Binding of the specific ligand to Fc receptors on *Trypanosoma cruzi* increases the infective capacity of the parasite

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Accepted for publication 7 September 1990

SUMMARY

The infective capacity of *Trypanosoma cruzi* was significantly increased after treatment with monoclonal IgG1 antibodies, whether or not specific for the parasite; minimal or no change in infectivity was seen after treatment with IgG2a, IgG2b or IgG3 monoclonal antibodies. The stimulatory effect was evidenced by elevated numbers of trypanosomes invading mammalian host cells *in vitro* compared to parasites treated with medium alone. Greater infectivity was also induced by pure human Fc, suggesting a role for Fc receptors on the organism. This inference received support in the fact that protein A inhibited the stimulatory effect of Fc. In addition, Fc-treated parasites incubated with fluorescein-labelled $F(ab')_2$ from goat anti-human IgG exhibited fluorescence detectable by both ultraviolet microscopy and flow cytometry. ^{125}I-Fc binding to *T. cruzi* was found to be saturable at 0° and was inhibited by cold Fc but not by bovine serum albumin (BSA) or orosomucoid. Interestingly, ^{125}I-Fc binding was greater at 37° and it was not saturable with the concentrations that did saturate at 0°. Possibly, Fc might up-regulate expression of its own receptor and greater endocytosis could take place at 37°. Significant increases in infectivity were detectable after a 40 min pretreatment with Fc—hinting that Fc could trigger a chain of biochemical events underlying the phenomenon—and were reversible, becoming undetectable 2 hr after Fc removal. The average number of Fc receptors per parasite, determined at 0° (at which binding saturation was possible), was estimated as 5×10^5, the dissociation constant was of the order of 10^{-6}–10^7M. The present results define an important biological role for an Fc-binding *T. cruzi* surface component and expose the capacity of this organism to exploit even elements of the immune system in its quest to attain intracellular localization, required for multiplication.

INTRODUCTION

A number of surface components of the protozoan *Trypanosoma cruzi* (etiological agent of Chagas' disease) has been identified by using monoclonal antibodies,[1] analysing parasite extracts,[2-4] testing the ability of the parasite to bind a variety of ligands,[5] parasite gene cloning[5] or monitoring adherence to the organism by erythrocytes coated with ligands of interest.[6] Whereas these efforts have included studies on the physical and chemical properties of the parasite surface components, very little is known about the biological significance of the latter. In preliminary experiments designed to screen possible inhibitors of *T. cruzi* infectivity we noted that a monoclonal antibody specific for a surface epitope of this flagellate enhanced rather than curtailed host cell invasion *in vitro*. This observation was unusual inasmuch as all of the antibody-mediated cytotoxic reactions tested to-date (e.g. antibody-mediated complement-dependent lysis, antibody-dependent cell-mediated cytotoxicity and phagocytosis of opsonized organisms) had been found to kill *T. cruzi*.[7-12] Moreover, the immune system has been reported to play a major role in containing the course of *T. cruzi* infection in both laboratory animals[13-16] and humans.[17] In the present study, we took a closer look at the noted enhancing effect and were able to establish that its occurrence was not linked to the specificity of the tested monoclonal antibody but, instead, appeared to result from binding to surface Fc receptors expressed by *T. cruzi*. The present results ascribe an important biological function to a *T. cruzi* surface component and demonstrate for the first time the ability of this pathogenic protozoan to increase its infectivity upon binding immunoglobulins.

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Abbreviations: BSA, bovine serum albumin; DMEM, Dulbecco's modified minimal essential medium containing penicillin and streptomycin; DMEM+BSA, DMEM supplemented with 1% BSA; DMEM+FBS, DMEM supplemented with 10% heat-inactivated foetal bovine serum; PBS, phosphate-buffered saline, pH 7·2.

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MATERIALS AND METHODS

Parasites

Trypomastigote forms of *T. cruzi* (Tulahuén isolate) were obtained from the blood of 5–6-week-old Crl-CD-1(ICR)BR Swiss mice (Charles River Breeding Laboratories, Portage, MI) infected subcutaneously with 1×10^6 parasites 8–10 days previously. The organisms were purified by centrifugation over a mixture of Ficoll and Hypaque of specific gravity 1·077,[18] followed by chromatography through a DEAE–cellulose column.[19] The eluted parasites (100% trypomastigotes) were centrifuged (800 *g*, 4°, 20 min) and resuspended at the desired concentration (see the Results) in Dulbecco's modified minimal essential medium containing 100 IU penicillin and 100 μg streptomycin per ml (DMEM) without or with 1% bovine serum albumin (DMEM + BSA).

