AMASTIGOTES OF *TRYPANOSOMA CRUZI* ESCAPE DESTRUCTION BY THE TERMINAL COMPLEMENT COMPONENTS

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In the absence of antibody, the alternative pathway of the complement system can function as a first barrier in preventing infection. However, certain microorganisms have developed mechanisms to escape attack and survive in the host's bloodstream (1). *Trypanosoma cruzi*, the causative agent of Chagas' disease, is a protozoan parasite that cycles between invertebrate insect vectors and mammalian hosts. During its development in the insect, *T. cruzi* assumes various forms and the infectivity of the parasite for the mammalian host is associated with the aquisition of resistance to lysis by complement (2–5). The epimastigote is the noninfective multiplicative form found in the gut of the insect. Epimastigotes transform into metacyclic trypomastigotes, which can invade cells of the mammalian host. While both epimastigotes and metacyclics activate the complement cascade, only epimastigotes are lysed. Metacyclics are not lysed because they have developed mechanism(s) to prevent the assembly of C3 convertase, a key amplifying enyzme of the complement system (6–9).

When trypomastigotes enter the host cells, they transform into amastigotes; the amastigotes then mulitply, transform again into trypomastigotes, and are released into the bloodstream to continue the cycle. Contrary to the conventional view that amastigotes are exclusively the intracellular multiplicative stage of the parasite, recent studies demonstrate that amastigotes can be found in circulation during the acute stages of the infection and can enter and develop in cells (10). In vitro infection of monocytes by amastigotes occurs in the presence of fresh human serum, indicating that they also avoid destruction by complement. These studies, however, did not discriminate between lack of complement activation and protection from attack.

Here, we address this question and show that amastigotes are extremely efficient activators of the cascade and that they bind terminal components. Functional channels, however, are not formed and the parasites are not destroyed.

Materials and Methods

Parasites. Strain Y of *T. cruzi* was maintained in monolayers of LLC-MK_2 cells in DMEM containing 5% FCS at 37°C in a 5% CO_2 atmosphere. Amastigotes were obtained as follows. Trypomastigotes were collected from a 5-d culture of infected cells within 24 h after changing the medium. As determined by light microscopy, most trypomastigotes transformed into

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amastigotes after incubation in liver infusion tryptose (LIT)[1] medium containing 10% FCS (LIT-FCS) for 24 h (11). If necessary, the remaining trypomastigotes were removed by centrifuging the parasites and incubating them for 2 h at 37°C. During this incubation, the contaminating trypomastigotes accumulated in the supernatant and the amastigotes remained in the pellet. Epimastigotes were cultured in LIT-FCS at 28°C with continuous agitation.

Complement Reagents and Hemolytic Assay. C8- or C9-depleted human serum (C8dpl, C9dpl) and C7 were purchased from Cytotech (San Diego, CA). Guinea pig C1 (12), human C2 (13), human C4, human C3, human C5 (14), and human C9 (15) were purified as described. C6–9 was prepared from guinea pig serum (16). EAC142 and EAC1423 cells were prepared with sensitized sheep erythrocytes and purified components and used for titration of C3 and C5. Normal human serum (NHS), diluted with medium 199 containing 10 mM EDTA or 4 mM $MgCl_2$ and 10 mM EGTA, are referred to as EDTA serum and EGTA serum, respectively.

Assay for the Presence of Lytic Antibodies in Chagasic Sera. Sera from 43 individuals with chronic Chagas' disease were obtained from Dr. M. Camargo, University of Sao Paulo, Brazil. ^{86}Rb-labeled amastigotes (2×10^6) were mixed with 10 μl of heat-inactivated patients' serum and 25 μl of NHS (1:1.25) in a total volume of 100 μl. The mixture was incubated for 30 min at 37°C, centrifuged, and ^{86}Rb in the supernatants was counted. A pool of patients' sera was prepared by mixing equal volumes of serum from 43 individuals, and was heat inactivated at 56°C for 30 min.

