

## ANTI-TRYPANOSOMA CRUZI IgM ANTIBODIES AS SEROLOGICAL EVIDENCE OF RECENT INFECTION

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### SUMMARY

By means of immunofluorescence techniques, IgM antibodies to *T. cruzi* were searched for, in chronic and in acute cases of Chagas' disease. In chronic latent cases, or in patients with cardiac or digestive involvement, the IgM-fluorescent test was negative as a rule, whereas in 20 acute cases studied, it was consistently positive, with titers ranging from 1:40 to 1:2,560. Therefore, immunofluorescence techniques could probably stand for a serological tool in epidemiological studies, to determine both the prevalence and the incidence of the infection, through anti-globulin and anti-IgM tests, respectively.

### INTRODUCTION

Immunofluorescence antibody tests are a practical procedure for the serologic diagnosis of *T. cruzi* infection, since they are very sensitive, specific and easy to perform. Also, besides detecting different kinds of antibodies when antiglobulin conjugates are used, they allow the identification of such antibodies according to immunoglobulin classes, through the use of fluorescent conjugates specific for each class. In several infectious diseases specific IgM antibodies are found mostly in recent infections, their presence being, thus, of help to detect diseases in their acute phase, as for example in toxoplasmosis<sup>1,7</sup> or rubella<sup>5</sup>. In this paper, we are reporting our initial results with the anti-*T. cruzi* IgM fluorescent test in chronic and in acute cases of Chagas' disease.

### MATERIAL AND METHODS

*Serum samples* — One hundred samples from chronic cases of Chagas' disease were

taken at random, part from our serum collection, kept for varying periods of weeks or months at  $-20^{\circ}\text{C}$ , and part from recent sera collected from patients presently under serologic diagnosis. All these sera exhibited positive immunofluorescence tests with anti-globulin conjugates<sup>2</sup> and positive hemagglutination tests<sup>3</sup> for *T. cruzi* antibodies. Clinical data indicated patients with no detectable signs or symptoms of cardiac or digestive impairment and others with such evidences. Most patients were residents in the city of São Paulo, living away from endemic areas for long periods. Several had positive xenodiagnoses. Serum samples from 20 cases with clinical and parasitological evidences of acute Chagas' disease were included in the lot studied. Fifteen were from our serum collection and 5 from cases being followed-up at present. They included both, patients living in endemic areas and residents of São Paulo with post-transfusional *T. cruzi* infections. In all of them, positive parasitemia had been recorded within periods of days to a few months before serum collection for the fluorescence tests.

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*Anti-T. cruzi* IgM immunofluorescence tests — Performed according to the technique employed for the antiglobulin immunofluorescence test<sup>2</sup>, with specific anti-IgM fluorescent conjugates (Hyland Travenol Laboratories International, USA). These were diluted for maximal reactivity, which was still obtained at a 1:100 dilution. Serum samples were diluted from 1:20 on, in two-fold dilutions, but only the sera reacting at least at 1:40 were considered positive.

#### RESULTS

Negative results were obtained with all the 100 sera from chronic cases. Doubtful or weakly positive readings at 1:20 dilution were occasional, occurring in two cases corresponding to infections diagnosed as acute 1 1/2 and 2 years earlier. In opposition, every sample from the 20 acute cases, yielded a positive IgM test, the titers ranging from 1:40 to 1:2,560 (Table I).

TABLE I

Results of the IgM and antiglobulin immunofluorescence tests for *T. cruzi* antibodies, in serum samples from 20 patients of acute Chagas' disease.

Patients	IgM-fluorescence test titers (*)	Antiglobulin-fluorescence test titers (*)
A.D.	80	320
A.C.	1,280	640
C.P.S.	320	320
D.N.S.	640	640
F.C.S.	160	160
F.M.E.	640	320
M.A.G.	80	20
M.S.G.F.	2,560	640
M.S.S.	640	320
O.M.B.	160	320
O.T.O.	40	80
O.A.L.S.	80	160
T.G.M.	40	320
W.V.C.	640	160
M.A.M.	1,280	320
M.A.	640	160
A.B.	160	80
S.C.	1,280	640
W.S.	640	640
O.R.A.	2,560	640

(\*) Expressed as reciprocal of the serum dilution

#### DISCUSSION

As it is currently known in regard to other infections, IgM antibodies to *T. cruzi* were found only in cases presenting evidences of recent infection. Follow-up studies are necessary to establish the length of time for IgM-test positivity, in acute cases. As a matter of fact, we were able to obtain serum samples from four of our patients, in later opportunities. Patient T.G.M. had a positive IgM-test 4 months after the first test, and a negative test after further 5 months. Patients C.P.S., A.C. and M.A.G., when tested again respectively at 9 months, 1 and 3 years, had negative IgM-tests. Our present results do not agree with those found by MAGNANI et al.<sup>6</sup>, who observed anti-*T. cruzi* IgM antibodies in 61 out of 71 chronic cases of Chagas' disease, with titers of 1:50 or higher. Such divergences could be due to different specificities of the conjugates to immunoglobulin chains, in the dilutions used. The recognition of acute cases of American trypanosomiasis on clinical grounds is not always easy, as apparently only a small percentage of the patients present clear-cut manifestations. Thus, a serological identification of such cases could be of large epidemiological value. A confirmation of our results would, thus, point the immunofluorescence techniques as useful resources for the epidemiologists to determine not only the prevalence of *T. cruzi* infections, through the common antiglobulin test, but also its incidence, with the help of the IgM-test. It is to be remembered, however, that occasional false positive results may be expected in the IgM-test, due to rheumatoid factors in the serum, as we have observed in tests for toxoplasma antibodies<sup>4</sup>. Absorption of serum with aggregated globulin can remove such non-specific reactions.

#### RESUMO

*Anticorpos IgM anti-Trypanosoma cruzi como evidência sorológica de infecção recente*

Anticorpos IgM anti-*T. cruzi* foram pesquisados, por imunofluorescência, em soros de casos crônicos e de casos agudos de infecção chagásica. Em 100 casos crônicos, tomados ao acaso, a reação anti-IgM foi sistematicamente negativa. Porém, em 20 casos

agudos, o teste foi positivo em todos, com títulos variando de 1:40 a 1:2.560. Pela dificuldade frequentemente observada na identificação clínica de casos agudos, a pesquisa de anticorpos IgM anti-*T. cruzi* poderá representar excelente recurso inclusive para fins epidemiológicos, pois a técnica de imunofluorescência permite a avaliação, não apenas da prevalência, mas ainda da incidência da infecção tripanossômica.

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