SERUM IgM ANTIBODY RESPONSE TO THE GALACTOSE-INHIBITABLE ADHERENCE LECTIN OF ENTAMEBOA HISTOLYTICA

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Abstract. An ELISA for detection of serum IgM antibodies to the galactose-inhibitable adherence lectin of Entamoeba histolytica revealed that 2.8% of uninfected controls, 0.0% of controls infected with other parasites, 13.4% of asymptomatic amebic infections, 55% of colitis patients, and 77% of amebic liver abscesses from Cairo, Egypt and Durban, South Africa had serum anti-lectin IgM antibodies. Of acute amebic colitis patients with symptoms for less than one week, only 6% possessed serum IgG anti-lectin antibodies, yet 45% had serum IgM antibodies to the amebic lectin. This compares with 65% of sera in acute colitis patients positive for lectin antigen as determined by ELISA with anti-lectin monoclonal antibodies. In conclusion, an ELISA for serum anti-lectin IgM antibodies appears to have greater clinical utility in the setting of acute amebic colitis than an ELISA for anti-lectin IgG antibodies, but is no more sensitive than an ELISA for detection of lectin antigen in sera.

In some protozoal infections, the measurement of IgM antibodies is helpful in identification of current and postinfection states, such as giardiasis and toxoplasmosis. Detection of anti–Entamoeba histolytica IgM antibodies may constitute an important strategy for early diagnosis. Regression of the serum anti-amebic IgM antibody response may constitute an important prognostic factor. The relatively short duration of the anti-amebic IgM antibody response has also been confirmed by Jackson and others compared with the persistence of anti-amebic IgG serum antibodies for years after cure of invasive amebiasis. Serum IgM antibodies to amebic plasma membrane antigens were detected previously with high specificity and sensitivity by an ELISA procedure. Reactivity of anti-amebic IgM antibodies to small (5–15 kD) cytoplasmic membrane antigens has been demonstrated.

The use of well-characterized native amebic surface antigens may improve the diagnostic utility of serologic tests. The galactose-inhibitable lectin of E. histolytica mediates adherence of trophozoites to colonic mucins, epithelial cells, and host inflammatory cells. Galactose-inhibitable lectin binding is absolutely required for amebic lysis of mammalian cells. We used an ELISA with purified native amebic lectin, which contains a highly conserved 170-kD antigen subunit that is recognized by serum IgG antibodies from patients worldwide with invasive amebiasis. Lectin antigenemia has been demonstrated in patients with invasive amebiasis. Amebic lectin has been detected in feces from patients with E. histolytica or E. dispar intestinal infections. Previous studies have demonstrated that subjects with other parasitic infections do not generate antibodies to the amebic lectin and that anti-lectin monoclonal antibodies are specific for E. histolytica and E. dispar lectin epitopes. In our study, we determined whether detection of serum anti-lectin IgM antibodies may be more useful than IgG antibodies for diagnosis of acute invasive amebiasis.

SUBJECTS AND METHODS

Subjects. All studies were approved by the Human Subjects Institutional Review Boards at the University of Virginia, the University of Minnesota, the University of Natal, and El-Hussein University as applicable. Informed consent was obtained in Zulu with English translation and documented by approved consent forms. Normal American control subjects were healthy employees of the University of Virginia without any history of amebic infection. Egyptian control subjects were healthy relatives of patients attending El-Hussein University Hospital (Cairo, Egypt) who denied a history of amebic disease and had negative stool examination results for E. histolytica. Cases with asymptomatic amebic infection (presumed E. dispar), acute amebic colitis, and amebic liver abscess were obtained from the outpatient clinic and inpatient section of the Tropical Medicine service of El-Hussein University Hospital. Colitis patients were characterized by having diarrhea, hematophagous trophozoites on microscopy, negative fecal culture for Shigella species, and a positive clinical response to metronidazole. Patients with amebic liver abscess had a characteristic clinical syndrome, including a defect in the liver on imaging and a negative abscess aspirate for bacterial culture, and a positive response to metronidazole. In addition, we studied sera from South African subjects who had stool cultures with zymodeme analysis by starch gel electrophoresis. Sera were from 41 controls, 73 cases with asymptomatic E. dispar infection, four cases with asymptomatic E. histolytica infection, and 12 amebic liver abscess patients. A final control group consisted of sera from patients with other parasitic diseases including Giardia lamblia, Cryptosporidium, Endolimax nana, Trichinella, Schistosoma mansoni, Filariae, malaria, and Iodamoeba butchli. These control groups had at least one negative stool microscopy for Entamoeba.

