Amebiasis is the third leading parasitic cause of death worldwide, and it is not known whether immunity is acquired from a previous infection. An investigation was done to determine whether protection from intestinal infection correlated with mucosal or systemic antibody responses to the *Entamoeba histolytica* GalNAc adherence lectin. *E. histolytica* colonization was present in 0% (0/64) of children with and 13.4% (33/246) of children without stool IgA anti–GalNAc lectin antibodies (*P* = .001). Children with stool IgA lectin-specific antibodies at the beginning of the study had 64% fewer new *E. histolytica* infections by 5 months (3/42 IgA+ vs. 47/227 IgA−; *P* = .03). A stool antilectin IgA response was detected near the time of resolution of infection in 67% (12/18) of closely monitored new infections. It was concluded that a mucosal IgA antilectin antibody response is associated with immune protection against *E. histolytica* colonization. The demonstration of naturally acquired immunity offers hope for a vaccine to prevent amebiasis.
worker, or hospitalized when appropriate. Four children with new E. histolytica infections developed dysentery and were treated for amebiasis with metronidazole. There is a low incidence of human immunodeficiency virus infection among high-risk groups in Dhaka (<3% in sex workers and injection drug users), so we did not test children for this disease [19]. Height and weight of the children were assessed by use of standard World Health Organization procedures [20].

Diagnosis of E. histolytica infection. Infection was diagnosed by use of an E. histolytica antigen–specific test (E. histolytica II; TechLab), which was designed for use with stool samples. The test was used according to the manufacturer’s instructions [21]. Stool samples were also cultured for Entamoeba species in Robinson’s medium within 6 h of collection [22]. A new E. histolytica infection was defined as a positive E. histolytica stool antigen or culture result preceded by ≥2 monthly surveillance stool samples with negative results. Colonization with E. histolytica was defined as infection without dysentery or extraintestinal amebiasis.

ELISA for detection of serum and stool antilectin antibodies. The procedure used to detect anti-GalNAc lectin serum IgG and stool IgA antibodies by ELISA was modified from the procedure of Kelsall et al. [15], who used microtiter plates coated with purified GalNAc lectin. A sample was considered positive for serum antilectin IgG if the optical density (OD) reading was ≥0.5, which is a cutoff point ≥3 SD above the mean of uninfected children in Dhaka and of negative serum samples from a country where E. histolytica is nonendemic. IgA was purified from stool samples, using anti–human IgA (α-chain–specific agarose, no. A22691; Sigma) according to the manufacturer’s instructions. Stool samples containing 5 μg of total IgA were tested for GalNAc lectin–specific IgA. A sample was considered to be positive if the OD reading was ≥0.188, a cutoff point ≥2 SD above the mean of infected Dhaka children.

Statistical analysis. All data collected were computer-coded and analyzed by use of SPSS software (release 7.5; SPSS). Means of different variables were compared by use of Student’s t test or, if the variables were not normally distributed, a nonparametric test. The χ² test and Fisher’s exact tests were used for categorical variables, to compare proportions between 2 groups. For all methods, a value of P < .05 was considered to be significant.

Results

As determined by antigen detection, asymptomatic E. histolytica infection was present in 4.3% (50/1164) of 2–5-year-old children in Mirpur. As we reported earlier, 100% of samples with positive culture results were also positive by antigen detection, and the test was not affected by the presence of antilectin IgA antibodies in stool (data not shown) [21]. We used polymerase chain reaction (PCR), which has a sensitivity of ~75% compared with antigen detection, to test the 34 samples with culture-negative and antigen-positive results: 79% (27/34) of these specimens were positive by PCR, demonstrating that most of these samples represented true E. histolytica infection [21].

Results of the cross-sectional survey of 310 children. Lectin-specific serum IgG was present in 14.6% (170/1164) of the children. Presence or absence of antilectin IgA in stool was determined in a subset of 310 of these children (approximately divided between those with and without lectin-specific serum IgG). Stool IgA antilectin antibodies were detected in 25.3% (37/146) of the children with and in 16.5% (27/164) of the children without serum IgG antilectin antibodies (P < .05). The level of serum IgG antilectin antibodies was significantly higher in the children with stool antilectin IgA (0.88 vs. 0.49 OD units; P < .0001). All antilectin IgA–positive stool samples tested contained antilectin secretory IgA, IgA1, and IgA2 and blocked lectin-mediated trophozoite adherence in vitro (data not shown).

The presence of stool antilectin IgA was associated with the absence of E. histolytica colonization: 0% (0/64) of children with stool IgA antilectin antibodies (IgA+ and 13.4% (33/246) of children without stool IgA antilectin antibodies (IgA−) were infected, as determined by antigen detection (P = .001; table 1). Although culture was a less sensitive test than antigen detection, it too demonstrated the absence of E. histolytica infection in children with stool IgA antilectin antibodies: 0% (0/64) of IgA+ versus 6.5% (16/246) of IgA− children who had culture-positive results (P = .04; table 1). In contrast, E. histolytica colonization was more frequent in children with serum IgG antilectin antibodies: 17.8% (26/146) of IgG+ versus 4.3% (7/164) of IgG− children (P < .0001).

