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IL-17 Is Necessary for Host Protection against Acute-Phase Trypanosoma cruzi Infection

Yoshiyuki Miyazaki,* Shinjiro Hamano, †‡ Seng Wang,* Yohei Shimanoe,* Yoichiro Iwakura,§ and Hiroki Yoshida*

IL-17A is a key cytokine that induces inflammatory responses through the organized production of inflammatory cytokines, such as IL-6, TNF-α, and GM-CSF, and induces neutrophil migration. The roles of IL-17A in infection of intracellular protozoan parasites have not been elucidated, although augmented immune responses by IL-17A are important for the resolution of some bacterial and fungal infections. Therefore, we experimentally infected IL-17A-deficient (IL-17A−/−) mice with Trypanosoma cruzi. IL-17A−/− mice had a lower survival rate and prolonged worse parasitemia compared with control C57BL/6 wild-type (WT) mice postinfection. In the infected IL-17A−/− mice, multiple organ failure was observed compared with WT mice, as reflected by the marked increase in serologic markers of tissue injury, such as aspartate aminotransferase, which resulted in increased mortality of IL-17A−/− mice. Expression of cytokines, such as IFN-γ, IL-6, and TNF-α, was lower in liver-infiltrating cells from the IL-17A−/− mice compared with WT mice. A similar defect was observed in the expression of neutrophil enzymes, such as myeloperoxidase and lipooxygenase, whereas cellular infiltration into the infected tissues was not affected by IL-17A deficiency. These results suggested that the efficient activation of immune-related cells critical for the killing of T. cruzi was impaired in the absence of IL-17A, resulting in the greater susceptibility of those mice to T. cruzi infection. From these results, we conclude that IL-17A is important for the resolution of T. cruzi infection. The Journal of Immunology, 2010, 185: 1150–1157.

Interleukin-17A is a proinflammatory cytokine, largely produced by activated T lymphocytes, and was originally called CTL-associated Ag-8 (1). The mouse IL-17 family consists of six members, including IL-17A (called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F, all of which share 16–45% homology with IL-17A (2). Among them, IL-17A and IL-17F are mainly produced by activated memory CD4+ T cells, which are now classified as Th17 cells (3, 4). However, the production of IL-17A and IL-17F is limited to Th17 cells; CD8+ T cells, γδT cells, NK cells, and neutrophils have also been identified as sources of these cytokines (5–7). The receptor for IL-17A is expressed in various tissues, such as lung, kidney, liver, and spleen, and by various types of cells, including fibroblasts, epithelial cells, endothelial cells, monocytes/macrophages, lymphocytes, and marrow stromal cells. These cells produce diverse proinflammatory cytokines and chemokines in response to IL-17A stimulation (3). Yao et al. (8) reported IL-17A to be a potent inducer of IL-6 and IL-8 (CXCL8) by human fibroblasts. In subsequent experiments, it was shown that IL-17A could stimulate the expression of CSFs (G-CSF and GM-CSF), chemokines (CXCL1, CXCL10, CCL2, CCL7, and CCL20), and matrix metalloproteinase-3 and -13. These cytokines and chemokines augment local inflammations by inducing the recruitment of neutrophils and leukocytes. IL-17A also induces the production of IL-1β and TNF-α from macrophages (9), and these cytokines cooperate with each other to induce the production of IL-6 and chemokines (10, 11) and to augment inflammatory reactions (12).

Trypanosoma cruzi, an intracellular protozoan parasite, is the etiologic agent of American trypanosomiasis or Chagas’ disease and affects ∼16–18 million people in Central and South America (13). Innate and acquired cell-mediated immune responses are induced after experimentally infected acute T. cruzi infection, and combined mobilization of NK cells, CD4+ T cells, CD8+ T cells, γδT cells, and Ab-producing B cells are required for establishing host resistance (14, 15). Production of IL-12 by macrophages is triggered by the invasion of blood trypomastigotes of T. cruzi early postinfection, and IL-12 induces Th1 differentiation and subsequent IFN-γ production (16, 17). IFN-γ is a critical cytokine in host resistance to T. cruzi infection (18, 19); it is produced by NK cells at the early phase of infection and by CD4+ and CD8+ T cells later during the infection (20, 21). IFN-γ, synergistically with TNF-α, induces NO synthesis by macrophages, a critical mediator for killing of causal organisms during the acute phase of infection (22, 23). Furthermore, it is known that T. cruzi infection stimulates production of proinflammatory cytokines, such as IL-1β/β, IL-6, and TNF-α (24, 25). Infected-induced inflammatory reactions mediated by these cytokines against T. cruzi result in effective expulsion of the...
parasites, although immunopathology from excessive inflammation also has a significant impact on the pathogenesis of experimental Chagas’ disease (26, 27).

