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Immunoglobulins and other serological parameters in Chagas' disease: evidence for increased IgA levels in the chronic digestive form

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SUMMARY

Immunoglobulin levels were measured in serum samples from 36 patients with different clinical forms of chronic Chagas' disease. Increased IgA levels were observed in 50% of the patients in the chronic digestive group and there was a significant correlation with the severity of the disease. IgG and IgM levels were within the normal range. Anti-ssDNA antibodies and EVI (endothelium, vessels and interstitium) antibodies were found in some patients with different clinical forms of the disease.

INTRODUCTION

American trypanosomiasis (Chagas' disease), caused by *Trypanosoma cruzi*, is characterized by a variety of clinical forms. Although trypomastigotes can be easily detected in the acute phase they are scanty in the circulation in both latent and chronic phases. The latent form is further characterized by the absence of clinical symptoms, while a progressive chronic myocarditis and/or involvement of the digestive tract resulting in megalesophagus and/or megacolon characterizes the chronic phase. However, trypanosome antibody activity is present in all stages of the disease.

Multifactorial mechanisms seem to be involved in the pathogenesis of this disease. However, immunological events seem to play an important role (Brener, 1980).

In the present study we investigate possible alterations in serum immunoglobulin levels and the occurence of autoimmune phenomema. By correlating clinical and immunological aspects we may obtain a better understanding of the pathology of Chagas' disease.

MATERIALS AND METHODS

Patients. Thirty-six serum samples from patients with Chagas' disease were collected in an endemic region (Mambaí, Goiás, Brazil). They were classified according to three different clinical forms: latent, cardiac and digestive, as described elsewhere (Rezende, Lauar & Oliveira, 1960; Macedo, 1976). The diagnosis was confirmed by positive xenodiagnosis (Nussenzweig & Sontag, 1952) and by the presence of specific antibodies in circulation detected by indirect immunofluorescence and passive haemagglutination (Camargo, 1966; Camargo, Hoshino-Shimizu & Siqueira,

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1973; Hoshino-Shimizu, Camargo & Nagasse, 1978). The latent group included seven patients and the cardiac group consisted of 15. In the digestive group 14 patients with different degrees of oesophagus alterations were studied. They were classified in three subgroups (DI, DII and DIV; Rezende *et al.*, 1960). The subgroup I (DI) included nine patients with an oesophagus of a normal diameter but with delayed emptying. The second subgroup (DII) consisted of four patients with a moderate enlargement of the oesophagus associated with motor unco-ordination. Only one patient with oesophagic alterations of the fourth grade (DIV), having dolicomegaoesophagus, was studied.

Control groups consisted of 11 clinically healthy Brazilians living in the endemic area in similar social conditions to the patients and 16 blood donors from Rio de Janeiro, Brazil, all matched for age and sex.

Serum samples were immediately frozen on a mixture of dry ice and ethanol, shipped on dry ice and kept at −70°C for less than 6 months.

Specific serology for Chagas' disease. Indirect immunofluorescence was performed according to a previously described technique (Camargo, 1966) using anti-human IgG (Behring Institute, Marburg, West Germany) or anti-human total Ig (Pasteur Institute, Paris, France) conjugated with fluorescein isothiocyanate (FITC). IgA antibodies were assayed using a rabbit anti-human IgA and an anti-rabbit total Ig conjugated with FITC (Behring Institute). Results were expressed as the $\log_2$ of the reverse of the highest dilution to give specific fluorescence.

Immunoglobulin levels. Serum levels of IgA, IgG and IgM were determined by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) using Tripartigen immunodiffusion plates (Behring Institute). Results were expressed in mg/100 ml and the values for the endemic area controls were used as a reference for the statistical analysis.

Other serological studies. The determination of circulating ssDNA antibodies was carried out by a modification of the Farr-DNA binding radioimmunoassay (Izui, Lambert & Miescher, 1976).

The presence of antibodies to endothelium, vessels and interstitium (EVI antibodies) was determined following a previously described technique (Cossio *et al.*, 1974). Titres corresponding to serum dilutions higher than 1:16 were considered as positives.

Statistical analysis. Results were analysed statistically according to the Willcoxon's rank sum test and to the Kendall's coefficient of concordance. The 90th normal percentile of the endemic region controls was used as a reference.

RESULTS

Levels of immunoglobulins

There was no statistically significant differences in total IgG or total IgM levels among the groups studied. However, IgA levels were significantly ($P < 0{\cdot}02$, Wilcoxon's rank sum test) increased in

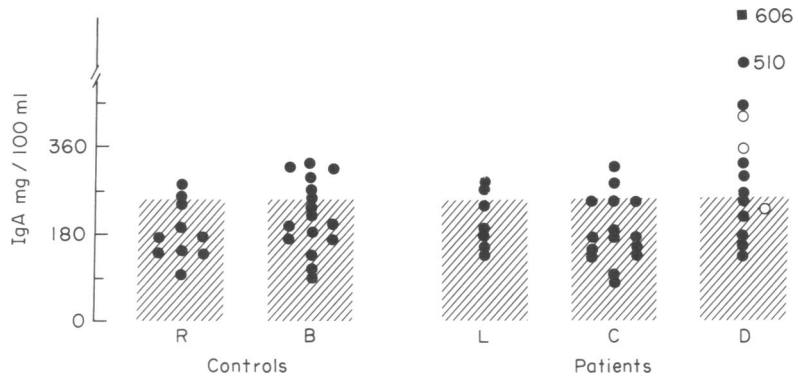

Fig. 1. IgA levels in serum samples from patients with chronic Chagas' disease. (R) endemic region controls, (B) Brazilian blood donors, (L) latent form, (C) cardiac form and (D) digestive form. (●) DI-patients with oesophagic alterations of the 1st grade; (○) DII-patients with oesophagic alterations of the 2nd grade and (■) DIV-patient with dolicomegaoesophagus. The normal range calculated from the endemic region control group is indicated by ■.