Radiolabeling. C3, C7, and C9 were labeled with ^{125}I using Iodogen (Pierce Chemical Co., Rockford, IL). The specific activities were between 1 and 10×10^6 cpm/μg protein. The hemolytic activity of C3 after labeling was 60% of that of the native C3, while the hemolytic activities of C7 and C9 did not change. ^{86}Rb incorporation into parasites was performed by incubating 5×10^7 parasites with 50 μCi of ^{86}Rb in 0.5 ml of LIT-FCS for 2 h at 37°C. Parasites were washed once, resuspended in medium 199, and overlaid onto 1 ml of FCS and centrifuged. They were washed once more immediately before they were used as target cells in complement-mediated lysis.

^{125}I-C3 Binding and Structural Analysis. Parasites (10^8/ml) were incubated at 37°C for 30 min in either 10% C8dpl or 10% heat-inactivated serum containing ^{125}I-C3 in a total volume of 200 μl. The reaction was stopped by adding 800 μl of cold medium 199 containing 5 mM EDTA, 1 μg/ml pepstatin, 100 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 1 mM PMSF (Sigma Chemical Co., St. Louis, MO), and 20 μg/ml synthetic elastase inhibitor Suc(OMe)-Ala-Ala-Pro-Val-MCA (Penninsula Laboratories, Inc., San Belmont, CA). The parasites were washed three times by centrifugation in medium 199 containing 1 mg/ml BSA, and radioactivity was counted in a gamma counter. To analyze the structure of C3 associated with parasites, the pellet was treated with 20 μl of 2% SDS in 0.08 Tris buffer (pH 8.8) for 20 min at 37°C. To each parasite lysate, 20 μl of 2 M hydroxylamine in 0.05 M carbonate buffer (pH 9.0) was added, and the mixture incubated further for 1 h at 37°C. After removal of insoluble materials by centrifugation, 30 μl of 10% glycerol was added. Samples were analyzed by SDS-PAGE using a 2.5%–15% gradient gel under reducing conditions, followed by radioautography.

^{125}I-C7 and -C9 Binding and Structural Analysis. Parasites (10^8/ml) were incubated with either ^{125}I-C7 or ^{125}I-C9 in NHS diluted as described in the text. After incubation, parasites were centrifuged at 12,000 *g* for 15 min, washed two times with medium 199 containing 10% FCS, followed by two further washes with 1 M NaCl. Parasites were then treated with SDS sample buffer by boiling for 5 min at 100°C and the bound complement molecules were analyzed in SDS-PAGE using a 2.5%–10% gradient gel. For immunoprecipitation experiments, the ^{125}I-C9 bound to the surface of 2.5×10^7 parasites was extracted with 300 μl of 1% 3-[(3-chloroamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) (Bio-Rad Laboratories, Richmond, CA) in 10 mM Tris buffer containing 0.15 M NaCl, 100 μg/ml leupeptin, and 1 mM PMSF for 1 h at 4°C. After removal of the insoluble material by centrifugation,

[1] *Abbreviations used in this paper:* CHAPS, 3-[(3-chloroamidopropyl) dimethylammonio]-1-propanesulfonate; LIT, liver infusion tryptose medium; NHS, normal human serum.

aliquots were immunoprecipitated with 50 μl (10 μg/ml) of an mAb that recognizes the neoantigen of poly-C9, kindly provided by Dr. R. J. Falk, University of Minnesota (17), with goat antisera to C9 (Cytotech) (diluted 1:20) or with control antibodies. After a 4-h incubation, 20 μl of protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) was added, and the incubation continued overnight. The Sepharose beads were washed four times with PBS containing 1% BSA and 0.5 mM PMSF, and the bound ^{125}I-C9 was counted.

^{86}Rb Release. Parasites labeled with ^{86}Rb were incubated with complement reagents. When kinetic studies were performed, parasites (10^8/ml) were incubated with equal volumes of 5% NHS. At indicated times, 100 μl of the reaction mixture was withdrawn into 200 μl of cold medium 199, and centrifuged for 3 min at 10,000 *g*. The supernatants were removed, the pellets lysed with 100 μl of 1% NP-40, and ^{86}Rb in supernatants and pellets were counted. The percent release was calculated using the formula: percent release = [% (T) − % (T_0)]/[(100 − % (T_0)]; where % (T) stands for percent ^{86}Rb released from the test sample incubated for the indicated period of time and % (T_0) for percent released from the control with identical reagents without incubation. When dose-dependent release was measured (Fig. 5), and the percent release was shown without subtraction.

