Clin Exp Immunol 1993; 93:85–92

Trypanosoma cruzi infection enhances polyreactive antibody response in an acute case of human Chagas' disease

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(Accepted for publication 2 March 1993)

SUMMARY

The kinetics of antibody response in an acute case of human Chagas' disease was investigated. Hypergammaglubulinaemia appeared at day 17 of infection, and persisted after 66 days of infection, at which time parasitaemia became undetectable. Titration of immunoglobulins showed that the three principal isotypes were involved in the response, emphasizing polyclonal B cell activation. Total IgA was detected before total IgM, and the latter before total IgG. High titres of autoantibodies were found among IgM and IgG subclasses. IgA was also the first isotype to be detected among specific anti-*Trypanosoma cruzi* antibodies. However, the maximal parasite antibody response was attained after 30 days of infection for all isotypes. With regard to possible cross-reactivity between molecules of host and parasite, adsorption experiments on *T. cruzi*-specific immunosorbent were designed. Specific antibodies, present in the eluates, also recognized natural antigens, especially laminin. In order to characterize the α-galactose epitope of laminin, adsorption experiments on sheep erythrocytes were performed, and revealed the possible presence of another epitope on the glycoprotein. Our results indicate that in the case of Chagas' disease investigated here, polyclonal activation occurred; moreover, they suggest that molecular mimicry may play a role by increasing autoantibodies, probably via a parasite-driven mechanism.

Keywords autoimmunity natural autoantibodies *Trypanosoma cruzi* Chagas' disease anti-α-Gal antibodies

INTRODUCTION

Chagas' disease (American trypanosomiasis) is due to infection with the protozoan haemoflagellate *Trypanosoma cruzi*, transmitted by blood-sucking reduviid bugs and blood transfusions from infected individuals. An initial, brief acute phase is followed by a chronic lifelong phase in which cardiac and/or digestive lesions may appear years after the infection. The disease constitutes a challenge for immunological research: cross-reactive epitopes between the host and the parasite have been described, as well as the presence of autoreactive antibodies and T cells [1–5]. Moreover, in animal models, the host reaction during the acute phase involves polyclonal B cell activation with subsequent antibody production [6, 7]. The antibody response has been shown to be mainly autoreactive, and very weakly specific for the parasite [8]. These facts argue in favour of autoimmune processes and may explain some features of the chronic disease [2].

The question has been raised as to the role of synthesized antibodies; naturally occurring polyreactive autoantibodies have been described in normal individuals [9,10], corresponding to a substantial part of the B cell production [11]. They derive mainly from germline genes and frequently exhibit multireactive activities: they are able to bind to several highly conserved natural molecules—so called 'natural antigens'—showing no specificity for a particular host [11]. It is still unclear whether this repertoire of 'natural' autoantibodies is devoted to a first-barrier immune defence concerning self-recognition and/or to immune regulation through the idiotypic network. This natural preimmune repertoire could mature through an antigen-driven mechanism to give high-affinity antibodies [11]. This would explain why non-specific autoantibodies increase just after infection, but then decrease as specific antibodies are produced [12]. Strikingly, in experimental *T. cruzi* infection, persistence of polyclonal B cell activation and high levels of IgM and IgG autoantibodies are observed [7,13]. Two mechanisms have been evoked to explain the autoimmunity of Chagas' disease: polyclonal B and T cell activation occurring during the acute phase, and/or antigen-driven reactions due to molecular mimicry of parasites.

Our knowledge of Chagas' disease derives from experimental models, due to the difficulties which exist in defining the acute phase in terms of the date, parasite strains and clinical

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declarations. We have undertaken a study of the acute phase of Chagas' disease in an accidentally infected human subject. In order better to understand the humoral response in human disease, we first investigated the kinetics of serum immunoglobulin production and the specificities of antibodies toward a panel of antigens. We next isolated *T. cruzi*-specific antibodies by passing sera through a parasite-affinity column, and studied the reactivities of effluents and eluates on the same panel employed in whole serum analysis. Finally, we focused our study on antibody activity directed against the epitope galactosyl α(1–3) galactose (α-Gal) found on laminin, on sheep and rabbit erythrocytes, and on *T. cruzi* [14,15].