The roles of IL-17A in host defense against intracellular protozoan parasites remain to be fully elucidated, although IL-17A has a role in the host’s protection against fungal and bacterial infection (28–31). Therefore, we investigated the role of IL-17A in T. cruzi infection. Infection by T. cruzi led to increased IL-17A production by CD4 + T, CD8 + T, NK T, and γδT lymphocytes. IL-17A-deficient (IL-17A−/−) mice infected with T. cruzi exhibited more severe parasitemia and mortality accompanied by the attenuated production of antiparasitic cytokines, including IFN-γ, IL-6, and TNF-α, compared with wild-type (WT) mice. These results clearly indicated that IL-17A is necessary for the host’s protection during acute-phase T. cruzi infection.

Materials and Methods

Animals

The generation of IL-17A−/− mice on the C57BL/6 background was described previously (32). Briefly, a targeting vector for deleting exons 1 and 2 of the IL-17A gene was electroporated into ES (E14.1) cells and selected in the presence of G418. Targeted clones, screened by Southern blot-hybridization analysis, were treated with adenovirus carrying the cre gene to delete the neo gene. Chimera mice were generated by the aggregation method, using C57BL/6 blastocysts as the recipients, and were mated with C57BL/6 female mice for germline transmission. Mutant mice were backcrossed onto the C57BL/6 background more than eight times (considered as the background in the experiment). Mice were housed in microisolator cages and were used between 8 and 12 wk of age. Male and sex-matched WT C57BL/6 mice (Kyudo, Saga, Japan) were used as controls. All experiments were approved by the institutional animal research committee of Saga University and conformed to the animal care guidelines of the American Physiological Society.

Parasites

T. cruzi (Tulahuen strain) was maintained in vivo in IFN-γ−/− mice by every 2 wk passages. For the experiments, IL-17A−/− and WT mice were injected i.p. with the plasma containing trypomastigotes. Mice were infected with 2000 trypomastigotes. The number of parasites in the blood was counted for each animal using 4 counts by centrifugal fractionation. Serum alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, and creatine kinase were measured with the Cytoxys/Cytoperm Plus kit (BD Biosciences), according to the manufacturer’s instructions. The sequence of PCR primers were as follows: IFN-γ: 5'-ACA GGA GAA GGG ACG CCA T-3', TNF-α: 5'-TGG GAG TAG ACA AGG-3'.

Histological analysis

Tissues were removed and fixed with 10% formaldehyde neutral buffer solution (Nacalai, Kyoto, Japan) at the indicated days after infection. Then, specimens were embedded in paraffin and stained with H&E, and structural changes and cellular infiltrations were evaluated with a light microscope.

In vitro assessment of IL-17A and Th17 function on macrophage and neutrophil activation

Spleen CD4 + cells were isolated from WT or IL-17A−/− mice at 14 d after T. cruzi infection by MACS using anti-CD4 mAb. In contrast, peritoneal exudate macrophages were collected from WT mice treated with 15% thioglycolate (Difco, Detroit, MI) for 3 d and seeded in 48-well culture plates at 5 × 10^5 cells/well. These macrophages were infected with 2.5 × 10^4 T. cruzi trypomastigotes and cocultured with the isolated WT or IL-17A−/− CD4 + cells for 18 h. After the culture, cytokines and nitrite (NO) levels in culture supernatant were measured with the DuoSet ELISA Development System (R&D Systems, Minneapolis, MN) and the Griess Reagent Kit for Nitrite Determination (Molecular Probes, Eugene, OR), respectively. In another experiment, the thiglycolate-induced peritoneal exudate macrophages (5 × 10^5 cells/well) were treated with 25 ng/ml recombinant murine IL-17A for 18 h, and NO secretion was measured. NO production was also evaluated after stimulation with 100 ng/ml LPS, 25 ng/ml IFN-γ, or 1 × 10^6 soluble Th17 cytokines (STAb) with or without 25 ng/ml LPS. STAb was prepared by repeated freeze and thaw of 1 × 10^6 trypomastigotes in 100 μl culture medium. For the preparation of neutrophils, peritoneal exudate cells were collected at 4–16 h after 3% thiglycolate injection. The cell suspension was put on 60% Percoll (GE Healthcare, Uppsala, Sweden) and centrifuged at 1000 × g for 30 min. The cell pellet, containing >80% neutrophils (7–4 Ly6G'), was resuspended with culture medium and used in in vitro experiments as follows. The prepared neutrophils (5 × 10^5 cells) were treated with indicated stimuli, with or without recombinant mouse IL-17A (25 ng/ml) for 2 h. These cells were washed with PBS two times and then re suspended in PBS containing 10 μM 5-(and-6)-chloromethyl-2', 7'- dichlorodihydrofluorescein diacetate (Inviogen, Carlsbad, CA), a detection reagent for reactive oxygen species (ROS). After incubation for 30 min at 37°C, the isolation of neutrophils was performed using the lysis buffer (Nacalai, Kyoto, Japan) according to the manufacturer’s instructions. The sequence of PCR primers were as follows: IL-17A: 5'-GAA GCC GCAC AAG GGT GC3' and TCR-Z: 5'-CCA AGG AGC GGA TAG TTC AGG-3'.

