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Research Article

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Inhibition of the Complement Membrane Attack Complex by the Galactose-specific Adhesin of *Entamoeba histolytica*

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Abstract

The human complement system is an important early host defense against infection. *Entamoeba histolytica* activates the complement system but is resistant to killing by complement C5b-9 complexes deposited on the membrane surface. Our aim was to identify components of the amebic plasma membrane that mediate resistance to human complement C5b-9 by screening for neutralizing monoclonal antibodies. A monoclonal antibody was identified that abrogated amebic resistance to C5b-9, and the mAb was shown to recognize the parasite's galactose-specific adhesin. The purified adhesin bound to C8 and C9 and conferred C5b-9 resistance to sensitive ameba upon reconstitution; these activities of the adhesin were inhibited by the antiadhesin mAb. The *E. histolytica* adhesin shared sequence similarities and antigenic cross-reactivity with CD59, a membrane inhibitor of C5b-9 in human blood cells, suggesting both molecular mimicry and shared complement-inhibitory functions. (*J. Clin. Invest.* 1992. 90:1131–1137.) Key words: complement • lectin • CD59 • adherence • amebiasis

Introduction

Entamoeba histolytica is a protozoan parasite that infects half a billion people annually and is one of the leading parasitic causes of death (1). *E. histolytica* trophozoites are continuously exposed to the host serum complement system during invasion of the colon and hematogenous spread to the liver. Although these pathogens activate the alternative pathway of complement, the trophozoites are resistant to killing by the C5b-9 membrane attack complex of complement that is consequently deposited on the amebic plasma membrane (2). The mechanism by which these organisms evade destruction by the cytolytic complement components in human blood remains unknown. Recently, it has been shown that *Trypanosoma cruzi* and potentially other microbial parasites express cell surface molecules that mimic the activity of the complement regulatory proteins CR1 and decay accelerating factor, which are cell surface components of human blood cells that, respectively, inhibit assembly and accelerate decay of the C3/C5-convertases required for complement activation (3). This raises the possibility that pathogenic strains of *E. histolytica* use a similar strategy to evade lysis by the C5b-9 proteins of the complement system.

In addition to membrane inhibitors of the C3/C5-convertase enzymes, human blood cells and vascular endothelium express cell surface glycoproteins that serve to prevent assembly of the C5b-9 complement pore in the plasma membrane, protecting these cells from complement-mediated lysis. These inhibitors of the membrane attack complex exhibit species selectivity, and show greatest activity when the C8 and C9 components of the C5b-9 complex derive from human serum. Plasma membrane proteins reported to exhibit this activity include a 65-kD protein designated "homologous restriction factor" (4), and an 18–21-kD leukocyte antigen, designated CD59 (5, 6). These proteins have been shown to be attached to the cell surface by a glycosylphosphatidyl-inositol anchor and are deficient in the most hemolytically sensitive erythrocytes of patients with the stem cell disorder paroxysmal nocturnal hemoglobinuria (5, 6). The complement-inhibitory activity of these proteins appears to be related to their capacity to prevent activation of the C9 component of the C5b-9 complex, restricting assembly of the cytolytic membrane pore (6, 7). The role of the CD59 antigen in protecting human cells exposed to complement from lysis by the C5b-9 proteins was confirmed by transfer of resistance to human complement upon transfection of a nonhuman cell line with the cDNA encoding CD59 (8).

The role of the CD59 antigen and other homologous restriction factors as membrane inhibitors of C5b-9 was identified by screening for mAb capable of neutralizing the complement-inhibitory activity of the human red cell membrane (5). We took a similar approach and produced mAb against *E. histolytica* to identify a candidate C5b-9-inhibitory protein in the amebic membrane.

Methods

Cultivation of E. histolytica. Axenic *E. histolytica,* pathogenic strain HM1-IMSS, were grown in medium TYI-S-33 (trypticase, yeast extract, iron, and 10% heat-inactivated adult bovine sera) (9–11). Increased resistance to complement lysis was induced in strain HM1-IMSS by the gradual addition of pooled human sera (antiamebic antibody negative, final concentration 10%) to the TYI-S-33 medium, or by using axenic amebae recently (< 8 wk) cultured from liver abscesses produced in gerbils by intrahepatic injection of strain HM1-IMSS, or by xenic (bacterial associate) culture of the HM1-IMSS strain (9, 10).