MATERIALS AND METHODS

Human sera

Six serum samples were collected from a patient from blood group A infected by the Cl strain of *T. cruzi* at days 15, 17, 30, 45, 66 and 93 post-infection. Treatment with benznidazol was prescribed: 5·5 mg/kg per day for 2 months, from day 21 on. Normal sera included a sample from the same patient before infection and another from a blood donor. Sera from two patients with known autoantibody titres—one systemic lupus erythematosus (SLE) and one rheumatoid arthritis (RA)—were used as controls. All donors were of the same age, sex and blood group as the patient. Patient consent was required.

ELISA measurements

Polystyrene microwell plates (CML, Nemours, France) were coated with different antigens under the following conditions: *T. cruzi* antigen from cultured trypomastigotes, prepared as described elsewhere [13], 1·25 μg/well; goat anti-human immunoglobulins (anti-IgGAM) (Biosys, Compiègne, France), 2 μg/ml; laminin from the murine EHS tumour (BRL, Herblay, France), 1 μg/ml; bovine muscle actin (Sigma, St Louis, MO), 5 μg/ml; rabbit muscle myosin (Sigma), 5 μg/ml; bovine serum albumin (BSA) (Sigma), 5 μg/ml; rabbit IgG (Sigma), 10 μg/ml; pig brain tubulin (purified in our laboratory), 3 μg/ml; mouse central nervous system myelin, kindly provided by Ben Younes-Chennoufi (La Salpêtrière, Paris), 1 μg/ml; tri-nitrophenyl-albumin (TNP-BSA) (prepared according to Little & Eisen [16]), 5 μg/ml. All these antigens were dissolved in 0·1 M carbonate buffer, pH 9·6; native and heat-denatured DNA from calf thymus (Sigma), 10 μg/ml in 0·5 M citrate buffer, pH 6; human epidermis keratin (Sigma), 10 μg/ml in 0·1 M Tris buffer containing 8 M urea and 1% β-mercaptoethanol, pH 8·6.

Plates were coated with 100 μl of the appropriate antigen dilutions and kept overnight at 4°C, except for rabbit IgG which required a 37°C incubation. After five washes with PBS (GIBCO, Paisley, UK) 0·15 M pH 7·4 containing 0·1% Tween 20 (PBS-Tw), saturation was accomplished with 50 μl/well of PBS-Tw containing 0·5% gelatin; 50 μl of samples were added and kept overnight at 4°C. After extensive washes, plates were incubated with goat antibodies: β-galactosidase-labelled anti-human IgGAM (Biosys) at 1 μg/ml—for total serum determinations—or peroxidase (PO)-labelled anti-human γ (Immunotech, Marseille, France) diluted 1:50 000, anti-human μ (Sigma) diluted 1:1000, and anti-human α (Sigma) diluted 1:1000. The β-galactosidase activity was revealed by *o*-nitrophenyl-β-D-galactopyranoside (Sigma) (80 mg/ml, 100 μl/well). After 3-h incubation at 37°C, absorbances were read at 414 nm. PO activity was revealed by *o*-phenylenediamin (Kit Diagnostic Pasteur, Marne-la-Coquette, France). All measurements were made in a 'Titertek Multiskan' ELISA reader (Labsystem, Les Ulis, France).

Optimal dilutions of the samples were determined with standard curves: 1:400 serum dilutions were used in reactions revealed with β-galactosidase-conjugate, while 1:1000 serum dilutions were used for reactions revealed by PO conjugates.

The results represent the mean of duplicate values in two different determinations, and are expressed as units of optical density (OD) or as percentage of OD in relation to S0 considered as 100%. When results were determined through a series of experiments, and in order to standardize values, 'Units of antibody activity' ('U Ab activity') equivalent to $100 \times (OD/(\mu g/ml))$ were used.

Purification of serum immunoglobulins

Immunoglobulins were purified from serum samples by selective precipitation in $(NH_4)_2SO_4$ 47% saturation, pH 7·5. Precipitates were dissolved and dialysed against PBS. The concentration of proteins was evaluated by spectrophotometry at 280 nm in a Carl Zeiss instrument.

Separation of immunoglobulins with immunosorbent

Trypanosoma cruzi-specific immunosorbent was prepared by fixing *T. cruzi* antigen to amino-hexyl-sepharose (AHS) (Pharmacia, Uppsala, Sweden) at a ratio of 8 mg of antigen/g of AHS. Serum immunoglobulin at a concentration of 6 mg/ml in a final volume of 2·5 ml was added; after 2 h of contact, the column was washed in PBS until absorbance $<0{\cdot}01$ OD units. Afterwards, antibodies were eluted with a 0·1 M Tris-glycine-HCl buffer, pH 2·8, neutralized and immediately dialysed in PBS. Thereafter, vacuum concentration was operated up to a final volume of 1 ml and kept at −20°C.