Results of the prospective study. A 1-year prospective study was conducted on 289 of the 310 children, 93% (269/289) of whom were still in the study at 12 months. Of the 254 participating families, 29 had 2 children and 3 had 3 children participating. Among the 4 groups of children, there were no significant differences at the time of enrollment in age or nutritional status, although age was greater in seropositive children (P = .004) and height was greater in seronegative children (P = .01; table 2). The 289 children included subgroups of 145 children with and 144 without serum antilectin IgG at enrollment and 46 children with and 243 without stool IgA lectin-specific antibodies at enrollment (figure 1; table 2). During the 12-month study, 39% (105/269) of the children had ≥1 new E. histolytica infection, with 3.1% (4/129) of the new infections associated with dysentery.

<table>
<thead>
<tr>
<th>Immune status</th>
<th>E. histolytica infection as determined by</th>
<th>Stool antigen</th>
<th>Stool culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antilectin IgA in stool</td>
<td>Positive</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>33</td>
<td>213</td>
</tr>
<tr>
<td>Antilectin IgG in serum</td>
<td>Positive</td>
<td>26</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>7</td>
<td>157</td>
</tr>
<tr>
<td>Total children</td>
<td>33</td>
<td>277</td>
<td>16</td>
</tr>
</tbody>
</table>

NOTE. Plus (+) and minus (−) signs indicate presence or absence of E. histolytica infection, respectively.
Table 2. Baseline characteristics at enrollment for Bangladeshi children in a study of *Entamoeba histolytica*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>According to serum antilectin IgG</th>
<th>According to stool antilectin IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum IgG positive (n = 145)</td>
<td>Stool IgG positive (n = 46)</td>
</tr>
<tr>
<td></td>
<td>Serum IgG negative (n = 144)</td>
<td>Stool IgG negative (n = 243)</td>
</tr>
<tr>
<td>Excluded</td>
<td>44.5 ± 0.4</td>
<td>Not done</td>
</tr>
<tr>
<td>Age, months</td>
<td>51.3 ± 0.85</td>
<td>47.7 ± 1.8</td>
</tr>
<tr>
<td>Sex, % boys</td>
<td>51</td>
<td>54</td>
</tr>
<tr>
<td>Height, cm</td>
<td>95.9 ± 0.9</td>
<td>96.8 ± 1.6</td>
</tr>
<tr>
<td>Height-for-age, %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.5 ± 0.5</td>
<td>95.2 ± 0.9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>13.5 ± 0.6</td>
<td>13.2 ± 0.4</td>
</tr>
<tr>
<td>Weight-for-age, %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.0 ± 3.7</td>
<td>80.8 ± 1.5</td>
</tr>
<tr>
<td>Weight-for-height, %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.2 ± 3.7</td>
<td>87.9 ± 0.9</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SEM.

<sup>a</sup> Values are percentages of World Health Organization standards [20].

Children with stool IgA lectin-specific antibodies at the beginning of the study had 64% fewer new *E. histolytica* infections by 5 months (3/42 children IgA<sup>+</sup> vs. 47/227 children IgA<sup>-</sup>; *P* = .03; figure 2). In contrast, children with serum IgG lectin-specific antibodies by 12 months had 53% more new *E. histolytica* infections (63/133 children IgG<sup>+</sup> vs. 42/136 children IgG<sup>-</sup>; *P* = .005; figure 3). All 4 new *E. histolytica* infections associated with dysentery occurred in the IgA<sup>-</sup> children, but this was not statistically significant.

Antilectin IgA responses were observed in 79% (15/19) of new *E. histolytica* infections, which is consistent with findings in an earlier study [16] (figure 4). In contrast to children with pre-existing *E. histolytica* colonization (table 1), children with new *E. histolytica* infection were frequently positive for stool lectin-specific IgA. Lectin-specific IgA was detected in the month during which resolution of infection was achieved in 67% (12/18) of cases and persisted at detectable levels for an average of 17 days (range, 10–56 days). (In 3 additional infections, antilectin IgA was detected but at levels below the predetermined cutoff value.) Purified fecal IgA (20 µg/mL) from

![Image](1164 Children were screened)

850 antilectin serum IgG<sup>+</sup> excluded
18 excluded for stool IgA<sup>+</sup>
832 excluded to equalize IgG<sup>+/−</sup>
25 antilectin serum IgG<sup>+</sup> declined

![Image](133/145 Completed 12-month flu)

63/133 with new *E. h.* infection
16/133 with 2 new *E. h.* infections
34/133 children IgA<sup>−</sup> with new *E. h.*

![Image](136/144 Completed 12-month flu)

42/136 with new *E. h.* infection
8/136 with 2 new *E. h.* infections
8/136 children IgA<sup>+</sup> with new *E. h.*

Figure 1. Results of cross-sectional screening, reasons for nonenrollment, and design and results of a 12-month prospective study of *Entamoeba histolytica* (*E. h.*) infection in Bangladeshi children. Plus (+) and minus (−) signs indicate presence or absence of antilectin antibodies, respectively. f/u, Follow-up.
Discussion

The major discovery of this study is the existence of acquired immunity to amebiasis. The presence of naturally acquired immunity to *Entamoeba histolytica* infection gives hope for the development of a colonization-blocking vaccine. Such a vaccine could eradicate amebiasis, as humans are the only known reservoir of infection.