Biological reagents

An IgG1 monoclonal antibody which recognizes a surface epitope of *T. cruzi* epimastigotes and trypomastigotes (MAb-10)[20] was kindly provided by Dr A. Alcina, New York University Medical Center. Other monoclonal antibodies, namely an IgG1 (anti-slp; specific for a mouse complement component), an IgG3 (VIF-8; which recognizes an epitope of amastigotes but does not bind to trypomastigotes of *T. cruzi*), an IgG2a (HB-5, specific for mouse complement receptor type 2), and an IgG2b (G3E; specific for C3), were generous gifts from Drs K. Ito, V. Nussenzweig and V. Ley, New York University Medical Center. An additional irrelevant monoclonal IgG1 (AB1-2, specific for the T15 idiotype) was kindly provided by Dr K. Brooks, Michigan State University. Orosomucoid, BSA and protein A (binding capacity = 6–8 mg human IgG/mg) were purchased from Sigma Chemical Company (St Louis, MO). Fc purified from human IgG was obtained from Accurate Chemical and Scientific Corporation (Westbury, NY).

Rat heart myoblasts (RHM)

For host cell maintenance, monolayers of RHM (American Type Culture Collection CRL 1446; Rockville, MD) were grown in sterile plastic flasks at 37°, 10% CO_2 and 100% humidity, using DMEM supplemented with 10% heat-inactivated (56°, 1 hr) foetal bovine serum (FBS; Hyclone Sterile Systems, Logan, UT). This medium will be referred to in the text as DMEM + FBS. Cultures of RHM to be used in cell infection assays (see below) were set up under the same conditions on 3-mm diameter wells cut on sterilized, Teflon-coated microscope slides (Cel-Line, Newfield, NJ) as described in detail previously.[21,22] The RHM were allowed to adhere and grow overnight in the CO_2 incubator and, after washing three times with serum-free DMEM, the cultures were used immediately. Under these conditions, the number of cells in each culture was approximately 10,000.

Parasite and RHM treatments, and RHM infection assay

Suspensions of *T. cruzi* at 2×10^6 trypomastigotes/ml, containing or lacking the reagent(s) to be tested, were incubated at 37° for various periods of time (described in the Results). The parasites were then washed with DMEM by centrifugation (800 *g*, 4°, 10 min) and resuspended at 1×10^7 organisms/ml in DMEM or DMEM + BSA. Fifteen microtitres of this suspension were added to each RHM culture. Unless otherwise indicated, the RHM-*T. cruzi* co-cultures were incubated at 37° and 10% CO_2 for 24 hr, at which time the free trypanosomes were removed by washing with DMEM. In experiments designed to establish whether noted effects were reversible, the parasites were treated as described above, washed with and resuspended in DMEM, and incubated at 37° for various periods of time before being added to RHM cultures. In some experiments, RHM cultures were incubated with DMEM alone or containing purified human Fc for 2 hr (37°, 10% CO_2) and washed with DMEM before addition of untreated, purified *T. cruzi*. These cultures were also incubated for 24 hr before removing the free organisms. At termination time, all cultures were washed with PBS, fixed with absolute methanol, stained with Giemsa and examined microscopically ($\times$ 1000). Not less than 200 RHM were randomly screened in each culture, recording the number of screened RHM, the number of RHM containing one or more parasites and the total number of organisms in the screened cells. From these data, the percentage of infected RHM and the average number of *T. cruzi* per 100 RHM were calculated. All control and experimental conditions were set up in triplicate and the results expressed as the mean $\pm$ standard deviation. In all experiments, the average number of RHM per microscopic field in control and experimental cultures were comparable. When screened, all parasites in the cultures were in the intracellular, amastigote stage.

Indirect immunofluorescence

Parasites purified as described above and resuspended in phosphate-buffered saline solution, pH 7·2 (PBS), supplemented with 1% BSA were mixed with the same solution alone or containing Fc so that the final concentration of this protein would be 100 μg/ml. After incubation at 37° for 30 min, the trypanosomes were washed three times by centrifugation with PBS containing 0·1% sodium azide, resuspended in a 1/40 dilution in PBS of fluorescein-labelled $F(ab')_2$ from goat anti-human IgG (gamma chain specific; Cappel, Malvern, PA) and incubated at 37° for 30 min. After three washings with PBS plus sodium azide, the organisms were fixed with 1% formaldehyde and examined by fluorescence microscopy.