Pronase Treatment of Amastigotes. Amastigotes (10^8/ml) labeled with ^{86}Rb were incubated with pronase (Boehringer Mannheim Biochemicals, Indianapolis, IN) at a final concentration of 1 mg/ml for 10 min at 37°C in medium 199; FCS (50%) was added and the parasites were pelleted by centrifugation. After further washings, parasites were counted and suspended to 10^8/ml and tested for ^{86}Rb release by incubation with either NHS or heat-inactivated human serum.

Results

Activation of the Complement Cascade by Amastigotes and Epimastigotes. The parasites were first tested for their ability to activate the complement cascade. They were incubated with 10% NHS at 37°C for various periods of time, and the remaining C3 and C5 hemolytic activities in the supernatants were titrated. Both forms of parasites consumed C3 and C5 with similar efficiencies, and more efficiently than sheep erythrocytes sensitized with antibody (EA), used as a positive control. Consumption of C3 by epimastigotes and amastigotes was observed also in EGTA serum, indicating that they are activators of the alternative pathway, whereas under the same conditions, consumption of C3 by EA, an activator of the classical pathway, was negligible (Fig. 1).

Consumption of C3 and C5 lead to the binding of C3 fragments to the parasites. Parasites were incubated with 10% C8dpl containing ^{125}I-C3 for 30 min at 37°C. After repeated washings, 5.8% and 4.0% of the ^{125}I-C3 remained bound to amastigotes and epimastigotes, which corresponds to 2.6×10^6 and 1.8×10^6 of C3 molecules per parasite, respectively. Structural analysis by SDS-PAGE showed that C3b and iC3b were the two major forms of C3 on both stages of *T. cruzi* (data not shown). The effective consumption of C5 and the binding of C3b indicate that C5-convertase is assembled on the surface of both forms of the parasite.

Binding of Terminal Components and Channel Formation in Membranes of Amastigotes and Epimastigotes. We next examined whether the terminal complement components are activated. Parasites were incubated with various dilutions of NHS containing ^{125}I-C7 for 30 min at 37°C, and washed twice with medium 199 containing 10% FCS, and twice with 1 M NaCl. 30–40% of the ^{125}I-C7 counts were removed during the two washes with 1 M NaCl. The remaining ^{125}I-C7 associated with the parasites was then determined. Both stages bound similar amounts of C7 (Fig. 2). Next, the kinetics of ^{125}I-C9 binding was examined. Parasites were incubated in 10% NHS

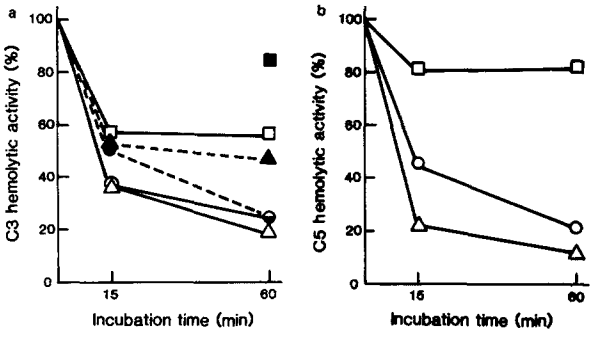

FIGURE 1. Consumption of C3 and C5 by amastigotes and epimastigotes of *T. cruzi*. 10^7 amastigotes (○), epimastigotes (△), or sensitized sheep erythrocytes (□) were incubated at 37°C in 200 μl of 10% NHS. Aliquots were withdrawn at indicated times and centrifuged. The hemolytic activities of C3 (*a*) and C5 (*b*) in the supernatant were titrated and expressed as percent of a control NHS incubated with medium at 37°C. In one series of experiments, the consumption of C3 in EGTA-serum by amastigotes (●), epimastigotes (▲), or sensitized erythrocytes (■) was measured. There was no loss of hemolytic activity when the incubation medium contained EDTA.

containing ^{125}I-C9 for various periods of time at 37°C. They were washed as above, but there was no significant release of labeled C9 during the washings with 1 M NaCl. As shown in Fig. 3, both stages bound C9, and the plateau was reached after 10 min of incubation. In contrast to the results with C7, epimastigotes bound four to six times more C9 than did amastigotes.

