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Adsorption of *Trypanosoma cruzi* proteins to mammalian cells in vitro

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Summary

It has previously been shown using immunological techniques that antigens from *Trypanosoma cruzi* adsorb to mammalian cells. Here we have used radioactively-labelled *T. cruzi* antigens to investigate the nature of the antigens bound. We have demonstrated that numerous *T. cruzi* polypeptides rapidly become adsorbed to mammalian cells in tissue culture. These polypeptides dissociate from the mammalian cells only at a slow rate. We found no indication that the binding of the polypeptides was mediated by the specific binding of any one protein.

Key words: *Trypanosoma cruzi*, Chagas' disease; immunopathogenesis; antigen adsorption.

Introduction

Human infection with the protozoan parasite *Trypanosoma cruzi* has a variable outcome. In many patients it is asymptomatic but in others overt Chagas' disease may follow (for review, see Santos-Buch, 1979). The mechanisms by which *T. cruzi* infection produces the pathology of the disease have yet to be fully elucidated. However, evidence has been accumulated in the past few years which suggests that the immune response to *T. cruzi* is involved at critical stages in the development of the disease. Immunological cross-reactivity between *T. cruzi* and some mammalian cells has been demonstrated (Teixeira et al., 1978; Wood et al., 1982). In addition, it has been shown that antibodies raised against *T. cruzi* react with host cells which have been exposed to *T. cruzi* antigens (Ribeiro dos Santos and Hudson, 1980). Related studies have indicat-

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ed that such adsorption of parasite antigens might also play an important role in the destruction of uninfected host cells (Ribeiro dos Santos and Hudson, 1981; Lopez et al., 1983). Here we report an investigation of the nature and diversity of this parasite material.

Materials and Methods

Preparation of parasites and radiolabelled parasite material

Y strain epimastigotes of *T. cruzi* were grown in continuous flow chemostat culture to ensure high viability prior to radioactive labelling of parasite proteins (Williams and Hudson, 1982).

Intrinsic labelling: Epimastigotes, at 3×10^7 /ml, were incubated at 27°C for 5 h in methionine-free Eagle's Minimal Essential Medium (Flow Laboratories, Irvine, Scotland) containing bovine serum albumin (100 µg/ml) and 200 µCi/ml ^{35}S -methionine (specific activity 1400 Ci/mmol, 11 mCi/ml, Amersham International plc).

Antigen isolation. Unlabelled and ^{35}S -methionine labelled parasites were washed 5 times in phosphate buffered saline (PBS) by centrifugation ($600 \times g$ for 10 min at 4°C). Parasites were disrupted by 3 cycles of freezing and thawing, and the supernatant collected after centrifugation at $30,000 \times g$ for 60 min at 4°C to remove cell debris (Ribeiro dos Santos and Hudson, 1980).

Extrinsic labelling. Soluble antigen prepared as above, but from unlabelled parasites, was iodinated using the chloramine-T technique (Greenwood et al., 1963) at the rate of 1 mCi ^{125}I (carrier free, Amersham International plc) per 150 µg protein. Protein-bound radioactivity was recovered as the first peak eluting after the void volume from a column of Sephadex G-50.

In studies on the binding of detergent-solubilised antigens, the non-ionic detergent, Renex 30 (Atlas Chemical Industries [U.K.] plc, Leatherhead) was added to a final concentration of 0.1, 0.5 or 1.0% w/v.

Binding of parasite material to mammalian cells

The mouse muscle tumour line S2 was maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) heat-inactivated foetal calf serum (Flow) and 160 µg/ml gentamycin (Roussel plc., Dublin, Ireland) in tissue culture flasks (3075 Costar plastics, 25 cm² growth area). S2 cells for use in antigen binding experiments were released from the tissue-culture flasks by trypsinisation, resuspended in complete DMEM at 9×10^5 cells/ml and allowed to adhere to precleaned 13 mm diameter circular glass cover slips (Chance Proper plc, Smethwick, U.K.), in 24-well Costar plates. After 12 h at 37°C, the culture medium was removed and 50 µl of radio-labelled parasite antigen 100 µg/ml in PBS with 0.1% w/v bovine serum albumin (BSA; Sigma Chemical Company, London, U.K.) was added to each coverslip culture. Coverslips were incubated for 1 h at 37°C unless stated otherwise. After incubation, coverslips were washed 3 times with PBS. S2 cells were removed from the coverslips by vigorous aspiration using a Pasteur pipette, resuspended in 10 ml of PBS and concentrated by centrifugation at $200 \times g$ for 10 min at 4°C.

Analysis of adsorbed materials

Cell pellets were resuspended in 1 ml of PBS and 9 ml of 90% (v/v) ethanol was added. After 72 h at -20°C, alcohol-insoluble material was pelleted by centrifugation. The radioactivity contained in the protein pellet was determined either directly by γ counting or after solubilisation in Soluene 350 (Packard Instruments Co.) for β counting. Alcohol-insoluble pellets to be analysed by electrophoresis were redissolved in sample buffer (2% sodium dodecyl sulphate, 10% glycerol, 0.1 M dithiothreitol, 80 mM Tris-HCl, pH 6.8) and analysed by sodium dodecyl sulphate-polyarylamide gel electrophoresis (SDS-PAGE; Laemmli, 1970). The distribution of ^{35}S in the gel was determined by fluorography (Bonner and Laskey, 1974).