Flow cytometry

Parasites stained as described in the preceding paragraph were analysed in a FACS IV flow cytometer. Cellular debris were excluded by thresholding on forward light scattering. A total of 10,000 parasites was accumulated in each histogram. Calculations were made after subtracting the background due to non-specific staining with fluorescein-labelled $F(ab')_2$ from goat anti-human IgG.

Iodination of human Fc

Human Fc was radio-iodinated with $Na^{125}I$ (specific activity = 16 mCi/μg of iodine; Amersham Life Science Products, Arlington Heights, IL) in the presence of Iodogen (Pierce, Rockford, IL) at 0° for 20 min. Unbound radioactivity was removed by filtration through Sephadex G-25 (Pharmacia, Piscataway, NJ) equilibrated with PBS. The specific activity of ^{125}I-Fc was determined by measuring the radioactivity of protein precipitated with 5% trichloroacetic acid.

Table 1. Effects of pretreatment with different IgG isotypes on the infective capacity of *T. cruzi**

Exp. no.	Parasites treated with	% infected RHM	Parasites, no. per 100 RHM
1	DMEM	29·2±2·3	72·0±13·0
	MAb-10 (IgG1)	24·9±3·5	116·0±11·0
	Irrelevant IgG1	34·4±4·8	130·0±18·9
	Irrelevant IgG2a	22·5±0·8	69·0±7·9
	Irrelevant IgG2b	23·3±2·2	71·3±25·0
2	DMEM	25·5±4·0	31·6±2·1
	DMEM+BSA	22·4±2·6	24·4±2·3
	Irrelevant IgG1	29·2±5·9	62·0±6·9
	Irrelevant IgG2a	22·5±5·4	39·4±6·8
	Irrelevant IgG2b	24·5±1·7	24·8±2·3
	Irrelevant IgG3	27·8±1·0	29·7±1·5
3	DMEM+BSA	28·6±2·1	40·3±7·9
	Irrelevant IgG1	24·4±12·8	147·0±26·0
	Irrelevant IgG2a	27·7±4·4	91·0±25·0
	Irrelevant IgG2b	25·4±2·7	34·1±3·6
	Irrelevant IgG3	29·5±4·6	37·4±0·4

* *T. cruzi* trypomastigotes were incubated at 37° with medium alone or containing 50 ng/ml (exp. no. 1), 10 ng/ml (exp. no. 2) or 100 ng/ml (exp. no. 3) of the indicated monoclonal antibody for 40 min and washed with DMEM before being co-cultured with RHM for 24 hr. DMEM was the medium used in exp. no. 1. In exp nos 2 and 3, except for the DMEM control, all other co-cultures were performed with DMEM+BSA. In all experiments only the increases in the number of parasites per 100 RHM resulting from treatment with MAb-10 or irrelevant IgG1 antibodies were statistically significant ($P<0·05$, Mann–Whitney *U*-test).

Table 2. Effects of *T. cruzi* treatment with varying concentrations of human Fc on infectivity*

Fc, ng/ml	% infected RHM	Parasites, no. per 100 RHM
0	25·8±4·7	41·8±3·6
0·001	21·3±2·2	38·7±4·2
0·01	24·3±3·0	43·5±5·0
0·1	29·2±5·1	56·4±3·3
1	33·9±3·7	106·0±9·1
10	33·2±1·4	131·3±7·2
100	39·4±3·1	138·8±14·8
1,000	42·4±4·2	155·9±20·0
10,000	43·2±5·1	156·0±15·4

* *T. cruzi* trypomastigotes were incubated at 37° in DMEM alone or containing the indicated concentration of Fc for 40 min, and washed before being co-cultured with the RHM for 24 hr. In this experiment, the increases in % infected RHM obtained with Fc concentrations ≥100 ng/ml, and the increases in the no. parasites per 100 RHM caused by Fc concentrations ≥0·1 ng/ml were statistically significant ($P\leq0·05$, Mann–Whitney *U*-test).

Table 3. Effect of protein A on the enhancing effects of Fc*

Parasite treatment	Parasites, no. per 100 RHM
Medium alone	35·3±7·5
Protein A, 250 μg/ml	44·8±10·1
Fc, 100 ng/ml	93·9±19·0
Fc, 100 ng/ml+protein A, 250 μg/ml	18·0±5·1

* *T. cruzi* trypomastigotes were incubated at 37° for 40 min with the indicated reagents, washed and co-cultured with the RHM for 24 hr. Only the increase produced by Fc in the absence of protein A was statistically significant ($P<0·05$, Mann–Whitney *U*-test).