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Production and screening of antiamebic monoclonal antibodies. Strain A/J mice were immunized intraperitoneally with axenic strain HM-1:IMSS *E. histolytica* trophozoites rendered complement resistant by growth in TYI-S-33 medium supplemented with 15% pooled normal human sera (9, 10). Spleen cells were then fused to Sp2/O-Ag-14 myeloma cells on day 81 (after boosting the animals on days 75, 76, and 77 with 10^6 amebae intraperitoneally) and ~ 200 hybridomas producing antiamebic mAb identified by ELISA. The mAb were tested for their effects on complement resistance by adding 75 μl of heat-inactivated hybridoma cell culture medium to 10^5 strain HM1:IMSS amebae in 1 ml of 50% normal human serum. After a 60-min incubation at 37°C, lysis was calculated by counting the remaining refractile viable amebae on a hemocytometer (2, 9, 10).

Lysis of E. histolytica with purified terminal complement components. Complement proteins were purified as previously described (7). Amebae (3×10^5) in 25 μl of PBS were incubated with 2.5 μg of C5b6 on ice for 5 min. After 5 min C7 (0.5 μg) was added with immediate mixing, and after an additional 10-min incubation on ice these amebae (amebae-C5b67) were split into two tubes. Amebae-C5b67 were incubated for 10 min on ice with 40 μg of mAb 3D12 or 40 μg of an unrelated control murine IgG_1 mAb. The amebae-C5b67 were then suspended in 200 μl of PBS and C8 (5 μg) and C9 (5 μg) sequentially added at 5-min intervals. The percentage of amebic lysis after 30 and 60 min of incubation at 37° was then measured microscopically.

Measurement of C9 binding to E. histolytica. C9 binding to strain HM-1 amebae (grown in medium containing 15% normal human sera) was measured by incubating amebae-C5b-8±mAb 3D12 (200 μg/ml) with 1.25 μg/ml of ^{125}I-labeled C9 for 40 min at 37°C. Nonspecific binding or pinocytosis of ^{125}I-labeled C9 to amebae was measured by incubating amebae with labeled C9 in the absence of C5b-8. After incubation, the amebae were pelleted at 150 *g* and washed once in PBS before centrifugation through 0.25 ml of oil (four parts silicon oil [Accumetric Inc., Elizabeth KY] and one part mineral oil [Sigma Chemical Co., St. Louis, MO]) at 9,000 *g* for 1 min. The tips of the microfuge tubes containing the cellular pellet were cut off with a razor blade and cell-associated radioactivity counted in a gamma counter.

Purification and reconstitution of the galactose adhesin. The adhesin was purified from strain HM1-IMSS trophozoites by antiadhesin monoclonal antibody affinity chromatography as previously described (12). Relatively complement sensitive trophozoites (strain HM-1:IMSS grown in axenic medium without normal human serum) were sequentially interacted with C5b6 and C7, as described for Fig. 1, and then 1.2×10^5 amebae-C5b67 incubated on ice in 200 μl of PBS with 0 or 40 μg of the purified adhesin from complement-resistant animal-passaged HM1 *E. histolytica*. Antiadhesin mAb 3D12 or a control IgG_1 mAb (40 μg/200 μl) was then added for an additional 30-min incubation on ice. C8 (5 μg) and C9 (5 μg) were sequentially added at 5-min intervals and the percentage of amebic lysis after a 60-min incubation at 37°C determined.

Statistics. Results are expressed as the mean±SE (n = 3 unless otherwise specified).

Results

Production of monoclonal antibodies that block complement resistance. Since ~ 50% greater resistance to lysis by complement is achieved in strain HM1 amebae adapted to growth in medium containing human sera (or by animal passage or growth with bacteria) (9, 10, 13), strain HM1-IMSS amebae cultured with human sera were used to immunize strain A/J mice. Functional screening of hybridomas derived from this mouse identified two clones (3D12 and 5F9) that augmented killing of *E. histolytica* trophozoites by human serum, from control lysis of 66%, to lysis of 93% and 83% in the presence of 3D12 and 5F9, respectively. mAb 3D12 (IgG_1 isotype) was successfully subcloned, and purified from ascites by protein A chromatography for further studies.

Serum complement-dependent killing of strain HM-1 *E. histolytica* trophozoites (grown in medium containing 15% human serum to increase their complement resistance) (9, 13) increased more than threefold in the presence of purified mAb 3D12 (either intact IgG or Fab fragments) (Table I). Maximal effects of mAb 3D12 were seen at a concentration of 200 μg/ml, with 50% of its inhibitory activity observed at 50 μg/ml. MAb 3D12 had no effect on amebic viability when the complement components in the human sera were destroyed by heat-inactivation. Fab fragments of 3D12 increased serum killing, indicating that cross-linking or capping of the antigen recognized by the mAb was not required for abrogation of complement resistance (Table I). The lysis of strain HM-1 amebae rendered complement resistant by animal-passage or by growth in xenic (bacterial associate) culture medium free of human sera (9, 10) was also increased by mAb 3D12 (from control lysis of 17±0.5% to 44±3.2% lysis with mAb 3D12 for xenic amebae, and from control lysis of 21±1.0% to 63±4.3% with mAb 3D12 for animal-passaged amebae). The observation that mAb 3D12 caused increased lysis of amebae made complement resistant by xenic culture suggested that the cell surface component affected by the antibody was intrinsic to the amebae plasma membrane, and did not derive from human serum.