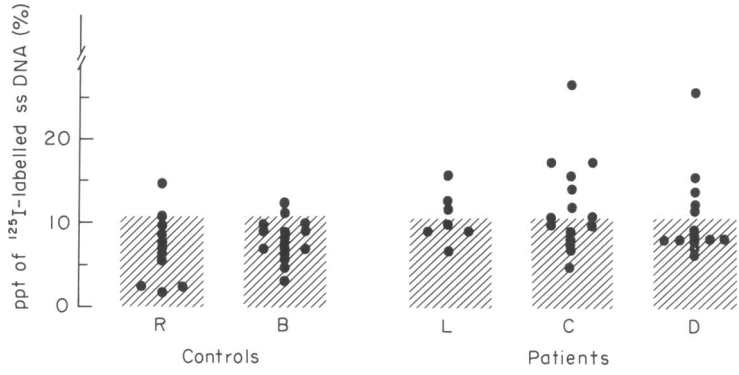

Fig. 2. Serum ^{125}I-labelled ssDNA binding activity in patients with chronic Chagas' disease. (R) endemic region controls, (L) latent form, (C) cardiac form and (D) digestive form. The normal range calculated from the endemic area control group is indicated by ▨.

seven out of 14 patients, with the digestive form of the disease. Moreover, there was a positive correlation of these levels with the severity of oesophagus involvement. DI patients had lower levels of IgA than patients (DII–DIV) with more severe oesophagus pathology ($P < 0{\cdot}05$, Wilcoxon's rank sum test; Fig. 1).

Specific antibodies to T. cruzi *by indirect immunofluorescence*

Positive results were obtained in all patients, using both anti-human IgG or anti-human Ig, the titres varying from $2{\cdot}5 \pm 1{\cdot}3$ to $3{\cdot}3 \pm 0{\cdot}8$ for IgG and from $3{\cdot}4 \pm 0{\cdot}9$ to $3{\cdot}7 \pm 0{\cdot}9$ for total Ig in the different groups. However, IgA antibodies were detected in only half of the patients approximately, without correlation with the clinical form of the disease (mean titres of $2{\cdot}5 \pm 1{\cdot}5$ to $3{\cdot}3 \pm 0{\cdot}8$). No significant correlation was observed between total IgA levels and IgA *T. cruzi* specific antibodies in the patients of the chronic digestive group.

DNA and EVI antibodies

Although circulating anti-DNA antibodies were slightly increased in 41·6% of the patients, without correlation with the clinical form of the disease, there was no statistically significant differences relative to control groups (Fig. 2). EVI antibodies were detected in 27·7% of all patients without correlation with the clinical form of the disease. Titres ranged from 5 to 7, with a mean of $5{\cdot}9 \pm 0{\cdot}9$.

DISCUSSION

In spite of the great deal of information available on the levels of different immunoglobulins in Chagas' disease, this subject is still a matter of controversy. The present work demonstrates increased IgA levels in the chronic digestive form of the disease and it suggests the presence of higher levels of that immunoglobulin in the patients with a more severe involvement of the oesophagus.

The increased IgA levels in these patients may be due to several mechanisms. First, it could reflect high levels of trypanosome antibodies of the IgA class. However, the absence of correlation between specific and total IgA does not support this hypothesis. Secondly, local damage to the mucosa, as a result of irritating effects of the oesophagic contents trapped in the area with altered motility, would favour the subsequent passage to the circulation of nutritional and bacterial antigens that do not normally cross the epithelial barrier (Walker & Isselbacher, 1974). These antigens would stimulate precursors of IgA producing plasma cells located in the lamina propria of the gastrointestinal tract. In addition, an associated deficiency in the production of the IgA secretory component, probably due to functionally altered epithelial cells, would prevent the

normal secretion of the IgA molecules into the oesophagic lumen and lead to their accumulation in the circulation (Tomasi, 1972; Waldman *et al.*, 1970).

Although we do not have any information about the involvement of other segments of the gastrointestinal tract in our patients, we should point out that oesophagic changes are frequently accompanied with other digestive alterations, especially those at the colon (Rezende, 1960), which would probably also play an important role in the increase of IgA levels.

Similarly to previous reports (Marsden *et al.*, 1970; Freitas *et al.*, 1976), we have observed no changes in IgG and IgM levels in any of the different chronic clinical forms. However, other authors (Lelchuk *et al.*, 1970; Vattuone, Szarfman & González-Cappa, 1973) have reported small but significant increases in IgG levels in chronic patients. These contradictory results might be attributed to methodological variations related to the control samples used in the experiments (Brener, 1980). Namely, the authors who found increased IgG levels in the chronic phase did not use controls from the endemic region.

A pathological role of autoimmunity in Chagas' disease has been suggested (Brener, 1980). In the present study we have detected EVI and DNA antibodies in a small number of patients, without correlation with the clinical form of the disease, which would suggest that these autoantibodies do not play an important pathogenetic role. However, we cannot rule out a possible role for EVI antibodies in the worsening of lesions, as it has been previously suggested (Cossio *et al.*, 1974).

The importance and significance of our findings remain to be determined. Sequential studies involving larger number of cases, with a more detailed investigation of some aspects, will have to be carried out.

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