Results and Discussion

Rate of adsorption of parasite material

S2 cells on coverslips were incubated with ^{35}S -labelled parasite proteins at 37°C . After various periods, the S2 cells were washed and the radioactivity associated with the mouse cells determined. Fig. 1 shows the time course of adsorption of the parasite protein. Only a small proportion – about 1% after 60 min – of the parasite protein was bound. It is clear that the rate of binding was rapid over the first 5 min of incubation. However, saturation was not reached at this time, and the quantity of protein binding to the S2 cells continued to increase for at least 60 min. The protein adsorbed was strongly bound and was not readily removed even by resuspension of cell pellets in PBS at 4°C and recentrifugation. The proportion of total protein bound was $1.1\% \pm 0.06$ after 3 such washes, and $1.0\% \pm 0.07$ after 4 washes.

Adsorption of parasite material was not significantly affected by reducing the temperature to 4°C . The amount of ^{125}I -labelled parasite material adsorbed after 60 min incubation at this temperature was 103.3% (range 95.5% to 111.0%) of that bound at 37°C . The association of the parasite material with the mammalian cells could not therefore be explained by pinocytosis.

Stability of the protein-S2 cell interaction at 37°C

S2 cells on coverslips were incubated for 30 min with the ^{125}I -labelled parasite preparation. The cells were washed and incubated in 5 ml of PBS at 37°C . After 30 min, the coverslips were washed and the amount of radioactivity still adsorbed was determined as described in Materials and Methods. The results shown in Table 1 indicate that only a small proportion of the bound material was released during the incubation. The slow loss of adsorbed protein

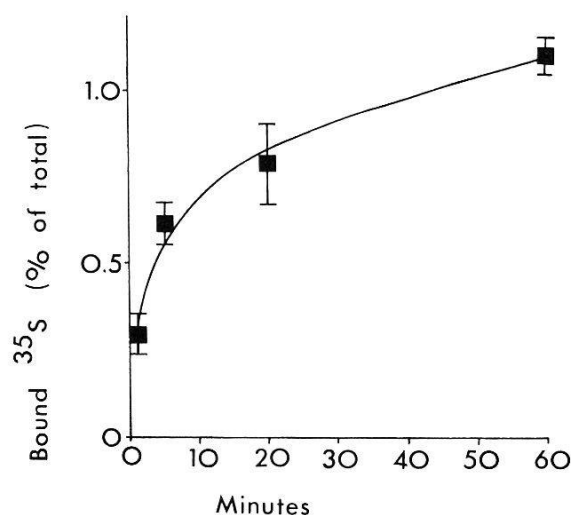


Fig. 1. Adsorption of ^{35}S -labelled *T. cruzi* proteins to S2 cells. $50\ \mu\text{l}$ of the radiolabelled material was applied to S2 cells on coverslips, which incubated at 37°C . The cells were washed after various periods of time and the proportion of the applied antigen which had bound to the cells was measured. The mean and standard error of three samples is shown in each case.

Table 1. Dissociation of adsorbed *T. cruzi* material

Period of dissociation (min) at 37° C	% of applied ¹²⁵ I-labelled material bound*
0	1.70 ± 0.03
30	1.48 ± 0.13
30 (in 10 mM azide)	1.32 ± 0.15

* mean ± standard error of 3 determinations

was not inhibited by 10 mM sodium azide indicating that no energy-requiring process, such as active internalisation, digestion and subsequent release, was involved. It is more likely that the loss of radioactivity simply represented dissociation of the bound polypeptides from the cell surface. However, the low rate at which this occurred provided a further indication that the parasite material was strongly bound.

