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Restriction of the Alternative Pathway of Human Complement by Intact *Trypanosoma brucei* subsp. *gambiense*

DANA V. DEVINE,[1]* RONALD J. FALK,[2] AND ANDREW E. BALBER[1]

Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710,[1] and Division of Nephrology, Department of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514[2]

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We studied the interaction of African trypanosomes with human complement. Bloodstream forms of *Trypanosoma brucei* subsp. *gambiense* isolated from mice activated the alternative pathway of complement during a 30-min incubation in vitro. In human serum, all cells remained intact and motile during this period. C3 was detected on the surface by a direct binding assay with a monoclonal antibody which recognizes C3b and iC3b. C3 deposition could also be detected by this radioimmunoassay when parasites were incubated with purified C3. Such C3 binding was enhanced by factor B, factor D, and magnesium. Surface deposition of factor B was demonstrated both by flow immunofluorescence analysis and binding of radiolabeled factor B. C3 binding and factor B binding were inhibitable by EDTA but not by ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA). The inhibited binding could be restored by addition of magnesium. No human immunoglobulin G or mouse immunoglobulin was detected on the trypanosome surface. By flow cytometry, neither human C5 nor polymerized C9 was detected on trypanosomes incubated in serum, although this assay was able to detect C5 and C9 on the surface of complement-treated human erythrocytes. Using a radioimmunoassay which measures C5b-9 in serum, we found that there was no generation of SC5b-9 in serum which had been incubated with trypanosomes. We concluded that, although trypanosomes activate the alternative pathway of complement, they are not lysed, because the cascade does not continue beyond the establishment of C3 convertase.

African trypanosomiasis (sleeping sickness) in humans is characterized in part by decreased levels of C4 and C3 and elevated levels of immunoglobulin M (IgM) in serum (12). In humans, as well as in animal models, hypocomplementemia has generally been attributed to activation of complement by immune complexes containing parasite antigens (19). Studies of patients with *Trypanosoma brucei* subsp. *gambiense* infection (12) demonstrate low C4 levels in both early- (before central nervous system involvement) and late-stage disease. This C4 depletion is attributed to activation of the classical pathway by immune complexes. There is also a marked decrease in C3 levels in the early stages of Gambian trypanosomiasis accompanied by a decrease in factor B levels (12). Using a rhesus monkey model, Nagle et al. (23) demonstrated the deposition of properdin in glomerular capillary walls of *T. brucei* subsp. *rhodesiense*-infected animals. Thus, in trypanosomiasis, the alternative pathway of complement may also be activated by circulating immune complexes.

To date, it has not been established whether the observed hypocomplementemia in humans with *T. brucei* subsp. *gambiense* or *T. brucei* subsp. *rhodesiense* infections is due, at least in part, to activation of complement by the parasites themselves. However, there is evidence that other African trypanosomes may directly activate complement. Variant surface glycoprotein purified from *T. brucei* subsp. *brucei* activates the classical pathway in an antibody-independent fashion (22). *T. congolense* directly activates both the classical (24) and alternative pathways (32) in the serum of its specific host, cattle. It is unclear whether this activation is related to the decreased levels of early complement components in serum seen in infected calves (25).

Complement is apparently unable to prevent the establishment of either *T. brucei* subsp. *rhodesiense* or *T. brucei* subsp. *gambiense* in the bloodstream. The aim of this study was to examine why the human complement system is apparently ineffective in mediating killing of *T. brucei* subsp. *gambiense*. We demonstrated for the first time that live *T. brucei* subsp. *gambiense* are capable of activating the alternative pathway of complement in human serum. There is, however, no lytic activity, because the cascade does not continue beyond establishment of C3 convertase on the trypanosome surface.

MATERIALS AND METHODS

Trypanosomes. *T. brucei* subsp. *gambiense* used in these experiments were from the TxTat 1 line obtained from J. R. Seed, University of North Carolina, Chapel Hill. BALB/cCr or C3HeB/FeJ mice were inoculated with sufficient numbers of trypanosomes from frozen stocks to produce fulminating parasitemia 30 to 60 h postinoculation. Blood was drawn by cardiac puncture with 3.2% sodium citrate (9 parts blood to 1 part sodium citrate) as an anticoagulant.

