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

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

Yoshiyuki Miyazaki, Shinjiro Hamano, Seng Wang, Yohei Shimano, 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<sup>−/−</sup>) mice with Trypanosoma cruzi. IL-17A<sup>−/−</sup> 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<sup>−/−</sup> 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<sup>−/−</sup> mice. Expression of cytokines, such as IFN-γ, IL-6, and TNF-α, was lower in liver-infiltrating cells from the IL-17A<sup>−/−</sup> mice compared with WT mice. A similar defect was observed in the expression of neutrophil enzymes, such as myeloperoxidase and lipoxygenase, 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.

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Abbreviations used in this paper: 5-LOX, 5-lipoxygenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; MLN, mesenteric lymph node; MNC, mononuclear cell; MPJ, myeloperoxidase; ROS, reactive oxygen species; STA, soluble Trypanosoma cruzi 2Ag; WT, wild-type.

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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, NKT, 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 germine transmission. Mutant mice were backcrossed onto the C57BL/6 background more than eight times (corrected backcross) before use in experiments. Mice were housed in microisolation cages and were used between 8 and 12 wk of age. 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-γR−/− mice by every 2 wk passages. For the experiments, IL-17A−/− and WT mice were infected i.p. with the plasma containing trypanosomes. Mice were infected with 2000 trypanosomes. The number of parasites in the blood was counted for each animal using 4 μl venous blood. For measuring parasitism, DNA was purified from 50 mg tissue specimen with a Maxwell 16 Tissue DNA Purification Kit (Promega, Madison, WI). The content of infected parasites was measured with a PCR using microglobulin (μMHC) primers (TczR-5′-CTC AAA ATT CGA GTG ACA A-3′ and TczF-5′-GGT GCT GCC CAC AAG GGT GC-3′) and β2-microglobulin gene as an internal control for DNA input using primers Beta2-F: 5′-TGG GAA GCC GAA CAT ACT G-3′ and Beta2-R: 5′-GCA GGC GTA TGT ATC AGC TCT A-3′.

Assay for serum chemistry

Blood was collected at indicated days postinfection, and serum was prepared by centrifugal fractionation. Serum alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, and creatine kinase were determined using Test WAKO kit (Wako Pure Chemical Industries, Osaka, Japan), according to the manufacturer’s directions.

Flow cytometric analysis

Prepared cells were stained with FITC-labeled anti-CD4 mAb, PE-labeled anti-CD62L mAb, and PE-Cy5-labeled anti-CD44 mAb (all from BD Biosciences, San Jose, CA) for the detection of effector CD4+ T cells (CD4+CD45R-B220−CD62L−), and PerCP-Cy5.5-labeled anti-CD69 mAb (BD Biosciences) for the detection of activated CD4+ T cells (CD4+CD69+). The following Abs against surface markers (BD Biosciences) were used to stain cells in the tissue: PE-labeled anti-CD4, FITC-labeled anti-CD8, PE-labeled anti-NK1.1, FITC-labeled anti-CD3, PE-labeled anti–Mac-1, FITC-labeled anti–Ly6G (1A8), FITC-labeled anti–neutrophils (7/4), and FITC-labeled anti–Gr-1 mAb. For the analysis of intracellular cytokines, the isolated cells (1×10^6) were cultured for 4 h with 10 ng/ml PMA (Sigma-Aldrich, St. Louis, MO) plus 500 ng/ml ionomycin (Sigma-Aldrich) in the presence of 2 μM monensin (Sigma-Aldrich) plus GolgiPlug (BD Biosciences). Cells were stained with FITC- or PE-labeled anti-CD4 mAb, FITC-labeled anti-CD8, NK1.1, γδ TCR, or Gr-1 mAb and PerCP or allophycocyanin-labeled anti-CD3 mAb (BD Biosciences), fixed, and permeabilized with the Cytofix/Cytoperm Plus kit (BD Biosciences), according to the manufacturer’s directions. Cells were then stained with FITC-labeled anti–IFN-γ mAb or PE-labeled anti–IL-17 mAb (BD Biosciences). The expression of surface markers and cytokines was analyzed using FACS Calibur (BD Biosciences).

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 neutrophils were collected from infected WT or IL-17A−/− mice treated with 1 g of thioglycollate (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 trypanosomes and cocultured with the isolated WT or IL-17A−/− CD4+ cells for 18 h. After the culture, cytokines and nitrite (NO) levels in culture supernatants 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 thioglycollate-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 1×10^3 soluble T. cruzi Ag (STA), with or without 25 ng/ml IL-16. STA was prepared by repeated freeze and thaw of 1×10^6 trypanosomes in 100 μl culture medium. For the preparation of neutrophils, peritoneal exudate cells were collected at 4–16 h after 3% thioglycollate 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 (7-4Ly6G−), was resuspended with culture medium and used 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 resuspended in PBS containing 10 μM 5-(and-6)-chloromethyl-2′,7′- dichlorodihydrofluorescein diacetate (Invitrogen, Carlsbad, CA), a detection reagent for reactive oxygen species (ROS). After incubation for 30 min at 37˚C in the dark, levels of ROS production were evaluated by flow cytometry. Induction of mRNA expression of myeloperoxidase (MPO) and 5-lipoxygenase (5-LOX) in the stimulated neutrophils for 16 h was analyzed by RT-PCR, as described below.