Determination of Fc receptors on T. cruzi

Mixtures (final volume = 250 μl) containing $0·8–2\times10^6$ *T. cruzi* and increasing amounts of ^{125}I-Fc (see the Results) were incubated at 0° or 37° for 40 min. The organisms were then washed three times by centrifugation with PBS and radioactivity bound to the pelleted parasites was determined using a gamma counter. Free radioactivity was calculated by subtracting bound from total radioactivity added.

Fc binding to T. cruzi *and binding competition*

Fc binding competition was studied at 0° under the conditions described above, except that a constant amount of ^{125}I-Fc was added to all tubes (1–3 μg in the various repeat experiments) and 500 μg of cold Fc, orosomucoid or BSA were present in some of the reaction mixtures.

RESULTS

Effects of immunoglobulin isotypes on the infective capacity of *T. cruzi*

Our initial experiments tested the effect of an IgG1 monoclonal antibody specific for an epitope present on both epimastigotes and trypomastigotes of *T. cruzi*, termed MAb-10,[20] on the infective capacity of bloodstream forms of the parasite. This antibody increased infectivity to an extent comparable to that produced by an irrelevant IgG1 monoclonal antibody (Table 1, exp. No. 1). This observation was readily reproduced in repeat experiments, which also revealed that immunoglobulin isotypes other than IgG1 had minimal or no detectable effect on infectivity. Whereas in all experiments stimulation by IgG1 was evidenced by a marked increase in the number of parasites per 100 RHM, a relatively small but nevertheless statistically significant elevation in the proportion of infected RHM was also observed in a few instances using 100 ng IgG1/ml (data not shown). It should be noted that, unlike MAb-10, the tested IgG3 (VIF-8) recognizes an epitope of *T. cruzi* amastigotes and does not cross-react with the trypomastigote form (Dr Kyoko Ito, personal communication).

Table 4. Kinetics of the induction of the Fc effect on *T. cruzi* infectivity*

Treatment with	Treatment duration (min)	Parasites, no. per 100 RHM
DMEM	10	10·0±2·8
Fc	10	14·1±1·5
DMEM	25	11·5±6·9
Fc	25	19·3±6·6
DMEM	40	26·5±7·2
Fc	40	56·7±12·0
DMEM	60	15·1±1·4
Fc	60	52·6±3·6

* The parasites were incubated with DMEM alone or containing 100 ng Fc/ml for the indicated times, washed and immediately added to the RHM cultures. Only the increases caused by the 10- and 25-min Fc treatments were not statistically significant ($P \leq 0{\cdot}05$, Mann–Whitney *U*-test).

Table 5. Reversibility of the Fc effect on *T. cruzi* infectivity*

Treatment with	Time after washing (min)	Parasites, no. per 100 RHM (% increase)
DMEM	30	21·1±0·5
Fc	30	79·1±4·9 (275)
DMEM	60	30·9±7·0
Fc	60	53·7±7·0 (74)
DMEM	90	27·5±5·9
Fc	90	43·5±10·0 (58)
DMEM	120	21·5±4·1
Fc	120	18·2±1·8 (−15)

* The parasites were incubated with DMEM alone or containing 100 ng Fc/ml for 40 min, washed with and resuspended in DMEM for the indicated periods of time before addition to the RHM cultures. The 30-, 60- and 90-min values for Fc-treated *T. cruzi* represent statistically significant increases over the corresponding control value ($P \leq 0{\cdot}05$, Mann–Whitney *U*-test).

