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Cytokines in innate and acquired immunity to Trypanosoma cruzi infection

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Abstract

Resistance to *Trypanosoma cruzi* infections is critically dependent on cytokine-mediated activation of cell-mediated immune effector mechanisms. This review focuses on the role of IL-10, TNF-α, IFN-γ and IL-12 in controlling *T. cruzi* replication by the innate and specific immune systems of the vertebrate host. A study performed on mice with disrupted recombinase-activating genes (RAG/KO), which lack *T* and B lymphocytes, revealed the importance of IL-12, IFN-γ and TNF-α in the resistance against *T. cruzi* mediated by the innate immune system. In addition, data from experiments using IL-10 KO, RAG/KO and double RAG/IL-10 KO mice indicating an in vivo regulatory role of IL-10 in innate and *T. cruzi*-specific immunity are discussed.

Key words
- Trypanosoma cruzi
- Interferon-gamma
- Interleukin-12
- TNF-alpha
- Interleukin-10
- Innate immunity
- Knock-out mice

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The digenetic protozoan *Trypanosoma cruzi* is the etiologic agent of Chagas' disease in man. The parasite can potentially infect any mammalian species and, in its sylvatic cycle, the infection has been detected in wild mammals captured in North America. The parasite is most commonly transmitted to man by strictly hematophagous insects of the Reduviidae family. *T. cruzi* infection is prevalent in South and Central America and the number of infected individuals is estimated to be about 18 million; thirty percent of the infected persons will eventually develop the cardiac and/or digestive manifestations characteristic of Chagas' disease. The infection has a self-limited acute phase, detected only in a minority of infected persons. The immune response controls parasitism yet fails to completely eradicate the parasite and the patients remain infected for life. Systematic vector control and improvement of housing conditions are the most important preventive measures that were effective in controlling insect transmission of *T. cruzi* infection in several South American countries including Brazil.

T. cruzi can infect a variety of host cell types including macrophages; intracellular replication as amastigotes is followed by the release of trypomastigotes that can virtually reach all host organs through the bloodstream.

Efficient control of parasite load and host survival rely on *T* cell-mediated immunity via *T* helper (*T*H) cell-dependent protective antibody responses and macrophage activation for intracellular killing of the protozoan. In addition to class II-restricted *T* cells, class I-dependent effector mechanisms (1-5) participate in immune resistance to *T. cruzi*. Natural killer (NK) cells have also been shown to play a role in host defense against the infection (6). In spite of the vigorous immune response, small numbers of parasites persist in the host.

Control of *T. cruzi* parasitism during the

first weeks of infection is considered to be critically dependent on effective macrophage activation by cytokines. Evidence has accumulated over the years showing that in vitro treatment of macrophages with IFN-γ (7-10) and/or TNF-α (10-12) results in more efficient intracellular killing of intracellular amastigotes whereas addition of TGF-ß (13) or IL-10 (14,15) to cultures inhibits the trypanocidal action of IFN-γ-activated macrophages. Treatment of infected mice with recombinant IFN-γ (rIFN-γ) (16) or rTGF-ß (13) respectively increases resistance and aggravates parasitism, consistent with the effects of these cytokines on macrophage trypanocidal activity observed in vitro. Early treatment of infected mice with anti-IFN-γ (17,18) neutralizing antibodies increases parasitism whereas treatment with anti-IL-10 (19) or anti-IL-4 (20) monoclonal antibodies (mAbs) results in a better control of the infection. Moreover, early in vivo activation of parasite-specific IL-10- and IL-4-secreting TH2 cells eliminates resistance to the infection, indicating that imbalances of TH1/TH2 cell activation might lead to increased or longer persisting tissue parasitism with consequent worsening of inflammation and tissue damage (21).

Multiple genes outside the H-2 locus determine the outcome of infection in the mouse, although a spectrum of resistance patterns is found among inbred strains (22,23). Unlike the situation observed in murine Leishmania major infections in which resistant and susceptible mouse strains exhibit an overwhelming dominance of either TH1- or TH2-type cytokine responses (24), respectively, both *T. cruzi*-resistant and -susceptible mouse strains show elevated production of IFN-γ during infection (25). IL-2 and IL-4 production is very low and often undetectable in stimulated lymphocyte culture supernatants (25-29). Increased mouse strain susceptibility to the infection has been linked to the detection of IL-4 (30,31) or IL-10 production (15,19,31) by spleen or peritoneal cell populations, depending on the parasite and mouse strain combinations utilized. However, other studies have detected IL-10 production by spleen cells from susceptible and/or resistant mouse strains infected with *T. cruzi* (29,32, and Abrahamsohn IA, unpublished results). The concomitant synthesis of IL-10 with high levels of IFN-γ in lymphoid organs from *T. cruzi*-infected mice raises the question to what extent the endogenous production of IL-10 may affect parasitism control by the host.

Over the years it has become clear that cells of the innate (or natural) immune system contribute to the synthesis of the macrophage-activating and regulatory cytokines TNF-α, IFN-γ and IL-10 in the early phases of infection by several pathogens; as the immune response develops, antigen-specific TH cells become the most important source of these cytokines. In addition, IL-12 synthesized by infected or LPS-stimulated macrophages, in addition to other actions, stimulates cytokine synthesis by both NK and T helper cells and promotes the activation and expansion of these lymphocyte subpopulations (33). Reciprocal regulatory interactions among cytokines secreted by the innate and acquired immune systems ultimately control the activation of each system and its cytokine-mediated effector functions.