Trypanosomes were separated from blood cells and plasma proteins by DEAE-cellulose chromatography (18). All experiments were run with freshly isolated trypanosomes kept at 4°C in Lanham's phosphate-buffered saline (60 mM $NaPO_4$, 44 mM NaCl [pH 8.0] containing 1.5% glucose) (LPBSG). In all cases, the concentration of cells was determined by automated cell counting with a Coulter counter (70-μm aperture). One-in-five hundred dilutions were counted five times, and the mean of the corrected counts was used as the cell count.

Complement sources. Normal human serum (NHS) was collected by venipuncture from volunteer donors and stored at −70°C for no longer than 60 days before use. For some experiments, NHS was made 20 mM with respect to either EDTA or ethylene glycol-bis(β-aminoethyl ether)-

* Corresponding author.

N,N,N',N'-tetraacetic acid (EGTA) by incubating serum for 5 min at 37°C with 0.1 volume of a 0.2 M neutralized (pH 7.2) stock of the appropriate chelator. Such sera are referred to as E-NHS or EG-NHS, respectively. E-NHS that had been made 25 mM $MgCl_2$ by addition of 0.1 volume of 0.25 M $MgCl_2$ is referred to as E-NHS-Mg^{2+}. C3 (33) and factor B (27) were purified from NHS. Functionally pure human factor D was made by the method of Lambris et al. (17). Factor B was radiolabeled with ^{125}I (Amersham Corp.) with Iodogen (Pierce Chemical Co.) and the manufacturer-recommended methodology. ^{125}I-labeled factor B was used within 24 h of labeling.

Antisera. Mouse monoclonal anti-human C3c was the gift of Gordon D. Ross, University of North Carolina, Chapel Hill. This antibody reacts with cell-bound C3b or iC3b. Mouse monoclonal anti-human polymerized C9 (poly-C9) (clone MBM-5) recognizes a neoantigen expressed by MC5b-9 and poly-C9 but not monomeric C9 (7). Anti-C3c and anti-poly-C9 were purified from ascitic fluid by ammonium sulfate precipitation and DEAE-cellulose chromatography. Mouse monoclonal anti-human IgG (Fc) (clone GG-5) was purchased as ascitic fluid (Miles Scientific) and purified by caprylic acid-ammonium sulfate precipitation (29). For some experiments, these monoclonal antibodies were labeled with ^{125}I as described above.

A ^{125}I-labeled $F(ab')_2$ fragment of sheep anti-mouse immunoglobulin was purchased from Amersham. Polyclonal goat anti-human factor B (immunoglobulin fraction) was purchased from Miles Scientific. Goat anti-human C5 was prepared with purified C5 (33). Fluorescein isothiocyanate (FITC)-conjugated probes included FITC-$F(ab')_2$ rabbit anti-goat IgG (Miles-Yeda), FITC-$F(ab')_2$ rabbit anti-goat IgG (Jackson Immunoresearch Laboratories), and FITC-goat anti-mouse IgG (Cappel Laboratories).

Alternative pathway activation. We studied the activation of the alternative pathway by *T. brucei* subsp. *gambiense* in whole serum by incubating equal volumes of trypanosomes at 2×10^7/ml and NHS, E-NHS, or EG-NHS for 30 min at 37°C, washing them three times by centrifugation at 4°C with ice-cold LPBSG, and then assaying them for the presence of bound complement components as described below. Deposition of C3 onto trypanosomes was also studied in a purified component system by incubating 250 μl of trypanosomes at 2×10^7/ml for 30 min at 37°C with 250 μl of LPBSG containing either: (i) 3 mg of C3, 500 μg of factor B, 50 μg of crude factor D, and 20 mM Mg^{2+} or (ii) 3 mg of C3 alone. Cells were then washed and assayed for cell-bound C3 as described below.

