Characterization of a Monoclonal Antibody That Selectively Recognizes a Subset of *Entamoeba histolytica* Isolates

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Monoclonal antibody 2D7.10 recognized an antigen present in seven of nine isolates of axenically cultured *Entamoeba histolytica* and absent in all other *Entamoeba* isolates studied. The antigen was absent in two isolates: 200:NIH and Rahman. All nine isolates belonged to pathogenic zymodeme II. Western blot (immunoblot) analysis and treatment with periodate and the proteolytic enzyme trypsin suggest that the antigen recognized by 2D7.10 is a carbohydrate moiety.

*Entamoeba histolytica* has been shown to have a number of antigenic determinants both on the cell surface and intracellularly (3–5, 13, 17, 23, 26). Some of these antigens have been implicated in cytotoxic killing by *E. histolytica* (2, 23). Though antigens specific for *E. histolytica* have been shown before, isolate-specific antigens have not yet been characterized. In order to analyze the molecular complexity of *E. histolytica*, we generated hybridoma antibodies to strain HM-1:IMSS, which belongs to a pathogenic zymodeme (25). In this report, we describe some of the characteristics of one of the monoclonal antibodies which recognizes an antigen that is present in some isolates of *E. histolytica* and absent in all other *Entamoeba* isolates tested.

The *Entamoeba* strains and species used in this study are described in Table 1. All amebae were maintained axenically in TYI-S-33 medium (8). *E. histolytica* was cultured at 36°C, and the other *Entamoeba* isolates were cultured at 24°C. Amebae were harvested by chilling cultures in ice water for 10 min, collecting the amebae by centrifugation at 275 × g for seven min at 4°C, and washing them twice with phosphate-buffered saline no. 8 (K2HPO4 3.7 g; KH2PO4 1.1 g; NaCl 9.5 g; distilled H2O to 1,000 ml; pH 7.2; 360 mosmol/kg). The washed cells were suspended in lysis buffer (20 mM Tris chloride, pH 7.5; 2 mM phenylmethylsulfonyl fluoride; 5 mM p-chloromercuribenzoate; 100 μg of leupeptin per ml) to give 2.5 × 10⁶ cells per ml. The suspension was incubated on ice for 30 min and disrupted by sonication for 30 s to give whole-cell lystate antigen.

Hybridoma cell lines were generated by fusing P3x63.Ag8.653 myeloma cells with spleen cells from hyperimmunized mice (14, 27). Mice were immunized alternately with purified plasma membrane (1) and whole cells. Approximately 10⁶ cells were used for each immunization. The hybridoma line 2D7.10 was expanded, and the ascitic fluid was generated by injecting 5 × 10⁶ hybridoma cells intraperitoneally into pristane-primed BALB/c mice. The antibodies were stored at −70°C.

Antigen-coated wells (5 μg per well) were treated with 50 μl of 1.5-mg/ml trypsin for 30 min at room temperature. The reaction was stopped by adding an equal amount of trypsin inhibitor. Controls had either no trypsin or trypsin plus trypsin inhibitor added together.

Sodium-m-periodate-mediated modification of sugar residues was carried out essentially as described previously (31). Periodate concentrations ranging from 1 to 50 mM were added to 10 μg of whole *E. histolytica* lystate coated on microtiter plates. Oxidation was carried out in 0.1 M sodium acetate buffer, pH 4.5, in the dark at room temperature for 30 min. The reaction was stopped by adding 50 μl of 0.02 M sodium sulfite, and the plate was washed twice with phosphate-buffered saline.

Enzyme-linked immunosorbent assay (ELISA) was carried out essentially as previously described (11). Briefly, *E. histolytica* lystate at 5 μg per well was used to coat 96-well microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.). After being blocked with gelatin, the antigen-coated wells were incubated with monoclonal antibody 2D7.10. A horseradish peroxidase-based biotin-streptavidin system and o-phenylene diamine as the color reagent were used for quantitation of bound 2D7.10 as recommended by the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Color intensity was estimated by measuring optical density at 492 nm.

