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Cytokine production profile of heart-infiltrating T cells in Chagas' disease cardiomyopathy

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Abstract

The hallmark of chronic Chagas' disease cardiomyopathy (CCC) is the finding of a T cell-rich inflammatory mononuclear cell infiltrate in the presence of extremely few parasites in the heart lesions. The scarcity of parasites in affected heart tissue casts doubt on the direct participation of Trypanosoma cruzi in CCC heart tissue lesions, and suggests the possible involvement of autoimmunity. The cells in the infiltrate are presumably the ultimate effectors of tissue damage, and there is evidence that such cells recognize cardiac myosin in molecular mimicry with T. cruzi proteins rather than primary reactivity to T. cruzi antigens (Cunha-Neto et al. (1996) Journal of Clinical Investigation, 98: 1709-1712). Recently, we have studied heart-infiltrating T cells at the functional level. In this short review we summarize the studies about the role of cytokines in human and experimental T. cruzi infection, along with our data on heart-infiltrating T cells in human Chagas' cardiomyopathy. The bulk of evidence points to a significant production of IFN-γ and TNF-α which may be linked to T. cruzi-induced IL-12 production.

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Cytokine interplay during acute infection: data from murine models

It has been shown by several investigators over the last decade that certain cytokines, notably IFN-γ, can enhance macrophage killing of T. cruzi in vitro and increase resistance to infectious challenge in vitro (1). More recently, this effect has been shown to depend on the de novo synthesis of tumor necrosis factor-α (TNF-α) and nitric oxide (2,3) by infected macrophages. It has also been demonstrated that parasite-induced IFN-γ produced during T. cruzi infection by T and NK cells is involved in resistance to infection in mice (4-6). Interleukin-12 (IL-12) is induced by T. cruzi (7,8) and has been reported to have a protective effect against T. cruzi infection (6,7). This protection seems to be dependent on the IFN-γ/TNF-α pathway (9).

Another set of cytokines, however, has been associated with increased susceptibility to T. cruzi infection. The susceptibility effect of IL-10 (10-13) and transforming growth factor-ß (TGF-ß) seems to operate by inhibiting the TNF-α-dependent synthesis of nitric oxide (10). In spite of the reported

potentiation of macrophage killing of T. cruzi in vitro by IL-4 (14), situations associated with increased in vivo synthesis of IL-4 were reported to result in increased susceptibility to T. cruzi infection (13,15). Other investigators were unable to detect increased IL-4 levels in susceptible mice (16). Immunohistochemical studies of cytokine production in the heart infiltrate of experimentally T. cruzi-infected mice showed persistent production of TNF-α, TGF-ß, IL-1α, and IL-6 and occasional production of IFN-γ and IL-10; IL-2, IL-4 and IL-5 were never detected (17). Taken together, the results obtained with T. cruzi-infected mice establish that Th1 cytokines, which are involved in delayed-type hypersensitivity, are induced during acute infection with T. cruzi and seem to play an obligatory role in parasite clearance. T. cruzi-induced IL-12 is likely to be the driving force behind the production of IFN-γ through innate (NK cells) and adaptive immunity (induction of differentiation of T1/Th1 phenotype T cells) effectors.

Cytokine studies in human Trypanosoma cruzi infection

Peripheral blood

Co-culture of peripheral blood mononuclear cells (PBMC) from normal individuals with live T. cruzi has been shown to induce IL-1ß, IL-2, IL-5, IL-6, IFN-γ, and TNF-α mRNA, but not IL-4 or IL-10 mRNA (18). Studies of PBMC from chronically T. cruzi-infected humans have shown that incubation with T. cruzi homogenates induces high levels of IFN-γ and low levels of IL-10 mRNA as detected by RT-PCR (19).

Insights from the scene of action: heart-infiltrating mononuclear cells and target organ environment

In human chronic Chagas' disease cardiomyopathy (CCC), heart failure is correlated with active myocarditis (20). The inflammatory infiltrate in the heart of CCC patients is rich in $CD4^+$ and $CD8^+$ T cells with a 2:1 predominance of the $CD8^+$ subset (21). TNF-α^+ mononuclear cells have also been identified in hearts of CCC patients in cytokine immunohistochemical studies (22). Another immunohistochemical study showed that IFN-γ was the most abundant cytokine in CCC hearts, followed by TNF-α and IL-6, whereas IL-4 and IL-2 were detected in a smaller number of cells (23). Histiocytes and endothelial cells display increased expression of human leukocyte antigen (HLA) class I and class II molecules; endothelial cells expressed ICAM-1 and E-selectin; CCC cardiomyocytes only display increased levels of HLA class I (24). All of these changes are consistent with in situ production of cytokines such as IFN-γ and TNF-α.