Although both stages of the parasite bound C7 and C9, the morphology of amastigotes was not altered when examined by light microscopy, whereas epimastigotes were destroyed. To determine whether small functional channels were formed on amastigotes, parasites were labeled with ^{86}Rb and incubated with 10% NHS. 60% of the trapped ^{86}Rb was released from epimastigotes within 10 min after the addition of complement, whereas no significant release from amastigotes was observed even after 1 h of incubation (Fig. 4).

In other experiments, we measured the release of ^{86}Rb as a function of the number of ^{125}I-C9 bound to the parasites. In one set of tubes, amastigotes and epimastigotes that had been labeled with ^{86}Rb were incubated with serially diluted NHS for 30 min at 37°C. In another set of tubes, parasites were incubated with

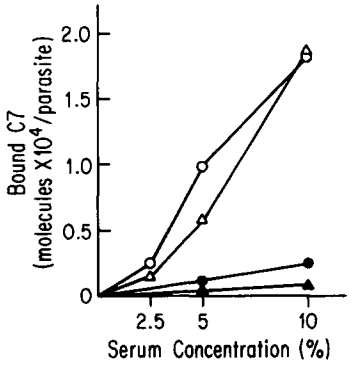

FIGURE 2. Binding of ^{125}I-C7 to *T. cruzi*. Amastigotes (○, ●) and epimastigotes (△, ▲) were incubated in indicated concentrations of either NHS (○, △) or heat-inactivated serum (●, ▲) containing ^{125}I-C7 for 30 min at 37°C. Final concentration of the parasite in the reaction mixture was 5×10^7/ml.

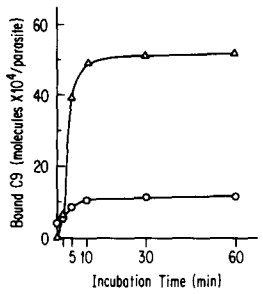

FIGURE 3. Kinetics of ^{125}I-C9 binding to *T. cruzi*. Amastigotes (O) and epimastigotes (Δ) were incubated with NHS containing ^{125}I-C9 for the indicated period of time at 37°C. Final concentrations of parasites and NHS were 5 × 10^7/ml and 10%, respectively.

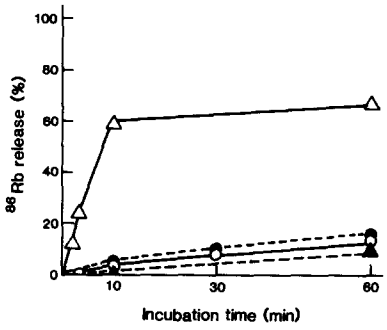

FIGURE 4. Release of ^{86}Rb from parasites. Amastigotes (O, ●) and epimastigotes (Δ, ▲) labeled with ^{86}Rb were incubated with NHS (O, Δ) or heat-inactivated serum (●, ▲) at 37°C for the indicated periods of time. Final concentrations of parasites and sera were 5 × 10^7/ml and 10%, respectively.

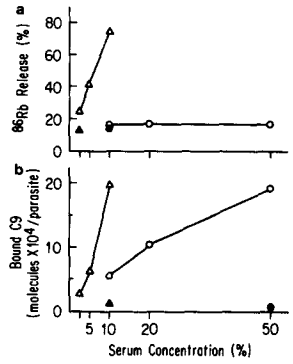

FIGURE 5. Correlation between C9 binding and ^{86}Rb release. Amastigotes (O) and epimastigotes (Δ), at a final concentration of 5 × 10^7/ml, were incubated with various dilutions of NHS, at final concentrations as indicated, containing ^{125}I-C9 for 30 min at 37°C (*a*). ^{86}Rb release experiments were performed as in Fig. 3, except that the incubation time was 30 min (*b*). Closed symbols represent controls of parasites incubated with heat-inactivated serum.

NHS diluted identically but containing ^{125}I-C9. At the end of incubation the amounts of ^{86}Rb released from, and ^{125}I-C9 bound to, the parasites were determined. The release of ^{86}Rb from epimastigotes was proportional to the bound C9, whereas ^{86}Rb was not released from amastigotes, even when 1.8 × 10^5 molecules of C9 had bound per parasite (Fig. 5).

