The Role of the Immune Response on the Development of Severe Clinical Forms of Human Chagas Disease

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Key words: Chagas disease - Trypanosoma cruzi - immunology

Understanding the role of the immune response on morbidity caused by the Trypanosoma cruzi infection has long challenged investigators in this field. It is well known that morbidity in Chagas disease develops long after the initial infection (Brener 1980). The more severe forms of the disease include the development of cardiomyopathy as well as of gastrointestinal forms more commonly known as mega-syndrome (Dias 1989). In all clinical forms of Chagas disease the involvement of cell-mediated immunity is undoubtedly of major importance. In this context, recent reports strongly support a role for cytotoxic immune mechanisms on the development of the severe clinical forms of Chagas (Reis et al. 1993 a,b).

Studies on the characterization of the inflammatory infiltrates in the chronic cardiac form of Chagas disease have demonstrated that they are composed predominantly of small lymphocytes, macrophages, plasma cells and segmented leukocytes (Reis et al. 1993a). Immunohistochemical studies performed by these investigators demonstrated that the cells present in the tissues are mainly CD8+ many of which express granzyme A and a few macrophages that express TNFα. These observations are in agreement with previous suggestions that the lesion in Chagas cardiomyopathy involve both cytolysis and fibrosis. Furthermore, it has also been demonstrated that the presence of the parasite is crucial for the maintenance of the inflammatory response. This was evidenced by amplification of parasite DNA in the inflammatory lesions in the hearts of chagasic patients (Jones et al. 1993, Higuchi et al. 1993). Other investigators have demonstrated that individuals with the cardiac form of the disease, show significant cellular reactivity to the heart tissue (Cunha-Neto et al. 1995). Although these studies have demonstrated the importance of the immune response on the development of the severe cardiac lesion, little is known about the role of soluble factors on the development of this pathology.

In recent studies, we have evaluated the different cell populations present in the peripheral blood of patients with varying degrees of cardiopathy as well as of indeterminate individuals. In this initial evaluation on the role of the various cytokines we demonstrated that secretion of IFNγ can be correlated with the severe cardiac form of Chagas disease. This was evidenced by the detection of higher levels of IFNγ secretion after in vitro stimulation with parasite derived antigens of PBMC of cardiac patients than of indeterminate individuals. The opposite was observed for in vitro secretion of IL-10. These results are important since they suggest a direct correlation between the secretion of IFNγ and the development of the severe pathology in Chagas disease and that IL-10 plays an important role in controlling morbidity. A detailed analysis of the individual levels of IFNγ secretion allowed us to separate the individuals into high and low IFNγ producers. This data demonstrated the group of indeterminate patients 59% of them were high producers while in the group of cardiac patients...
this percentage increased to 83 (Bahia-Oliveira et al. 1998). To further investigate whether alterations on cardiac function could be related to different levels of IFNγ and IL-10 secretion, a detailed clinical evaluation was performed and the patients divided into different groups. These studies demonstrated that as the cardiac function worsens, IFNγ secretion by PBMC increases with a parallel decrease on secretion of IL-10. Thus, these data clearly reinforce our previous hypothesis that IFNγ secretion can be correlated with the development of the severe cardiac form of the disease.

Identification of the cell population secreting the various cytokines was performed by flow cytometry. Our results demonstrate that IL-10 is being produced mainly by macrophages/monocytes (CD14+high) whereas the majority of the IFNγ producers are the CD3+ cells, both αβ and γδ. It is important to emphasize that while in the cardiac patients the majority of the CD3+ cells were IFNγ+, the same was not true for the indeterminate patients where CD3+/IFNγ+ were significantly lower. On the other hand, the percentage of CD14+high/IL-10+ cells were higher in the group of indeterminate patients (Gomes et al. manusc. in prep.).