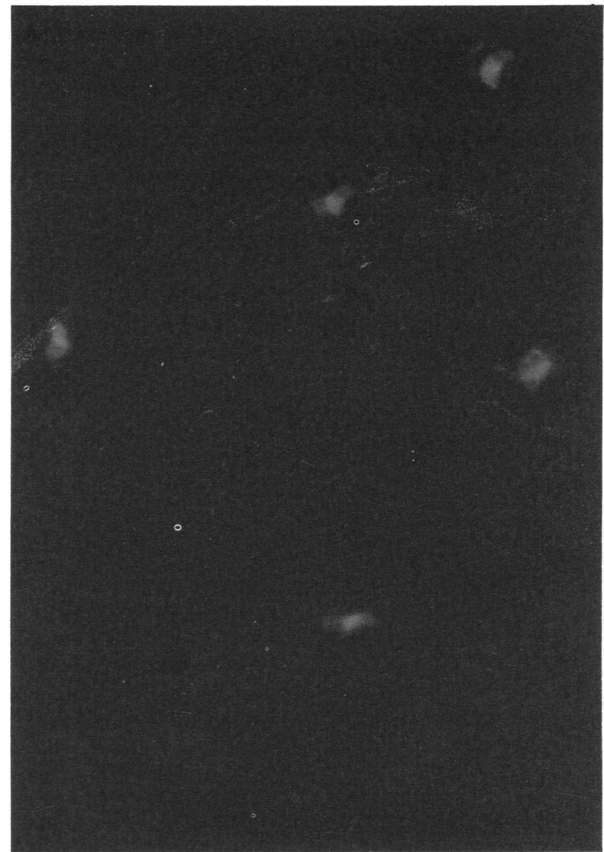

Figure 1. Detection of Fc binding to *T. cruzi* by indirect immunofluorescence. The flagellates were incubated with human Fc, washed with PBS, incubated with fluorescein-labelled $F(ab')_2$ specific for the gamma chain of human IgG, washed again with PBS and fixed with formaldehyde before examination by ultraviolet microscopy. Magnification ×400.

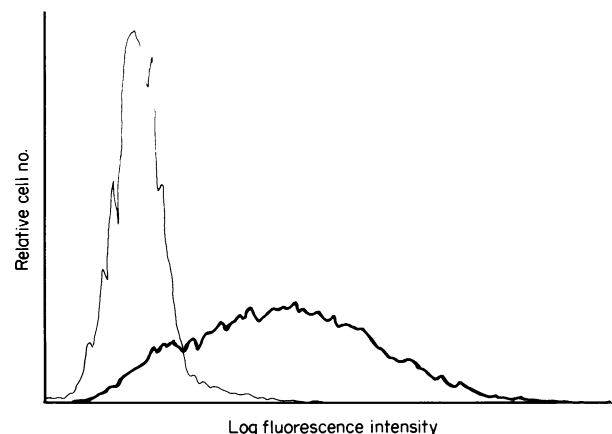

Figure 2. Flow cytometric analysis of *T. cruzi* trypomastigotes. The parasites were incubated with DMEM alone (thin curve) or containing Fc (thick curve), washed with PBS, treated with fluorescein-labelled $F(ab')_2$ specific for the gamma chain of human IgG, washed again with PBS and fixed with formaldehyde before analysis.

Effects of human Fc on *T. cruzi* infectivity

The lack of relationship between antibody specificity and ability to enhance infectivity suggested that the stimulatory activity could reside in a common structure such as, for example, the Fc portion. To explore this possibility, we treated *T. cruzi* with purified Fc and observed again enhanced infectivity (Table 2). Significant increases were elicited with 1 ng Fc/ml and, in a few experiments, with as little as 0·1 ng/ml (Table 2). The lowest concentration of Fc required to produce maximal stimulation fluctuated in the various repeat experiments between 10 and 100 ng/ml (intermediate concentrations were not tested). In these assays, the number of organisms per 100 host cells was found to be a more sensitive parameter than the percentage of infected RHM. Whereas increases in the percentage of infected RHM

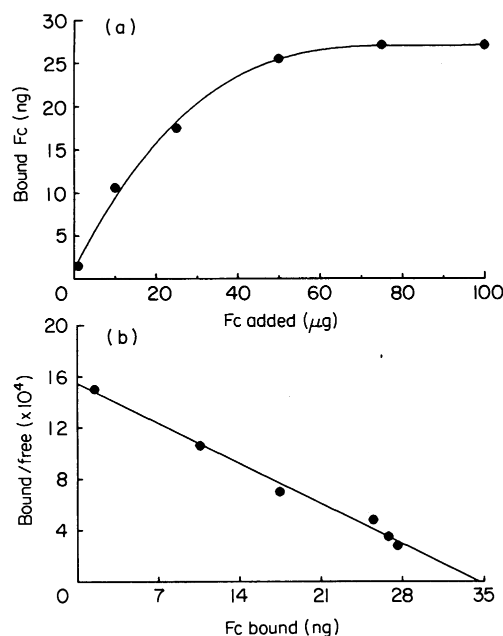

Figure 3. Binding of ^{125}I-Fc to *T. cruzi* trypomastigotes at 0°. (a) Saturation curve; (b) Scatchard plot. These data are from a typical experiment repeated three times. For this set of results, the calculated number of Fc molecules bound per organism at saturation (at 0°) was $4{\cdot}1 \times 10^5$ and $K_d = 1{\cdot}8 \times 10^6$ M.