Effect of mAb 3D12 on lysis by terminal complement components. To exclude the possibility that the observed effect of mAb 3D12 on complement-dependent lysis resulted from increased C5b formation due to initiation of the complement activation pathways by the bound antibody, C5b67 complexes were first deposited on the plasma membrane (by incubation of 5×10^4 amebae in 50% C8-deficient human serum), before amebae were incubated with 3D12. Human serum containing 10 mM EDTA was then added, providing the C8 and C9 components of the C5b-9 complex without further conversion of C5 to C5b. Under these conditions, increased lysis due to mAb 3D12 was again observed (from control lysis of 32±8.3% to 73±2.0% lysis with mAb 3D12), suggesting an mAb 3D12-induced increase in sensitivity of amebae to the lytic properties of the C5b-9 proteins per se, irrespective of any potential effect of

Table I. Effect of mAb 3D12 on E. histolytica Susceptibility to Complement-mediated Lysis

Condition	Lysis
	%
NHS	17±3.2
NHS + 3D12 mAb	58±9.7
NHS + 3D12 Fab	63±4.3
Heat-inactivated NHS	4±0.1
Heat-inactivated NHS + 3D12 mAb	4±0.1

E. histolytica strain HM-1:IMSS trophozoites grown in medium containing 10% normal human sera (NHS) were preincubated for 30 min at 4°C with protein A-purified mAb 3D12 (200 μg/ml) or 100 μg/ml of Fab fragments of 3D12 in PBS. Amebae were then exposed to 20% NHS or 20% heat-inactivated NHS and percent lysis measured at 1 h.

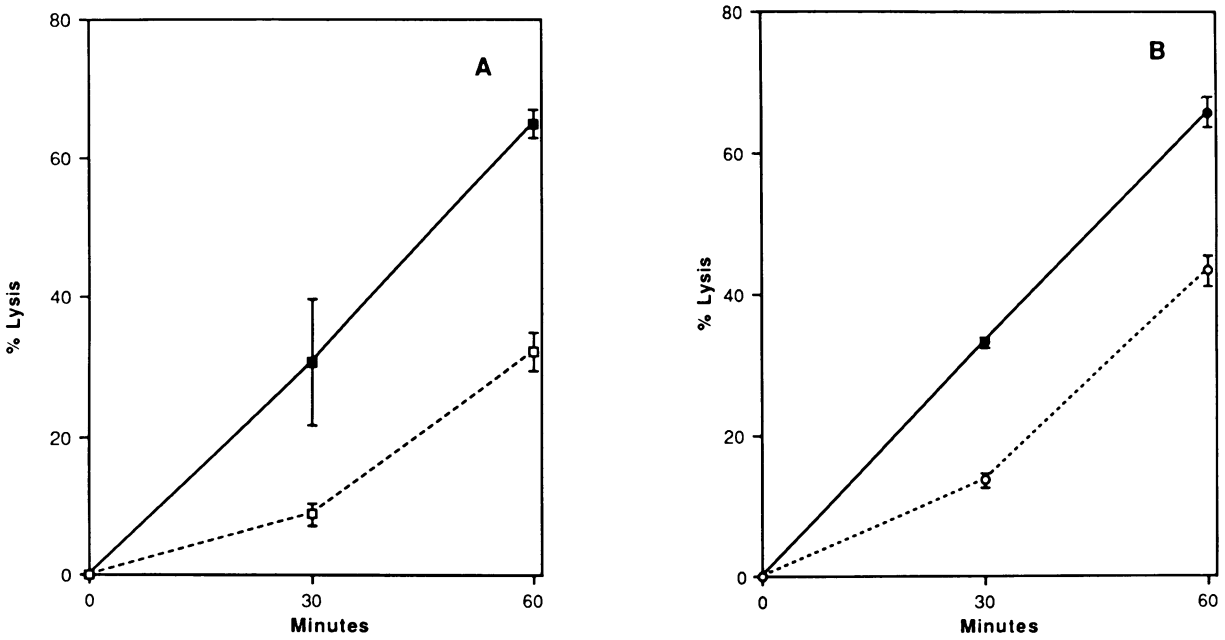

Figure 1. Monoclonal antibody 3D12 potentiates lysis of *E. histolytica* by purified complement C5b-9. (*A*) MAb 3D12 (■) or control IgG_1 mAb (□) were added to amebae preexposed to C5b67, and lysis measured after the subsequent addition of C8 and C9. (*B*) MAb 3D12 (●) or control IgG_1 mAb (○) were added to amebae preexposed to C5b678, and lysis measured after the subsequent addition of C9.