Isolation and culture of cells

Splenocytes, mesenteric lymph node (MLN) cells, and liver mononuclear cells (MNCs) were isolated from IL-17A−/− and WT mice at the indicated days after T. cruzi infection. Single-cell suspensions were prepared with RPMI 1640 medium (RPMI 1640 Sigma-Aldrich) supplemented with 10% FBS (ThermoTrace, Melbourne, Australia) and penicillin/streptomycin (Invitrogen). Prepared cells (5 × 10^5 cells/well) were cultured in 96-well culture plates (Nunc, Roskilde, Denmark) for 3 d, and the culture supernatants were analyzed for cytokine production with ELISA, according to the manufacturer’s directions (DuoSet ELISA Development System, R&D Systems).

Quantitative real-time PCR analysis

Total RNAs were extracted from cells using Trizol reagent (Invitrogen) and reverse-transcribed with a ReverTra Plus kit (TOYOBO, Osaka, Japan). Expression levels of cytokines and neutrophilic enzymes were determined relative to that of β-actin with HotStar Taq DNA polymerase (Qiagen, Valencia, CA), supplemented with SYBR Green ( Molecular Probes), using an ABI PRISM 7000 sequence-detection system, according to manufacturer’s instructions. The sequence of PCR primers were as follows: IL-17, 5'-CTG TTT GTT GGA GAA GTA G-3' and 5'-TGG CTC TTC AGG ATT TTC ATC-3'; IL-4, 5'-ACA GGA GAA GGG ACG ACA T-3'; IL-6, 5'-GAA GCC CTAG AGC AGT CAC A-3'; IL-8, 5'-TCAT CTT CTC AAA ATT CCA GTG ACA A-3'; and 5'-TGG CAG TAG ACA AGG.
TAC AAC CC-3'; IL-6, 5'-GAG GAT ACC ACT CCC AAC CC-3' and 5'-AAG TGC ATC GTT GTT CAT ACA-3'; MPO, 5'-ATC ACC GCC TCC CAG GAT ACA ATG-3' and 5'-ACC GCC CAT CCA GCT CAA CCA AAC-3'; and 5'-GCG ATA CCA AAC ACC TCA GAC ACC-3'.

Data analysis

Experiments were repeated at least two times. Values are expressed as mean ± SEM. Differences among groups were analyzed using unpaired Student t tests. A value of p < 0.05 was considered statistically significant.

Results

Induction of IL-17A expression by T. cruzi infection

First, we assessed IL-17A expression during T. cruzi infection. In uninfected mice, percentages of IL-17A–expressing cells were very low (<0.2%) in spleen and MLN. After T. cruzi infection, percentages of IL-17A–producing (CD3+CD4+ and CD3+CD4−) cells in spleen and MLN were increased; they reached a peak at 21 d after the infection and then decreased to basal levels (Fig. 1A). IFN-γ–producing cells also increased after T. cruzi infection as similar time-course in the case of IL-17A-producing cells (Fig. 1A). In contrast, although IL-17A–expressing cells existed in uninfected liver MNCs (≈0.5%), the percentages were relatively constant during T. cruzi infection (Fig. 1A). Furthermore, CD8+ T, NKT, and γδT cells in spleen, MLN, and liver also produced IL-17A against T. cruzi infection at 21 d after the infection (Fig. 1B). Therefore, it was demonstrated that T. cruzi infection induced production of IL-17A by various cell lineages.