Characterization of C9 Bound to Amastigotes and Epimastigotes. Although binding of C7 and C9 to amastigotes did not lead to the channel formation, the bound C9 had the characteristic properties of polymerized C9 (poly-C9). By SDS-PAGE analysis under reducing conditions, it remained at the top of a 2.5–10% gradient gel, with an apparent M_r >500,000 (Fig. 6). Furthermore, the bound C9 expressed a unique epitope of poly-C9. This was determined as follows. Parasites were incubated with

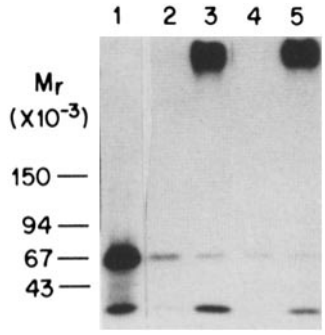

FIGURE 6. SDS-PAGE analysis of C9 bound to parasites. Amastigotes (lanes *2* and *3*) and epimastigotes (lanes *4* and *5*) were incubated with either C8dpl (lanes *2* and *4*) or C9dpl (lanes *3* and *5*) containing ^{125}I-C9, at final concentrations of 10^8 parasites/ml, 10% serum, and 5 μg/ml ^{125}I-C9 for 30 min at 37°C. Parasites were lysed and analyzed by SDS-PAGE using a 2.5–10% gradient gel under reducing conditions. Lane *1* is a control containing ^{125}I-C9.

10% NHS containing ^{125}I-C9 for 30 min at 37°C, washed as described, and extracted with 1% CHAPS. 85% of the bound ^{125}I-C9 counts were solubilized from amastigotes and epimastigotes. The extracts were then immunoprecipitated with an mAb to the C9 neoantigen. 42% and 28% of the counts were removed from the amastigote and epimastigote CHAPS extracts, respectively. A control antibody removed 1% of the counts.

To verify whether the bound poly-C9 was inserted into the membrane of the parasites, we treated the parasites with trypsin. Others have shown that as the terminal complement components insert deeper in the membrane, they become less susceptible to release by proteolytic enzymes (18). Amastigotes and epimastigotes were incubated with NHS containing ^{125}I-C9, washed, and then exposed to 100 μg/ml of trypsin at 37°C for 15 min. 63% of the counts were released from amastigotes, while only 8% were specifically released from epimastigotes (Table I). Almost all of the released C9 was of high molecular weight, migrating at the top of the 2.5–10% gradient gel.

Conversion of Amastigotes into Complement-sensitive Organisms. In an attempt to overcome the resistance of amastigotes to complement lysis, we incubated ^{86}Rb-labeled parasites with 10% NHS in the presence of pooled sera from patients with chronic Chagas' disease. 56% of ^{86}Rb was released during 30 min of incubation at 37°C. When tested individually, however, only seven of these sera were highly lytic, but the majority of them (24:43) did not induce significant lysis. All sera contained antibodies to Ssp-4, the major surface glycoprotein of amastigotes, as determined by immunofluorescent staining and a two-site immunoradiometric assay (19). Amastigotes pretreated with proteolytic enzymes also became susceptible to complement. Pronase-

TABLE I

^{125}I-C9 Release from the Surface of T. cruzi by Trypsin Treatment

T. cruzi	Incubated with:	^{125}I-C9 released
		%
Amastigotes	Trypsin	75
	Medium	12
Epimastigotes	Trypsin	20
	Medium	12

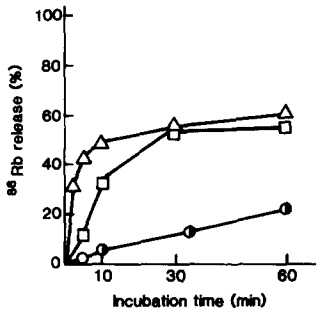

FIGURE 7. Loss of resistance to complement attack. ^{86}Rb release from pronase-pretreated amastigotes by incubation with NHS (△), or from nontreated amastigotes by incubation with NHS in the presence of a pool of Chagasic sera (□) was measured kinetically. As controls, amastigotes incubated either with NHS (○), or with heat-inactivated serum (●) are shown. Final concentration of NHS, of heat-inactivated serum, and of the pool of Chagasic sera was 10%.

treated amastigotes released 60% of ^{86}Rb by incubation with 10% NHS (Fig. 7). However, when the pronase-treated amastigotes were incubated at 37°C in medium for 24 h before complement treatment, they again became resistant to lysis (not shown).

