

ANTI-T (THOMSEN-FRIEDENREICH) AGGLUTININ IN CHAGAS'DISEASE

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S U M M A R Y

Serum samples of patients with Chagas'disease and controls from an endemic area of this country were titrated against neuraminidase treated blood group 0 (OT-activated cells) and normal A and B (ABO) red cells. In addition, some agglutination tests with peanut (anti-T + anti-Tk), soybean (anti-T) lectins and human anti-T antibody were performed on red cells from cardiomyopathic patients. The average anti-T score of patients was similar to that of the control group. However, the average score of individuals with the cardiac form was significantly higher than that of asymptomatic patients, and also higher than that of the control sera. The comparison between individuals with positive versus negative serology for Chagas'disease was not significant. Neither diagnostic serology nor the variables race, sex, age, age squared, intestinal parasitic infection, serum protein level, packed red cell volume, degree of bucal mucous membrane paleness, cardiomyopathy and the interaction terms sex x age, sex x age squared, were significantly associated with log titre or log score. The ABO agglutinin scores fell within the normal range of variation. Neither T and Tk receptors nor immunoglobulins (Direct Coombs'test could be detected on the red cell membrane of patients with the cardiac form. The fact that the increase of anti-T levels has been detected only in one of the forms of the disease in patients of the same endemic area strongly argues against the possibility of a concomitant infection as the cause of the observed variations.

KEY WORDS: Tripanosomiasis — Chagas' disease
— Anti-T (Thomsen-Friedenreich) agglutinin test.

I N T R O D U C T I O N

Chagas'disease or American Trypanosomiasis is one of the most important infections of man in Latin America. In Brazil there are about ten million people affected by the disease. During the acute phase the parasite multiplies in the tissues and invades the circulatory system. This phase is followed by a long-lasting "latent", "undetermined" or "laboratorial" phase without signs or symptoms.

The diagnosis in this period is made through various serological methods and xenodiagnosis⁸. Most of the infected individuals remains in this phase indefinitely, while some develop the chronic forms of the disease. The chronic forms — with a low level of parasitemia — are characterized by either cardiac or digestive malfunction or both. The cardiac involvement can result in myocarditis, myocardial fibrosis

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and other lesions of the heart whereas digestive involvement can cause megaesophagus, megacolon, etc. attributable to the destruction of the autonomic nervous system of these organs¹.

It has been reported previously by PALATNIK et al.⁵ that the mean anti-T (Thomsen-Friedenreich) serum titres from adult patients with the chronic form of Chagas' disease are significantly lower than those from the normal control sera. It has also been suggested that the decreased titres could be related to binding of the agglutinin to an excess of T-like antigens liberated either from the membrane of *Trypanosoma cruzi* or by the enzymatic action of the parasite on the human host's invaded tissues.

T-polyagglutination of red cells, either *in vivo* or *in vitro*, is caused by microorganisms that produce neuraminidase. Neuraminidase (sialidase or Receptor Destroying Enzyme, RDE) cleaves terminal sialic acid (N-acetylneuraminic acid, NANA) residues from red cell membrane glycoproteins and glycolipids, and exposes hidden T-receptors which are recognized by a specific anti-T polyagglutinin present in most of normal human sera, chiefly in the IgM immunoglobulin class. Tk-polyagglutination of red cells has been shown to be caused by enzymes produced by certain strains of *Bacteroides fragilis* (beta-galactosidase). Only T-activated red cells have reduced NANA levels. Peanut lectin agglutinates T- and Tk-activated cells while soybean lectin agglutinates only T-activated cells. T-activated red cells aggregate and Tk-activated cells fail to aggregate in Polybrene^R solution (a polycation molecule)².

We report here the study of the anti-T agglutinin of a sample of sera of patients and controls living in an endemic area of this country in addition to some tests of T and Tk antigens performed on the red blood cells of patients with the cardiac form of chronic Chagas' disease from a hospital in Rio de Janeiro.

MATERIAL AND METHODS

The population — Serum samples from subjects from an endemic area (Bambui, MG)

were investigated. Those samples were randomly selected from a population of 390 individuals who were being surveyed for a study on the genetics of Chagas' disease. The diagnosis of patients and controls was made through a specific immunofluorescence test for Chagas' disease. The cardiac form was diagnosed through the pathological findings of a routine electrocardiogram. The digestive form was diagnosed through an X-ray film after ingestion of opaque substance.