this antibody on the complement activation pathways leading to C5 conversion to C5b.

mAb 3D12 was also found to increase cell lysis when amebae were exposed to the purified C5b-9 proteins in the absence of any other serum components (Fig. 1). Increased lysis due to 3D12 was observed both under the circumstance that this antibody was added to the cells before addition of the terminal complement components, as well as when C5b-8 was first assembled on the plasma membrane, before addition of 3D12 and C9 (Fig. 1, *A* and *B*). Fab fragments of mAb 3D12 also increased amebic lysis by purified C5b-9 (from control lysis of 32±1.3% to lysis of 56±0.8% with mAb 3D12 Fab fragments). Again, no increase in cell lysis was observed due to the antibody alone or when C9 was omitted. These results suggested that mAb 3D12 potentiated C9 activation by membrane C5b-8, possibly by neutralizing a cell-surface inhibitor of this step in C5b-9 pore formation. Consistent with this hypothesis, mAb 3D12 increased the binding of ^{125}I-labeled C9 to complement resistant strain HM-1 ameba. In one experiment (representative of four similarly performed experiments) mAb 3D12 increased ^{125}I-C9 binding to ameba pretreated with C5b-8 to 14,300±2,070 cpm, versus 7,090±2,650 cpm for C5b-8 cells in the absence of mAb 3D12. Under these conditions, nonspecific binding of ^{125}I-C9 to amebae lacking C5b-8 was 5,169±2,770 cpm.

Effect of mAb 3D12 on lysis by rabbit C8 and C9. Human CD59 preferentially restricts lysis of cells by human C8 and C9, with little or no inhibitory activity if rabbit C8 and C9 are substituted after assembly of human C5b67 on the erythrocyte surface (7). To address whether the resistance to C8 and C9 from other species is abrogated by mAb 3D12, human C5b67 complexes were first deposited on the plasma membrane by incubation of 5×10^4 amebae (animal-passed strain HM1-IMSS) in 50% C8-deficient human serum at 37°C for 30 min. The amebae were then washed three times in PBS before the addition of mAb 3D12 or an IgG_1 control mAb and rabbit or human C8 and C9 (added as rabbit or human sera containing 10 mM EDTA to prevent further activation of the alternative and classical complement pathways). Under these conditions, lysis of ameba-C5b-$7_{human}C89_{human}$ increased from 38±5% to 65±3% with mAb 3D12, and lysis of ameba-C5b-7_{human} $C89_{rabbit}$ increased from 36±4% to 52±9% with mAb 3D12.

Identification of the antigen recognized by mAb 3D12. Amebic trophozoites metabolically labeled with 100 μCi/ml of [^{35}S]methionine and cysteine for 2 h in TYI-S-33 medium were solubilized in detergent as previously described (11). mAb 3D12 immunoprecipitated a ^{35}S-labeled protein that comigrated with the 170-kD subunit of the *E. histolytica* galactose-specific lectin or "adhesin" immunoprecipitated by mAb 3F4 (Fig. 2 *A*). Western blots of the affinity-purified galactose adhesin isolated from strain HM-1 amebae demonstrated that mAb 3D12 is directed against the 170-kD subunit of the adhesin (Fig. 2 *B*). The adhesin is a major cell surface heterodimeric glycoprotein that consists of 170-kD and 35-kD subunits linked by disulfide bonds, with a nonreduced molecular mass of 260 kD in SDS-PAGE (12). The galactose adhesins purified from both the relatively complement sensitive (axenic) and resistant (animal-passed) HM1-IMSS amebae were recognized by mAb 3D12 (data not shown).

Effect of galactose on lysis by terminal complement components. We evaluated whether inhibition of the lectin-like function of the adhesin also blocked its putative complement inhibitory function. Animal-passed strain HM-1 amebae were incubated for 60 min at 37°C in 30% normal human sera, or purified C5b-9 terminal complement components. Galactose (25 mM) was added to the incubation to inhibit the lectin activity of the adhesin (11), with 25 mM glucose used as an osmotic control. Inhibition of the lectin activity of the adhesin