Cytokine production by heart-infiltrating T cell lines from endomyocardial biopsies from Chagas' disease cardiomyopathy patients

In order to investigate the effector role of T cells in the heart infiltrate, we studied the cytokine production pattern of T cell lines obtained from routine pre-transplant endomyocardial biopsies from 8 CCC patients. T cell lines were obtained by incubating minced tissue fragments with IL-2 for 10 days followed by re-stimulation with phytohemagglutinin (PHA; 5 µg/ml), irradiated mononuclear cells and IL-2. After 12 days, cells (500,000/well) were incubated with an equal number of irradiated mononuclear cells in the presence or absence of PHA (5 µg/ml). Culture supernatants were collected at 12 and 48 h and assayed for IL-2, IL-4, IL-10, IL-12, IFN-γ and TNF-α. Results showed that T cell lines from 7/8 biopsies produced IFN-γ, cell lines from 6/8 biopsies produced TNF-α, cell lines from 3/8 biopsies produced IL-2, and cell lines from 3/8 biopsies produced IL-10. Production of IL-4 or IL-12 was not detected. The presence of IFN-γ and

TNF-α in the absence of IL-4 indicates an inflammatory Th1 pattern and is in agreement with the immunohistochemistry findings.

For one of the biopsies, we had paired biopsy samples on which T. cruzi trypomastigotes grew spontaneously in vitro. Upon comparison between T. cruzi-positive and T. cruzi-negative T cell lines from the same patient, we found that the presence of T. cruzi was correlated with increased levels of IFN-γ, TNF-α, and IL-12, while it decreased the production of IL-2 and IL-10. When T. cruzi was deliberately added to CCC heart-derived T cell lines, similar changes in cytokine production were observed. Although T. cruzi could induce IL-12 directly on irradiated PBMC, IL-12 production was potentiated 10- to 100-fold in the presence of PHA-activated T cell lines. Preliminary evidence indicates that this potentiation is dependent on both IFN-γ and CD40L expression by activated T cell lines.

Given all evidence that T. cruzi infection leads to high production of IFN-γ and TNF-α secondary to stimulation of IL-12 production by T. cruzi components (7,8), it seems likely that the dominant production of IFN-γ and TNF-α in the heart lesions of human CCC is related to lifelong infection with T. cruzi. The presence of T. cruzi in acute myocarditis, as well as lifelong low-grade tissue and heart T. cruzi parasitism (25), by providing a persistent stimulus for IL-12 production may shape the cytokine profile of heart-infiltrating T cells into an inflammatory, Th1-like pattern during the chronic phase. The finding of predominant Th1 cytokine production by heart-infiltrating T cells in human CCC agrees with the bulk of the literature on cytokines induced by T. cruzi infection, which points to the concept that protection and pathology share the same underlying immunological effector mechanisms. The finding also challenges the possible participation of Th2 cells/cytokines in tissue damage, as suggested before in murine models of T. cruzi infection (26,27). The Th1 profile is consistent with a delayed-type hypersensitivity mechanism of tissue damage in human CCC and may open the possibility of immune deviation therapy as a possible means of controlling tissue damage in Chagas' disease.

The endocytosis of T. cruzi components provides a macrophage with costimulatory activity (28), high production of IL-12 (7,8) and presentation of T. cruzi epitopes known to be in molecular mimicry with heart-specific epitopes (29,30). This unique combina-

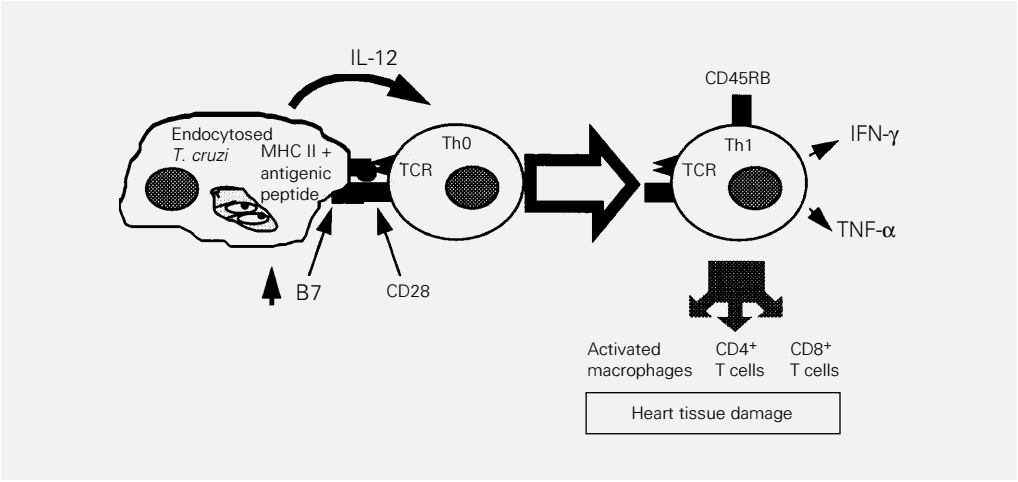

Figure 1 - Antigen presentation of *T. cruzi* by macrophages and induction of "experienced" Th1 T cells. TCR, T cell receptor.

tion of factors in T. cruzi may be the link between low-grade pathogen persistence and heart tissue damage in areas devoid of parasite antigens, as follows (see Figure 1): T cells recognizing T. cruzi antigens in the presence of costimulation and IL-12 become "experienced" Th1 T cells capable of recirculating to peripheral nonlymphoid tissues with enhanced avidity for reacting with molecular mimicry epitopes. This may lead to an encounter with self-antigen and Th1 cytokine production with the initiation of a delayed-type hypersensitivity process leading to tissue damage.

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