The epidemiological characterization of this whole population was done by one of us (KRIEGER), and some of its parameters are: sex-ratio, 0.60; positive Chagas serology, 76%; other parasitic infections, 72%; goiter, 3%; Chagas' disease cardiomyopathy, 37%; digestive form of Chagas' disease, 9%.

Serum and red blood cell samples — Serum samples of 82 individuals were studied, 65 of them had a positive serology for Chagas' disease, 17 had a negative serology (controls). Of the former, 30 had the cardiac form of the disease (three of them had also the digestive form), one had the digestive form and the remaining 34 were in the undertermined phase. Among the controls, three had electrocardiographic pathological findings and two, X-ray digestive abnormalities. Due to the small number and to the somewhat difficult diagnosis of the digestive form of the disease in a field study, this trait was not considered in the following analysis.

Red blood cells from 10 patients with chronic Chagasic cardiopathy from a hospital in Rio de Janeiro were examined by testing their agglutinability with anti-T and anti-Tk lectins, human anti-T agglutinins and rabbit polyspecific anti-human globulin serum (Direct Coombs' test).

Serum titration and red blood cell studies — Progressive twofold dilutions of the sera were incubated with normal suspension of blood group OT-activated red cells following the method already described by PALATNIK et al.⁶. Parallel titrations of ABO agglutinins (anti-A and anti-B), mainly IgM immunoglobulins. The results were recorded as titres and agglutination scores. Titre is the reciprocal of the highest serum dilution at which macros-

copic agglutination is still observed. The agglutination score is the total sum of empirical values assigned to the intensity or avidity of reactions in each tube with a serum dilution. A score obtained in this way is a more meaningful index of antibody/antigen activity than the titre, following the criteria established by MARSH³.

The red cells were examined for the presence of T and Tk antigens by testing with several normal adult sera (anti-T), cord sera (without anti T), peanut (*A. hypogaea*), soybean (*G. soja*) vegetal lectins and 0.1% Polybrene^R solution. The specificity of lectins was confirmed by their agglutination with OT-activated and OTk-activated cells. These latter cells were obtained with the supernatant of a culture of a selected strain of *Bacteroides fragilis* following the method of MOULDS⁴.

Statistical analysis — As the agglutination scores or titres are not normally distributed they were first converted to logarithms and then statistically treated. The average anti-T-score is the arithmetic mean of the logarithms of the agglutination scores, finally expressed by its respective antilogarithm (Mean \pm 1 S.D.). Two tests for significance levels were used: the Student's t test and a non-parametric technique (Kruskal-Wallis'). A parametric stepwise multiple regression was also applied to test the effects of cardiomyopathy and concomitant variables on the anti-T agglutination scores and titres.

RESULTS

The average anti-T score of chagasic patients (28.8 ± 2.6) was similar to that of the control group (23.9 ± 2.7) (probability for t, .4 — .5). However the distribution curve of patient agglutination scores showed to be bimodal (Fig. 1); the average score of individuals with the cardiac form (40.7 ± 1.6) being significantly higher than that of asymptomatic patients (22.4 ± 3.2) (p for critical t, significant at the 1% level). The average score of cardiomyopathic patients was also higher than that of the control sera (p less than .05). The difference of average scores between cardiomyopathic and asymptomatic patients was also significant for the Kruskal-Wallis'H test

(6.72, one degree of freedom, p less than .01). The comparison between individuals with positive versus negative serology was not significant ($H=1.77$, p higher than .25).

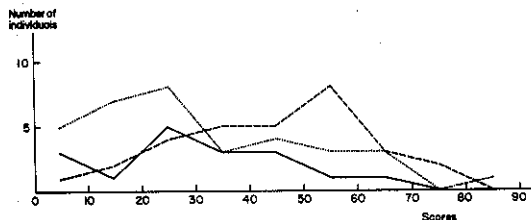


Fig. 1 — Anti-T (Thomsen-Friedenreich) agglutination scores in Chagas'disease. Cardiomyopathic patients — — — — — Asymptomatic patients Controls —————

Neither diagnostic serology for Chagas nor the variables race, sex, age, age squared, intestinal parasitic infection, serum protein level, packed red cell volume, degree of bucal mucous membrane paleness, cardiomyopathy, and the interaction terms sex x age, sex x age squared, were significantly associated with log titre or log score when a sample of 60 individuals with positive serology for Chagas was treated by multiple regression analysis.