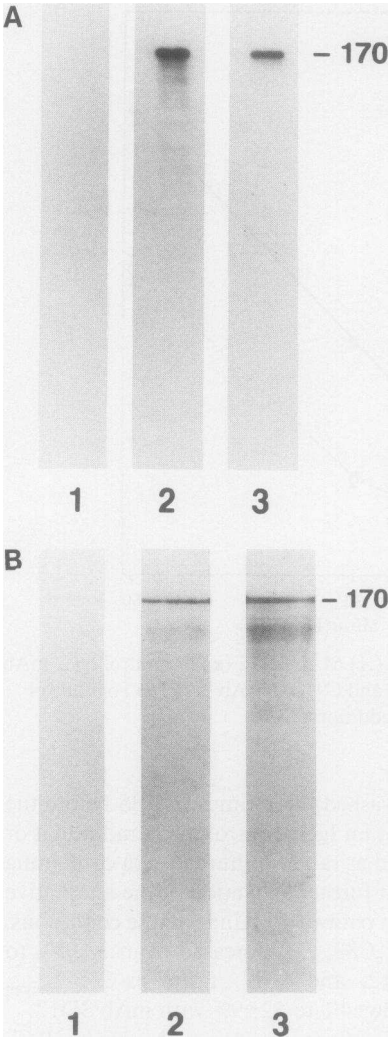

Figure 2. Monoclonal antibody 3D12 is directed against the 170-kD subunit of the galactose adhesin. (*A*) [^{35}S]-methionine-cysteine-labeled *E. histolytica* proteins immunoprecipitated with a control IgG_1 mAb (lane *1*), mAb 3D12 (lane *2*), and antigalactose adhesin mAb 3F4 (lane *3*), and then analyzed by reducing SDS-PAGE and autoradiography. (*B*) Purified galactose adhesin electrophoresed in reducing SDS-PAGE and transferred to Immobilon membranes (Millipore Corp., Bedford, MA) and probed with control IgG_1 mAb (lane *1*), mAb 3D12 (lane *2*), and antigalactose adhesin mAb 3F4 (lane *3*). The position of the reduced 170-kD subunit of the galactose adhesin (which has migrated one-tenth of the total distance of the SDS-PAGE running gel) is indicated.

did not markedly increase lysis of the ameba by normal human sera (control lysis of 43±3.0% compared to 45±1.4% with galactose and 40±0.8% with glucose), or by purified C5b-9 (control lysis of 34±2.6% compared to 40±0.7% with galactose and 31±4.9% with glucose).

Epitope specificity of antiadhesin mAb abrogation of C5b-9 resistance. With the knowledge that mAb 3D12 recognized the galactose adhesin, we tested mAbs directed against adhesin epitopes 1-6 (14) for their effects on amebic susceptibility to complement lysis. As shown in Table II, mAb to adhesin epitope 1 had no significant effect on lysis of *E. histolytica* by sera, while epitope 2–5 mAb caused a modest increase, and epitope 6 mAb H85 a dramatic increase in *E. histolytica* susceptibility to lysis. Lysis by purified C5b-9 was also increased by mAb H85 (from 41% lysis without antibody to 83% with mAb H85). Competitive binding studies of antiadhesin mAbs demonstrated that mAb 3D12 binds to a unique epitope (epitope 7) on the 170-kD subunit (data not shown).

Measurement of C5b-9 inhibitory activity of galactose adhesin. In addition to its role in amebic adherence to colonic mucins, the expression of the 3D12 epitope by the 170-kD subunit of the galactose adhesin raised the possibility that this protein also exhibits a C5b-9-inhibitory function in the ameba

Table II. Epitope Specificity of Antiadhesin mAbs Increasing E. histolytica Susceptibility to Complement-mediated Lysis

Epitope	mAb	Lysis
		%
1	3F4	2±4.1
2	8A3	20±3.5
3	7F4	27±14
4	8C12	18±6.0
5	1G7	14±12
6	H85	80±4.1

Protein A–purified mAb directed against adhesion epitopes 1–6 (as defined in reference 14) were tested at an IgG concentration of 200 μg/ml for their effects on amebic susceptibility to complement lysis, as described in Table I. Data are expressed as percent lysis above control lysis in the absence of added mAb. None of the antiadhesin mAb had an effect on amebic viability in the absence of complement.

plasma membrane, analogous to the function of CD59 and other putative "homologous restriction factors" that are present on the surface of human erythrocytes and other blood cells: first, as indicated by data of Fig. 1, antibody against the 3D12 epitope of adhesin caused increased lysis by the purified C5b-9 proteins. This effect was epitope specific in that it was not observed for mAb directed against epitope 1 on the adhesin. Second, a membrane-destabilizing effect of the antibody itself, as might potentially arise through induced capping of cell surface adhesin, was considered unlikely as: (*a*) we observed no effect of the antibody on cell viability (in the absence of the C5b-9 proteins); (*b*) because Fab fragments prepared from mAb 3D12 shared the property with intact IgG of augmenting C5b-9-mediated lysis; (*c*) since mAb 3F4 directed against epitope 1 of the galactose adhesin caused cell surface capping of the adhesin but had no effect on resistance to C5b-9; and (*d*) since increased complement sensitivity has not been observed to occur upon antibody-induced capping of an unrelated 30-kD surface antigen (15). Despite these considerations, the possibility that antibodies to the adhesin were altering the plasma membrane to permit more effective insertion of C5b-9 could not be excluded. Accordingly, the C5b-9 inhibitory activity of the adhesin purified from the plasma membrane was directly tested.