The ability of human serum to lyse trypanosomes by complement activation was assayed by incubating trypanosomes with NHS in microtiter plates for 30 min at 37°C as previously described (9). Wells were examined for possible lysed cells with a Zeiss inverted-phase microscope. Killed cells were defined as those which were nonmotile or of abnormal shape.

Assay of complement binding with ^{125}I-labeled antibodies. One hundred microliters of trypanosome suspension treated as described above was incubated with 100 μl of ^{125}I-labeled monoclonal antibody. Anti-C3c and anti-human IgG were used at a concentration of 10 μg/ml, and anti-poly-C9 was used at 15 μg/ml. After incubation for 20 min at room temperature, three 50-μl samples were removed and spun through a mixture of phthalate esters (1.5 parts *n*-butyl phthalate [Fisher Scientific Co.] to 1.0 part bis(2-ethylhexyl) phthalate [Eastman Kodak Co.]) in 400-μl Microfuge (Beckman Instruments, Inc.) tubes to separate cell-bound antibody from unbound antibody. The tube tips containing the pelleted trypanosomes were cut off and counted in a gamma counter.

The number of monoclonal antibody molecules bound per cell was calculated from cell counts and the specific activity of the antibody used. Specific antibody binding was defined as molecules bound per cell in samples exposed to serum minus molecules bound per cell in samples incubated in buffer. Monoclonal anti-C3c shows a 1:1 binding stoichiometry with erythrocyte-bound C3b (27). At the concentration of anti-C3c used in these experiments, typical nonspecific background binding is about 1,000 molecules per human erythrocyte or about 2,000 molecules per human platelet. Monoclonal anti-IgG shows a 1:1 binding stoichiometry with erythrocytes (S. Atwater-Boyd and W. F. Rosse, manuscript in preparation) and detects about 67% of platelet-bound IgG (30). Nonspecific background binding is about 600 molecules per erythrocyte and 1,200 molecules per platelet. The binding stoichiometry of anti-poly-C9 has not yet been determined. The ability of the assay to specifically measure poly-C9 or MC5b-9 was assessed by measuring antibody binding to normal human erythrocytes exposed to serum and different amounts of complement-fixing cold agglutinin anti-I. Typical background binding at the concentration of anti-poly-C9 used is about 600 molecules per erythrocyte and about 1,350 molecules per platelet. Such antibody binding was not inhibitable by a 50× molar excess of unlabeled anti-poly-C9. Data from binding studies were analyzed with the Wilcoxon paired *t* test (37).

Trypanosomes were assayed for the presence of mouse immunoglobulin on their surfaces by the method described above with ^{125}I-labeled $F(ab')_2$ sheep anti-mouse immunoglobulin; however, the trypanosomes were not pretreated with any human serum or complement components.

Assay of complement binding by cytofluorography. Cell-associated fluorescence was measured with an Ortho Diagnostics, Inc., 50H cytofluorograph. Cells were illuminated with the 488-nm line of an argon ion laser. Right-angle light signals were focused onto appropriate fiber optics with a Leitz 32×-0.40 NA objective mounted in a carrier of local design. Fluorescence was measured behind a 515- to 530-nm band-pass filter. Fluorescence signals were gated on the basis of a right-angle versus forward light scatter cytogram to restrict analysis to signals from single cells. Amplifiers were set in the linear area mode. Data were analyzed with the software in an Ortho 2140 data handling system. For statistical analysis of histograms, two regions were defined. Region 1 encompassed channels 1 to 999 to exclude material in the last channel; this effectively eliminated unweighted off-scale data from statistical analysis. Fewer than 2% of total gated counts were in channel 1000. Region 2 was arbitrarily set with the lower channel at the base of the right shoulder of the histogram of the negative cell population. Cells incubated with nonimmune serum and then FITC-conjugated second antibody were used to define negative populations.