Discussion

Parasites have developed different strategies to avoid destruction by the complement system, and in many instances they acquire this ability only at stages of development that are infective for the mammalian host. In the case of *T. cruzi*, the noninfective epimastigotes disintegrate when treated with complement, while infective forms, that is, metacyclics (4–8), blood stage trypomastigotes (3, 8, 9), and amastigotes, are not lysed when incubated in fresh human serum. The main finding of this paper is that the amastigotes activate the alternative complement pathway effectively, but are not killed because the C5b-9 complexes that bind to the parasite surface fail to insert into the plasma membrane.

As shown in Fig. 1, a large proportion of C3 and C5 is rapidly activated when either amastigotes or epimastigotes are incubated in fresh serum. The efficiency of the consumption of these complement components is even greater than that of an equivalent number of sheep erythrocytes optimally sensitized with antibodies. Complement activation leads to the deposition of similar amounts of C3 fragments on the surface of both stages of the parasite. In fact, because epimastigotes have a much larger surface area than amastigotes, the density of C3 fragments must be greater on the latter. Although precise surface areas have not been determined, the spherical amastigotes have an approximate diameter of 3–4 μm, while the polymorphic fusiform epimastigotes have width and length of 0.5–3 and 30–50 μm, respectively.

Assembly of C5-convertase is followed by the rapid deposition of a large number of C7, C9, and presumably C8 molecules on the membrane of epimastigotes and amastigotes. Contrary to the results of C3 and C7 binding, four to six times more C9 is bound on epimastigotes (Fig. 3). Thus, the C9/C5b67 ratios are much larger on the complement-sensitive epimastigotes. This difference could be functionally significant; for example, killing of *Escherichia coli* by complement is only optimal when the C5b-9 complex contains a critical number of C9 molecules (20). Channel size increases with increasing numbers of C9 molecules per C5b67 complexes, and larger channels are presumably more efficient in inducing lysis (21, 22).

An unexpected finding is that the C9 molecules found on the resistant amastigotes have properties of poly-C9, that is, they migrate as high M_r complexes after boiling

in SDS in the presence of urea, or even after trypsin treatment (23). It could be argued that the high M_r material represents monomer C9 bound to a large parasite surface molecule. This is, however, unlikely, since monomer C9 is trypsin sensitive and, moreover, a large proportion of the high M_r C9 can be immunoprecipitated with an mAb directed against the neo-antigen expressed on poly C9.

High M_r C9 is also found on the epimastigotes but it differs from that found on amastigotes with respect to one important property: the C9 bound to epimastigotes is not removed by treatment with trypsin (Table I), while most of the C9 on amastigotes is accessible and is removed from the parasite surface by treatment with the enzyme. The C9 released from amastigotes by trypsin is of high M_r, which is in agreement with the observation that poly-C9 is mostly resistant to proteolysis (24). The site of trypsin cleavage is not known. We favor the idea that C5b-9, containing poly-C9, binds to a surface molecule of the parasite, and that it is this molecule that is cleaved by trypsin. Alternatively, the C5b-9 complex may be bound to the membrane in such a way that trypsin cleaves one or more of its components. In either case, the C5b-9 complexes are not inserted in the lipid bilayer of amastigote membrane in the same molecular orientation as they are in the membrane of epimastigotes. In fact, they may not be inserted into the amastigote lipid bilayer at all. This is consistent with our observation that there is no ^{86}Rb release from amastigotes (implying a lack of channels) despite binding of C5b-9 to the membrane (Fig. 5).

Activation of terminal complement components in the absence of lysis has been also reported in the case of promastigotes of *Leishmania* parasites. However, in this instance, although C9 is efficiently consumed, there is minimal C9 deposition on the parasite surface (25, 26). This is in contrast to our results with the amastigotes of *T. cruzi* in which large amounts of C9 are bound stably to the surface (Fig. 3).

