

Serum cytokines in chronic Chagas' disease

Citocinas séricas na forma crônica da doença de Chagas

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Abstract We studied the serum levels of IL-2, IFN- and TNF in different clinical forms of Chagas' disease and in patients clinically compensated and decompensated. Cytokines measured in 91 patients with the chronic form of the disease did not differ from those of 13 normal individuals, suggesting the absence of activation of the TH1 pattern of lymphocyte response. There were no statistical differences among the 17 patients in the indeterminate form of the disease, the patients presenting either early (n = 4) or well-developed signs of cardiomyopathy (n = 62), the digestive (n = 4) or the mixed (n = 4) forms of the disease. Serum TNF was undetectable and IFN- levels did not differ between clinical forms and severities of Chagas' disease. However, we found IL-2 higher levels in the 25 non-controlled patients than in the 66 controlled individuals (p < 0,001). We suggest that IL-2 dosage may be useful as an indicator of the need for more aggressive procedures.

Key-words: Cardiomyopathy. Clinical form. Severity. Cytokines. Chagas' disease.

Resumo Estudamos os níveis séricos da IL-2, IFN- e do TNF- de portadores da doença de Chagas em suas diferentes formas clínicas, compensados e descompensados. As citocinas medidas nos 91 pacientes com a forma crônica da doença não diferiram dos níveis de 13 indivíduos controle. Não houve diferença estatística entre os 17 portadores da forma indeterminada da doença e os portadores de cardiopatia insipiente (n = 4), de cardiopatia bem estabelecida (n = 62), da forma digestiva (n = 4) ou da forma mista (n = 4) da doença. Os níveis séricos de TNF foram indetectáveis e IFN- não diferiu nas diferentes formas clínicas ou com a severidade da doença. No entanto, encontramos níveis mais elevados de IL-2 nos 25 pacientes não-controlados do que nos 66 pacientes bem compensados (p < 0,001). Sugerimos que a dosagem de IL-2 possa servir como indicadora da necessidade de terapêuticas mais agressivas nestes pacientes.

Palavras-chaves: Miocardiopatia. Forma clínica. Severidade. Citocinas. Doença de Chagas.

Chagas' disease is estimated to affect 18 million people in Brazil⁶. The disease is one of the most important causes of morbidity and mortality in a broad area ranging from Argentina to the South of the United States^{16 28}. Characteristically, after a self-limited acute

phase, the immune response controls parasitism yet fails to completely eradicate the protozoan parasite. It is interesting that in the chronic phase, Chagas' disease becomes clinically manifested in less than 60% of the infected individuals who develop the characteristic cardiac and/or digestive

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manifestations of the disease¹⁷. The heart is the organ most frequently affected, followed by the autonomic nervous system, the lesion of which leads to the digestive form of the disease¹⁷. The pathogeny of the disease is still unclear, but three main mechanisms have been proposed for it: the neurogenic, the microvascular and the immunological-autoimmunity theories^{12 22}. The few existing studies of the acute human infection demonstrate an abnormal immunological response to the parasite^{25 26}. Immunologic findings in the chronic phase are more difficult to interpret, particularly considering the different clinical forms of the disease. A series of manifestations of immune-suppression have been demonstrated in experimental animals and in human, but the mechanism by which they develop remains obscure^{13 20 21 26}. Depressed responses were reported in patients with either the cardiac or the digestive form of Chagas' disease although parasite antigen-specific and/or mitogen-induced PBMC proliferative responses were reported to be normal⁴. Normal levels of NK cell activity and normal IL-2 activated cytotoxicity (LAK) were shown in both symptomatic and non-symptomatic cardiac patients²³. On the other hand, NK cell

activity was found to be enhanced by *in vitro* exposure of PBMC to *T. cruzi* antigen in all but symptomatic patients³. Increased HLA-DR expression by peripheral blood T-cells and a higher frequency of CD28T cells have been described in both symptomatic and indeterminate phase patients but neither IL-2 or IL-4 message in PBMC nor serum IL-2r levels were found to be augmented^{4 7 8 9}.

Because of their well-known actions in the inflammatory response, some investigators have attempted to relate cytokines levels to the severity of diseases^{10 21 24 27}. We and others also demonstrated that chagasic patients with different clinical forms of the disease have peripheral blood mononuclear cells, which respond to *T. cruzi* antigen with a cytotoxic response that is selectively up regulated by IL-12^{2 3}. However, we found no difference in the increment caused by cytokine treatment among the different forms of Chagas' disease².

In the present study, we have examined some important cytokines levels in patients of chronic Chagas' disease with different clinical forms and severity.

MATERIAL AND METHODS

Controls. The Medical Ethics Committee of the Hospital das Clínicas (FCM/UNICAMP) granted permission to study 20 healthy medical students who volunteered to provide normal parameters for the cytokine assays. After careful clinical examination, a blood sample was obtained from each subject for serum IL-2, IFN and TNF determinations. All individuals were observed for a period of four to eight weeks and when they were suspected of any immune or inflammatory disease, their data were eliminated from analysis of the results. At the end of the observation period, only six males and seven females were selected (median age 24 years, range 21 to 27 years) and denoted group N.