Adsorption on sheep erythrocytes

Fractions with IgG anti-laminin activity > 100 U at a concentration of 5 μg/ml were adsorbed twice on sheep erythrocytes: first, for 2 h at 20°C, and thereafter, overnight at 4°C. Control sera (S0, NHS and SLE) were diluted 1:1500. After final centrifugation at 1400 *g* for 15 min, the samples were analysed by ELISA.

RESULTS

Antibody production kinetics

In order to study the kinetics and magnitude of total antibody production in a *T. cruzi*-infected human subject, we investigated whole serum response with a panel of antigens, consisting of *T. cruzi* extract, cytoskeleton proteins, double-stranded and denatured DNA and rabbit IgG (for rheumatoid factor antibodies), during a period of 93 days post-infection. Relative titres of total immunoglobulin showed an important increment in comparison with basal state (S0) during this period. Antibody activity was found to be generally increased; however, variations were observed in the response to the different antigens (Fig. 1): titres increased and stayed high for anti-*T. cruzi*, anti-laminin, anti-DNA and rheumatoid factor antibodies, while those corresponding to actin, keratin, myelin, myosin, tubulin, BSA and TNP-BSA responses declined at the end of the 93-day period. In further studies, tubulin was taken as representative of

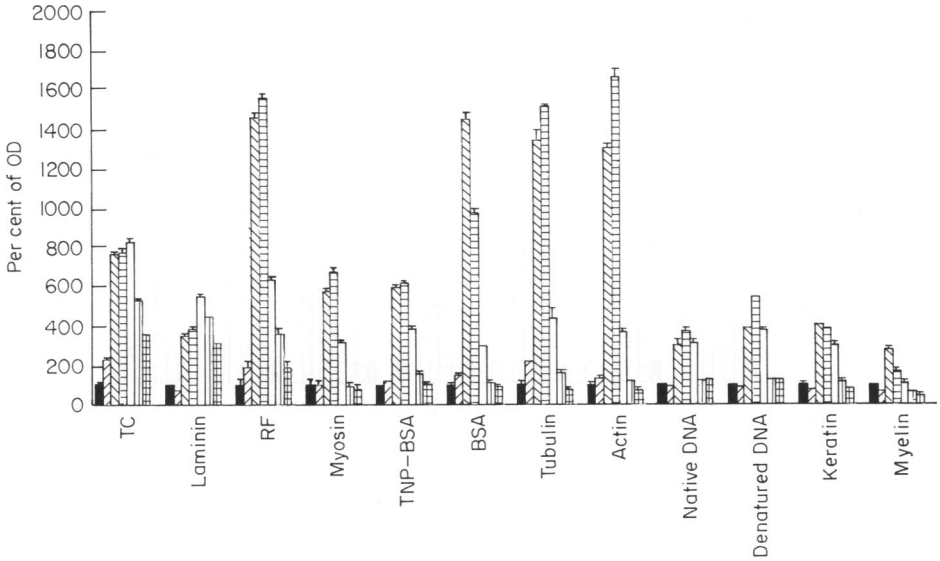

Fig. 1. Kinetics of whole serum antibody production tested on the panel of indicated antigens. The curves express OD_{492} variations in antibody activity in comparison with the basal level (S0), measured by ELISA. Sera diluted 1:400. OD corresponding to the buffer without serum was subtracted. ■, S0 day 0; ■, S1 day 15; ▨, S2 day 17; ▤, S3 day 30; □, S4 day 45; ■, S5 day 66; ▦, S6 day 93. TC, *Trypanosoma cruzi*; RF, rheumatoid factor; TNP-BSA, tri-nitrophenyl-albumin.

Table 1. Titration of whole serum IgM, IgG and IgA

Serum (S)	Titre IgM: 1/dil	Ratio S/S0	Titre IgG: 1/dil	Ratio S/S0	Titre IgA: 1/dil	Ratio S/S0
S0	12000	1	2600000	1	66000	1
S1 (day 15)	10500	0·88	2100000	0·81	72000	1·09
S2 (day 17)	160000	13·33	2500000	0·96	115000	1·74
S3 (day 30)	310000	25·83	3000000	1·15	115000	1·74
S4 (day 45)	220000	18·33	3800000	1·46	110000	1·67
S5 (day 66)	19000	1·58	2300000	0·89	92000	1·39
S6 (day 93)	10600	0·88	1600000	0·62	60000	0·91

Titres of total immunoglobulins were determined by ELISA and express the reciprocal dilution needed to give 50% of maximum OD when serial dilutions of serum samples were performed. Ratios are taken in comparison with the basal level (S0). Samples were diluted 1:1000. OD were read at 414 nm. Values corresponding to the buffer without serum were subtracted.

cytoskeleton antigens, and BSA as representative of both BSA and TNP-BSA antigens.