The polypeptides were separated in 10% polyacrylamide gels according to the method of Laemmli (15). After separation, the gels were either stained in 0.125% Coomassie brilliant blue R250 or transferred to nitrocellulose paper electrophoretically (31). Gels were stained with Coomassie brilliant blue after transfer to ascertain completion of transfer. Molecular weights were determined by using prestained molecular weight markers (Bio-Rad Laboratories, Richmond, Calif., or Sigma Chemical Co., St. Louis, Mo.). The blot was probed with 2D7.10, and reactive antigens were detected with goat anti-mouse immunoglobulin and conjugated to alkaline phosphatase. Color development utilized 5-bromo-4-chloro-3-indolyl phosphate and p-Nitro Blue Tetrazolium as substrates. All protein concentrations were determined with the BCA reagent (Pierce Chemical Co., Rockford, Ill.).

Hybridoma lines were screened for isolate- and species-specific antibodies by indirect ELISA. Antibodies reacting with *E. histolytica* isolates, but not other *Entamoeba* species, were further characterized. One monoclonal antibody, 2D7.10 (γδ isotype), showed some isolate specificity. Representative assays are shown in Fig. 1. Table 1 summarizes all the studies. The 2D7.10 antigen was found to be present only in *E. histolytica* and not in other *Entamoeba* isolates tested. It was not detected in two (200:NIH and Rahman) of the nine *E. histolytica* isolates studied.

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In order to determine the molecular size of the antigen recognized by 2D7.10, Western blot analysis was carried out (30). Results of the experiment are shown in Fig. 2. On HM-1:IMSS antigen, a number of broad bands were seen (lane 9). No reactivity was observed in an *E. histolytica*-like ameba, Laredo strain (lane 5). Molecular weights of the antigens recognized by the antibody varied. All isolates tested showed a similar diffuse pattern; however, both the KCG strains had extra bands (lanes 6 and 7) compared with both HK-9 (lane 8) and HM-1 strains.

In order to determine the nature of the epitope recognized by 2D7.10, the sensitivity of the antigen toward proteolytic cleavage and periodate-mediated oxidation was studied. Treatment of antigen with trypsin (up to 1.5 mg/ml) had no effect on 2D7.10 reactivity as determined by ELISA (Fig. 3a). The 2D7.10 antigen is sensitive to periodate. Increasing periodate concentrations lead to increased loss of antigenicity. At 50 mM periodate, 91% of antigenicity is lost (Fig. 3b).

Monoclonal antibodies reported to date mainly recognize determinants present in all *E. histolytica* (22, 29). Antibodies which recognize determinants correlating with amebal isoenzyme patterns have also been reported (28). To our knowledge, this is the first report of an antibody that can distinguish among isolates of *E. histolytica* belonging to the same zymodeme (II, the most common pathogenic zymodeme).

Western blot analysis of *E. histolytica* strains with 2D7.10

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**TABLE 1. *E. histolytica* and other *Entamoeba* isolates used in these studies**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Host</th>
<th>Clinical diagnosis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Zymodeme</th>
<th>Reference</th>
<th>Reactivity with 2D7.10</th>
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<tbody>
<tr>
<td><em>E. histolytica</em> Schaudinn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200:NIH</td>
<td>Human</td>
<td>D</td>
<td>II</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>HM-1:IMSS, cl 6</td>
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<td>D</td>
<td>II</td>
<td>9</td>
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<tr>
<td>Rahman, cl 1</td>
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<td>NDAC</td>
<td>II</td>
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<td>–</td>
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<tr>
<td>KCG:0986:11</td>
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<td>NDAC</td>
<td>II&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>KCG:1287:16</td>
<td>Human</td>
<td>NDAC</td>
<td>II&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>SAW 1734 R cl AR</td>
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<td>20</td>
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</tr>
<tr>
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<td>6</td>
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<tr>
<td>HK-9, cl 2</td>
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<td>NDAC</td>
<td>II</td>
<td>18</td>
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**Other Entamoeba isolates**