Cells treated with NHS, E-NHS, or E-NHS-Mg^{2+} were washed as described above and then incubated with unlabeled goat anti-human factor B or nonimmune goat serum for 30 min at 4°C. After three washings with cold LPBSG, the samples were incubated with FITC-labeled rabbit anti-goat IgG antibody for 30 min at 4°C. Following two washings in cold LPBSG, the trypanosomes were suspended at 10^6/ml, and the suspension was made 1% formaldehyde to fix the cells.

A similar assay was used to examine MC5b-9 and poly-C9

TABLE 1. Binding of ^{125}I-labeled monoclonal antibodies to *T. brucei* subsp. *gambiense*—data are reported as molecules of antibody per cell

Monoclonal antibody against	Molecules of antibody/cell on trypanosomes incubated with:				
	NHS	E-NHS	E-NHS-Mg^{2+}	EG-NHS	LPBSG
Human IgG (Fc)					
No. 1	4,321	5,532	5,388		4,028
No. 2	1,679		3,147	3,817	6,096
Human C3c					
No. 1	9,547	2,486[a]	13,524		2,709
SB[b]	6,838	0	10,815		
No. 2	17,867	11,372[a]	39,742		9,908
SB	7,959	1,464	29,834		
No. 3	12,848			10,250	4,160
SB	8,688			6,090	
No. 4	15,804	2,971[a]	88,928		2,595
SB	13,209	376	86,333		
Human poly-C9					
No. 1	2,768	2,679	1,296		1,389
No. 2	1,470	1,180	1,753		1,301

[a] The difference between the value and that of the LPBSG control was not statistically significant.
[b] SB, Molecules of antibody specifically bound.

binding and C5 binding. In these experiments, the first antibody was either monoclonal anti-poly-C9 (10 μg/ml) or polyclonal goat anti-C5 (diluted 1:200) described above, followed by FITC-conjugated goat anti-mouse IgG or FITC-$F(ab')_2$ rabbit anti-goat IgG, respectively. Incubations and washings were as described above.

Positive control cells for detection of C5 and poly-C9 were made by depositing complement on the surface of normal human erythrocytes. This was done by incubating equal volumes of NHS, a dilution of cold agglutinin anti-I, and normal human erythrocytes at a cell concentration of 5×10^7/ml for 20 min at 0°C and 60 min at 37°C. Washed erythrocytes were then incubated with first and second antibodies as described before. Human erythrocytes were chosen as the control for antibody binding studies because they have a surface area of 145 μm^2 (36), which is comparable to the 113-μm^2 estimated surface area of trypanosomes (2).

Binding of ^{125}I-labeled factor B. To measure activation of the alternative pathway by a direct method, we incubated 200 μl of *T. brucei* subsp. *gambiense* (2×10^7/ml in LPBSG) for 30 min at 37°C with 200 μl of NHS, E-NHS, E-NHS-Mg^{2+}, or LPBSG, each containing 10 μg of purified ^{125}I-factor B. Three 120-μl samples were removed from each incubation mixture and spun through phthalate esters as described above. The molecules of factor B bound per cell were calculated from the specific activity of the reagent.

Measurement of C5b-9 in serum. Sera which had been incubated with *T. brucei* subsp. *gambiense* were assayed for the presence of soluble SC5b-9 complexes by radioimmunoassay. Trypanosomes at 2×10^7/ml in LPBSG were incubated with an equal volume of NHS or E-NHS. Samples (200 μl) were removed at 0, 1, 2, 5, 10, 15, and 30 min after incubation at 37°C. Cells were removed by centrifugation, and supernatant fluids were frozen at −70°C until assayed.