IgG, IgM and IgA production kinetics

The titration of the different serum isotypes showed that IgM, IgG and IgA activities peaked on days 30, 45, and between 17 and 30, respectively (Table 1).

Levels of all three isotypes increased for every antigen (Fig. 2), with different kinetics for each: for IgM activity, specific anti-*T. cruzi* antibodies showed an important increase from S3 on (30 days); IgM antibodies directed against the 'natural' antigens already showed very high levels from S2 on (17 days); neither anti-*T. cruzi* nor anti-DNA IgM antibodies returned to basal level at the end of the period studied. Anti-*T. cruzi*-specific IgA antibodies were the first to be detected (15 days); among non-*T. cruzi*-specific IgA responses, only anti-tubulin and anti-laminin antibody activities reached levels higher than 200% of basal values. Anti-*T. cruzi* and anti-tubulin IgA antibodies remained at levels higher than 100%. Anti-*T. cruzi*, but also anti-laminin, IgG antibodies peaked on day 30, simultaneously with the corresponding IgM. For the remaining IgG antibodies, only those directed against tubulin showed significant values. Most IgG values remained at levels higher than the basal level at the end of the 93-day period.

Thus, specific anti-*T. cruzi* IgA were the first to be detected; IgG and IgM peaked thereafter, and none of them returned to basal level at the end of the period; anti-laminin IgG and IgM were detected before IgA of the same specificity, with IgG being the most important; for other activities, the most important isotype was IgM, while IgG and IgA were mainly directed against tubulin.

Antibody specificity

The mono- or polyreactivity of anti-*T. cruzi* antibodies that appear during Chagas' disease is a controversial subject. To