Immunity is linked to a mucosal anti–adherence lectin IgA response. The association of mucosal antilectin IgA with protection was demonstrated in 3 ways. First, in a cross-sectional analysis, *E. histolytica* colonization was absent in all 64 children with stool antilectin IgA. Second, children with stool antilectin IgA acquired fewer new *E. histolytica* infections over a prospective period of observation. Last, the appearance of a stool IgA antilectin response coincided with the resolution of infection. Mucosal antilectin IgA is, therefore, an indicator of immune protection and may prove effective as a surrogate marker of vaccine efficacy.

It is tempting to speculate that stool IgA antilectin antibody is a mediator and not merely a marker of acquired immunity. The intestinal mucosa contains the highest number of immunoglobulin-producing cells of any organ, and its synthesis of IgA outstrips the body’s production of all other immunoglobulins combined. Mucosal IgA has little inflammatory or complement activation activity, with its primary function thought to be the prevention of adherence of microorganisms to the gut epithelium [23]. IgA antibodies purified from stool samples from the children in this study neutralized the *E. histolytica* adherence lectin. This lectin mediates trophozoite adherence to human colonic mucin glycoproteins and colonic epithelium, the sites of natural infection, and is required for in vivo virulence [10–12, 23–26].

It seems likely that a cellular immune response acting at the mucosal level also contributes to the acquired immunity that we observed. A less than stringent requirement of cellular immunity for protection is suggested by the lack of any evidence that amebiasis is exacerbated in patients with AIDS [27]. However, activation of human macrophages and neutrophils with interferon-γ and tumor necrosis factor (TNF)-α endows them with the ability to kill *E. histolytica* trophozoites, and in murine macrophages, TNF-α has been shown to activate macrophages for nitric oxide–dependent cytotoxicity against *E. histolytica* [28, 29]. It is important that future studies characterize the mucosal cellular immune responses in humans with acquired immunity.

There was no association of a systemic immune response, as monitored by serum IgG antilectin antibodies, to immunity from intestinal *E. histolytica* infection. This is potentially important information for the rational design of a vaccine, which, on the basis of the results of this study, should be targeted to the mucosal immune system. Others have noted a lack of correlation between mucosal and systemic responses in humans to the GalNAc lectin and the absence of an association of serum IgG antibodies with decreased colonization rates [15, 30]. In our study, we actually observed an increased frequency of new *E. histolytica* infections in children with serum IgG antibodies to the lectin. This increase could be due to the previously described presence of adherence-enhancing antilectin antibodies in serum leaking into the intestinal site of infection or to serum IgG antibodies identifying a population with increased risk for amebic infection within Mirkpur (despite our attempts to match seropositive children with seronegative children) [17]. As an emphasis of the importance of mucosal immunity, the mucosal antilectin IgA response was associated with protection even in children with serum antilectin IgG; at 5 months, the cumulative incidence of infection was 5.9% (2/34) for IgA+ and IgG+ children versus 20.4% (48/235) for the rest of the children (*P* = .04).

Acquired immunity to most enteric infections is not under-
Figure 4. Dynamic relationship of stool antilectin IgA response and *Entamoeba histolytica* infection in Bangladeshi children. Bars: *red*, *E. histolytica*–positive stools; *green*, *E. histolytica*–negative stools; *yellow*, antilectin IgA–positive stools. Hatch marks indicate sampling intervals.

stood. In the few cases in which it has been studied, evidence is split on the importance of systemic and mucosal responses. Vaccine-provided protection against polio colonization is associated with a duodenal IgA but not a serum IgG response [31]. Immunity to enterotoxigenic *Escherichia coli* is also linked to a local intestinal immune response and not to serum antibody responses to the bacterium [32]. Protection against rotavirus infection and disease correlates with both stool IgA antibody levels and serotype-specific serum-neutralizing antibody [33, 34]. A correlate of protection against reinfection with cholera is serum vibriocidal antibody [35–37]. Last, serum antibodies to *Clostridium difficile* toxin have been associated with protection from disease but not from colonization [38]. Subtle differences in the immunopathogenesis of these enteric infections may contribute to the relative importance of mucosal and systemic immunity in protection.

The acquired immunity that we observed was incomplete, with a decreased rate but not an absence of infections in children with IgA antilectin antibodies. Potential explanations include the short duration of detectable stool IgA production (as has also been seen with shigella and rotavirus infections), the ability of the parasite’s cysteine proteinase to degrade IgA, and the presence of genetically distinct strains of *E. histolytica* in children in Mirpur (R.H., I.M.A., and W.A.P., unpublished data) [33, 39–41]. Further investigations on both host immune response and parasite genetic diversity will be required to design an effective *E. histolytica* vaccine, which now seems possible with the discovery of acquired immunity.
Acknowledgments

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