Details of this solid-phase competitive inhibition radioimmunoassay have been described elsewhere (6). Briefly, 50-μl immunobeads (Bio-Rad Laboratories) bearing rabbit anti-mouse immunoglobulin were incubated with monoclonal anti-poly-C9 for 1 h at room temperature in gelatin-coated polypropylene tubes. The beads were then washed three times with phosphate-buffered saline containing 0.05% Tween (Sigma Chemical Co.). Sixty microliters of sample serum and 90 μl of phosphate-buffered saline containing 1% sodium desoxycholate (Fisher) were added to each assay tube. After incubation for 1 h at room temperature, 1 ng of ^{125}I-labeled poly-C9 was added, and the incubation was continued for 90 min. The beads were then counted, washed, and recounted, and the ratio of counts bound to counts added was determined. A standard curve was constructed with NHS containing increasing amounts of unlabeled purified poly-C9. The positive control for the NHS used was to incubate 1 ml of serum with 300 μg of endotoxin for 30 min at 37°C to generate SC5b-9. All samples were run in duplicate. SC5b-9 values are expressed in units equivalent to units of poly-C9, where one unit of poly-C9 is approximately 50 ng.

RESULTS

Trypanosomes incubated in NHS or NHS treated with EDTA, EGTA, or magnesium were not lysed. All cells seen in the lytic assay were motile and of normal shape. Nevertheless, C3 deposited on the surfaces of these trypanosomes when the parasites were incubated with NHS or EG-NHS but not when they were incubated with E-NHS. The addition of 25 mM Mg^{2+} to E-NHS restored the ability of C3 to bind to trypanosomes; C3 binding in E-NHS-Mg^{2+} was always at least as great as the level of NHS and often significantly greater than binding in NHS. The amount of C3 deposited was the same after 20 or 30 min of incubation in serum. All results reported here are from 30-min incubations. Because the amount of complement components detected on the surface of a trypanosome varied from one experiment to another and one donor serum to another, representative experiments are shown in Table 1. The amount of anti-C3c bound to NHS- or E-NHS-Mg^{2+}-treated trypanosomes was significantly greater than the amount bound to either E-NHS-treated cells or LPBSG buffer controls ($T' = 0$, $P < 0.005$).

In two experiments, trypanosomes incubated in purified C3 were able to deposit 8,000 or 9,500 molecules of C3 on their surfaces as measured by ^{125}I-labeled monoclonal anti-C3c binding. The addition of factor B, factor D, and magne-

TABLE 2. Binding of ^{125}I-labeled factor B to *T. brucei* subsp. *gambiense*[a]

Expt. no.	Molecules/cell ± SD on trypanosomes incubated with ^{125}I-labeled factor B plus:				
	NHS	E-NHS	E-NHS-Mg^{2+}	1 mg of C3 + 20 mM Mg^{2+} + 20 μg of factor D	LPBSG
1	212 ± 29	137 ± 23	254 ± 24	562 ± 43	17 ± 12
2	278 ± 27	101 ± 17	273 ± 34	664 ± 38	28 ± 8

[a] Two representative experiments are shown; data are reported as molecules per cell ± one standard deviation.

sium ions increased deposition to 30,000 or 27,000 molecules per cell, respectively.

Radiolabeled factor B binding experiments demonstrated that incubation in NHS, E-NHS-Mg^{2+}, or the C3-factor D-Mg^{2+} mixture containing ^{125}I-factor B resulted in an increased amount of factor B bound to the surface compared with those cells incubated in E-NHS (Table 2). A nearly sixfold increase in factor B binding was seen in purified component experiments in which there was no unlabeled factor B to compete with the labeled factor B for binding sites.

Deposition of factor B on the surface of trypanosomes which had been exposed to NHS or E-NHS made 25 mM Mg^{2+} (E-NHS-Mg^{2+}) was detected by flow cytometric analysis. No binding of anti-factor B antibody was detected on trypanosomes incubated in E-NHS (Fig. 1) or LPBSG (data not shown).

