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Modulation of a Surface Antigen of *Entamoeba histolytica* in Response to Bacteria

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Changes in the cell surface of *Entamoeba histolytica*, a human intestinal parasite and the causative agent of amebic dysentery, were examined with a monoclonal antibody, 2D7.10, which selectively recognizes carbohydrate epitopes in some axenic amebic strains. While high-level expression of this epitope was observed in axenic amebae, it was either absent or present only in small amounts in xenic amebae. Furthermore, reassociation of the axenic amebae with intestinal flora resulted in loss of the 2D7.10 epitope. Our data suggest that surface antigens of *E. histolytica* can be modulated in response to bacteria and may provide an explanation for the observed influence of bacteria on amebic virulence.

Infection with *Entamoeba histolytica* results either in a noninvasive, commensal state in the colon or in a diseased state with characteristic intestinal and/or extraintestinal lesions. However, the vast majority of the infections (more than 90%) are asymptomatic (25).

A number of studies have suggested a synergistic effect between *E. histolytica* and bacteria in amebic virulence. Treatment with antibacterial drugs resulted in marked improvement in the clinical profile of amebiasis in infected patients (10). Axenic amebae inoculated into germ-free guinea pigs did not produce lesions. However, lesions were produced when the animal or the amebae were reassociated with bacteria (19, 20, 26). The virulence of most strains, in animal models, decreases significantly on continuous axenic cultivation. In some strains virulence could be restored by reassociation with bacterial flora (18). The interaction of bacteria with amebae was shown to be mediated by specific sugar-binding molecules present on bacteria (16). These studies suggest that an association between bacteria and *E. histolytica* may have a profound effect on the physiology of the latter cells, leading to changes in virulence.

Sargeaunt and colleagues have studied isoenzyme profiles of thousands of cultured *E. histolytica* isolates; strains from symptomatic cases had distinct profiles compared with those from asymptomatic cases (23). The former were termed pathogenic and the latter nonpathogenic strains. Both pathogenic and nonpathogenic strains can be isolated in xenic culture. However, axenic cultures have been obtained only from those amebae which display pathogenic isoenzyme forms.

We have begun studies aimed at understanding the symbiotic relationship between bacteria and amebae. In this report we present evidence which suggests that cell-surface glycoconjugates differ between strains growing with and without bacteria.

All axenic strains, HM-1:IMSS clone (cl) 6 (14), SAW 1734 R cl AR (17), and HI 1295:AIIMS (isolated from a patient from India with invasive disease), were maintained in TYI-S-33 medium at 36°C (8). All xenic strains, 401:NIH (isolated from a patient at the National Institutes of Health; nonpathogenic isoenzyme profile isolate), SAW 891 R cl B (obtained from P. Sargeaunt), SAW 1734 R cl AR, and HI 1295:AIIMS, were grown in TYSGM-9 at 36°C (6). Bacterial flora were grown under the same conditions as the xenic cultures. Antigens were prepared according to published procedures (2, 3). Total-cell lysate was used as an antigen without further fractionation. The amount of amebic antigen in each cell lysate was determined by measuring proteins with BCA reagent (Pierce Chemical Company). There may be an error in determining amebic antigen concentration in xenic amebic lysates because of the presence of bacteria.

The antibodies used in this study were as follows. Monoclonal antibody (MAb) 2D7.10 has been described recently. It recognizes a carbohydrate determinant present on the cell surface of a number of axenic *E. histolytica* strains (3). MAb 2F3.4 recognizes a polypeptide determinant on the surface of all *E. histolytica* strains (11a). Polyclonal antibody aEhM was raised in rabbits against the Triton X-114 detergent phase-separated fraction of the HM-1:IMSS strain and recognizes mainly carbohydrate epitopes on *E. histolytica*. It competed with 2D7.10 in binding to amebic antigens, suggesting that these antibodies recognize a common epitope (11a). Polyclonal antibody aEhT.KCG was raised against a total-cell lysate of 200:NIH and was a kind gift of Shiv Pillai (21). We have used aEhM and aEhT.KCG as control antibodies to estimate the contribution of difference in amebic antigen concentration, antigen masking, and nonspecific degradation in different antigen preparations derived from different strains. Enzyme-linked immunosorbent assay (ELISA) and Western immunoblotting were carried out by published procedures (3, 11, 24). Polyacrylamide gels (10%) were used for all separations (12).