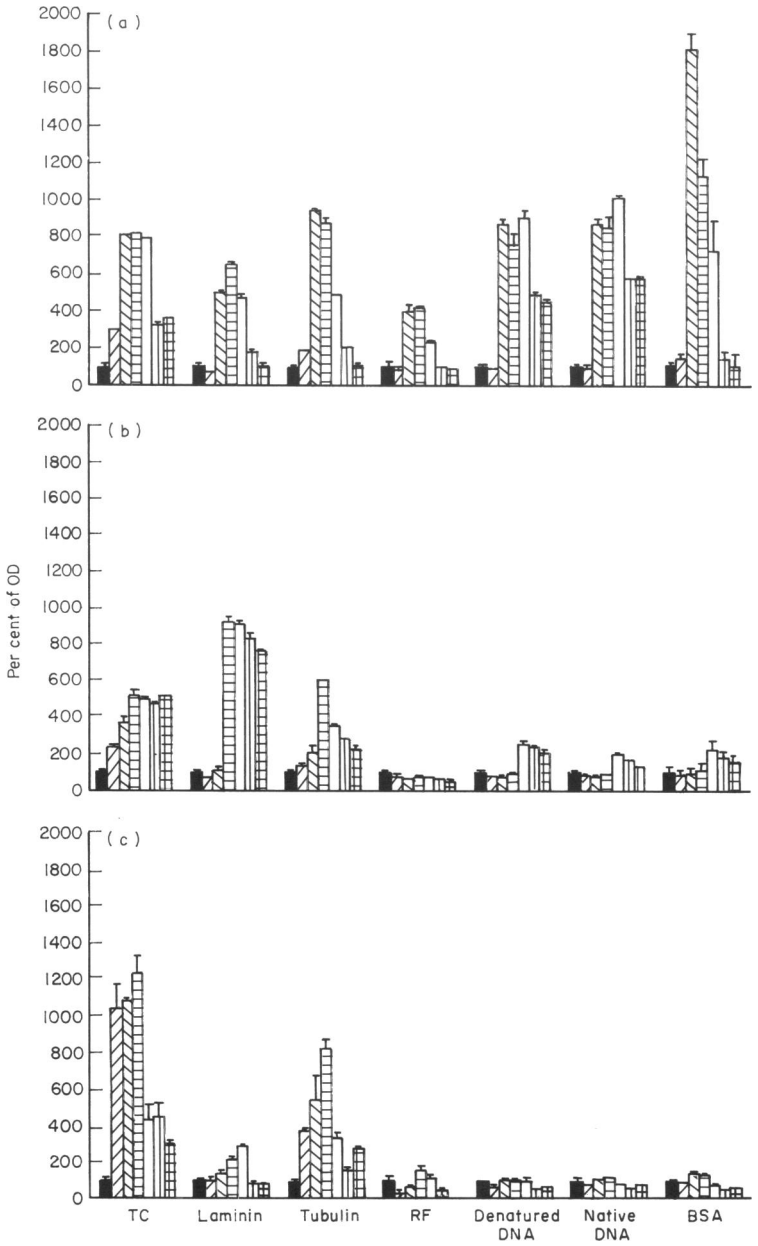

Fig. 2. Kinetics of whole serum IgM (a), IgG (b) and IgA (c) antibody production. Tested antigens were as indicated. The curves express OD_{414} variations in antibody activity in comparison with the basal level (S0), measured by ELISA. Sera diluted 1:1000. OD corresponding to the buffer without serum was subtracted. ■, S0 day 0; ▨, S1 day 15; ▧, S2 day 17; ▤, S3 day 30; □, S4 day 45; ▥, S5 day 66; ▦, S6 day 93. TC, *Trypanosoma cruzi*; RF, rheumatoid factor; BSA, bovine serum albumin.

approach this question, we adsorbed purified serum immunoglobulins on parasite antigens immobilized on AHS and tested effluent (Eff) and eluate (Elu) activities against our panel of antigens. Table 2 shows, for each blood sample, the anti-*T. cruzi*-specific IgM, IgA and IgG activities present in both fractions. Results expressed in units of antibody activity ('U Ab activity') indicate that the kinetics of antibody production in the eluate followed the pattern of unfractioned serum study. For specificities different from anti-*T. cruzi*, eluted IgM and IgA exhibited little cross-reactivity (data not shown). Effluent IgA reacted with the parasite extract in ELISA tests, probably because of the polyreactivity of natural autoantibodies. Neither rheumatoid factor (RF) nor anti-BSA nor anti-DNA IgM reactivities expected from the unfractioned serum analysis were found in the eluates. The absence, in the effluents, of these antibodies, which are polyreactive and of low affinity, suggests denaturation during elution [17]. *Trypanosoma cruzi*-eluted IgG reacted with other antigens, and particularly with laminin (Table 3), indicating their polyreactivity. Interestingly, IgG rheumatoid factors appeared to be more important than expected from the whole serum study.

Assessment of cross-reactivities between T. cruzi *and laminin*

As anti-laminin antibodies appearing during Chagas' disease recognize the epitope galactosyl α-(1–3) galactose ('α-Gal') [14,15,18], fractions showing anti-laminin IgG activity > 100 U

Table 2. Specific anti-*Trypanosoma cruzi* IgG, IgM and IgA activities in effluent (Eff) and eluted (Elu) fractions after adsorption on parasite antigens

Fraction origin	Fraction	IgG	IgM	IgA
S0	0 Eff	30	0	18
	0 Elu	50	0	12
S1	1 Eff	0	0	30
(15 days)	1 Elu	339	4	23
S2	2 Eff	0	0	27
(17 days)	2 Elu	473	433	9
S3	3 Eff	0	0	33
(30 days)	3 Elu	447	85	22
S4	4 Eff	4	0	1
(45 days)	4 Elu	666	123	17
S5	5 Eff	0	4	5
(66 days)	5 Elu	566	27	24
S6	6 Eff	16	8	0
(93 days)	6 Elu	433	62	14

Values are expressed in 'U Ab activity' equivalent to $100 \times (OD/(\mu g/ml))$. Immunoglobulins were incubated with a *T. cruzi* immunosorbent and antibodies were eluted with a Tris-Glycine-HCl buffer as described in Materials and Methods. After concentration up to 1 ml, all samples were tested by ELISA.

Ab activity were adsorbed on sheep erythrocytes (on the surface of which α-Gal is present [14,19]). Subsequently, ELISA was performed to assess the exhaustion of anti-laminin antibodies and to test residual anti-*T. cruzi* activity. Two normal human sera (from a blood donor and 'S0' from our patient), a third from a patient with SLE, and both fractions (Eff and Elu) corresponding to S0 serum, were employed as controls. Normal human erythrocytes from group A, lacking the α-Gal epitope, were taken as controls for sheep erythrocytes.