Binding studies with ^{125}I-labeled monoclonal anti-poly-C9 demonstrated that C9, in its activated form as either MC5b-9 or the polymer form, was not found on the trypanosome surface under any conditions (Table 1). Antibody binding could not be inhibited by a 50× molar excess of unlabeled antibody; therefore, the bound antibody represented nonspecific binding. At the same concentration used in these experiments, human erythrocytes exposed to cold agglutinin anti-I antibody and NHS bound as many as 9,500 anti-poly-C9 molecules. On erythrocytes containing amounts of C3 similar to that on the trypanosomes, 4,000 to 5,000 molecules of anti-poly-C9 were bound. Greater than 70% of this binding was inhibited by addition of a 50× molar excess of unlabeled anti-poly-C9 (data not shown). In addition, poly-C9 was not detected on serum-treated trypanosomes by flow cytometry with unlabeled monoclonal anti-poly-C9 and FITC-conjugated anti-mouse IgG. The mean channel number (± the standard deviation) of region 1 of the nonimmune ascites control was 231.9 (± 100.2), while the region 1 mean of the anti-poly-C9-treated sample was 236.8 (± 104.7). Anti-poly-C9 binding to complement-containing erythrocytes could be detected by flow cytometry (Fig. 2).

By flow cytometry, C5 was not detected on trypanosomes exposed to NHS, E-NHS, or E-NHS-Mg^{2+}. For NHS-treated cells, the region 1 mean channel number was 27.1 for nonimmune goat serum incubation and 56.5 for goat anti-C5 incubation. The control mean for E-NHS-treated cells was 25.0, and the anti-C5 mean was 23.9. E-NHS-Mg^{2+}-treated cells incubated with nonimmune goat serum had a mean fluorescence of 24.5, while anti-C5-treated cells had a region 1 mean of 36.9. With the identical first and second antibody regimen, C5 could readily be detected on erythrocytes treated with various dilutions of anti-I and NHS as described above (Fig. 2). The amount of C3 bound to these erythrocytes as measured by ^{125}I-anti-C3c binding was similar to the amount on the surfaces of trypanosomes (6,000 to 50,000 molecules per cell).

The radioimmunoassay for serum C5b-9 failed to detect the generation of SC5b-9 in either NHS or E-NHS incubated with *T. brucei* subsp. *gambiense* which had been incubated for up to 30 min. This experiment was repeated on three different occasions with identical negative results. Sample duplicates were within 10% of their mean, and the standard curves run with each experiment gave *r* values of greater than 0.95 with virtually identical slopes. In control experiments designed to demonstrate that the complement pathways in these sera were active and that we could detect SC5b-9 complexes if they were formed, two of the same three serum samples not exposed to trypanosomes were treated with bacterial endotoxin as previously described (6) and assayed for SC5b-9. Significant amounts of SC5b-9 were detected (18 U).

Two types of experiment were done to evaluate the possible contribution of immunoglobulin to activation of complement by trypanosomes. First, we determined that the freshly purified *T. brucei* subsp. *gambiense* used for these experiments did not have detectable amounts of murine immunoglobulin on their surfaces. The amount of ^{125}I-labeled sheep anti-mouse immunoglobulin per cell was less than that bound to erythrocytes or platelets incubated with nonimmune mouse monoclonal IgG. Second, we could not

