, spleens and MLNs were harvested, and single-cell suspensions were cultured without or with T. gondii extract. Supernatants were collected after 24–96 h to test inflammatory and anti-inflammatory cytokine production.

IFN-γ serum levels were significantly increased in IL-17RA–

![Figure 3](https://academic.oup.com/jid/article-abstract/202/3/427/830922/4)

**Figure 3.** Reduced systemic inflammation in the absence of interleukin (IL)-17RA. Macroscopic observations of liver (A), spleen (B) and mesenteric lymph node (C) 7 days after infection are shown alongside corresponding microscopic observations. Wild-type BL6 (WT) animals are shown in the left panels, whereas IL-17RA–deficient mice (KO) are shown in the right panel. Tissue lesions in WT liver as well as cellular infiltration in all organs are indicated with arrows. Histological studies were conducted on formaldehyde-fixed organs, which were stained with hematoxylin and eosin. Scale bars represent 1 cm.
Figure 4. Augmented cytokine production in the absence of interleukin (IL) 17RA. Seven days after infection, blood samples were collected before mice were sacrificed and spleens were harvested. Splenocytes were cultured with *Toxoplasma gondii* extract (10 μg/mL) or medium alone. Supernatants were collected at 24–96 h to determine cytokine levels (IL-4 and MCP-1 at 24 h, IL-2 at 48 h, IL-10 at 72 h, and IFN-γ at 96 h). Cytokine secretion was evaluated in serum samples (A) or supernatants (B–F) from BL6 (white bars) and IL-17RA–deficient mice (black bars) ( ). Results are presented as mean values ± standard error of the mean and are representative of 3 independent experiments (*P < .05).

deficient mice (P < .05) (Figure 4A), as was IFN-γ production in cultured splenocytes from IL-17RA–deficient mice (325 pg/mL), compared with BL6 mice (123 pg/mL; P < .05) (Figure 4B). The same profile was seen for IL-2 production (Figure 4C) (P < .05). Anti-inflammatory cytokine profiles were also augmented in IL-17RA deficient mice. IL-10 secretion in splenocytes (Figure 4D) was 31 pg/mL for BL6 mice and 111 pg/mL for IL-17RA KO deficient mice (P < .05). IL-4 production was not significantly increased (3 pg/mL for BL6 mice and 17 pg/mL IL-17RA deficient mice; Figure 4E). MCP-1 production was slightly increased in IL-17RA–deficient mice, compared with BL6 mice, but the difference was not statistically significant (638 and 225 pg/mL, respectively) (Figure 4F). IL-12 p70 as well as TNF-α could not be detected in supernatants from spleen and MLN cells (data not shown). The results obtained from restimulated MLNs showed the same profile as that for spleens (data not shown). The results indicate that the splenic cytokine responses are augmented in the absence of IL-17RA signalling during the acute phase of the infection.

**Reduced inflammation and parasite load in the brain of IL-17RA KO mice.** Forty days after *T. gondii* infection—that is, during the chronic phase of the disease—surviving mice were killed and their brains were either processed for morphological analysis or for the determination of the number of cysts. The microscopic investigations reveal abundant inflammatory lesions with mononuclear cells and neutrophils in infected BL6 mice (Figure 5A). However, the severity and extent of the inflammatory infiltrates in the brain are significantly reduced in IL-17RA–deficient mice, as revealed by the relative inflammatory scores (4 and 1.3 for wild-type and IL-17RA KO–infected mice, respectively; Figure 5B). Respective brain cyst loads were evaluated in brain homogenates (Figure 5C). IL-17RA KO mice had significantly fewer cysts than did BL6 mice (2285 vs 5245 cysts; P < .01). The results suggest that disrupted IL-17 signalling attenuates the inflammatory response, reduces parasite growth, and protects mice with long-term infection.

**DISCUSSION**

The role of IL-17 in various infectious diseases is an emerging area of interest [14]. Using IL-17RA–deficient mice, we provide strong evidences for a deleterious role of the IL-17 receptor signalling pathway during *T. gondii* infection.

We found that IL-17RA-deficient mice survived longer than did infected BL6 mice. Reduced ileitis and inflammation in other organs correlate with reduced mortality in IL-17RA–deficient mice. Kelly et al [7] reported that IL-17R signalling has a protective effect, because IL-17RA–deficient mice succumb
Figure 5. Reduced inflammation and parasite load in brains of interleukin (IL) 17RA deficient mice. A, Forty days after infection, the inflammation in the central nervous system was evaluated by microscopy of brain sections from infected wild-type (WT), IL-17RA KO, and noninfected BL6 mice (WT control). B, The inflammation was graded semiquantitatively on samples from noninfected BL6 mice (WT NI) and infected wild-type (WT) and IL-17RA KO BL6 mice. C, Protection of BL6 (white bar) and IL-17RA-deficient (black bar) mice was evaluated via their brain cyst load. Cumulative results of 3 independent experiments (12 mice) are given and are presented as mean number of cysts ± standard error of the mean (**P < .01). AU, arbitrary units.

more rapidly to T. gondii infection. Our repeat studies (conducted 4 times) and IL-17A antibody neutralizing studies in BL6 mice reveal reduced ileitis and enhanced survival in the absence of IL-17A. However, we agree with the previous work, that inflammation is drastically reduced in the ileum and other organs, and no effect on parasite burden is found at day 7, as shown by Kelly et al [7]. Reduced inflammation and mortality are also found in T. gondii–infected BL6 mice upon IL-17A antibody administration. These data suggest that endogenous IL-17A enhances T. gondii–induced inflammatory pathology and may enhance spread of the parasites at later time points, as evidenced in the brain at 40 days. Although we cannot fully reconcile the discrepancies between data from our study and data from the previous study [7], the main outcome of a reduction in inflammation is comparable.