High mortality accompanied by sustained severe parasitemia and aggravated multiple organ failure in IL-17A−/− mice

To clarify the role of IL-17A, we infected IL-17A−/− and WT mice with T. cruzi. As shown in Fig. 2A, the survival rate was markedly decreased in the IL-17A−/− mice compared with WT mice 21 d after the infection. In WT mice, expansion of the parasites showed a small peak at ∼14 d postinfection but returned to sublethal levels in almost all mice (Fig. 2B). In contrast, IL-17A−/− mice showed prolonged, more severe parasitemia compared with WT mice up to 28 d after the infection (Fig. 2B). At 33 d postinfection, although the parasitemia in IL-17A−/− mice seemed similar to that in WT mice (†; Fig. 2B), this resulted from the fact that data were obtained only from a few survivors in the IL-17A−/− mice group. The survivors maintained the parasitemia at relatively milder levels than did others in the group during the acute phase of the infection, the parasitemia did not worsen again, and the mice...
survived >3 mo postinfection (data not shown). This may indicate that some factors, such as IL-17F, may compensate for IL-17A during the infection. Nonetheless, it was shown that IL-17A−/− mice were essentially more sensitive to T. cruzi infection than WT mice, and lethal expansion of the parasites occurred in more than half of the infected IL-17A−/− mice (Fig. 2A, 2B). These results indicated that IL-17A plays an important role in the successful resolution of T. cruzi infection.

In the acute phase of infection, T. cruzi replicates and infects various target organs, which leads to tissue injury. Therefore, we assessed the tissue parasitism and the degree of tissue damage by measuring serum concentrations, including aspartate aminotransferase and alanine aminotransferase for liver injury, blood urea nitrogen and creatinine for renal damage, and creatine kinase for heart failure. As shown in Fig. 2C, IL-17A−/− mice showed significantly greater parasitism in liver, heart, and kidney compared with WT mice. Furthermore, all markers measured were higher in IL-17A−/− mice compared with WT mice (Fig. 2D). These data suggested that T. cruzi infection leads to severe multiple organ failure resulting from physical damage by the parasites in IL-17A−/− mice, which was assumed to be a reason for the increased mortality.

Equivalent cellular infiltration in the liver of WT and IL-17A−/− mice

We then explored mechanisms of IL-17A resistance to T. cruzi infection. Liver is a secondary infection site of T. cruzi, and it is well known that IL-17A induces the recruitment of immune-related cells to infected sites. We initially assumed that the cellular accumulation in the infected liver was impaired in IL-17A−/− mice. However, cellular infiltration was unexpectedly equivalent between WT and IL-17A−/− mice (Fig. 3A). Also, microscopically discernible differences between WT and IL-17A−/− mice were not observed in the heart, which is another target organ of the infection (data not shown). As shown in Fig. 3B, the ratio of CD4+ and CD8+ T cells, NK and NKT cells, macrophages and B cells, and neutrophils in the infected liver of IL-17A−/− mice was similar to that of WT mice 21 d after the infection. Similar results were obtained ∼14 d after the infection (data not shown). Total numbers of liver-infiltrated MNCs were also similar in WT and IL-17A−/− mice on days 14 and 21 (data not shown). To address whether IL-17A deficiency affected cell migration at very early phases of infection, cellular infiltration into the peritoneal cavity during the infection was evaluated. The results showed that the number of liver-infiltrated MNCs increased over time and was similar in WT and IL-17A−/− mice (Fig. 3C). These data suggested that IL-17A may regulate the recruitment of immune-related cells to infected sites, and the recruitment of immune-related cells was not impaired in IL-17A−/− mice.
cavity was examined at 3 d after i.p. injection of T. cruzi. Despite our initial assumption, there was no significant difference between WT and IL-17A−/− mice with regard to the number of cells infiltrating the peritoneal cavity or the percentage of innate immune cells, such as NK/NKT (CD3−NK1.1+ or CD3−NK1.1+) cells, γδT (CD3+γδTCR+) cells, macrophages (CD11b+Ly6G−), and neutrophils (CD11b+Ly6G+) (Supplemental Fig. 1). Furthermore, expression levels of activation markers, such as CD69, CD44, and CD62L, on CD4+ T cells in the infected liver were similar between WT and IL-17A−/− mice on day 16 (Fig. 3C).