IgG anti-*T. cruzi* activity remained relatively unchanged after adsorption, indicating that the anti-α-Gal response represented a low proportion of the anti-*T. cruzi*-specific antibodies. The slight decrease in activity observed was also present in controls (Fig. 3). Since IgM and IgA values were too weak, the reduction in activity was within the limits of significance (data not shown). On the other hand, most of the anti-laminin antibodies produced during the disease were actually directed against the α-Gal epitope, since they decreased after adsorption on sheep erythrocytes. Strikingly, there remained a residual anti-laminin activity—which coincided with the normal control level—even after the second adsorption. SLE serum activity was not affected by adsorption (Fig. 4). A control experiment carried out on tubulin (lacking sugar residues) did not show any variations in antibody activities before or after sheep erythrocyte adsorption (data not shown).

DISCUSSION

Accidental laboratory contamination is an infrequent cause of *T. cruzi* infection. In the very rare cases reported in the literature [20,21] authors have generally considered the production of specific anti-*T. cruzi* antibodies and their kinetics. We addressed the question of distortion of the overall B cell response that such an infection can induce, thereby leading to autoreactivity. Indeed, mechanisms underlying anti-self-reactions have not yet been studied in humans. Previous results elicited two possible means of provoking autoimmunity: polyclonal activation described in mice [6,7], and an antigen-driven mechanism via molecular mimicry, since molecular structures shared by the parasite and its host have been identified [2,22,23].

In the present report, using ELISA, we studied *T. cruzi*-specific and non-specific antibody responses produced during the 93 days which followed *T. cruzi* infection in a laboratory-acquired case. An increase in the response was not evident until day 17, before which it even appeared to decrease. This may correspond to the immunosuppression observed in experimental models during the first fortnight post-infection [13,24,25]. It then followed classical primo-infection kinetics, wherein total IgM and IgA increased before total IgG. The increasing levels of all three classes of serum immunoglobulins (IgM, IgG, IgA) suggest polyclonal B cell activation. The three isotypes reacted with the whole panel of antigens, and IgM showed the strongest reaction. Antibody titration revealed very high level increments compared with the basal state (S0) (Fig. 1): between eight- and 20-fold for the different IgM antibodies, between two- and 10-fold for IgG antibodies, and between two- and 12-fold for IgA.

Our results confirm previous reports showing increasing levels of IgM and IgA (measured by indirect immunofluorescence techniques) during *T. cruzi* acute infection [20,26].

Results of unfractioned serum analysis demonstrating B cell polyclonal activation are compatible with what is known from mouse models [13,27,28]. Indeed, these studies have shown massive antibody secretion directed against different antigen determinants, mainly non-*T. cruzi*-specific [6,8,13,29].

Our second question concerned the relationship between *T. cruzi*-specific antibodies—which could recognize similar structures in the parasite and in its host through a molecular mimicry mechanism—and polyreactive natural autoantibodies. If the autoantibodies detected were triggered by cross-reacting epitopes, we should have been able to adsorb those reactivities on *T. cruzi* antigens. IgM and IgA show few cross-reactions with molecules other than *T. cruzi* antigens. On the other hand, eluted IgG reacted to some extent with 'natural' antigens, suggesting their polyreactivity. These results suggest that molecular mimicry may play a role in the synthesis of autoantibodies detected here. Indeed, both mechanisms, polyclonal B cell activation and molecular mimicry, seem to take place in autoantibody production during Chagas' disease. Similar experiments carried out in untreated *T. cruzi*-infected mice have shown that the autoantibody response was not eliminated when the serum was passed through a *T. cruzi*-immunosorbent, suggesting that only polyclonal B cell activation is responsible for autoimmunity [13]. Moreover, polyclonal B cell activation during experimental Chagas' disease was demonstrated by the absence of cross-reactivity among hybridoma supernatants and the study of V_H families [6,8].