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 a culture 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 neutrophil 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 was as follows: IFN-γ, 5′-ACA ACT ATG GCC GTA GGA GAA GA-3′ and 5′-TGG CTC TGC AGG ATT TTC ATG-3′; IL-4, 5′-ACA GGA GGA GGG ACG CCA T-3′ and 5′-GAA GCC CTA CAG ACG AGC TCA-3′; TNF-α, 5′-CAT CTT CTC AAA ATG CTA ACA A-3′ and 5′-TGG GAG TAG AGC AAG AGG...
TAC AAC CC-3’; IL-6, 5’-GAG GAT ACC ACT CCC AAC CC-3’ and 5’-AAG TGC ATC ATC GTT GTT CAT ACA-3’; MPO, 5’-ATC ACG GCC TCC CAT ACA ATG-3’ and 5’-ACC GCC GAT GCC CAT GAT GTC GTA AAT G-3’; and 5-LOX, 5’-TGC 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+CD4-2) 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, NK, 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−/− mouse 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

FIGURE 1. Induction of IL-17A from CD4+ and other cell lineages during T. cruzi infection. A, left panels, Splenocytes, MLN cells, and liver MNCs prepared from WT mice on day 0 (upper panels) and day 21 (lower panels) of infection were stained for surface markers and intracellular IL-17A or IFN-γ and analyzed with FACS, as described in Materials and Methods. Numbers shown are the percentages of cells contained in gated CD3+ cells. Experiments were repeated three times with similar results. Right panels, Splenocytes (○), MLN cells (●), and liver MNCs (▲) were prepared from WT mice at the indicated days postinfection, and the percentage of cells expressing IL-17A and IFN-γ was assessed. B, Splenocytes (top row), MLN cells (middle row), and liver MNCs (bottom row) collected from WT mice on day 21 of infection were stained for lineage surface markers and intracellular IL-17A. Numbers shown in each square are the percentages of the cells contained in each gated lineage; percentages of whole living cells are given in parentheses.
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

**FIGURE 2.** Increased mortality accompanied by sustained severe parasitism and aggravated multiple organ failure in IL-17A−/− mice. A, WT (○) and IL-17A−/− (●) mice were infected i.p. with 2 × 108 blood trypomastigotes of T. cruzi. Mortality was monitored at the indicated days postinfection. Data are mean ± SEM from three independent experiments (n = 10–20 each). B, Parasitemia was monitored at the indicated days postinfection as in A. Data are mean ± SEM (n = 18–20). Experiments were repeated at least three times with similar results. C, Seventeen days postinfection, parasitism in liver, heart, and kidney was measured with a T. cruzi 195-bp-repeat DNA-specific-PCR, as described in Materials and Methods. Data are mean ± SEM (n = 4). Experiments were repeated at least two times with similar results. D, Serum was prepared from WT and IL-17A−/− mice on day 21 of infection and analyzed for parasitemia and serologic marker concentrations, as described in Materials and Methods. Experiments were repeated at least four times with similar results. Data are mean ± SEM (n = 4). *p < 0.05; **p < 0.01 versus WT samples; †, Mean parasitemia in survivors at 33 d postinfection. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase.

**FIGURE 3.** Equivalent cellular infiltration in the liver of WT and IL-17A−/− mice. A, Liver segments from WT and IL-17A−/− mice were stained with H&E for histological examination. Original magnification, ×200 (insets ×640). Experiments were repeated two times with similar results. B and C, Liver MNCs prepared from WT (B, upper row) and IL-17A−/− (B, lower row) mice on day 21 (B) or day 16 (C) of infection were stained for indicated surface markers and analyzed with FACS, as described in Materials and Methods. Numbers shown are the percentages of cells contained in each gated lineage. Experiments were repeated two times.
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 might have a strong effect.

**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) 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 systemically, as shown for splenocytes. This might reflect observations...
that producers of IL-17A, including NKT and γδT cells, exist and act in local sites, such as liver and intestinal tract. It is also known that marked pathologies of most autoimmune diseases caused by IL-17A are localized inflammation.

Furthermore, mRNA expression of neutrophilic enzymes, such as MPO and 5-LOX, was lower in IL-17A−/− mice compared with WT mice (Fig. 5D). Because MPO and 5-LOX participate in the biosynthesis of H2O2 and leukotrienes (34, 35), respectively, neutrophils in the infected IL-17A−/− mice are assumed to have weak trypanocidal activity compared with the WT neutrophils.

Neutrophils may also participate in the control of antiparasite immune responses as producers of IL-17A. As shown in Fig. 5E, CD3⁺ Gr-1⁺ cells from spleen, MLN, and liver produced IL-17A in response to T. cruzi infection. Production of IL-17A by neutrophils was also observed in Leishmania major infection, as reported by Lopez Kostka et al. (36). However, the percentages of IL-17A+ neutrophils were low, and production levels, as shown by intracellular cytokine staining, were also low compared with other types of cells (Fig. 1B). The contribution of neutrophil-derived IL-17A to defense against T. cruzi has not been examined in detail.

In conclusion, cytokine production and activation of lymphocytes, macrophages, and neutrophil were markedly impaired in the infected IL-17A−/− mice, whereas cellular migration was not affected by IL-17A deficiency. The attenuated immune activation in IL-17A−/− mice resulted in propagation of T. cruzi infection.

**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 antiparasosomal 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 introducing 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 protzoan 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. 4D). 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.

References
Supplementary Figure 1. Early phase cellular infiltration into the infection site of WT and IL-17A+ mice. Mice were intraperitoneally injected with the plasma containing 2000 of trypomastigotes. Three days after the infection, peritoneal exudate cells were prepared from WT (upper) and IL-17A+ (lower) mice. The collected cells were stained for indicated surface markers and analyzed with FACS. Numbers shown in each quadrant are percentages of the cells containing in each gated lineage. Experiments were repeated three times.