In vitro assay of capacity of Th17 cells and IL-17A for macrophage and neutrophil activation

Next, we performed an in vitro assay to assess the helper function of IL-17A−/−-producing CD4+ T (Th17) cells to activate infected macrophages directly (Fig. 4A). Peritoneal exudate macrophages from thioglycolate-treated WT mice were infected with T. cruzi in vitro and then cocultured with spleen CD4+ cells isolated from infected WT or IL-17A−/− mice. After 18 h of culture, the production of IL-6, TNF-α, and NO, which are mainly produced by activated macrophages, was enhanced by coculture with both CD4+ cells compared with that in the absence of CD4+ cells. However, there were no significant differences in the quantity of macrophage-derived factors between coculture with WT and IL-17A−/− CD4+ cells (Fig. 4A). Therefore, a CD4+ Th cell lineage other than Th17 was able to activate macrophages normally, even in the absence of IL-17A production. Furthermore, we examined the direct effect of IL-17A on macrophage activation; however, treatment with IL-17A did not enhance NO production by macrophages, even in the presence of costimulation with LPS and IFN-γ (Fig. 4B). In the thioglycolate-induced macrophages, STA could not induce NO production, whereas LPS and IFN-γ had a strong effect.

In addition, rIL-17A could not directly induce activation of neutrophils, as measured by upregulation of ROS production (Fig. 4C) and mRNA expression of MPO and 5-LOX (Fig. 4D). Interestingly, STA induced ROS production in the neutrophils more strongly than did 100 ng/ml LPS (Fig. 4C), and it was assumed that the T. cruzi infection immediately activated locally resident neutrophils or those recruited to the infection site. These results suggested that IL-17A produced from Th17 and other lineages in vivo is required for proper defense against T. cruzi by mechanisms other than the induction of cellular migration and direct activation of macrophages and neutrophils.

Cytokine production was impaired in IL-17A−/− mice

We then addressed the production of inflammatory cytokines, another important mechanism by which IL-17A plays a role during infection. As shown in Fig. 5A, cells from spleen, MLN, and liver of WT mice at 14 d postinfection produced substantial levels of IFN-γ and TNF-α in response to T. cruzi infection, which then decreased to 1/7 to 1/50 on day 21 (compare Fig. 5A, 5B), due to successful control of infection. At both time points, cytokine production by IL-17A−/− cells was impaired compared with that by WT cells derived from MLN and liver (Fig. 5A, 5B), demonstrating the influence of IL-17A on the production of inflammatory cytokines during T. cruzi infection. Of note, the high susceptibility of IL-17A−/− mice to infection was first apparent around day 14 and became more evident on day 21 and thereafter, as shown by greater mortality and more severe parasitemia (Fig. 2A, 2B). Similar results were obtained for mRNA expression in liver MNCs and MLN cells (i.e., expression of IFN-γ, TNF-α, and IL-6 were lower in IL-17A−/− mice than WT mice during T. cruzi infection) (Fig. 5C, data not shown). IL-4 expression was not affected by IL-17A deficiency. Interestingly, the impaired cytokine production was most evident in MLN cells and liver MNCs compared with splenocytes (Fig. 2A, 2B). These results indicate the differential requirement of IL-17A for cytokine production; IL-17A is required more in local sites, including draining lymph nodes and liver, rather than systematically, as shown for splenocytes. This might reflect observations...
FIGURE 5. Attenuated cytokine production in IL-17A−/− mice during T. cruzi infection. Splenocytes, MLN cells, and liver MNCs prepared from WT and IL-17A−/− mice on day 14 (A) and day 21 (B) of infection were cultured for 64 h and analyzed for cytokine production. Data are mean ± SEM (n = 4). C and D, Liver MNCs were isolated from WT (C) and IL-17A−/− (D) mice at the indicated days postinfection and analyzed for mRNA expression by RT-PCR, as described in Materials and Methods. Experiments were repeated at least three times with similar results. Data are mean ± SEM (n = 4). E, Splenocytes (top panel), MLN cells (middle panel), and liver MNCs (bottom panel) collected from WT mice on day 21 of infection were stained for indicated surface markers and intracellular IL-17A. Numbers shown are the percentages of cells contained in each gated lineage. *p < 0.05; **p < 0.01 versus WT samples.