On the other hand, the identification of several molecular epitopes shared by the parasite and its host which induce specific antibodies, attests to molecular mimicry. Thus, the cross-reactivity existing between *T. cruzi* and mammalian MAP-proteins or glycolipids [2,23], as well as a 13-amino acid peptide from *T. cruzi* ribosomal P protein which defines the specificity shared between severe chronic Chagas' heart disease and SLE

Table 3. Non-specific IgG activity in effluent (Eff) and eluted (Elu) fractions

Fraction origin	Fraction	Anti-lam	Anti-tub	RF	Anti-BSA	Anti-DNA
S0	0 Eff	60	29	87	35	21
	0 Elu	74	13	90	21	13
S1	1 Eff	0	0	9	11	0
(15 days)	1 Elu	19	12	98	38	11
S2	2 Eff	1	2	31	3	2
(17 days)	2 Elu	12	21	224	28	1
S3	3 Eff	2	1	24	3	3
(30 days)	3 Elu	34	32	230	35	28
S4	4 Eff	2	0	13	2	2
(45 days)	4 Elu	169	45	267	46	26
S5	5 Eff	7	2	15	5	2
(66 days)	5 Elu	165	26	174	44	17
S6	6 Eff	268	14	21	19	21
(93 days)	6 Elu	358	39	152	82	59

Anti-lam, anti-laminin; anti-tub, anti-tubulin; RF, rheumatoid factor; anti-BSA, anti-bovine serum albumin.

Immunoglobulins were incubated with a *Trypanosoma cruzi* immunosorbent and antibodies were eluted with a Tris-glycine-HCl as described in Materials and Methods. After concentration up to 1 ml, all samples were tested by ELISA. Values are expressed in 'U Ab activity' to $100 \times (OD/(\mu g/ml))$.

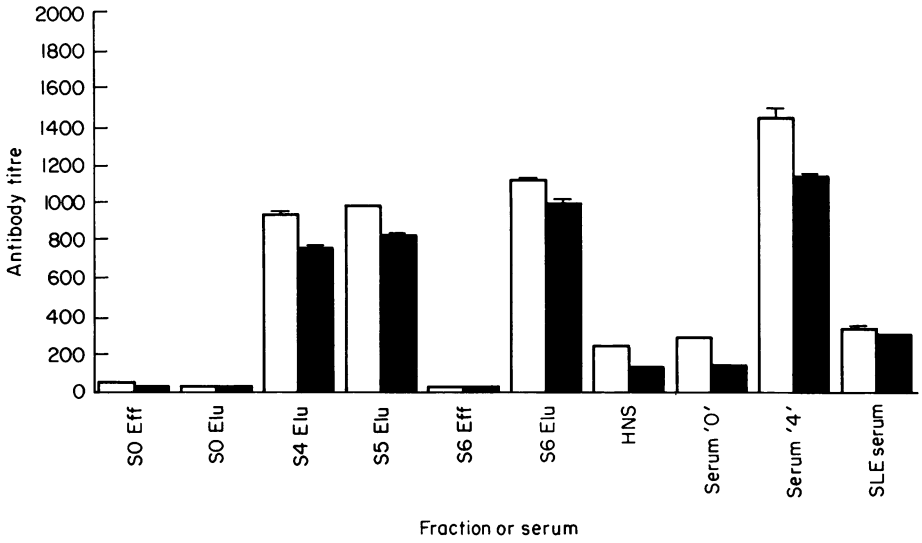

Fig. 3. Anti-*Trypanosoma cruzi* IgG titre before (□) and after adsorption (■) on sheep erythrocytes. Tests were carried out on fractions with anti-laminin IgG activities higher than 100 U Ab activity and in normal and pathological control sera. Samples were diluted at a final concentration of 2·5 μg/ml. OD were read at 414 nm; values corresponding to the buffer were subtracted. SLE, Systemic lupus erythematosus.

anti-P protein antibodies [3,22], have been described. Towbin *et al.* have, in turn, shown that the galactosyl α(1–3) galactose ('α-Gal') epitope is shared by *T. cruzi* and widespread natural structures, such as laminin and sheep erythrocytes [14,15].

In our case, the autoimmune activity was practically removed by passing the serum through the affinity column, thus suggesting that, at least for this human case, autoantibodies mainly accounted for molecular mimicry.