The ABO agglutination scores fell within the normal range of variation. Neither T and Tk receptors nor IgG and IgM immunoglobulins could be detected on the red cell membrane of patients with the cardiac form.

DISCUSSION

Our observations clearly indicate that the anti-T agglutinin average level is increased in patients, from an endemic area, with the cardiac form of Chagas'disease. This antibody level is higher than that of asymptomatic patients with positive serology for Chagas'disease and also than that of control persons from the same area. The fact that the increase of the anti T levels has been detected in only one of the categories of patients strongly argues against the possibility of a concomitant infection as the cause of the observed phenomena. The data suggest that the anti-T average score increases as a result of the presence of exposed T receptors in the cells of patients with Chagasic myocardopathy.

Anti-T titres are increased in cirrhosis, chronic granulocytic leukemia, human malaria, and decreased in chronic lymphocytic leukemia⁹. The T antigen has been described on human tumour cells of breast, colon and gastric carcinoma and the anti-T titres were severely depressed in patients with breast carcinoma. When the tumours were removed the antibody showed a significant increase^{6,9}.

Live trypanosomes release NANA from human erythrocytes and from plasma glycoproteins. Erythrocytes from mice infected with *Trypanosoma cruzi* were shown to agglutinate with peanut lectin. The degree of agglutination showed a correlation with the observed parasitemia⁷.

The results here reported suggest T activation of cells, other than erythrocytes. Indeed, red cells are not host cells for *T. cruzi* and our findings, showing the absence of T-activated red cells of chronic Chagasic patients, are in agreement with this statement. Moreover, it could be hypothesized that the absence of exposed T receptors is related to the low level of parasitemia, contrasting with the findings in the experimental animal⁵. It may be finally argued that the red cell T receptors are activated and recovered by an excess of anti-T antibody giving false negative reactions with the specific lectins. However, the negative direct Coombs'test discloses the absence of both IgG and IgM immunoglobulins on the red cell membrane.

RESUMO

Aglutinina anti-T (Thomsen-Friedenreich) na Doença de Chagas

Uma amostra de soros de pacientes e controles de uma área endêmica da Doença de Chagas do País foi titulada contra hemácias de grupo O tratadas pela neuraminidase (células OTativadas) e hemácias normais de grupo A e B (sistema ABO). Também foram feitos alguns testes de aglutinação de hemácias de pacientes com cardiomiopatia com as lectinas de amendoim (anti-T + anti-Tk) e com anticorpo humano anti-T.

O escore médio da aglutinina anti-T dos pacientes foi similar ao do grupo controle. En-

tretanto, o escore médio dos indivíduos com a forma cardíaca foi significativamente maior que o dos pacientes assintomáticos e também maior que o do grupo controle.

A comparação entre indivíduos com sorologia diagnóstica positiva versus negativa foi não significante.

Nem a sorologia para Chagas nem as variáveis raça, sexo, idade, idade ao quadrado, infecção intestinal parasitária, teor de proteínas do soro, volume globular eritrocitário (hematócrito), grau de palidez da mucosa bucal, cardiomiopatia e os termos de interação sexo x idade, sexo x idade ao quadrado, estão associados significativamente com o logaritmo do título ou com o logaritmo do escore de aglutinação.

Os escores de aglutinação das aglutininas anti-A e anti-B do sistema de grupo sanguíneo ABO estão dentro da faixa de variação normal.

As hemácias de pacientes com a forma cardíaca não são aglutinadas nem pelas lectinas de amendoim (anti-T + anti-Tk) e da soja (anti-T) nem pela aglutinina anti-T de origem humana. A prova de Coombs direta foi negativa em todos os pacientes, demonstrando a aparente ausência de imunoglobulinas IgG e IgM na superfície eritrocitária.

O fato de que o aumento dos níveis de anti-T só foi detectado em uma das formas clínicas da doença em pacientes da mesma área endêmica constitui um argumento contra a possibilidade de que a variação observada seja produzida por uma infecção concomitante.

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