The C5b-9 inhibitory activity of the galactose adhesin was investigated by examining the effect of galactose adhesin (immunoaffinity purified from animal-passed amebae) on the lytic susceptibility of axenically cultured amebae. Because of potential effects of this membrane-derived protein on the efficiency of membrane binding of C5b67 complexes assembled in the fluid phase, the amebae were first incubated with C5b6 and C7 before addition of adhesin. As summarized by the data of Table III, adhesin caused as much as a 90% reduction of lysis mediated by the C5b-9 components. This effect of adhesin was partially reversed by addition of mAb 3D12, an effect that was not observed for isotype-matched control antibody.

Interaction of the adhesin with C8 and C9. We next tested for a direct interaction of the galactose adhesin with the human complement components comprising the membrane attack complex. As illustrated by the data of Fig. 3, ligand blotting of the terminal complement proteins with ^{125}I-labeled galactose

Table III. Reconstitution of the Galactose Adhesin Confers C5b-9 Resistance to Complement-sensitive E. histolytica

Adhesin	mAb	Lysis
		%
–	–	80±1.7
–	IgG_1 control	75±4.4
+	—	7.3±2.5
+	IgG_1 control	11±3.5
+	mAb 3D12	55±5.3

Complement-sensitive trophozoites (strain HM-1:IMSS grown in axenic medium without normal human serum) were sequentially interacted with C5b6 and C7, as described for Fig. 1, and then 1.2×10^5 ameba-C5b67 incubated on ice in 200 μl of PBS with 0 or 40 μg of the purified adhesin from complement-resistant animal-passaged HM1 *E. histolytica*. Antiadhesin mAb 3D12 or a control IgG_1 mAb (40 μg/200 μl) was then added for an additional 30-min incubation on ice. C8 (5 μg) and C9 (5 μg) were sequentially added at 5-min intervals and the percentage of amebic lysis after a 60-min incubation at 37°C determined.

adhesin revealed specific binding to C8 αγ, C8 β, and to C9, as well as a low level of binding to C5b. The interaction of the adhesin with both C8 and C9 was inhibited to a greater extent by mAb 3D12 than by a control IgG_1 mAb (Fig. 4).

Structural similarities of adhesin with CD59. The similarity of function observed for the galactose adhesin isolated from *E. histolytica* and the CD59 complement inhibitor purified from human erythrocyte membranes led us to consider whether these two proteins might be structurally related. Analysis of the sequence of the cDNAs from two members of the

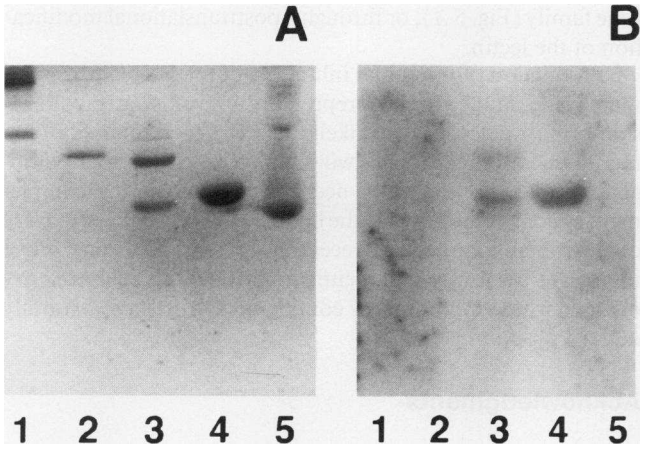

Figure 3. Recognition of human complement components C8 and C9 by ^{125}I-labeled galactose adhesin. Purified proteins C5b6, C7, C8, C9, and BSA (lanes *1–5*, respectively) were electrophoresed without reduction by SDS-PAGE (3 μg/lane) and transferred to polyvinylidene difluoride membranes. The membranes were blocked in PBS containing 1% BSA and 0.05% Tween before being probed with ^{125}I-labeled galactose adhesin (2×10^5 cpm/ml). (*A*) Coomassie blue protein stained gel. (*B*) Autoradiograph of gel transferred to PVDF membrane and probed with ^{125}I-labeled adhesin. Estimated molecular masses are: C5b, 190 kD; C6, 120 kD; C7, 110 kD; C8 αγ, 86 kD; C8 β, 64 kD; C9, 69 kD; BSA, 69 kD.