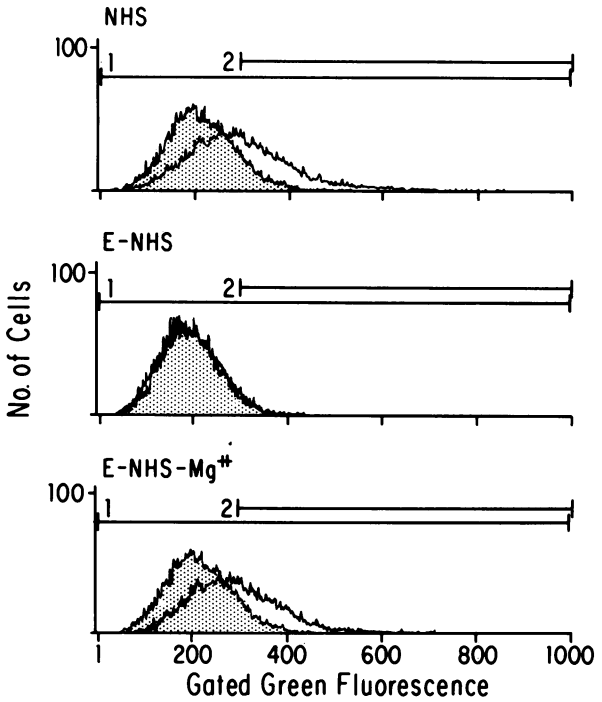

FIG. 1. Histograms of anti-factor B binding detected by flow cytometry. Shaded regions represent nonimmune serum controls. The mean channel number of region 1 ± the standard deviation and the percentage of cells in region 2, respectively, were as follows: NHS (top) control = 221.4 ± 83.7 and 14.6%, anti-factor B = 306.2 ± 127.4 and 47.1%; E-NHS (middle) control = 194.5 ± 71.6 and 6.9%; anti-factor B = 204.6 ± 83.1 and 9.5%; E-NHS-Mg^{2+} (bottom) control = 241.2 ± 83.0 and 22.3%; and anti-factor B = 325.7 ± 130.3 and 52.4%.

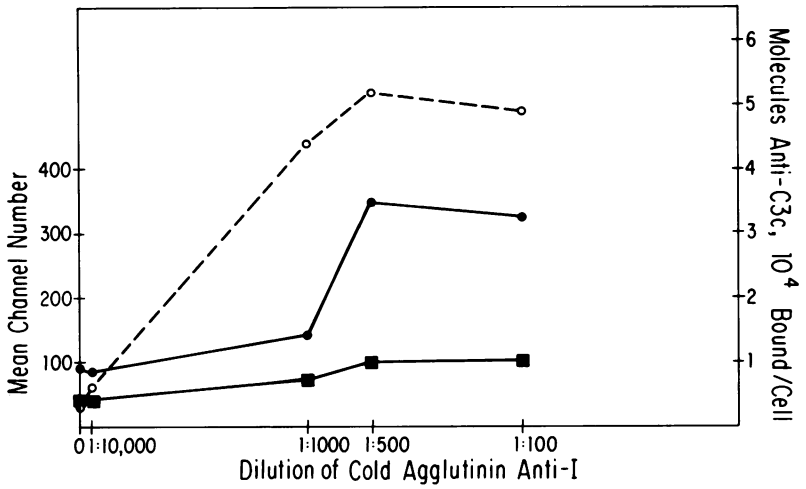

FIG. 2. Detection of polyclonal anti-C5 and monoclonal anti-poly-C9 binding to sensitized erythrocytes by flow cytometry and anti-C3c binding by radioimmunoassay. Anti-I concentration of 0 represents cells incubated in buffer and NHS under the same conditions as cells exposed to anti-I and human serum. Antibody incubations were performed as described in Materials and Methods. The mean channel number is the mean of region 1 (channels 1 to 999). Symbols: ●, anti-C5; ■, anti-poly-C9; ○, anti-C3c.

detect any human IgG bound to trypanosomes that had been incubated in NHS for 30 min at 37°C.

DISCUSSION

We have demonstrated that intact living African trypanosomes of the *T. brucei* subgroup can activate complement when incubated in NHS. Deposition of C3 onto the surfaces of these cells was inhibited by the addition of 20 mM EDTA and could be restored by 25 mM magnesium ion. However, C3 deposition was not inhibited by addition of 20 mM EGTA. Furthermore, C3 deposition in a purified component system was enhanced by addition of factors B and D. These observations suggest that the activation of complement is via the alternative pathway (11). This conclusion is further supported by the observation that factor B was also found on the surfaces of serum-treated trypanosomes. The number of molecules of factor B detected per cell in the direct binding assay was probably lower than the number actually present, as there was approximately four times as much unlabeled factor B (200 μg/ml) as labeled factor B in the serum used in the experiments (21). Trypanosomes incubated in E-NHS-Mg^{2+} showed a marked increase in C3 binding. The reason for this increase is unclear.