Our results lead us to think that both mechanisms take part in a unique process of 'specialization' of immune reactions. Naturally polyreactive non-specific B clones could mature under the 'pressure' of *T. cruzi* epitopes [11,30]: the more antigenic an epitope is, the more antibodies (specific and/or non-specific) will be produced. The specialization process would parallel the decreasing infection [12]. Our findings of increasing anti-α-Gal antibodies after 45 days, as parasitaemia decreased,

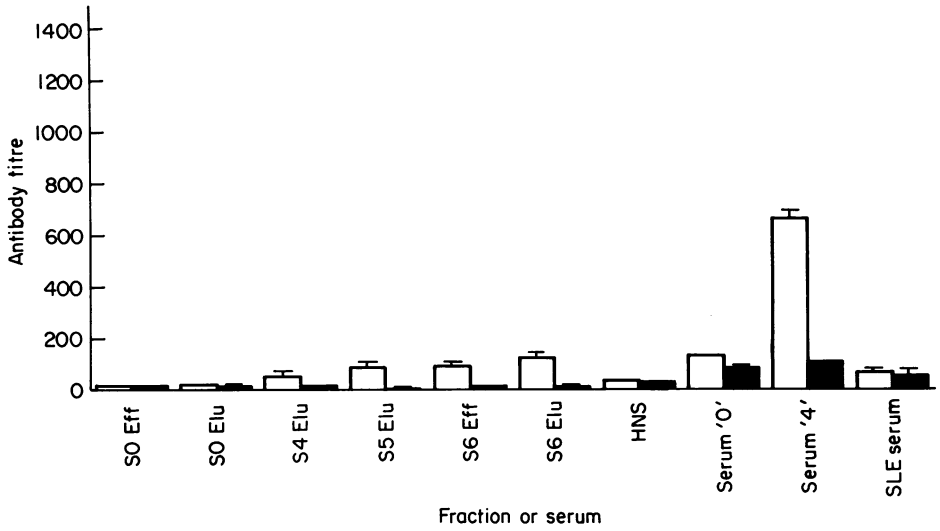

Fig. 4. Anti-laminin titre before ('non-adsorbed') (□) and after adsorption (■) on sheep erythrocytes. Tests were driven on fractions with anti-laminin IgG activities higher than 100 U Ab activity and in normal and pathological control sera. Samples were diluted at a final concentration of 2·5 μg/ml. OD were read at 492 nm; values corresponding to the buffer were subtracted. SLE, Systemic lupus erythematosus.

seem to confirm previous hypotheses. It is conceivable that polyclonal activation is, in this case, the first barrier of the defence mechanism, as it is for other infections. However, molecular mimicry may take over in the outbreak of autoimmune reactions as parasitaemia recedes. Alternatively, the chemotherapy could cause perturbations [31] in the autoimmune response, accelerating the antibody specialization process.

Taking into account the fact that mouse laminin and *T. cruzi* share the α-Gal epitope recognized by chagasic patients' sera [14,18], we looked for cross-reactivity between these two antigens. Our results show an anti-*T. cruzi* activity that increases with time and remains unchanged by sheep erythrocyte adsorption: this suggests an increased affinity toward the antigen, independently of the α-Gal epitope, since the low activity reduction observed is also present in controls (Fig. 4).

Concerning anti-laminin antibodies, our experiments offer us a glimpse of the difference between 'natural' and Chagas-associated antibodies. The latter seem to be primarily directed toward the α-Gal epitope and are exhausted after incubation with the sheep erythrocytes. Nevertheless, after adsorption in both groups of samples (controls and disease-associated), some remaining anti-laminin activity can be detected, suggesting a different specificity of both types of antibodies. Such dual reactivity has been demonstrated in the case of other natural antigens, namely tubulin [32] and thyroglobulin (Bouanani *et al.*, unpublished results). In this sense, it is interesting that anti-laminin residual activity was described when chagasic patients' sera were adsorbed on sheep erythrocytes [14]; on the other hand, anti-laminin residual activity of normal sera adsorbed on sheep erythrocytes showed higher values than that of patient sera after adsorption [14]. Another report states that a whole *T. cruzi* extract kept its α-Gal sites after adsorption with normal human serum [33]. It is possible that natural autoantibodies recognize other epitopes besides α-Gal. The fact that the SLE serum remains 'insensitive' to sheep erythrocyte adsorption seems to support this idea. The role of α-Gal epitopes in the triggering of autoimmunity during Chagas' disease remains to be confirmed.

The present work argues for the role of polyclonal activation and molecular mimicry as mechanisms involved in the onset of autoimmunity during Chagas' disease.

ACKNOWLEDGMENTS

We thank Guillaume Dighiero, Pasteur Institute, Paris, for his support and invaluable advice in discussions on autoimmunity, and Mrs Reine Kossoboutzky-Bouyssié for kindly collaborating in typing this manuscript. This work was partially supported by SAREC (Ref. BIL-URU-OO-A).

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