Patients. Ninety one Chagas' disease patients were sequentially selected among the individuals who routinely attend the outpatient clinic. After a careful clinical observation and a review of each patient medical records, the subjects were classified as (-) when they had few or no symptoms, i.e., when they did not present manifestations of Chagas' disease and/or of any concomitant pathology, and as (+) when they were symptomatic or in an advanced stage of heart disease (functional type IV by the criteria

of the Committee of the American Cardiology Association⁴¹. Again we discarded all data from patients suspected of any other concurrent active immune or inflammatory disease.

According to their clinical manifestation of the disease, the patients were divided into:

- **Group I (indeterminate form):** seventeen patients (8 M, 9 F) aged 21 to 71 years ($X = 48 \pm 15$ years) non-symptomatic, with no cardiac or visceral manifestations, although they had serologic tests confirming Chagas' disease. Eight of these patients had other diagnoses that included asthma (one case), arterial hypertension (four cases), coronary artery disease (two cases) and peptic ulcer (one case), but no one presented clinical manifestations during the period of observation of this study.
- **Group II (early cardiac form):** four patients (2M, 2F) aged 45 to 58 years ($X = 54 \pm 10$ years) showed abnormalities in the ECG but had normal cardiac function. Although the four had other concomitant diagnosis, including chronic obstructive pulmonary disease and coronary artery disease (two cases each), no one presented clinical manifestations during the observation period.

- **Group III (cardiomyopathy):** sixty-two patients (20 M, 42F) aged 34 to 71 years ($X = 55 \pm 8.5$ years) with Chagas' cardiac disease. These patients had been using amiodarone to control their arrhythmia at the dose of $M_o = 400\text{mg/day}$ for about 4 years ($M_o = 4$ years). At the time of blood collection, 24 of these patients presented decompensated heart disease with clear clinical manifestations (22 patients were functional type III, and two patients were classified as functional type IV).
- **Group IV (digestive form):** four patients (1 M, 3F) aged 38 to 62 years ($X = 51 \pm 13$ years) with megaesophagus or megacolon confirmed by radiological examination but no sign of cardiac disease. One of these individuals also had a well-controlled hypertension.
- **Group V (mixed form):** four patients (4 F) aged 37 to 67 years ($X = 54 \pm 7$ years) with the combined manifestations of groups III + IV. No patient was using amiodarone. One of these individuals presented signs of non-compensated heart disease at the time of blood collection.

Methods. Blood sampling took place between 9:00 and 13:00h. The samples were immediately processed and stored at -20°C until assayed. IL-2, -IFN and TNF- were measured by solid-phase enzyme immunoassays employing the multiple antibody sandwich principle (Interstest-2 Human Interleukin-2, Interstest-Human IFN- and Predicta Tumor Necrosis Factor- ELISA kits), obtained from Genzyme Diagnostics, Cambridge, MA, USA. IL-2 kit had a detection limit of 100pg/ml. IFN- kit also had a detection limit of 100pg/ml. TNF- detection limit was 10pg/ml.

Statistical analysis. The results are expressed as the mean \pm standard error. Since the data did not show a normal distribution, they were analyzed by the Kruskal-Wallis (H statistic) test, the χ^2 test or Wilcoxon's rank sum test (Z statistic), as appropriate. Statistical analysis that did not reach a level of significance of $p < 0.05$ were considered as non-significant (NS). For statistical purposes, undetectable cytokine levels were assigned an arbitrary level corresponding to the least detectable value of the method.

RESULTS

Normal individuals did not differ from Chagas' disease patients in terms of age or gender ($\chi^2 = \text{NS}$). There was also no difference among the patients grouped according to their clinical form (I to V) ($\chi^2 = \text{NS}$). The results of both normal individuals and Chagas' disease patients are shown in Table 1. IL-2 levels ranged from 200 to

5120pg/dL, -IFN ranged from 42 to 190 pg/dL, and TNF was undetectable in all individuals. There was no significant difference in serum cytokine levels between normal individuals and chagasic patients. Analysis of the 91 patients as a whole showed no correlation among IL2, TNF and IFN- levels. Considering the forms of clinical

Table 1 - Serum thyroid hormones and cytokines levels of normal individuals (group N) and Chagas' disease patients divided into groups I (indeterminate form), II (early cardiac form), III (cardiomyopathy), IV (digestive form) and V (mixed form). Values are reported as Mean \pm SEM.