No murine or human immunoglobulin was detected on the trypanosomes; therefore, complement activation was not due to classical pathway activation. It is possible that some amount of murine C3 was covalently bound to the surfaces of column-purified trypanosomes, but we did not investigate this possibility. If some of this C3 was in the C3b form, it could possibly serve as a nidus for the formation of an alternative-pathway C3 convertase by human complement components. However, the trypanosomes are not killed by complement in vivo, and we showed in vitro that convertases formed in human serum are quickly restricted. These observations suggest that C3b bound to trypanosomes in vivo is rapidly inactivated.

Complement was not lytic for even a subpopulation of trypanosomes exposed to human serum. This is consistent with the observation that bloodstream forms of African trypanosomes with an intact surface coat are not lysed by complement (10). Complement was not lytic, because there was no formation of the membrane attack complex, C5b-9. The assays used in these studies could readily detect C5 and MC5b-9 on the surface of a cell with a surface area similar to that of a trypanosome. However, we were unable to detect either C5 or MC5b-9 on the surfaces of *T. brucei* subsp. *gambiense* exposed to human serum. Arguably, the binding stoichiometry of monoclonal anti-poly-C9 on the parasite surface may be very different from that on erythrocytes; however, it is quite unlikely that polyclonal anti-C5 would fail to detect C5 bound to trypanosomes. Indeed, our control experiments demonstrated that our assays could readily detect C5 on erythrocytes bearing similar amounts of C3. Thus, the alternative-pathway C3 convertase apparently is not able to form C5 convertase with neighboring C3b molecules on the trypanosome surface; hence, no C5b is generated. This is unlike some bacterial systems in which the membrane attack complex is formed but is not able to penetrate the bacterial cell wall to insert into the cell membrane and lyse the cell (14, 15). Sialic acid on the surface of certain bacteria (5), as well as on mammalian cells (8, 16, 26), plays a role in destabilizing C3 convertase by promoting the binding of factor H. Although sialic acid has not been described to be among the surface carbohydrates of *T. brucei* subgroup trypanosomes (13), it has been reported to account for the charge heterogeneity of the variable surface glycoprotein of *T. congolense* (28). Trypanosome surface components may destabilize alternative-pathway C3 convertase directly or by potentiating factor H binding. In a preliminary report, Joiner et al. (K. A. Joiner, A. Sher, L. V. Kirchhoff, T. Gaither, and C. H. Hammer, Fed. Proc. **44**:1173, 1985) have recently stated that factor H binding is promoted on the surface of metacyclic trypomastigote forms of *T. cruzi*. Whether classical pathway activation by trypanosomes (24), trypanosome fractions (22), or specific antibody can be regulated by similar mechanisms is unknown.

Our studies suggest a new interpretation of the interaction between complement and the trypanosome surface. It has been previously demonstrated that an intact complement system is required for killing of *T. rhodesiense* in immune rat serum (4, 19). Trypanosomes are known to be able to cap parasite-specific antibody (31) or otherwise inactivate surface immune complexes (1) when incubated at 37°C. Binding

of antibody may cause the trypanosome to shed or reorganize enough of its surface coat to be lysed by complement. The procyclic forms of *T. brucei*, which lack a surface coat, are susceptible to complement-mediated cytolysis (34, 20). Ferrante and Allison (10) described a coatless form of *T. congolense* which is lysed by human serum, whereas the coated parent strain is not. These observations have led to the conclusion (35) that the surface coat protects the bloodstream (trypomastigote) forms from cytolysis by covering underlying molecules which activate the alternative pathway. The data reported here demonstrate that viable bloodstream forms with an intact surface coat still display activator surfaces for the alternative pathway of complement. Rather than masking underlying molecules, the variant surface glycoprotein or other surface molecules may protect against cytolysis by regulating complement activation on the trypanosome surface. This possibility is particularly significant in light of data from animal models suggesting that terminal complement components play little role in controlling *T. rhodesiense* infections (3).

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