Brazilian Journal of Medical and Biological Research (1998) 31: 111-115
ISSN 0100-879X

The role of IL-12 in experimental Trypanosoma cruzi infection

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Abstract

Host resistance to *Trypanosoma cruzi* infection is dependent on both natural and acquired immune responses. During the early acute phase of infection in mice, natural killer (NK) cell-derived IFN-γ is involved in controlling intracellular parasite replication, mainly through the induction of nitric oxide biosynthesis by activated macrophages. We have shown that IL-12, a powerful inducer of IFN-γ production by NK cells, is synthesized soon after trypomastigote-macrophage interaction. The role of IL-12 in the control of *T. cruzi* infection in vivo was determined by treating infected mice with anti-IL-12 monoclonal antibody (mAb) and analyzing both parasitemia and mortality during the acute phase of infection. The anti-IL-12 mAb-treated mice had higher levels of parasitemia and mortality compared to control mice. Also, treatment of infected mice with mAb specific for IFN-γ or TNF-α inhibited the protective effect of exogenous IL-12. On the other hand, TGF-ß and IL-10 produced by infected macrophages inhibited the induction and effects of IL-12. Therefore, while IL-12, TNF-α and IFN-γ correlate with resistance to *T. cruzi* infection, TGF-ß and IL-10 promote susceptibility. These results provide support for a role of innate immunity in the control of *T. cruzi* infection. In addition to its protective role, IL-12 may also be involved in the modulation of *T. cruzi*-induced myocarditis, since treatment of infected mice with IL-12 or anti-IL-12 mAb leads to an enhanced or decreased inflammatory infiltrate in the heart, respectively. Understanding the role of the cytokines produced during the acute phase of *T. cruzi* infection and their involvement in protection and pathogenesis would be essential to devise new vaccines or therapies.

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Presented at the International Meeting on Cytokines, Angra dos Reis, RJ, Brasil, November 24-28, 1996.

Publication supported by FAPESP.

Received September 4, 1997
Accepted October 6, 1997

Key words
- IL-12
- Trypanosoma cruzi
- NK cells
- IFN-γ
- Nitric oxide

Trypanosoma cruzi, a hemoflagellate protozoan parasite, is the causative agent of human Chagas' disease, a widely distributed debilitating infection which constitutes a major health problem in many Latin American countries. Following infection, the parasites are able to survive and replicate in a variety of nucleated cells, including non-activated macrophages. Cytokines that enhance or inhibit parasite replication in macrophages seem to influence the outcome of infection, as well as the pathology of the disease.

The host's resistance during experimental Chagas' disease is dependent on both innate and acquired immunity, requiring the

combined efforts of a number of cells including natural killer (NK) cells (1), $CD4^+$ (2) and $CD8^+$ (3) *T* cells as well as antibody production by B cells (4). Cytokines play key roles in regulating both parasite replication and immune responses in infected animals. IFN-γ has been most closely associated with host resistance during the acute phase of infection. Whereas treatment with IFN-γ is protective, the neutralization of endogenously produced IFN-γ results in increased susceptibility during the acute stage of infection with *T.* cruzi. We have suggested that IFN-γ is important in the control of acute infection when produced shortly after parasite injection and that IFN-γ may limit early parasite replication by inducing macrophage activation, through stimulation of tumor necrosis factor secretion (5) and activation of nitric oxide synthase (6,7), or by down-regulating *T*h2 differentiation, leading to a decrease in the levels of secreted IL-10. *T*he down-regulatory cytokines IL-10 and *T*GF-ß have been associated with susceptibility to *T.* cruzi infection (8,9) by inhibiting IFN-γ-mediated macrophage activation. We have shown that inhibition of IL-10 by anti-IL-10 monoclonal antibody (*m*Ab) leads to an increased *T.* cruzi-induced IFN-γ production in vitro (5) and in vivo (10), suggesting that this cytokine may be a potent inhibitor of IFN-γ production during *T.* cruzi infection in mice and that early resistance to *T.* cruzi infection may be mediated by the pattern of IFN-γ/IL-10 produced.

Our previous study demonstrated that supernatants from euthymic or athymic mouse macrophages cultured with live trypomastigotes induced IFN-γ production by spleen NK cells. *T*reatment with anti-IFN-γ *m*Ab exacerbated parasitemia only if administered before or early after infection. Both IFN-γ neutralization and NK-cell depletion resulted in an increased susceptibility to infection and increased IL-10 production. *T*aken together, these observations suggest that NK cells are the major source of IFN-γ in the early acute phase of infection (5).

Since a) IFN-γ produced by NK cells in the early acute phase of *T.* cruzi infection is important in mediating resistance, b) IL-12 has been described as a potent inducer of IFN-γ production by NK cells and different subsets of *T* cells (11), and c) IL-12 is required for the establishment of *T* cell-dependent protective immunity in immunocompetent mice infected with a variety of microorganisms (12), we investigated the ability of trypomastigotes to trigger IL-12 production by mouse macrophages and the involvement of the IL-12-dependent pathway of IFN-γ production in resistance to acute infection with *T.* cruzi. Live *T.* cruzi trypomastigotes, but not epimastigotes or parasite lysates, were able to induce IL-12 production by mouse macrophages. *T*his production was closely correlated with the ability of macrophage supernatants to induce IFN-γ secretion by normal murine splenocytes and was completely inhibited in the presence of anti-IL-12 *m*Ab (13). *T*hese results suggest that IL-12 is produced as the active *p*70 heterodimer and therefore there is no modulation by the IL-12 *p*40 monomer or homodimer. IL-12 production was associated with the adherent spleen cell population. Moreover, inflammatory macrophages and bone marrow-derived macrophages also produced *p*40 and induced IFN-γ production in response to infection with *T.* cruzi. *T*herefore, it seems that the macrophage is capable of producing IL-12 when faced with infection by *T.* cruzi. *T*his is of the utmost importance, since IL-12 elicits IFN-γ production by both *T* and NK cells (14), and IFN-γ is essential for resistance to this parasite (9,15). Macrophages are probably the first cells *T.* cruzi encounters and infects, and the fact that these cells can respond with the production of a protective cytokine soon after infection favors the host, leading to a chronic benign infection.