Resolution of bound polypeptides by electrophoresis

50 µl of the ³⁵S-labelled parasite protein preparation was applied to each coverslip with its S2 cells. After 30 min at 37° C, the S2 cells were washed and the antigen associated with the cells analysed by SDS-PAGE and fluorography, as previously described. Fig. 2, track B, shows that the bound antigen was composed of many different polypeptides. Since this material was labelled by incorporation of ³⁵S methionine, it was clear that at least some of the *T. cruzi* material which had been found to bind to mammalian cells was protein. The heterogeneity of the bound material could be explained either by relatively non-specific interactions between the S2 cell surface and numerous parasite proteins, or by a specific interaction with a single antigen, or a small number of antigens, which was bound to other proteins in small micelles or protein complexes. In order to distinguish between these two possibilities the proteins binding from detergent-treated preparations were analysed. For this part of the experiment, S2 cells on coverslips were treated with 0.05% formaldehyde for 5 min at room temperature and washed 3 times with PBS. The adsorption of *T. cruzi* polypeptides to these lightly fixed cells was similar to that occurring with unfixed cells both qualitatively (Fig. 2 B and C) and quantitatively (the ³⁵S-labelled parasite material adsorbed after 60 min was 89.7% (range 83.6–95.7%) of that binding to unfixed cells). The S2 cells were incubated with the ³⁵S-methionine labelled *T. cruzi* preparation in PBS, 0.1% BSA (Fig. 2 C), in PBS, 0.1% BSA, 0.1% (w/v) Renex 30 (Fig. 2 D), in PBS, 0.1% BSA, 0.5% (w/v) Renex 30 (Fig. 2 E), or in PBS, 0.1% BSA, 1.0% (w/v) Renex 30 (Fig. 2 F). The binding of the *T. cruzi* proteins was progressively reduced by increasing concentrations of detergent. This reduction in binding may have been due either to interference with the binding of *T. cruzi* proteins to the cell surface, or possibly to limited loss of S2 surface proteins.

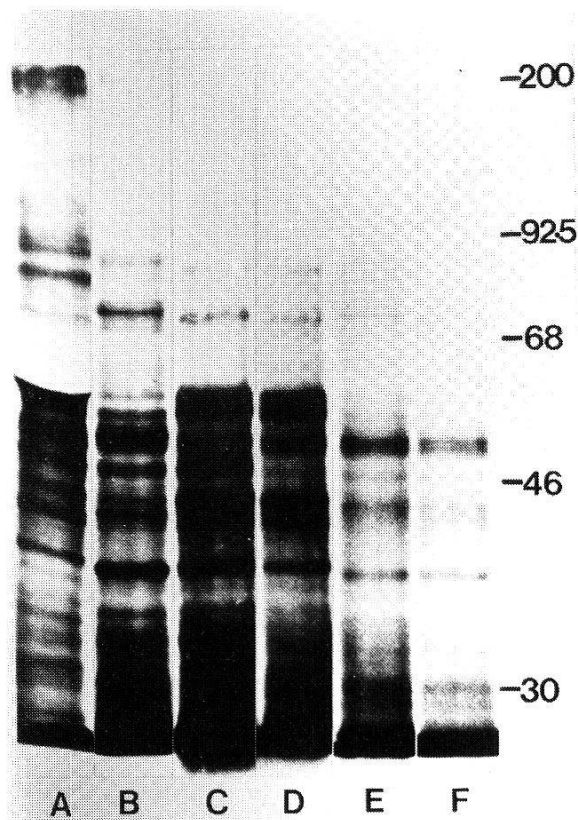


Fig. 2. Radiolabelled *T. cruzi* polypeptides binding to S2 cells. 50 μ l of the 35 S-labelled preparation, with or without detergent, was applied to fixed S2 cells on coverslips. After 30 min at 37°C, unbound radioactivity was washed away and the material bound to the S2 cells was analysed by SDS-PAGE and fluorography. A. Applied material (the mobility of polypeptides of apparent molecular weight around 70 kD has been affected by bovine serum albumin added as a carrier for precipitation). B. Polypeptides bound to S2 cells. C. Polypeptides bound to 0.05% formaldehyde-fixed S2 cells. D. Polypeptides solubilised in 0.1% (w/v) Renex 30 which bound to 0.05% formaldehyde-fixed S2 cells. E. Polypeptides solubilised in 0.5% (w/v) Renex 30 which bound to 0.05% formaldehyde-fixed S2 cells. F. Polypeptides solubilised in 1.0% (w/v) Renex 30 which bound to 0.05% formaldehyde-fixed S2 cells. The molecular weights in kD of standard protein markers are shown.

Whatever the mechanism by which the binding was reduced, no indication of selective retention of any particular polypeptide could be obtained. This suggested either that the binding of the *T. cruzi* antigens to the S2 cell surface was relatively non-specific, or that the specific interactions with particular polypeptides which normally occurred were prevented even by the non-ionic detergent used, and could not be distinguished from non-specific interactions.

The observed adsorption of a large number of *T. cruzi* proteins with high avidity for the surface of mammalian cells suggests that cells exposed to such antigens in vivo could become targets for the host's own immune response against the parasite. Previous studies have shown that anti-*T. cruzi* antibodies and cytotoxic T-cells do indeed bind to mammalian cells treated with parasite antigen (Ribeiro dos Santos and Hudson, 1980a, b). Cells to which parasite antigens have adsorbed can also be killed by eosinophils and neutrophils

(Lopez et al., 1983). The potential importance of this mechanism in the development of Chagas' disease has recently been reviewed (Hudson, 1983).

Since we have observed no indication of a specific interaction between the mammalian cell surface and any one particular parasite protein, it may be that similar interactions between the host cell surface and proteins from other parasites also occur. Synergism between this effect and other relevant mechanisms, such as immunological cross-reactivity between parasite and host proteins (Teixeira et al., 1975; Wood et al., 1982) may therefore play a crucial role in the immunopathogenesis characteristic of Chagas' disease.

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