- *E. histolytica*-like Laredo
- *E. invadens* Rodhain 165, cl 3
- *E. moshkovskii* Tshalaia FIC

<table>
<thead>
<tr>
<th>Strain</th>
<th>Host</th>
<th>Classification</th>
<th>Reference</th>
<th>Reactivity with 2D7.10</th>
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<tr>
<td>Human</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Terrapin</td>
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<td>6</td>
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<tr>
<td>KCG:1287:16</td>
<td>Sewage effluent</td>
<td></td>
<td>7</td>
<td>–</td>
</tr>
</tbody>
</table>


<sup>b</sup> Zymodeme pattern changed from nonpathogenic to pathogenic during the process of axenization.

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**FIG. 1.** ELISA of different isolates of *E. histolytica* to determine specificity of 2D7.10. Whole-cell lysates, prepared as described in the text, were used as antigens in microtiter plate-based ELISA. Bound antibody was detected using a biotin-streptavidin system. Antigens were used in different concentrations ranging from 10 to 0.625 μg; Symbols: ○, HM-1:IMSS with 2D7.10; ●, HM-1:IMSS with control ascites; △, Rahman with 2D7.10; ▲, Rahman with control ascites; □, KCG:1287:16 with 2D7.10; ■, KCG:1287:16 with control ascites.

**FIG. 2.** Western blot analysis of different isolates of *E. histolytica*. Total cell lysates of the following isolates were resolved in sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis. (A) Coomassie blue staining of gels; (B) enzyme immunostaining of 2D7.10-reactive antigens after electrophoretic transfer. Antibody was used at a dilution of 1:5,000. Lanes 4 and 9, HM-1:IMSS; lanes 3 and 8, HK-9; lanes 2 and 7, KCG:1287:16; lanes 1 and 5, *E. histolytica*-like ameba, Laredo strain; lane 6, KCG:0986:11. The molecular weight standards (in thousands) are indicated by arrowheads.
showed numerous bands extending from 30 to 200 kilodaltons. These different bands were not due to degradation of the antigens, because a number of different protease inhibitors were used in preparation of the lysate. In addition, the similar pattern obtained at all times also indicated that multiplicity of bands may be an inherent characteristic of the antigen. A similar pattern has also been observed with antibodies recognizing a carbohydrate epitope of glycoproteins of neuronal cells (21). It is likely that a common epitope present in different molecules may give rise to a similar pattern (16, 21). The difference in the Western blot pattern between the KCG strains and strains HM-1:IMSS and HK-9 may reflect the physiological changes in the cell brought about by changes in isoenzyme patterns induced during the process of axenization (20); however, we have to analyze more such strains before we can make definitive conclusions. The difference in Western blot patterns may be due to the addition of a common epitope to different polypeptides.

The experiments with periodate and proteolytic enzymes suggest that the antigenic determinant may be a carbohydrate molecule. The carbohydrate moiety is not a neuraminic acid derivative, because mild periodate oxidation (1.0 mM) has a nominal effect on antigenicity (31).

The importance of carbohydrates in virulence has been amply demonstrated with *E. histolytica* (19, 24). It now seems certain that the bacterial flora associated with the amebae in vitro influences their pathogenicity. Molecular mediators of ameba-bacterium interaction appear to be carbohydrates, especially the mannose-containing sugars (19). Recognition of target epithelial cells by *E. histolytica* is also mediated through interaction with specific sugars on the target cell (24). These studies indicate that sugars play an important role in the virulence of *E. histolytica*. Our finding that *E. histolytica* strains can be distinguished on the basis of altered carbohydrate antigens may have relevance in the studies on the mechanism of pathogenicity of the ameba.

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**LITERATURE CITED**