Our results could at first appear to be in contradiction with those of other studies [2, 15]. The authors reported that neutrophils are important during the first days of T. gondii infection, because mice treated with a neutrophil-depleting antibody or CXCR2-deficient mice are more sensitive to infection (eg, they experience more-severe lesions, greater parasite burden, and decreased Th1 response). The discrepancy between these
reports and our data is likely attributable to the use of different mouse and parasite strains and different routes of infection. Bliss et al [2] used intraperitoneal injections of an avirulent strain (ME49), whereas we used per os infections with the highly virulent RH strain. Del Rio et al [15] used intraperitoneal injections of the RH strain in resistant BALB/c mice, known to have responses that differ from those of susceptible C57BL/6 mice.

Furthermore, we showed that IL-17A-deficient mice are able to mount efficient T. gondii–specific T cell responses. IFN-γ and IL-10 are increased in IL-17RA-deficient mice without a distinct Th1/Th2 polarization, which suggest that the pathology would instead be associated with a prevailing neutrophil/IL-17 response. Previous studies have observed markedly increased expression of an inflammatory chemokine, MCP-1, in inflamed intestinal specimens from patients with ulcerative colitis and Crohn’s disease. Inflamed mucosa contained multiple cell types expressing MCP-1, including mononuclear and endothelial cells [16]. MCP-1 is a potent chemoattractant for monocytes but not for neutrophils, and thus, in our model, the setting of an overwhelming inflammation would be restricted by the combination of the local secretion of MCP-1 and of a lack of infiltrating neutrophils caused by IL-17A deficiency. However, reduced MIP-2 serum concentrations have been reported in infected IL-17RA-deficient mice, which may explain diminished neutrophil infiltration in the intestine [7].

IL-17RA signals both IL-17A and F, which are cytokines that are induced in the ileum upon T. gondii infection. Ileitis induced by an oral infection with T. gondii was significantly reduced in IL-17RA-deficient mice. Our results suggest that IL-17A mediates inflammation, because an IL-17A–specific neutralizing antibody attenuates ileitis. Similar results were observed in patients with inflammatory bowel diseases, such as Crohn’s disease or ulcerative colitis. Colorectal tissue samples from these patients showed inflamed mucosa associated with IL-17 expression by T cells and macrophages [17]. Thus, in this study, we showed that IL-17RA signalling pathway plays a major role in the setting of a strong inflammation after T. gondii infection and is potently implicated in inducing an uncontrolled lethal response in BL6 susceptible mice.

However, the other Th17 family member, IL-22, is also elevated in intestinal inflammation after T. gondii infection. Muñoz et al [18] recently reported an IL-23–dependent IL-22 secretion leading to intestinal inflammation. They also showed that this inflammation, contrary to our results, was independent of IL-17. However, they used the avirulent ME49 strain of parasite. It could be of interest to revisit the role of this cytokine in our experimental model, because IL-22 has already been shown to have a role in experimental models of colitis [19] and in clinical studies [20].

Our results also suggest that IL-17 could be important to resist the chronic phase of infection, given that IL-17RA-deficient mice are protected, as shown by decreased brain cysts, compared with the number of cysts in parental mice. Several explanations could account for this lower cyst load. Indeed, T. gondii is known to exploit mononuclear cells, such as dendritic cells and monocytes, to disseminate from the gut to the whole organism, including the brain [21]. Fewer intestinal parasites could have explained a decreased number of brain parasites. However, we could not see any difference between BL6 mice and IL-17RA-deficient mice. Another possibility would be an inhibition of the trafficking of the shuttle cells, leading to an impaired dissemination to the brain. Further investigations are needed to assess this hypothesis.

IL-17 has already been implicated in the brain pathology of experimental autoimmune encephalomyelitis (EAE), the murine model of human multiple sclerosis. In patients with multiple sclerosis, IL-17 mRNA and protein are increased in both brain lesions and mononuclear cells isolated from blood and cerebrospinal fluids. A recent study of EAE revealed that onset was delayed in IL-17–deficient mice and that these mice had less severe histological changes and early recovery. The authors demonstrated that IL-17 is secreted by CD4+ T cells and is important in the activation of antigen-specific T cells [22].

Because of the crucial implication of IL-17A in the induction of tissue damage and increased mortality following T. gondii infection, it would be of interest to target this cytokine in vivo as an efficient treatment to moderate the deleterious effect of IL-17 expression. IL-17A targeting has been investigated in several autoimmune pathologies in which IL-17 has a detrimental role. The use of a monoclonal antibody directed against IL-17 before the onset of EAE resulted in attenuated clinical course of the disease. Injection of a soluble IL-17R-Fc fusion protein to neutralize circulating IL-17 after onset of symptoms also resulted in improvement of clinical symptoms [23]. Immunization of mice with virus-like particles conjugated with recombinant IL-17 is another efficient strategy. Their use induced a lower incidence of disease, slower progression to disease, and reduced severity in both collagen-induced arthritis and EAE [24]. An indirect targeting of IL-17 activity can be accomplished by the neutralization of IL-23. Indeed, this cytokine is essential for the development of IL-17–producing T cells. Efficacy of such a strategy has already been proven in an experimental model of colitis in mice [25].

In conclusion, IL-17A is induced upon oral T. gondii infection. This results in neutrophil recruitment, severe inflammatory tissue damage, and early death, which are attenuated in the absence of IL-17R signalling or by IL-17A antibody neutralization.

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References