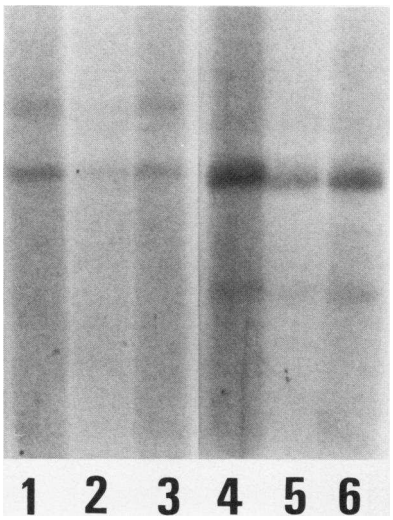

Figure 4. Inhibition of galactose adhesin's binding to complement components C8 and C9 by monoclonal antibody 3D12. Purified C8 (lanes *1–3*) and C9 (lanes *4–6*) were subjected to SDS-PAGE without reduction and transferred to PVDF membranes. The membranes were blocked in 1% BSA and 0.05% Tween and then probed with 10^6 cpm/ml of ^{125}I-labeled galactose adhesin without (lanes *1* and *4*) or with antiadhesin mAb 3D12 (lanes *2* and *5*) or an isotype-matched control mAb (lanes *3* and *6*). Estimated molecular masses are: C8 αγ, 86 kD; C8 β, 64 kD; C9, 69 kD.

adhesin 170-kD subunit gene family revealed regions of limited sequence identity within an area encompassing one-third of the sequence of the human inhibitor of C5b-9, CD59 (Fig. 5 *A*). We also tested whether the two proteins were antigenically related. Antiadhesin mAbs did not cross-react with CD59. However, as shown in Fig. 5 *B*, antibody to CD59 recognized the purified nonreduced (260 kD) adhesin, and this binding was completely competed by purified CD59 isolated from human erythrocyte membranes. When Western blotting was performed under reducing conditions, we were unable to detect binding of anti-CD59 to the 170-kD subunit of adhesin, suggesting that the cross-reactive epitope(s) is conformationally determined by disulfide bonding. A similar loss of immunoreactivity of anti-CD59 with CD59 is also observed upon reduction of the antigen (data not shown). Galactose (50 mM) did not block anti-CD59 binding to adhesin, suggesting that the apparent immunoreactivity was not due to the adhesin binding to carbohydrate residues on the antibodies via its lectin activity. The portion(s) of the adhesin heterodimer that is responsible for this antigenic cross-reactivity and functional similarity to the human CD59 antigen remains to be determined.

Discussion

E. histolytica resistance to the lysis by the membrane attack complex of complement was blocked by antiadhesin mAbs, and the purified galactose adhesin was shown to bind to C8 and C9 and to inhibit lysis of C5b-9 sensitive ameba at the step of C8 and C9 assembly. The importance of the identification of the adhesin as an inhibitor of C5b-9 lysis rests both in the insight that it may shed on the previously described ability of pathogenic *E. histolytica* to evade complement lysis, and as the first description of a microbial inhibitor of the terminal steps in assembly of the membrane attack complex.

The galactose-specific adhesin mediates adherence of trophozoites to human colonic mucins and epithelial cells, with adherence via this protein required for contact-dependent killing of mammalian cells (11, 12, 16). This adhesin is the dominant antigen recognized by immune sera, with > 95% of pa-

