REVIEW ARTICLE
Adherence-Blocking Vaccine for Amebiasis

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The Gal/GalNAc lectin is a candidate vaccine antigen for an amebiasis vaccine due to its mediation of parasite adherence to the human intestine, because partial immunity in humans is associated with a mucosal IgA response against it, and because it is effective as a vaccine against amebic colitis in the murine model. The LecA domain of the Gal/GalNAc lectin contains neutralizing antibody epitopes. LecA contains the active site of the lectin (the carbohydrate recognition domain or “CRD”) and has been an effective vaccine antigen in animal models of amebic colitis and liver abscess. Research needs include production of the LecA domain of the Gal/GalNAc lectin by a process that can be transferred to cGMP and optimization for immunogenicity, using adjuvants such as alum, MF59 or QS-21 adjuvants. Accomplishing this will enable testing of the ability of LecA immunizations to protect from amebic colitis in humans. © 2006 IMSS. Published by Elsevier Inc.

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Introduction
Amebic colitis and liver abscess are due to infection with the enteric protozoan parasite Entamoeba histolytica. This parasite has recently been separated using modern diagnostic techniques from the nonpathogenic parasite E. dispar, which is more common and identical in appearance to E. histolytica (1–3). The World Health Organization (WHO) estimates that approximately 50 million people worldwide suffer from invasive amebic infection each year, resulting in 40–100 thousand deaths annually (1–3). Carefully conducted serologic studies in Mexico where amebiasis is endemic demonstrated antibody to E. histolytica in 8.4% of the population (4). In the urban slum of Fortaleza, Brazil, 25% of the people tested carried antibody to E. histolytica, the prevalence of anti-amebic antibodies in children aged 6 to 14 years was 40% (5). A prospective study of preschool children in a slum of Dhaka, Bangladesh demonstrated new E. histolytica infection in 39% of children over a 1-year period of observation, with 10% of the children having an E. histolytica infection associated with diarrhea and 3% with dysentery (6). We conclude that there is an increasing recognition of the burden of infection due to this protozoan parasite.

Pathogenesis of Amebiasis: Gal/GalNAc Lectin-Mediated Adherence/Cytotoxicity
E. histolytica invades tissue and causes clinical disease through a well-defined sequence of events that starts with the ingestion of the infectious cyst form of the parasite from fecal contaminated food or water (7–13). Excystation of the amebic trophozoites occurs in the intestinal lumen. The trophozoites adhere to the colonic mucus and epithelial cells through interaction of a galactose and N-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin with host Gal/GalNAc-containing glycoconjugates (Figure 1) (7). The trophozoites kill host epithelial and immune cells at points of invasion in a process that requires the activity of the Gal/GalNAc lectin. Finally, E. histolytica resists the host’s immune response and survives to cause extra-intestinal infection such as amebic liver abscess. The Gal/GalNAc lectin is a 260-kDa heterodimer of disulfide-linked heavy (Hgl) and light (Lgl) subunits which is non-covalently associated with an intermediate subunit (Igl) (Figure 1).
All three subunits are encoded by gene families. The sequence of the hgl genes is nearly completely conserved in isolates of *E. histolytica* from different continents (14). The carbohydrate recognition domain (CRD) is a cysteine-rich region within Hgl (amino acids 895–998) recognized by adherence-inhibitory mAb and that when expressed in *E. coli* binds to Gal/GalNAc (15–17). We are focusing our immunization strategy on “LecA” (aa 578–1154) (Figure 1) as it contains CRD and adherence-inhibitory monoclonal antibody epitopes (Table 1), vaccination with it has provided protection from *E. histolytica* in animal models (Table 2), and in children anti-CRD IgA is a marker of immunity (6,18).

**Human Immunity Is Associated with Intestinal IgA against the Gal/GalNAc Lectin**

A critical recent advance by our team of investigators has been the discovery of acquired immunity in children to intestinal amebiasis (Figure 2) (6,18). Immunity is associated with a mucosal IgA response against the carbohydrate recognition domain of the Gal/GalNAc lectin: children with this response had 86% fewer new infections over 1 year of prospective observation (18). Anti-Gal/GalNAc lectin IgA has also been associated with immunity to *E. dispar* (19).