Discussion

Although IL-17A is known to participate in the induction of inflammation during infection of an intracellular protozoan parasite, Toxoplasma gondii (37), the pathogenic or protective roles of IL-17A in infection by other intracellular protozoan parasites are not well understood. In this study, we revealed that the production of IL-17A was induced against T. cruzi infection (Fig. 1A). As shown in Fig. 1B, although IL-17A was produced by a small population of CD4+ T (Th17 cells) and CD8+ T cells, its production was more potently induced in substantial numbers of NKT and γδT cells in response to T. cruzi infection (5–7). Some reports indicated that infection by Leishmania amazonensis and L. braziliensis induced the expression of IL-17A (38, 39), but there was no evidence for a relationship between IL-17A production and host protection in the protozoa infection. In this regard, we demonstrated, using IL-17A−/− mice, that IL-17A plays an important role in the successful resolution of T. cruzi infection (i.e., T. cruzi-infected IL-17A−/− mice showed prolonged, more severe parasitemia and exacerbated mortality compared with WT mice) (Fig. 2A, 2B).

Interestingly, the production of IFN-γ, which is a critical cytokine for achieving antitrypanosoma immunity (18, 19), was lower in IL-17A−/− mice during T. cruzi infection compared with WT mice (Fig. 5A–C). Therefore, the delay in parasite expulsion in IL-17A−/− mice might result from the weak IFN-γ responses against T. cruzi infection. Similar defects in IFN-γ production were observed in IL-17A−/− mice during Mycobacterium infection (30, 40). Furthermore, reduction of serum IL-17A by anti-IL-23p19 treatment also resulted in the attenuated production of IFN-γ, IL-6, and TNF-α in CNS during autoimmune encephalomyelitis (41). Unfortunately, the direct mechanisms for the decrease in IFN-γ production in IL-17A−/− mice have not been clarified. It was reported that TNF-α induces maturation of dendritic cells and the matured dendritic cells drive IFN-γ production of CD4+ T cells (42). Therefore, attenuated production of IFN-γ by IL-17A deficiency, as shown in Fig. 5, might result from the decrease in TNF-α production. Furthermore, activated production of TNF-α observed in steatohepatitis induced by fat/alcohol feeding with LPS injection was associated with increased expression of IFN-γ (43).

In addition to IFN-γ, IL-6 and TNF-α are well-known cytokines induced by IL-17A stimulation (8, 9). Production of these cytokines during T. cruzi infection was decreased in IL-17A−/− mice compared with WT mice (Fig. 5A–C). IL-6 and TNF-α were required for the successful resolution of T. cruzi infection (44–47). For example, IL-6 induces B cell terminal differentiation into plasma cells during T. cruzi infection, and IL-6−/− mice were
more susceptible to the infection (44, 45). TNF-α is known to act synergistically with IFN-γ on macrophages to augment killing of *T. cruzi* (22, 23), and a defect in TNF-α signaling by introduction of TNFR-Fc transgene or gene targeting of the receptor brought about increased susceptibility to *T. cruzi* infection (46, 47). Therefore, it is likely that poor production of IL-6 and TNF-α in *T. cruzi*-infected IL-17A−/− mice (Fig. 5A–C) was a factor contributing to the deviant expansion of the parasites and increase in mortality (Fig. 2A, 2B).

IL-17A induced during infection of bacteria and fungi and a protozoan parasite *Toxoplasma gondii* mobilizes neutrophils for elimination of the pathogens (28, 29, 31, 48). However, in the current study, deficiency in IL-17A did not affect the number of immune-related cells migrating into infected tissues (Fig. 3A, 3B). Therefore, IL-17A was not essential for the recruitment of neutrophils and other immune cells in *T. cruzi* infection. Presumably, cytokines, such as IL-17F, and other inflammatory cytokines produced during infection compensated for the lack of IL-17 for cell migration. Nevertheless, because neutrophilic enzyme activities were lower in *T. cruzi*-infected IL-17A−/− mice (Fig. 5D), IL-17A might be important for proper neutrophil activation required for killing of *T. cruzi* (34, 49). However, the activation of neutrophils by IL-17A seemed to be achieved indirectly, because IL-17A itself did not enhance the neutrophilic enzyme expression (Fig. 4). Interestingly, neutrophils were activated for the enhanced ROS production by stimulation with *T. cruzi* Ags (Fig. 4C). Therefore, it was supposed that neutrophils participate in the protection against *T. cruzi* infection as effectors in host immune systems.

In conclusion, we demonstrated that IL-17A is induced in response to *T. cruzi* infection and results in efficient activation of the immune system critical for the killing of infected *T. cruzi*, mainly through sufficient production of INF-γ and other inflammatory cytokines. IL-17A is required for the elimination of bacteria, fungi, and *T. cruzi*; it controls cytokine production by T cells and macrophages, as well as neutrophil activation.

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**Disclosures**

The authors have no financial conflicts of interest.

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