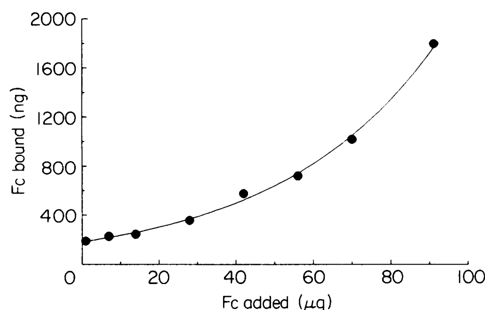

Figure 4. Binding of ^{125}I-Fc to *T. cruzi* trypomastigotes at 37°. A continuous increase in Fc binding was observed with up to 90 μg ^{125}I-Fc.

were occasionally observed using 100 ng Fc/ml, they were consistently recorded at higher concentrations. The Fc effect was inhibited by the presence of the Fc-binding reagent protein A, which by itself had no detectable consequence on infectivity (Table 3). Pretreatment of the RHM with Fc had no demonstrable effect on their susceptibility to infection by untreated *T. cruzi* (data not shown).

Kinetics of the Fc effect

Experiments designed to establish the minimum amount of time required for Fc to induce a significant increase in *T. cruzi* infectivity defined it as 40 min (Table 4). We also tested whether the Fc effect was reversible. Trypanosomes incubated with Fc, washed with and resuspended in fresh medium, were allowed to stand at 37° for increasing amounts of time before being added to RHM cultures. Controls were included for each time-point, using trypanosomes mock-treated with medium alone, to account for any spontaneous changes in infectivity that might have occurred during incubation. The results of a representative experiment are presented in Table 5, showing that the extent of the Fc effect started to subside after 60 min and became undetectable after 120 min.

Fc receptors on *T. cruzi*

Direct evidence for the binding of Fc to *T. cruzi* was obtained by indirect immunofluorescence staining. Parasites which had been incubated with Fc and washed, presented bright, diffuse immunofluorescence after incubation with fluorescein-labelled $F(ab')_2$ specific for the gamma chain of human IgG (Fig. 1). The binding of Fc to *T. cruzi* was confirmed by flow cytometric analysis (Fig. 2), which also revealed that approximately 80% of the trypomastigote population had Fc bound to their surface. We also measured parasite-bound radioactivity after incubating the trypanosomes with ^{125}I-Fc and washing off the free radiolabelled molecules. ^{125}I-Fc binding to *T. cruzi* was markedly inhibited in the presence of cold Fc. Thus, in a typical experiment, the value obtained by incubating the flagellates with ^{125}I-Fc alone at 0° was 937,289 c.p.m., whereas in the presence of cold Fc it was reduced to 292,902 c.p.m. No significant binding competition was observed when cold irrelevant proteins, such as orosomucoid and BSA, substituted for cold Fc in this type of binding competition assays (data not shown). ^{125}I-Fc binding to *T. cruzi* was saturable at 0° (Fig. 3); in three repeat experiments the number of Fc molecules bound per organism at saturation was $4{\cdot}1 \times 10^5$, $1{\cdot}6 \times 10^5$ and $9{\cdot}9 \times 10^5$; the corresponding K_d values, calculated by Scatchard analysis, were $1{\cdot}8 \times 10^{-6}$, $2{\cdot}3 \times 10^{-7}$ and $3{\cdot}6 \times 10^{-6}$ M, respectively. It is noteworthy that these determinations were made at 0° because saturation could not be attained at 37°, all other conditions remaining unchanged (Fig. 4).

DISCUSSION

These results demonstrate that ligand binding to Fc receptors on virulent *T. cruzi* trypomastigotes lead to increased parasite infectivity, and define a significant biological role for a surface component of this protozoan. The finding that immunoglobulins, mainly IgG1, can up-regulate *T. cruzi* infectivity is novel and striking in that it defines an antibody-induced effect on the parasite which, far from being deleterious, may benefit the pathogen by increasing its capacity to attain a sheltered intracellular localization.

Although MAb-10, an IgG1 specific for *T. cruzi*, induced increased infectivity, its activity did not differ significantly from that displayed by irrelevant IgG1 molecules (Table 1), indicating that antibody specificity was not a necessary condition to produce the enhancement. This conclusion found additional support in the stimulatory effect caused by purified Fc (Table 2), which also pinpointed the immunoglobulin portion responsible for the effect.