Group	TSH mU/L	T4I ng/dL	T3 ng/dL	T4 $\mu\text{g/dL}$	Tg ng/dL	IL-2 pg/dL	TNF pg/dL	IFN- pg/dL
N	1.34 \pm 0.37	1.04 \pm 0.52	117.8 \pm 7.8	8.9 \pm 1.7	12.2 \pm 3.3	1671.57 \pm 445.23	ND	112.35 \pm 14.54
I	4.68 \pm 1.13	1.19 \pm 0.28	128.66 \pm 31.20	6.5 \pm 1.5	12.05 \pm 2.92	1476.47 \pm 358.10	ND	122.31 \pm 29.66
II	5.40 \pm 2.70	1.53 \pm 0.76	181.96 \pm 90.98	10.6 \pm 5.3	10.25 \pm 5.12	1285.00 \pm 642.50	ND	118.75 \pm 94.37
III	5.05 \pm 0.70	1.28 \pm 0.17	116.17 \pm 16.26	8.5 \pm 1.1	80.88 \pm 11.32	1057.07 \pm 148.04	ND	122.62 \pm 17.17
IV	1.01 \pm 0.50	1.28 \pm 0.64	NP	NP	46.13 \pm 23.06	1000.00 \pm 500.00	ND	125.75 \pm 62.87
V	16.53 \pm 8.26	1.15 \pm 0.50	91.66 \pm 45.83	8.5 \pm 4.2	224 \pm 112.00	725.00 \pm 362.50	ND	100.00 \pm 50.00

ND = not detectable; NP= not performed.

presentation of the disease, we observed a tendency of IL-2 levels to decrease from group I to group V, while IFN levels were randomly distributed (H = NS for both IL-2 and IFN).

Sixty-six patients did not have concomitant pathologies or were considered to be clinically

compensated (-), whereas 25 not controlled were classified as (+). Their serum cytokine levels are presented in Table 2. There was no difference in the IFN- (Z = 0.74; p = 0.22). However, serum IL-2 levels were higher in the non-controlled patients (Z = 3.895, p < 0.001).

Table 2 - Serum thyroid hormones and cytokines levels of 66 patients with Chagas' disease with a compensated clinical picture (-) and of 25 non-compensated patients (+). Values are reported as mean \pm SEM.

Clinical presentation	TSH mU/L	T4I ng/dL	T3 ng/dL	T4 μ g/dL	Tg ng/dL	IL-2 pg/dL	TNF pg/dL	IFN- pg/dL
(-)	5.32 \pm 0.65	1.19 \pm 0.14	123.44 \pm 15.19	8.8 \pm 1.0	54.09 \pm 6.67	613.11 \pm 75.46	ND	120.82 \pm 14.87
(+)	5.37 \pm 1.07	1.43 \pm 0.28	110.66 \pm 22.13	7.6 \pm 1.5	27.98 \pm 5.59	2506.18 \pm 501.23	ND	134.91 \pm 26.98

ND = not detectable.

DISCUSSION

Like many other parasitic infections, *T. cruzi* induces alteration in the immunological system of the host to circumvent his defense mechanisms¹⁰. IL-2, IFN- and TNF- probably contribute to the control of parasite growth by triggering phagocytic cell activation and inflammation¹. There are also evidences that, in addition to mechanical damage related to the presence of the parasite, infection by *T. cruzi* induces proinflammatory cytokine production in the myocardium itself, which may further exacerbate the pathology^{5 12}.

Serum cytokine quantification has seldom been reported in Chagas' disease. One major reason for this is the widespread involvement of cytokines in inflammatory responses that may impair the interpretation of serum cytokine levels. In the present investigation, we studied the serum levels of IL-2, IFN- and TNF in different clinical forms of Chagas' disease. The serum cytokine levels in patients with the chronic form of the disease did not differ from those of normal individuals, suggesting a lack of activation of the TH1 lymphocyte response. Since most of our patients presented the cardiac form of the disease, these results agree with recent experimental studies suggesting that a lower level of TH1 activity may play a role in cardiac involvement, whereas an enhanced TH-1-type response is related to protective immunity and decreased pathogenesis^{14 18}. Serum TNF- levels were undetectable in both chronic chagasic patients and normal individuals. Serum IFN- levels also did not differ among clinical forms and the stage

of the disease. Variations have been reported between symptomatic versus indeterminate form and in patients with cardiac and indeterminate form. However, cytokine profile does not seem to differentiate the clinical presentations of the disease.

In contrast, serum IL-2 levels were significantly increased in patients with decompensated cardiac function. IL-2 is a glycoprotein of pleotropic effects including the activation and proliferation of T cells, natural killer cells and B cells. Suppression of the IL-2 gene with deficient serum IL-2 and IL-2 receptor expression, apparently mediated by suppressor macrophages, is a well documented phenomenon occurring during acute infection which possibly plays an important role in the evolution of the disease^{19 28}. Nonetheless, its role in Chagas' disease is still poorly understood. It is intriguing to note that IL-2 levels increase strikingly in non-compensated individuals, even though their distribution pattern in the different forms of the disease suggest an inverse correlation with more aggressive forms of the disease (Table 1).

It is frequently difficult to appraise the clinical condition of Chagas' disease patients, because of their misleading autonomic symptoms adjoined by specific social and cultural characteristics. Our results suggest that IL-2 may be related to the severity of the disease. Its increased level in non-compensated patients may be useful a useful tool in the clinical evaluation of these patients as an indicator of the need for more aggressive or invasive procedures.

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