**The Gal/GalNAc Lectin Is a Vaccine Candidate in Animal Models of Amebiasis**

Immunizations with native Gal/GalNAc lectin, native Igl, and with recombinant proteins containing parts of the cysteine-rich extracellular portion of Hgl, have been protective in the gerbil model of amebic liver abscess (Table 2).

**The C3H Mouse Model of Amebic Colitis**

An impediment to the development of a vaccine had been the lack of an animal model of intestinal infection. We have extended the work of Ghosh and colleagues to utilize a C3H mouse model of amebic liver abscess (20–21). Mice are infected

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### Table 1. Location of linear and conformational epitopes of the Gal/GalNAc lectin heavy subunit (Hgl)

<table>
<thead>
<tr>
<th>Epitope #</th>
<th>Effect on adherence</th>
<th>Effect on cytotoxicity</th>
<th>Effect on serum resistance</th>
<th>Epitope location in lectin Hgl</th>
<th>Epitope is conformational or linear</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Increases</td>
<td>Inhibits</td>
<td>No effect</td>
<td>909–1013</td>
<td>Conformational</td>
</tr>
<tr>
<td>2</td>
<td>Increases</td>
<td>Inhibits</td>
<td>No effect</td>
<td>909–1013</td>
<td>Conformational</td>
</tr>
<tr>
<td>3</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>1097–1154</td>
<td>Linear</td>
</tr>
<tr>
<td>4</td>
<td>Inhibits</td>
<td>Inhibits</td>
<td>No effect</td>
<td>909–1013</td>
<td>Linear</td>
</tr>
<tr>
<td>5</td>
<td>Inhibits</td>
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<td>No effect</td>
<td>611–833</td>
<td>Linear</td>
</tr>
<tr>
<td>6</td>
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<td>1048–1096</td>
<td>Linear</td>
</tr>
<tr>
<td>7</td>
<td>No effect</td>
<td>No effect</td>
<td>Inhibits</td>
<td>909–1013</td>
<td>Conformational</td>
</tr>
</tbody>
</table>

Note: LecA is amino acids 578–1154 and CRD is amino acids 895–998 of Hgl.
by injection of amebic trophozoites into the cecum. Chronic *E. histolytica* infection with ulcerative colitis develops in 60–90% of infected mice; histopathologically the lesions in the mouse colon closely resemble human amebic colitis (Figure 3). Without intervention *E. histolytica* infection and amebic colitis persist for months in C3H mice. Conversely, immunization of mice with the Gal/GalNAc lectin prevents amebic infection and colitis (Table 2) (23).

**Conclusions**

The Gal/GalNAc lectin has met all of the basic requirements as a subunit vaccine candidate. The lectin plays an essential role in adherence and cytotoxicity, as well as in resistance to serum complement. Importantly, fecal IgA against the Gal/GalNAc lectin is associated with acquired immunity to intestinal infection in children. In addition, the lectin’s cysteine-rich extracellular domain is highly conserved. Finally, the lectin is the only antigen demonstrated to be protective in animal models of both colitis (by far the most common form of disease due to *E. histolytica*) and amebic liver abscess. Extensive investigation by a number of independent laboratories has centered on the LecA portion of the Gal/GalNAc lectin heavy subunit as the most promising vaccine candidate. The challenge today is to identify means of immunization with LecA that are both protective and acceptable for use in humans.

**Figure 2.** The presence of stool IgA anti-CRD (lectin carbohydrate recognition domain) antibodies at study enrollment is associated with a lower incidence of new *E. histolytica* infections. Children from Mirpur with (*n* = 12) or without (*n* = 218) stool CRD-specific IgA at the time of enrollment were followed over 2 years for new *E. histolytica* infections, as detected by antigen detection in monthly stool samples. The two groups are statistically significantly different (*p* ≤0.05) at month 11 and months 14–24.

**Figure 3.** Chronic amebic colitis in C3H/HeJ mice. Three weeks after intracecal inoculation of *E. histolytica*, amebas were both ulcerating the epithelium and occupying the lumen (inset ×1000). Extensive intestinal mucosal hyperplasia is evident as well as a large ulceration (boxed area in A, enlarged in B). Note invasive trophozoites (arrowheads), many of which contain ingested erythrocytes (arrows).
References