Cultivation of E. histolytica and purification of adherence lectin. Axenic E. histolytica trophozoites of strain HMI-IMSS were maintained in our laboratory and grown in TYI-S medium. Amoebae were harvested within 72 hr of subculture in the logarithmic phase of growth. Entamoeba histolytica adherence lectin was prepared as described previously. Five mouse monoclonal antibodies (MAbs) to the adherence lectin, 8H5, 7F4, 3F4, 5B8, and 6D2, were purified from mouse ascites by protein A agarose (Sigma, St. Louis, MO) affinity chromatography. Purified MAbs were coupled to Sepharose 4B (Sigma). Di-isopropylfluorophosphate (Sigma)–treated amoebae were solubilized in 0.5% Nonidet P40 (Sigma), 50 mM Tris, 150 mM NaCl, pH 7.5.
Enzyme-linked immunosorbent assay for detection of serum anti-lectin antibodies. This procedure was performed as previously described. Briefly, 96-well microtiter plates (flat-bottom, polystyrene ELISA plates; Costar, Cambridge, MA) were coated with purified adherence protein (0.1 μg/well), incubated for 2 hr at room temperature, washed three times with phosphate-buffered saline (PBS)-1% Tween, and blocked with PBS-Tween, 1% bovine serum albumin (BSA) for 2 hr at room temperature. The test sera were added at a 1:1,000 dilution in PBS-Tween, 1% BSA (100 μl/well), incubated for 2 hr at room temperature, and washed four times with PBS-Tween. Peroxidase-conjugated goat anti-human IgMFc (μ chain fragment specific, Cod 109-035-043; Jackson Immunoresearch Laboratories, West Grove, PA) was added at a 1:25,000 dilution in PBS-Tween, 1% BSA (100 μl/well), incubated for 2 hr at room temperature, and washed three times with PBS-Tween. Enzyme substrate (100 μl of 0.1 M phosphate-citrate containing 10 mg of o-phenylenediamine dihydrochloride and 10 μl of 30% H₂O₂/25 ml, pH 5.0) was added. The reaction was developed in the dark for 90 min and the degree of color change (optical density [OD]) was measured in an ELISA plate reader at 492 nm. Results were corrected for nonspecific background by subtracting the OD of the paired wells not coated with adherence lectin but otherwise exposed to the identical procedure.

Statistical analysis. All results were expressed as the mean ± SD and the unpaired Student’s t-test was used to determine the significance. The following definitions were used: sensitivity = number of patients with positive test results/total number of patients; specificity = number of controls with negative test results/total number of controls; positive predictive value = true positive/true positive + false positive; and negative predictive value = false negative/true negative + true negative.

Each serum was tested in duplicate. The cut-off value for a positive test result was determined as being greater than the mean plus two standard deviations of the results in the control populations.

RESULTS

Anti-lectin IgM sera antibody responses for the Egyptian and South African study groups are presented in Figures 1 and 2, respectively. The cut-off point for a positive test result is defined by the mean plus two standard deviations of results from controls; subjects from the local endemic area are used for each group. A positive ELISA result for anti-lectin IgM was found in 2.8% of uninfected controls, 13.4% of asymptomatic cyst passers, 55% of colitis patients, 77% of amebic liver abscess (ALA) patients, and in none of the sub-
jects with other parasitic infestations (Figures 1 and 2; \( P < 0.01 \), colitis and ALA compared with all control groups). Of the amebic colitis patients who lacked serum anti-lectin IgG antibodies, 40.5% had serum anti-lectin IgM antibodies (Table 1). The ELISA for lectin antigen was more sensitive: 64.8% of the colitis sera were positive; importantly, all IgG (-) and IgM (+) sera contained lectin antigen. Of those subjects with a clinical diagnosis of amebic colitis at El-Hussein University Hospital, 23% were negative for serum lectin antigen, serum anti-lectin IgG, and IgM antibodies. In no cases of amebic colitis or liver abscess was the serum anti-lectin IgM ELISA result positive if either serum anti-lectin IgG or serum lectin antigen was not found (Table 1). In subjects with amebic liver abscess having at least seven days of symptoms, detection of sera anti-lectin IgM antibodies offered no advantage over the ELISA for IgG antibodies (Table 1).

The ELISA for serum anti-lectin IgM was of greatest diagnostic use in colitis patients with less than one week of symptoms (Table 2), being positive in 45.1%, compared with only 5.6% for the anti-lectin IgG ELISA \( (P < 0.001) \). However, the ELISA for lectin antigen had greater sensitivity, being positive in 64.8% of the acute colitis patients \( (P < 0.01 \), compared with IgM or IgG antibodies). In amebic colitis greater than one week in duration, detection of serum anti-lectin IgG antibodies was the preferred test with a sensitivity of 93% and a positive predictive value of 0.71. The combination of all three ELISAs for IgM, IgG, and lectin antigen in colitis patients, regardless of duration of symptoms, had a combined sensitivity of 77% and a positive predictive value of 0.82.