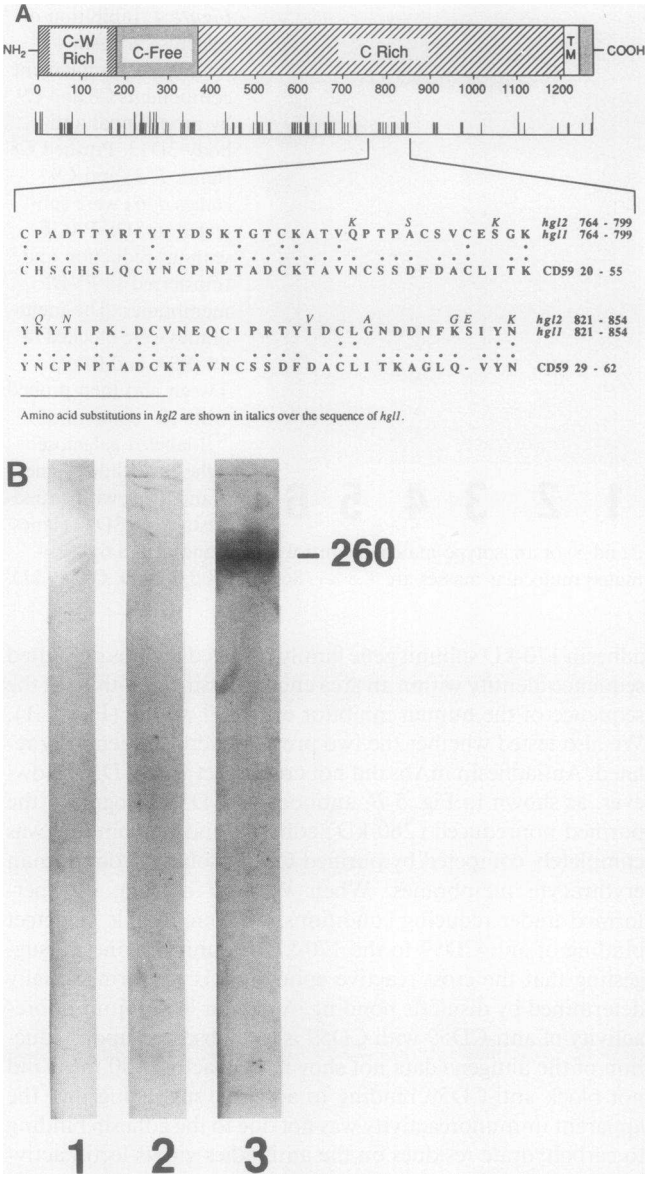

Figure 5. Sequence similarities and antigenic cross-reactivity of human CD59 and the galactose-specific adhesin of *Entamoeba histolytica.* (*A*) Schematic representation of the *E. histolytica* galactose adhesin gene family (*hgl1* and *hgl2*) highlighting the region (adhesin amino acids 764–854) with sequence similarity to CD59 (amino acids 20–62) (21–24). Identical amino acids in the adhesin and CD59 are indicated with two dots; conservative substitutions with one dot. The putative structural domains of the two adhesin gene family members sequenced to date and conservative (*short vertical lines*) and nonconservative (*tall vertical lines*) amino acid changes between the gene family members are also indicated. (*B*) The affinity-purified galactose adhesin (5 μg/lane), subjected to nonreducing SDS-PAGE and electrophoretically transferred to Immobilon membranes, was probed with a rabbit IgG control (lane *1*), rabbit IgG anti-CD59 preabsorbed with purified CD59 (lane *2*), and rabbit IgG anti-CD59 (lane *3*). The position of the 260-kD nonreduced adhesin on the stained SDS-PAGE gel is indicated.

tients who have recovered from amebic liver abscess producing antibodies against it (17). Human immune sera contains antibodies directed against epitope 6 on the adhesin, one of the two epitopes shown to be recognized by C5b-9 inhibitory mAbs (14). Whether the protection conferred by immunization of animals with the adhesin against amebic liver abscess (18) is due to neutralization of its adherence and/or C5b-9 inhibitory activities awaits further study.

The interaction of the adhesin with human C8 and C9 is consistent both with the known homology of structure between C8 α, C8 β, and C9 polypeptides (19), and with the observed inhibitory effects of adhesin on the interaction between C9 and C5b-8 required for functional pore formation (Table III). Of note, the functional activity of the human complement regulatory protein CD59 also appears to reside in its capacity to bind to the C8 and C9 subunits of the C5b-9 complex (7, 20). While much is left to be understood about the mechanism of action of the adhesin's inhibition of C5b-9 lysis and how it compares to that of CD59, one clear-cut difference is that the adhesin is not specific for human C8 and C9. In fact *E. histolytica* was equally resistant to lysis with, and mAb 3D12 equally increased lysis by, rabbit and human C8 and C9. That the mechanisms of action of CD59 and the adhesin are different is not surprising based on their very limited sequence identity, with the adhesin apparently unique amongst putative inhibitors of C8 and C9 in its ability to block nonprimate C8 and C9.

The mechanisms by which growth in human sera or animal passage of axenically cultured amebae increases complement resistance remains unresolved by our experiments, as the 3D12 epitope is expressed in strain HM-1 amebae grown in the presence and absence of human sera. This raises the possibilities that the ~ 50% increase in C5b-9 resistance in the animal-passed or human serum–grown amebae could be due to more than one mechanism related to expression of the adhesin, including (*a*) a quantitative increase in the cell surface expression of this protein, or (*b*) a qualitative change in the lectins expressed in the amebic plasma membrane, as might arise either through altered expression of different members of the lectin gene family (Fig. 5 *A*), or through a posttranslational modification of the lectin.

Production of microbial inhibitors of the late complement membrane attack complex represents a novel strategy of evasion of host defenses. It is likely that similar inhibitors of the membrane attack complex will be discovered in other pathogenic microorganisms that need to circumvent the complement system as they invade the body. In addition, the discovery of microbial complement receptors or inhibitors may prove important for future therapeutic approaches to inflammatory diseases where inhibition of complement with such naturally derived products is desired.

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