The general features of the interaction between amastigotes and complement resemble closely those described in studies using the serum-resistant and -sensitive strains of *Neisseria gonorrhoeae*. Both strains activate the complement, bind similar numbers of C9 molecules to their surfaces, and the C9 appears as a large aggregate (27). Also, a larger proportion of the C9 bound to the resistant strain is accessible to cleavage by trypsin, showing that the configurations of the terminal complement complexes are different in the two strains (28). Whether these similarities between *Neisseria* and *T. cruzi* amastigotes reflect the utilization of analogous molecular mechanisms by these pathogens to protect themselves against complement damage is not known.

A possible mechanism for accumulation of nonfunctional C5b-9 complexes is that hydrophobic domains of parasite surface molecules serve as nonspecific "traps" for nascent C5b67 complexes. The efficiency of these traps would be greater if the C5-convertase is assembled at a distance from the lipid bilayer. The anomalously bound C5b67 might then bind C8 and C9 inefficiently. This would explain the low C9/C5b67 ratios found on amastigotes.

Alternatively, if the C5b67 complexes reach the membrane of amastigotes, a surface component may function as a specific inhibitor of C8 and C9 incorporation and prevent channel formation and enlargement. That is, the putative inhibitor could be functionally analogous to the homologous restriction factor (HRF, or C8bp) of mammalian cells, which protects them from attack by autologous complement (29, 30). Both the trap hypothesis, and the HRF-like inhibitor hypothesis, postulate the

formation of complexes between parasite surface molecules and terminal complement components and are therefore subject to experimental verification. The surface molecule involved in this interaction is likely to be protein because amastigotes become sensitive to complement attack after pronase treatment (Fig. 7).

Others have shown that *T. cruzi* metacyclic trypomastigotes are poor activators of complement and that this can be explained by the production of an inhibitor that accelerates the decay of the C3-convertase (8, 9). While we have not specifically searched for this inhibitory activity in extracts of amastigotes, their resistance to complement cannot be explained in the same manner since large amounts of C3 fragments are deposited, and the C5-convertase is assembled. In the mammalian host, the insect-derived metacyclics transform into amastigotes. It appears, therefore, that the strategy used by this parasite to avoid destruction must change in different phases of the life cycle. This is compatible with the observation that *T. cruzi* parasites undergo profound morphological remodeling, and express novel sets of membrane antigens when they transform from flagellates into amastigotes (11). However, precise events at the molecular level need to be determined, before the definitive conclusion that mechanisms of resistance to complement are stage specific is reached.

Finally, regardless of the mechanism of resistance of amastigotes to complement, the finding that large amounts of C3 split products and C5b-9 accumulate on their surface membrane raises the possibility that some of these complement fragments enhance the parasite's survival in the mammalian host. In this respect, it is remarkable that the amastigotes were not destroyed by complement, even in the presence of antibodies from the majority of patients with chronic Chagas' disease.

Summary

We studied the effect of complement on two life cycle stages of the protozoan parasite *Trypanosoma cruzi*: epimastigotes, found in the insect vector, and amastigotes, found in the mammalian host. We found that while both stages activate vigorously the alternative pathway, only epimastigotes are destroyed.

The amounts of C3 and C5b-7 deposited on the amastigotes were similar to those bound to the much larger epimastigotes. Binding of C9 to amastigotes was four to six times less than binding to epimastigotes, resulting in a lower C9/C5b-7 ratio. Although a fairly large amount of C9 bound stably to amastigotes, no functional channels were formed as measured by release of incorporated ^{86}Rb. The bound C9 had the characteristic properties of poly-C9, that is, it expressed a neo-antigen unique to poly-C9, and migrated in SDS-PAGE with an apparent $M_r > 10^5$. The poly-C9 was removed from the surface of amastigotes by treatment with trypsin, indicating that it was not inserted in the lipid bilayer.

Modification of amastigote surface by pronase treatment rendered the parasites susceptible to complement attack. These results suggest that amastigotes have a surface protein that binds to the C5b-9 complex and inhibits membrane insertion, thus protecting the parasites from complement-mediated lysis.

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