The amounts of 2D7.10 antigen in different strains of *E. histolytica* were determined by ELISA. Since a comparison was being made between xenic and axenic strains, polyclonal antibodies aEhM and aEhT.KCG were used in the assays as controls to rule out the possibility that the difference in immunoreactivity is due to either bacterial masking of antigens or differences in the concentration of amebic antigens as a result of bacterial components. Binding of antibodies to antigens prepared from different strains of *E.*

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TABLE 1. Antigen expression in different strains of *E. histolytica*[a]

Strain and amt of antigen (μg/well)	OD		
	2D7.10	aEhM	aEhT.KCG
Expt 1			
HM1-IMSS, cl 6 (axenic)			
10.0	>2.00	0.56	0.49
2.5	>2.00	0.75	0.55
401:NIH (xenic)			
10.0	0.00	0.60	0.65
2.5	0.00	0.70	0.72
HI 1295:AIIMS (xenic)			
10.0	0.00	0.40	0.14
2.5	0.00	0.38	0.20
SAW891 R cl B (xenic)			
10.0	0.02	0.40	0.63
2.5	0.03	0.54	0.82
Bacterial flora			
10.0	0.00	0.15	0.01
2.5	0.00	0.10	0.00
Expt 2			
HI 1295:AIIMS (axenic)			
10.0	0.59	1.44	ND
5.0	0.66	1.35	ND
HI 1295:AIIMS (xenic)			
10.0	0.01	1.14	ND
5.0	0.00	1.16	ND

[a] All axenized strains were grown in Diamond's TYI-S-33 medium, and xenic strains were grown in TYSGM-9. Cells were harvested after 72 h at 36°C. Cultures were chilled at 4°C for 10 min and centrifuged at 275 × *g* for 7 min. The pellet was washed with phosphate-buffered saline 8 (K_2HPO_4, 3.7 g; KH_2PO_4, 1.1 g; NaCl, 9.5 g; distilled water, as much as needed to make 1 liter, pH 7.2, 360 mosmol/liter). Cells were incubated in lysis buffer (10 mM Tris-Cl, pH 7.5; 150 mM NaCl; 2 mM phenylmethylsulfonyl fluoride, 5 mM *p*-chloromercuribenzoate; 10 μg of leupeptin per ml) for 30 min on ice and lysed by sonication for 30 s. ELISA was carried out as described earlier (3). Briefly, antigen was coated overnight at 4°C onto wells of microtiter plates. After blocking nonspecific sites with gelatin, primary antibody was added. Detection of bound antibody was by anti-mouse immunoglobulin antibody (for 2D7.10) or anti-rabbit immunoglobulin antibody (for aEhM and aEhT.KCG) conjugated to horseradish peroxidase. Color reagent used as *o*-phenylene diamine or 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid). Controls had either preimmune serum, control ascites, or control culture supernatants as a first antibody. OD values are presented here after subtraction of values obtained with control antibodies.

histolytica is shown in Table 1. 2D7.10 antigen was present in significant amounts in axenic strains HM-1:IMSS and HI 1295:AIIMS, as revealed by high ELISA values. However, the antigen could not be detected in xenic strains. This difference between xenic and axenized strains is not due to interstrain variation, since the axenized strain derived from xenic line HI 1295:AIIMS showed high-level expression (Table 1, experiment 2).

The corresponding ELISA values obtained with aEhM and aEhT.KCG are also shown in Table 1. In contrast to 2D7.10, these antibodies recognized all strains tested. No major variation among xenic and axenized strains was observed. 2D7.10 and aEhT.KCG did not bind to bacterial flora at the same concentration as amebic lysates. A low level of binding was observed with aEhM under the same conditions (Table 1). Thus, binding to antigens from xenic strains by polyclonal antibodies is not due to binding to bacterial components. This datum suggests that there is no significant masking of epitopes. The small variation observed in the ELISA values with polyclonal antibodies may be due to our inability to accurately determine the concentration of antigens derived from amebae. These observations were also confirmed by Western blot analysis. While aEhM immunostained equally, 2D7.10 stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-separated antigens prepared from axenized cells but not xenic cells, as observed before (data not shown) (3).

TABLE 2. Changes in antigenicity on reassociation of *E. histolytica* axenized strain HM-1:IMSS with bacterial flora

Antigen concn (μg/ml)	OD_{405}[a]	
	HM-1, axenic	HM-1, flora[b]
1	1.23	0.01
2	1.23	0.01
5	1.14	0.01
10	1.17	0.01
20	1.04	0.02

[a] ELISA was performed with horseradish peroxidase as an enzyme label and *o*-phenylenediamine as the substrate. OD_{405}, OD at 405 nm.
[b] HM-1 cl 6 cells were adapted to grow with NRS flora for about 5 weeks.