The concentration-dependence of the Fc effect (Table 2) suggested that a threshold number of Fc receptors might have to be engaged by the specific ligand for increased infectivity to become detectable in our assay system. Maximal stimulation of infectivity, achieved with the highest Fc concentrations tested,

was accompanied by noticeable increases in the percentage of infected RHM. This suggested that host cell contacts with parasites were more likely to result in infection if the latter had been pretreated with adequate concentrations of Fc. If so, Fc binding to *T. cruzi* would be expected to be followed by the expression of new, or modulated expression of previously present, parasite surface components playing a role in parasite attachment to host cells. This possibility remains to be explored.

In our *in vitro* system, detection of significantly increased infectivity required a 40-min Fc treatment (Table 4). This lag time, for which an explanation is not available at present, suggested Fc binding might trigger a chain of biochemical events from which enhanced infectivity results. Biological regulatory mechanisms are usually subject to up- and down-regulation and are, therefore, usually reversible. In this context, the reversibility of the Fc effect (Table 5) suggested that Fc binding to its receptors may be an important element of the regulatory mechanisms governing *T. cruzi* infectivity.

The presence of Fc receptors on *T. cruzi* trypomastigotes was first reported by De Miranda-Santos & Campos-Neto,[6] who used a technique involving rosetting of antibody-coated erythrocytes by trypsinized parasites; very few (<1%) non-trypsinized organisms formed rosettes. However, in our work, Fc binding to non-trypsinized *T. cruzi* was readily demonstrable by both immunofluorescence (Fig. 1) and ^{125}I-Fc binding (Figs 3 and 4), and flow cytometric studies revealed that 80% of the organisms expressed Fc receptors (Fig. 2). We verified the specificity of Fc binding to the parasite through competition experiments, which showed that cold Fc but not irrelevant proteins reduced ^{125}I-Fc binding significantly. Moreover, protein A, an Fc-binding agent, inhibited Fc-enhanced infectivity (Table 3). The apparent discrepancy between the early work of De Miranda-Santos *et al.*[6] and our own probably has its roots in the different methodological approaches used to detect Fc receptors. Conceivably, the erythrocytes used as immunoglobulin carriers in the rosetting technique could have limited immunoglobulin accessibility to the untreated but not to the trypsinized trypomastigote surface.

Saturation of Fc receptors on *T. cruzi* was demonstrable at 0° (Fig. 3) but not at 37° (Fig. 4) under otherwise identical conditions. This contrast infers (i) that Fc up-regulated the expression of its own receptor, and (ii) that Fc endocytosis was increased at 37°, or a combination of these possibilities.

We previously reported that *T. cruzi* trypomastigotes express receptors for alpha- and beta-adrenergic agonists.[23] Specific ligands binding to these receptors were found to increase and decrease, respectively, parasite infectivity for RHM with respect to that of organisms which had been mock-treated with culture medium alone. From this point of view, the present results advance further the notion that the mechanism(s) involved in host cell penetration by *T. cruzi* is subject to regulation via interaction of parasite surface components with host molecules. The nature of the Fc receptor on *T. cruzi*, the signalling mechanism set in motion by Fc binding and the biochemical events underlying the enhancement of infectivity are exciting subjects deserving further attention.

ACKNOWLEDGMENTS

We are grateful to Dr M. B. Sztein, University of Maryland at Baltimore, for performing the flow cytometric analyses and Dr A. Alcina, New York University, Dr K. Brooks, Michigan State University, and Drs V. Ley, K. Ito and V. Nussenzweig, New York University, for providing monoclonal antibodies used in this work. This work was supported in part by a Biomedical Research Support Grant from the College of Osteopathic Medicine, Michigan State University and by Grants AI-26542 and AI-24874 from the United States Public Health Service.

