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IFN-γ in human Chagas' disease: protection or pathology?

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Abstract

An apparently paradoxical role for IFN-γ in human Chagas' disease was observed when studying the pattern of cytokine production by peripheral blood mononuclear cells (PBMC) obtained from two groups of chagasic patients after specific stimulation with Trypanosoma cruzi-derived antigens. The groups studied were 1) patients treated with benznidazole during the acute phase of Trypanosoma cruzi infection and 2) chronically infected untreated patients. In the treated group, higher levels of IFN-γ were produced by PBMC from individuals cured after treatment when compared to non-cured patients. In contrast, in the chronically infected group (not treated) higher levels of IFN-γ were produced by PBMC from cardiac patients in comparison with asymptomatic (indeterminate) patients. This apparently paradoxical role for IFN-γ in human Chagas' disease is discussed in terms of the possibility of a temporal difference in IFN-γ production during the initial stages of the infection (acute phase) in the presence or absence of chemotherapy. The maintenance of an immune response with high levels of IFN-γ production during the chronic phase of the infection may favor cure or influence the development of the cardiac form of the disease.

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Chagas' disease, which is caused by the intracellular protozoan parasite Trypanosoma cruzi, is an important endemic disease in Latin America. It is estimated that the disease affects about 16-18 million people and that more than 90 million are at risk to be infected with T. cruzi in the Western hemisphere (1). The infection is life-long and spontaneous cure is extremely rare or does not occur at all. When the disease is diagnosed during the acute phase, there is a consensus that specific chemotherapy may be beneficial for the patients, eventually leading to a complete cure. Most patients, even though untreated, recover from the acute phase and remain apparently asymptomatic for long periods of time (10-20 years) or sometimes for life. These patients are characterized by positive serological and/or parasitological tests for T. cruzi and are consid-

ered to have the "indeterminate" clinical form of Chagas' disease. No clinical signs or symptoms of the disease are detected and electrocardiograms as well as X-rays of heart, esophagus and colon are normal. Sectional epidemiological studies in endemic areas show that 50-60% of the chronic patients have the indeterminate form, 20-30% display variable degrees of heart disease (from minor electrocardiographic alterations to heart failure or sudden death), and 8-10% present a digestive form characterized by variable degrees of anatomic and functional alterations of the esophagus and colon (2).

Morbidity and mortality in patients with chronic Chagas' disease are secondary to inflammatory cardiomyopathy with attendant arrhythmias, conduction defects, congestive heart failure, and/or thromboembolic events (3,4). The disproportion between the number of parasites in the lesions and the extension of the cardiomyopathy indicates the participation of autoimmune reactivity in the pathogenesis of Chagas' disease. According to this concept, the morbidity in Chagas' disease results from misdirected effects of the humoral and/or cellular immune responses on the infected patients, induced by a breakdown of self-tolerance (2). Undoubtedly, cell-mediated immunity is important in the control of parasitemia in experimental disease and in human infection, as demonstrated in patients immunosuppressed for transplantation (5) or with HIV infection (6). The occurrence of acute myocarditis and meningoencephalitis in HIV patients infected with Trypanosoma cruzi was interpreted as being caused by relapse of chronic infection. Recent reports strongly support a role for cytotoxic immune mechanisms in the development of heart damage in chronic chagasic pathology (7-9).

To further investigate the role of the cellular response within the context of the pathology of Chagas' disease, we studied some aspects of this response to T. cruzi antigens in treated and untreated chagasic patients. The treated group (T group) included 26 patients treated with benznidazole during the acute phase of Chagas' disease and followed during the last 16 years. The success of the therapy was evaluated by parasitological and serological parameters and the original group of treated patients was subdivided into three subgroups based on the criteria established by Krettli et al. (10): a subgroup denoted C (cured) which presented with negative parasitological tests (xenodiagnosis and hemoculture), conventional serological tests (immunofluorescence, hemagglutination and complement fixation) and complement-mediated lysis test for living parasites (LMCo); a subgroup denoted D (dissociated), also considered by some investigators as cured, which included individuals whose parasitological tests and LMCo were both negative, but at least one of the conventional serological tests was positive, and the third subgroup (NC, not cured) in which all tests were positive. Only one of 26 patients from subgroup NC presented cardiac symptoms and this was probably due to hypertension and not to Chagas' disease. The remaining patients had no complaints or symptoms of cardiac disease. The untreated group (UT group) consisted of 40 chronic chagasic patients with the indeterminate (asymptomatic) or cardiac form of the disease.

We evaluated the profile of IL-10 and IFN-γ production by peripheral blood mononuclear cells (PBMC) from both the treated and untreated groups after stimulation with antigens derived from T. cruzi. The peak of IL-10 production occurred between 6 and 24 h, with the levels of this cytokine decreasing over time and being undetectable on day 6. Spontaneous secretion of IL-10 was observed in control cultures (not stimulated with parasite antigen) and the level was similar to those observed after antigenic stimulation (data not shown).

In contrast, increased levels of IFN-γ were observed in cultures after antigenic stimulation, with cytokine production being highest

on the 6th day. The levels of IFN-γ secretion in non-stimulated cultures were in general below the cut-off point of the PBMC response after stimulation for both groups.

Indeterminate and cardiac patients of the UT group were classified according to the amounts of IFN-γ produced by PBMC cultures stimulated with T. cruzi antigens as high (>5.0 ng/ml) and low (<4.9 ng/ml) producers. The percentage of "high producers" among the cardiac patients was higher (83%) than that observed among the indeterminate ones (59%). These data suggest a relationship between the production of IFN-γ and morbidity in Chagas' disease. The presence of IFN-γ in sera from mice chronically infected with T. cruzi and presenting heart lesions has also been reported (11).