Axenized strains, with high levels of 2D7.10 antigen, were found to lose reactivity when reassociated with bacteria. *E. histolytica* HM-1:IMSS was reassociated with the NRS bacterial flora (7). No antigen could be detected in reassociated cultures in contrast with high levels of antigen in the axenized strain (Table 2). This change is not due to masking or degradation of antigens by bacterial products, since short-term xenic cultures (2 to 3 days) or mixing of bacteria before harvesting do not cause any change in antigen levels (data not shown).

During the process of axenization *E. histolytica* undergoes gross morphological changes visible under a light microscope (13). It is likely that these changes may affect many cell surface molecules. In order to determine whether other antigens may be affected by bacterial association, immunoassays with another MAb 2F3.4 which recognizes a polypeptide antigen were carried out (Table 3). The binding reactions of 2F3.4 and 2D7.10 were compared. The ELISA values were presented as ratios of experimental over control (e/c) antibodies, in addition to optical density (OD) values after subtraction of control. There was no binding of 2D7.10 to xenic strains, as reflected in OD (>0.02) and e/c (1 to 1.6). On the other hand there was significant binding of 2F3.4 to the same strains, with OD values of around 0.21 and e/c values of >13. The corresponding values for axenic strains were as follows: OD > 0.6 and e/c > 35. These results suggest that while 2D7.10 antigen was totally absent, 2F3.4

TABLE 3. Binding of MAbs 2D7.10 and 2F3.4 to *E. histolytica*[a]

Strain	OD (e/c)	
	2D7.10	2F3.4
HM-1:IMSS, cl 6 (axenic)	2.00 (40)	0.62 (31)
SAW 1734 R cl AR (axenic)	0.77 (19)	0.90 (45)
SAW 1734 R cl AR (xenic)	0.03 (1.0)	0.21 (13)
401:NIH (xenic)	0.07 (1.4)	0.23 (23)

[a] ELISA was carried out by using antigens prepared as described in footnote *a* to Table 1. The plates were coated with 50 μl of antigen preparation (100 μg/ml) overnight at 4°C. 2D7.10 ascites were used at 1:5,000, 2F3.4 supernatant was used at 1:5, and aEhM was used at 1:800. Values for controls have been obtained by using appropriate dilutions of control antibodies (ascites, supernatant, or preimmune serum). Horseradish peroxidase-labeled anti-mouse immunoglobulins along with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) was used for immunodetection.

antigen was present in xenic strains, although in somewhat reduced amounts compared with those in axenic strains.

Our results show a phenotypic modulation of a surface epitope recognized by a specific MAb. The level of antigen was modulated in response to associated organisms. Bacteria may influence surface antigens in a number of ways. First, because of continuous phagocytosis, the cell surface of the ameba may turn over very rapidly, resulting in loss of surface molecules. This, however, may not contribute to bacterium-induced modulation, since the cell surface of axenic strains also turns over rapidly (1). Second, bacteria bind amebic cell surface molecules through either specific or nonspecific interactions and thereby do not allow antibodies to approach the antigen (masking effect). This is unlikely, since immunostaining after SDS-PAGE separation and Western blotting gave results similar to those from the ELISA. Since adaptation of cells to bacterial flora is necessary before loss of the epitope takes place, it appears to be a response of the amebae towards bacterial association. Presence of a 30-kDa antigen only in xenic strains suggests that there may be both loss and gain of epitopes when cells go from xenic to axenized forms (4).

A number of studies have shown that bacteria influence physiological behavior of the amebae. This may, in turn, affect virulence of these organisms (18, 19, 20, 26). Cell surface molecules, including carbohydrate groups, are known to be involved in amebic recognition of target cells and bacteria (16). Modulation of surface antigens could be one of the mechanisms by which amebae control interaction with bacteria. Alteration in surface carbohydrates has been associated with changes in pathogenic properties in the protozoan parasite *Leishmania major* and in schistosomes (9, 22).

Modulation and variation of surface antigens have been observed in a number of different parasites (5, 15). In many instances, these changes help the parasite to survive inside the host. Our study shows a unique modulation of antigen dependent on associated microbial flora. This may provide a mechanism by which the ameba modulates host-parasite interaction.

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