Mice with disrupted IL-10 genes (IL-10 KO) or with disrupted recombinase-activating genes that completely lack T and B cells (RAG/KO) provide a model to investigate the role of IL-10 in the regulation of immune responses and to directly evaluate the relative contribution of innate and acquired immunity and respective cytokines to in vivo resistance to *T. cruzi*.

Using this approach, Abrahamsohn and Coffman (34) have recently shown that IL-10 KO mice infected with the Y strain of *T. cruzi* have lower parasite numbers in blood and tissues than strain-matched infected wild-type (WT) mice, indicating that control of *T.*

cruzi replication was more efficient in the absence of IL-10. The higher IFN-γ and nitric oxide production in response to *T. cruzi* antigen stimulation found in these mice is consistent with a more effective control of parasitism than that observed in WT mice. Confirmation that IL-10 does indeed control *T. cruzi* parasitism comes from experiments in which the protection exhibited by IL-10 KO mice was reversed by treatment with the missing cytokine. Moreover, treatment of WT mice with recombinant IL-10 resulted in increased parasitemia. In order to determine the contribution of the innate and acquired immunity to IL-10 regulation of in vivo parasitism, the course of *T. cruzi* infection was compared between RAG/KO mice and double RAG/IL-10 KO mice. Both types of mice had superimposable parasitemia curves, indicating that in the absence of *T* and B cells endogenous IL-10 does not limit the efficacy of the innate immune system. Moreover, RAG/KO mice reconstituted with IL-10 KO spleen cells had lower blood parasite counts than those reconstituted with WT spleen cells (Abrahamsohn IA and Coffman RL, unpublished results), further indicating that the regulation of parasitism levels by IL-10 depends on an intact immune compartment. Although *T. cruzi*-infected IL-10 KO mice had lower blood and tissue parasitism, they did not survive longer and often died slightly earlier than infected WT or RAG/KO mice. IL-10 KO mice are extremely susceptible to endotoxemia by LPS as a consequence of their higher TNF-α production (35) and are prone to developing intermittent inflammatory bowel disease (36) that may contribute to Gram-negative toxemia. The importance of endogenous IL-10 synthesis in preventing cytokine-mediated immunopathology in intracellular infections was emphasized by the recent results of accelerated death of Toxoplasma gondii-infected IL-10 KO mice (37).

Direct evidence of the importance of innate immunity mechanisms in the early resistance to *T. cruzi* infection was recently obtained in experiments conducted on RAG/KO and WT mice (34). Parasitemia levels did not differ between RAG/KO and WT mice until day 11 of infection. From then on, the contribution of specific immunity to resistance became apparent since WT but not RAG/KO mice were able to control parasitism. These results may indicate that, during the first days after parasite inoculation, control of parasitism relies on mechanisms of innate immunity that precede the onset of the specific immune response by *T* and B cells. Most important, the increase in parasitemia levels and shorter survival time observed in RAG/KO mice treated with anti-IFN-γ, anti-IL-12 or anti-TNF mAbs provide direct evidence of the important contribution of innate immunity, via endogenous production of these cytokines, to the control of the parasite load early in the course of infection. Within this context, it was recently shown that in vivo depletion of NK cells aggravates the infection and reduces IFN-γ production to *T. cruzi* stimulation (6). That anti-IL-12 mAb treatment of RAG/KO mice resulted in increased parasitemia indicates that IFN-γ production by cells from the natural immune system (possibly by NK cells) is IL-12 dependent. IFN-γ, IL-12 and TNF-α also mediate *T. cruzi* resistance mechanisms in mice with an intact immune system, since the respective mAb treatments increased the parasitemia levels of infected WT mice. Moreover, anti-IFN-γ- or anti-TNF-mAb-treated *T. cruzi*-infected mice presented increased IL-10 and decreased nitric oxide production by spleen cells, whereas anti-IL-12 mAb treatment resulted in diminished IFN-γ and nitric oxide production, providing further evidence of mechanisms whereby endogenous production of these cytokines controls intracellular parasitism (34). These results confirm and extend recent reports on aggravation of *T. cruzi* infection in WT mice treated with anti-TNF or with anti-IL-12 neutralizing antibodies (38-40). Production of

IL-12 is stimulated by *T. cruzi* infection of cultured macrophages (39) and treatment of infected mice with rIL-12 during the first week of infection markedly reduced tissue parasitism (40).

Taken together, these recent results draw attention to the importance of innate immunity in the resistance to *T. cruzi* infection. Early in the infection, IL-12, IFN-γ and TNF-α primarily produced by cells of the innate immune system would trigger phagocytic cell activation and inflammation and thus contribute to the control of parasite growth. IL-12 and IL-12-induced IFN-γ also favor TH1 cell differentiation and IL-12 further stimulates optimal production of cytokines and cytotoxic T lymphocyte generation in response to parasite-antigen stimulation as the specific immune response expands (33). In this scenario, IL-10 plays an important in vivo regulatory role in the resistance to *T. cruzi* infection conferred by the specific immune response. However, IL-10 is also an essential regulator of immune and inflammatory responses that are detrimental to the host. This regulatory role may be very important in controlling pathology in situations of intense activation of TH1-type cytokine responses, as is the case in *T. cruzi* infection.

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