Although a clear demonstration of the correlation between IFNγ production and cardiomyopathy was observed in our studies, the fact that a large proportion of the indeterminate patients also produce high levels of IFN-γ after specific stimulation, raises the question of what is the role of this cytokine in these patients. One obvious explanation for is the fact that in chronic endemic diseases, the infection is asynchronous as well as the development of the disease. If our hypothesis that IFNγ production is a key factor on the development of severe cardiomyopathy, it is reasonable to speculate that the indeterminate patients that are high IFNγ producers will develop cardiomyopathy sooner than the lower producers. To determine whether changes in cardiac function parallel the alterations on IFNγ secretion, a longitudinal study of the group of indeterminate patients is needed.

Based on our studies and those previously described, we postulate that IFNγ is involved in the augmentation of the cytolytic potential of lymphocytes in the cardiac inflammatory infiltrate. Under these conditions, it is easily conceivable an over expression of MHC class I on myocytes induced by IFN-γ (Wang et al. 1991, Reis et al. 1993b), thus, increasing the possibility of recognition by CD8+ specific T-cells of the altered self cardiac antigens expressed by the heart muscles. Previous work by Cunha-Neto et al. (1998) have demonstrated a role for CD4+ T cells in reacting with self cardiac myosin.

Adad et al. (1991) demonstrated that patients with the gastrointestinal clinical form of the disease present lesions of the myenteric plexus. Tafuri et al. (1971) demonstrated that microscopically, the inflammatory infiltrates composed primarily of small lymphocytes that are found mainly in the muscularis propria and in the myenteric nervous system and are often associated with a striking reduction in the number of enteric neurons. Although the anatomo-pathological findings have been well described very little information is currently available on the immune response related to the development of gastrointestinal disease. Our group has initiated a study with the objective of investigating the role of the immune response on the development of gastrointestinal form of Chagas disease.

Our initial observations on the phenotypic analysis of peripheral blood mononuclear cells from patients with the gastrointestinal form of Chagas disease, demonstrated a significant decrease in the absolute number of CD3+ T cells as well as in CD19+ B lymphocytes. The most striking observation was an inversion of the CD4/CD8 ratio, contrasting with results from cardiac chagasic patients where the ratio of these cells is normal. A decrease of the percentage of CD4+CD28+ cells and an increase in the expression of HLA-DR both on CD4+ and CD8+ cells suggest that although these T cells express activation markers their function may be altered by the lack of CD28 expression (Lemos et al. 1998). The alteration on the percentage of CD4+ T cells in the PBMC from these patients have at least three possible explanations for these observations: (1) impaired maturation of CD4+ lymphocytes caused by the sub-nutritional state of these patients; (2) increased susceptibility of these cells to apoptosis; (3) migration of these cells to the lesions induced by the T. cruzi infection.

Initial studies on evaluating serum protein levels did not show any significant differences between the groups of patients. However, analysis of micronutrients is still needed. The third possibility was also investigated by histological of the megaeosophagus and megacolon. Staining with hematoxilin and eosin, showed the presence of inflammatory infiltrates in association with muscle fibers and neurons, composed of lymphocytes, macrophages, mast cells and occasionally eosinophils. Immunohistochemical analysis demonstrated a predominance of CD3+ T lymphocytes and CD68+ cells. Analysis of the lymphocyte subpopulations showed a higher number of CD4+ cells when compared to the CD8+ population. An interesting observation was the presence of cells expressing TIA-1, a granule associated protein expressed by cytotoxic T cells, suggesting a role for
these cells on the pathogenesis of the chagasic mega. Finally we also observed that all chagasic patients with mega syndrome presented denervation associated with inflammation, whereas patients without them can present or not denervation, but no inflammation is observed in the latter group, suggesting a role for the inflammatory process on the development of this pathology.

It is clear from the results described above that the immune mechanisms involved in the development of the cardiac form of Chagas disease may be significantly different from those of the gastrointestinal form. They also raise the question of whether these mechanisms act simultaneously in patients with both cardiac and gastrointestinal forms of the disease and how these responses are reflected系统ically.

REFERENCES