*T*he role of IL-12 in the control of *T.* cruzi infection in vivo was determined by treating

infected mice with an anti-IL-12 mAb and analyzing both parasitemia and mortality during the acute phase of infection. We concluded that mice treated with the anti-IL-12 mAb had a higher parasitemia and accelerated mortality when compared to control mice infected with *T. cruzi* and treated with normal rat IgG (13). Based on these observations and on our previous study (5), we postulate that early IL-12 secretion may induce IFN-γ synthesis by NK cells, and that the latter cytokine may in turn activate macrophages to increase parasite killing during the early acute phase of infection. Although our in vitro experiments suggest that NK cells are in fact the major source of IFN-γ during early infection with *T. cruzi*, we cannot exclude the possibility that both $CD4^+$ and $CD8^+$ *T* cells also contribute to IFN-γ synthesis during this phase. In this regard, $CD4^+$ *T* lymphocytes from mice on day 5 after infection with *Toxoplasma gondii* produce high levels of IFN-γ in an IL-12-dependent manner (16).

Another recently published paper also reported that IL-12 mediates resistance to *T. cruzi* infection in mice (17). The authors described that IL-12 treatment of infected mice resulted in reduced parasitemia and in a significantly prolonged survival compared with infected untreated controls. The protective effect of IL-12 treatment on mice was correlated with increased serum levels of IFN-γ and TNF-α. Since treatment of infected mice with a combination of IL-12 and anti-IFN-γ or anti-TNF-α mAb inhibited the protective effects of IL-12, it is possible that the effect of this cytokine is dependent on IFN-γ and TNF-α. In fact, IFN-γ and TNF-α play a role in amplifying nitric oxide production and parasite killing (7).

IL-10 and TGF-ß have also been shown to strongly influence the synthesis and/or effects mediated by IL-12 and are important regulators of IL-12-induced IFN-γ synthesis by NK cells (18,19). Whereas IL-10 appears to be a potent inhibitor of IL-12 synthesis by macrophages exposed to microbial products, the mechanism by which TGF-ß inhibits IFN-γ synthesis by NK cells is unknown. Nevertheless, it is clear that both TGF-ß and IL-10 are potent modulators of resistance during acute Chagas' disease (8,9). In contrast to IL-10 and TGF-ß, other macrophage-derived (i.e., IL-1ß and TNF-α) and *T* cell-derived (IL-2) cytokines potentiate the effects of IL-12. Recently, we showed that, while TNF-α and IL-1α potentiate the induction and/or effects of the IL-12 pathway during *T. cruzi* infection, TGF-ß and IL-10 inhibit it (Aliberti JCS and Silva JS, unpublished observations). Thus, *T. cruzi* belongs to a long list of microorganisms which elicit the synthesis of IFN-γ through the induction of IL-12 (12). In addition, the involvement of an IL-12-dependent pathway for IFN-γ production in the resistance to this parasite during the acute phase of infection appears to be clear. It is noteworthy that in other experiments we observed a more dramatic effect of treatment with anti-IFN-γ mAb than with anti-IL-12 mAb on the parasitemia levels and mortality of *T. cruzi*-infected mice. Thus, it is possible that during *T. cruzi* infection the parasite may trigger both IL-12-dependent and IL-12-independent pathways for IFN-γ synthesis and that they may have an additive effect on resistance to the parasite.

In terms of the parasite-vertebrate host interaction, several questions were raised by this study, including, for instance, whether the virulence and avirulence observed with different strains of *T. cruzi* are related to the parasite's ability to elicit or evade the induction of IL-12 synthesis by macrophages. We believe that *T. cruzi* could induce IL-12 to promote its own survival by protecting the host against a lethal infection. Also, IL-12 produced early during the infection may induce the differentiation of naive *T*h cells to the *T*h1 phenotype through its ability to maximize IFN-γ and curtail IL-4 production by stimulated naive *T*h cells (20) and thus favor macrophage activation and parasite killing.

However, the cardiac lesions observed during infection with *T. cruzi* are typically of a delayed hypersensitivity type (i.e., mediated by *T*h1 lymphocytes) and therefore it is possible that the induction of IL-12 may also mediate the resulting immunopathology during chronic Chagas' disease by favoring parasite-specific $CD4^+$ *T* cell differentiation in the direction of *T*h1 lymphocytes. This hypothesis could be valid since treatment of infected mice with anti-IL-12 mAb, although causing increased parasitemia and parasite nests in the heart, leads to decreased myocarditis during the acute phase of infection. Similarly, administration of IL-12 resulted in an increased cellularity associated with many of the foci of parasite replication in the hearts of treated mice compared with untreated infected controls (17). Also, since IFN-γ and IL-12 comprise an autocrine positive feedback system that amplifies the levels of IFN-γ for macrophage activation and IL-12 for the proliferation and activation of NK and *T*h1 cells (21), this may lead to increased nitric oxide production (6) which, in turn, induces apoptosis cell death in the acute phase of infection (Martins GA, Cardosa MAG, Aliberti JCS and Silva JS, unpublished data). We believe that elucidation of the role of IL-12 in the resistance to and in the pathogenesis of Chagas' disease may have important implications for the development of a vaccine and of a therapy designed to protect the host against the infection and immunopathology induced by *T. cruzi*.

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