However, if one assumes that there is a relationship between the production of IFN-γ and morbidity in Chagas' disease, how does one explain that a large proportion of patients with the indeterminate clinical form (asymptomatic) share, with the cardiac patients, the capacity to produce high levels of IFN-γ after specific stimulation? We argue that the asynchronous development of the disease in the indeterminate group may explain these findings. It is possible that the indeterminate patients classified as high IFN-γ producers will develop chagasic cardiomyopathy sooner than those presenting low IFN-γ secretion. Hence, if we consider that IFN-γ worsens the pathology of the disease, the indeterminate patients producing high levels of this cytokine could be considered as candidates for developing the cardiac form of the disease in the near future. The parallel clinical follow-up of indeterminate patients and in vitro evaluation of IFN-γ production by PBMC after specific stimulation will be of great value to clarify this question.

The modulation of IFN-γ production by PBMC of stimulated cultures from the UT group was also investigated by adding the respective monoclonal antibodies against cytokines IL-2, IL-12, IL-10, and IFN-γ to the culture media. We found that the neutralization of IL-2 or IL-12 alone, or simultaneously, down-regulated IFN-γ production. The addition of anti-IL-10 negligibly affected the production of IFN-γ in PBMC cultures stimulated by T. cruzi antigens. However, in the absence of antigenic stimulation (control cultures) the neutralization of IL-10 caused a significant elevation of IFN-γ production. These data support the hypothesis of two pathways of IFN-γ regulation in human Chagas' disease in which the presence or absence of a parasitic antigen stimulus would be important. In the absence of a specific stimulus, at the periphery for instance, IFN-γ produced by T cells specific for T. cruzi antigens may be controlled by the action of IL-10. Supporting this hypothesis are the data of ex vivo analysis demonstrating significantly higher levels of IL-10 mRNA in PBMC from chagasic patients when compared to the levels found in PBMC from non-infected control individuals (12). However, in cardiac inflammatory infiltrates, where the presence of T. cruzi as well as cross-reactive autologous antigens has been demonstrated, the role of IL-10 in inhibiting IFN-γ production may be less important. Supporting this hypothesis are the data on cardiac autopsies from chagasic patients demonstrating high expression of MHC class I molecules by myocardial cells, the presence of granzyme A+, CD8+ T cells, as well as the presence of large cells secreting TNF-α, resembling activated macrophages. All of these aspects are known to be governed by the action of IFN-γ (8,9,13).

Since PBMC from chagasic patients with heart lesions secrete large amounts of IFN-γ after specific antigenic stimulation, and considering the immunohistochemical aspects of the cardiac inflammatory tissue described above, we postulate a role for this cytokine in the increase of the cytolytic potential of lymphocytes in the cardiac inflammatory infiltrate. This condition may be facilitated by the overexpression of MHC class I (HLA-

ABC) molecules on myocytes which is also probably influenced by the presence of IFN-γ(13). In this respect, the chances of autoimmune reactions may be increased due to the possibility of recognition of altered self-cardiac antigens expressed by heart muscle by CD8+ specific T cells. Within this context, it is important to consider the role of CD4+ T cells since it has been demonstrated that T cell lines and clones (CD4+) recognizing self-cardiac myosin occur in biopsies from chagasic patients presenting myocardiopathy (14). The investigation of the cytotoxic capacity and the pattern of cytokine production of these cell lines and clones would be helpful in elucidating their role in the pathogenesis of Chagas' disease. The role of TH1 CD4+ T cells in organ-specific autoimmune diseases has been reported (15).

The production of IFN-γ by PBMC from T-group patients was also investigated. Surprisingly, despite the fact that the group of cured patients presented no detectable levels of antibodies specific to T. cruzi, they secreted very large amounts of IFN-γ and also displayed strong cellular proliferation after specific PBMC stimulation. Both the level of cytokine production and the magnitude of cellular proliferation were significantly higher than those observed in the individuals belonging to the NC group. The average level of IFN-γ produced by PBMC from dissociated individuals was intermediate between those observed in the cured and non-cured individuals of the T group (data not shown).

A relevant question is why all the treated individuals were not cured of the infection. This is important since all of them were homogeneous in terms of clinical symptoms, specific serological reactions and parasitological tests at the time of admission to the follow-up study. Based on the protecting role of IFN-γ against lethal disease in the early phase of experimental T. cruzi infection (16), we speculate that the combination of chemotherapy and the possible early production of IFN-γ during the acute phase may have favored the elimination of the parasites in cured patients. Obviously, since cured patients no longer harbor parasites, the risk of cardiac disease caused by the action of IFN-γ produced by PBMC as a consequence of antigenic stimulation is unlikely, unless an autoimmune process is eventually underway.

The specific cellular immune response against antigens from T. cruzi detected in dissociated and cured patients is illustrative of a long-term memory developed during the acute phase and conserved over the last 16 years. The long term memory of CD4+ and CD8+ T cells has been reported to occur in other human diseases (17,18). It is worth considering that none of the cured, dissociated or non-cured patients studied had been at risk of reinfection for at least the last 10 years.

The continuation of clinical follow-up studies associated with laboratory investigation of the cellular reactivity of the patients will be of great importance to clarify many aspects of the lesions caused by immune responses in human Chagas' disease. For instance, the putative heart lesions caused by the action of autoimmune reactivity without the interference of the parasite can be investigated in the group of cured and dissociated individuals.

In summary, we propose that a possible temporal difference in IFN-γ appearance during the course of the early stages of the infection combined with chemotherapy may be decisive in terms of disease progression and may also explain the apparently paradoxical dual role of this cytokine in human Chagas' disease: 1) promotion of protection during the acute phase and 2) augmentation of the cytolytic effects of lymphocytes during the chronic phase of the infection increasing the chances of myocellular destruction in heart tissue.

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