### Discussion

Serologic methods for diagnosis of amebiasis have been limited by the use of crude uncharacterized amebic antigen preparations, complex methodologies, and the high prevalence of seropositivity in uninfected controls residing in endemic areas. Use of an ELISA for detection of anti-lectin antibodies has the advantage of using a well-defined antigen. Since the genes encoding the lectin 170-kD subunit have been identified and sequenced,20,21 recombinant proteins are available for use. For example, a recombinant antigen designated LC3, which includes a 52-kD cysteine-rich section of the 170-kD subunit of the lectin,22 demonstrated promise for diagnostic application in both intestinal and extra-intestinal amebiasis.16 A number of other recombinant \textit{E. histolytica} antigens have been found to have potential use in serodiagnosis.23,24

The ELISA for detection of serum anti-lectin IgM antibodies can be helpful in distinguishing patients with invasive amebiasis from healthy controls and asymptomatic carriers in endemic regions of Egypt and South Africa. Correlation of IgM results with lectin antigenemia and detection of serum anti-lectin IgG antibodies improves the specificity, sensitivity, and predictive values for diagnosis. The ELISA for lectin antigenemia is the most sensitive test, followed by the ELISA for serum anti-lectin IgM antibodies, during the first week of colitis symptoms. Following one week of symptoms, the sensitivity of anti-lectin IgG antibodies is markedly improved. All cases of invasive amebiasis that were positive for serum anti-lectin IgM antibodies were subsequently positive for either serum lectin antigen or anti-lectin IgG anti-

### Table 1

Detection of serum anti-lectin IgM antibodies with correlation to an ELISA for detection of serum anti-lectin IgG antibodies and serum lectin antigen (Ag)*

<table>
<thead>
<tr>
<th>Group (total numbers)</th>
<th>IgG(+)</th>
<th>IgG(-)</th>
<th>Ag(+)</th>
<th>Ag(-)</th>
<th>IgG and Ag(+)</th>
<th>IgG and Ag(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (141)</td>
<td>0/10</td>
<td>4/131</td>
<td>2/9</td>
<td>2/132</td>
<td>0/0</td>
<td>2/122</td>
</tr>
<tr>
<td>Asymptomatic infection (127)</td>
<td>5/26</td>
<td>12/101</td>
<td>14/26</td>
<td>3/101</td>
<td>4/6</td>
<td>2/81</td>
</tr>
<tr>
<td>Amebic colitis (100)</td>
<td>27/31</td>
<td>28/69</td>
<td>38/57</td>
<td>17/43</td>
<td>10/11</td>
<td>0/23</td>
</tr>
<tr>
<td>Amebic liver abscess (18)</td>
<td>14/18</td>
<td>0/0</td>
<td>10/11</td>
<td>4/7</td>
<td>10/11</td>
<td>0/0</td>
</tr>
</tbody>
</table>

* Criteria of seropositivity were > 2 standard deviations above the mean of endemic area controls (Egyptian and South African).

### Table 2

Comparison of specificity, sensitivity, and predictive values for an ELISA in relation to duration of symptoms in patients with amebic colitis in Cairo, Egypt**

<table>
<thead>
<tr>
<th></th>
<th>Colitis symptoms for a week or less</th>
<th>Colitis symptoms for more than a week</th>
<th>All colitis subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>Lec.Ag</td>
</tr>
<tr>
<td><strong>Sensitivity (%)</strong></td>
<td>45.1</td>
<td>5.6</td>
<td>64.8</td>
</tr>
<tr>
<td><strong>Specificity (%)</strong></td>
<td>97.2</td>
<td>92.9</td>
<td>93.6</td>
</tr>
<tr>
<td><strong>Positive predictive value</strong></td>
<td>0.88</td>
<td>0.29</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Negative predictive value</strong></td>
<td>0.02</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Lec.Ag = lectin antigen.
bodies. Detection of fecal lectin antigen by ELISA\textsuperscript{15,17} can be more sensitive than serology in identifying \textit{E. histolytica} infection but does not differentiate acute invasive disease from asymptomatic infection with diarrhea due to another enteric pathogen.

Twenty-three patients with amebic colitis in Egypt had negative ELISA results for serum anti-lectin IgG and IgM antibodies and for detection of serum lectin antigen. Despite their classic presentations, diarrhea in these cases might be attributed to another pathogens, despite the reported presence of hemopathagous trophozoites on fecal microscopy. Cultures and zymoderm analysis were not performed on these specimens to differentiate \textit{E. dispar} from \textit{E. histolytica} infections. The difficulties in clinical diagnosis of amebic colitis by even highly experienced clinicians in developing countries point out the need for reliable, rapid, quantitative, diagnostic methods. In conclusion, the ELISA for serum anti-lectin IgM antibodies is more useful than that for anti-lectin IgG antibodies in the setting of acute amebic colitis, but is no more sensitive than the ELISA for detection of serum lectin antigen.

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