REFERENCES

1. Kirchhoff L.V., Engel J.C., Dvorak J.A. & Sher A. (1984) Strains and clones of *Trypanosoma cruzi* differ in their expression of a surface antigen identified by a monoclonal antibody. *Molec. Biochem. Parasitol.* **11**, 81.
2. Takle G.B., Young A., Snary D., Hudson L. & Nicholls S.C. (1989) Cloning and expression of a trypomastigote-specific 85-kilodalton surface antigen gene from *Trypanosoma cruzi. Molec. Biochem. Parasitol.* **37**, 57.
3. Snary D. & Hudson L. (1979) *Trypanosoma cruzi* cell surface proteins: identification of one major glycoprotein. *FEBS Lett.* **100**, 166.
4. Norris K.A., Harth G. & So M. (1989) Purification of a *Trypanosoma cruzi* membrane glycoprotein which elicits lytic antibodies. *Infect. Immun.* **57**, 2372.
5. Van Voorhis W.C. & Eisen H. (1989) A surface antigen of *Trypanosoma cruzi* that mimics mammalian nervous tissue. *J. exp. Med.* **169**, 641.
6. De Miranda-Santos I.K.F. & Campos-Neto A. (1981) Receptor for immunoglobulin Fc on pathogenic but not nonpathogenic protozoa of the Trypanosomatidae. *J. exp. Med.* **154**, 1732.
7. Budzko D.B., Pizzimenti M.C. & Kierszenbaum F. (1975) Effects of complement depletion in experimental Chagas' disease: immune lysis of virulent blood forms of *Trypanosoma cruzi. Infect. Immun.* **11**, 86.
8. Kierszenbaum F. (1979) Antibody-dependent killing of bloodstream forms of *Trypanosoma cruzi* by human peripheral blood leukocytes. *Am. J. Trop. Med. Hyg.* **28**, 965.
9. Kierszenbaum F., Lima M.F. & Wirth J.J. (1985) Effects on antiserum to *Trypanosoma cruzi* on the uptake and rate of killing of vector-borne, metacyclic forms of the parasite by macrophages. *Int. J. Parasitol.* **15**, 409.
10. Reed S.G., Douglass T.G. & Speer C.A. (1982) Surface interactions between macrophages and *Trypanosoma cruzi. Am. J. Trop. Med. Hyg.* **31**, 723.
11. Kipnis T.L., James S.L., Sher A. & David J.R. (1981) Cell-mediated cytotoxicity to *Trypanosoma cruzi*. II. Antibody-dependent killing of bloodstream forms by mouse eosinophils and neutrophils. *Am. J. Trop. Med. Hyg.* **30**, 47.
12. Krettli A.U., Weisz-Carrington P. & Nuzzenzweig R.S. (1979) Membrane-bound antibodies to bloodstream *Trypanosoma cruzi* in mice: strain differences in susceptibility to complement-mediated lysis. *Clin. exp. Immunol.* **37**, 416.
13. Kierszenbaum F. & Howard J.G. (1976) Mechanisms of resistance against experimental *Trypanosoma cruzi* infection: the importance of antibodies and antibody-forming capacity in the Biozzi high and low responder mice. *J. Immunol.* **116**, 1208.
14. Rodriguez A.M., Santoro F., Afchain D., Bazin H. & Capron A. (1981) *Trypanosoma cruzi* infection in B-cell deficient mice. *Infect. Immun.* **31**, 524.
15. Hayes M.M. & Kierszenbaum F. (1981) Experimental Chagas' disease: kinetics of lymphocyte responses and immunological control of the transition from acute to chronic *Trypanosoma cruzi* infection. *Infect. Immun.* **31**, 1117.
16. Krettli A.U. & Brener Z. (1976) Protective effects of specific antibodies in *Trypanosoma cruzi* infections. *J. Immunol.* **116**, 755.
17. Mocelin A.J., Brandina L., Gordan P.A., Baldi J.L.S. & Chieffi P.P. (1977) Immunosuppression and circulating *Trypanosoma cruzi* in a kidney transplant case. *Transplantation*, **23**, 163.
18. Budzko D.B. & Kierszenbaum F. (1974) Isolation of *Trypanosoma cruzi* from blood. *J. Parasitol.* **60**, 1037.

19. Mercado T.I. & Katusha K. (1979) Isolation of *Trypanosoma cruzi* from the blood of infected mice by column chromatography. *Prep. Biochem.* **9,** 97.
20. Alcina A. & Fresno M. (1988) A tubulin-related 55 kilodalton surface antigen recognized by different *Trypanosoma cruzi* stage-specific monoclonal antibodies from infected mice. *Molec. Biochem. Parasitol.* **19,** 181.
21. Wirth J.J. & Kierszenbaum F. (1987) Inhibition of mammalian host cell infection by insect-derived, metacyclic forms of *Trypanosoma cruzi* in the presence of human or rabbit anti-*T. cruzi* antibodies. *Int. J. Parasitol.* **17,** 841.
22. Kierszenbaum F., Wirth J.J., McCann P.P. & Sjoerdsma A. (1987) Inhibition of the capacity of *Trypanosoma cruzi* to infect and multiply within mammalian host cells by treatment with inhibitors of arginine decarboxylase. *Proc. natl. Acad. Sci. U.S.A.* **84,** 4278.
23. Connelly M.C., Ayala A. & Kierszenbaum F. (1988) Effects of alpha- and beta-adrenergic agonists on *Trypanosoma cruzi* interaction with host cells. *J. Parasitol.* **74,** 379.