Innate and Acquired Resistance to Amebiasis in Bangladeshi Children

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Entamoeba histolytica infection and colitis occurred in 55% and 4%, respectively, of a cohort of Bangladeshi preschool children observed for 2 years. DNA typing demonstrated that infecting E. histolytica isolates were genetically diverse. Innate resistance to infection in children was linked to the absence of serum anti-trophozoite IgG. Most children who lacked serum anti-trophozoite IgG failed to develop it in response to a new infection. The serum anti-trophozoite IgG response clustered in families, which is consistent with genetic inheritance. Acquired resistance to infection was linked to intestinal IgA against the carbohydrate-recognition domain of the E. histolytica galactose N-acetyl-D-galactosamine lectin. This was associated with an 86% reduction in new infection over 1 year. Amebiasis is a common and potentially serious infection in children from Dhaka, and both innate and acquired immune responses limit infection.

Amebic colitis and amebic liver abscess are estimated to account for 50 million cases of colitis and liver abscess and for 100,000 deaths worldwide each year [1–4]. The killing of host cells requires parasite adherence via an Entamoeba histolytica cell-surface lectin specific for galactose (Gal) and N-acetyl-D-galactosamine (GalNAc) [5–14]. Genetic “knockout” of the lectin abrogates in vivo virulence in a gerbil model of liver abscess [15–16]. Immunization with the Gal/GalNAc-lectin provides protection from amebiasis in animal models of the disease [17–22].

We found an association between an anti-lectin immune response and acquired immunity: children with preexisting mucosal IgA anti-Gal/GalNAc-lectin antibodies had 64% fewer new E. histolytica infections during 5 months of follow-up observation. Paradoxically, children with serum anti-lectin IgG had 53% more new infections [4]. The goals of the present study were to better define the acquired immunity associated with anti-lectin IgA antibodies and to explore why children with preexisting serum IgG antibodies to the lectin were more susceptible to amebic infections.

Methods

Study protocol. Preschool children (2–5 years old) from Mirpur, an urban slum in Dhaka, Bangladesh, were enrolled as described elsewhere [4]. Diarrhea was defined as ≥3 loose or watery stools in a 24-h period. Amebic colitis was defined as a diarrheal stool sample containing occult or gross blood that was positive for E. histolytica and negative for infection with Shigella, Salmonella, and Campylobacter species. We did not routinely test for Shiga-toxin-producing Escherichia coli (STEC), because our survey of 248 diarrheal stool samples from these study children revealed no STEC infections. We also did not test for human immunodeficiency virus infection, because of its low prevalence among high-risk groups in Dhaka [23]. Infection was diagnosed by detection of antigen in stool, by the E. histolytica II test (designed to detect specifically E. histolytica in stool) performed according to the manufacturer’s instructions (TechLab) [24–25]. Stool samples also were cultured for Entamoeba species in Robinson’s medium within 6 h of collection. Strain-specific DNA typing was performed as described elsewhere [26, 27].

Analysis of serum and stool anti-lectin antibodies. Serum IgG and stool IgA antibodies against the lectin lecA, the carbohydrate-recognition domain (CRD), or whole-trophozoite antigens were determined by ELISA using 96-well plates coated with 0.2 μg/well whole-trophozoite antigens or 0.1 μg/well purified Gal/GalNAc-lectin, CRD, or lecA [4, 22, 24, 28].

Statistical analysis. All data collected were computer coded and analyzed by SPSS software (version 7.5; SPSS). Comparisons among means of different variables were made with Student’s t-test or, if the variables were not normally distributed, a nonparametric test. The χ2 and Fisher’s exact tests were used for categorical var-
IgA E. histolytica 50 did not. PCR detected antigen: 50 contained anti–lectin IgA antibodies, and performed for 100 stool samples that were negative for amplification of the E. histolytica (PCR) results, for the identification of antigen detection was compared with polymerase chain reaction infection did not alter the statistical significance of the results presented here.

E. histolytica infections were detected by antigen capture from stool samples. Because of concern that anti–amebic IgA antibodies present in stool could decrease the sensitivity of antigen–detection tests used for monthly infection surveillance, antigen detection was compared with polymerase chain reaction (PCR) results, for the identification of E. histolytica. PCR amplification of the E. histolytica small-subunit rRNA gene was performed for 100 stool samples that were negative for E. histolytica antigen: 50 contained anti–lectin IgA antibodies, and 50 did not. PCR detected E. histolytica DNA in 3 of the 50 IgA+ samples and in 0 of the 50 IgA− samples (P = .12). We concluded that antigen detection was a reliable method, even in the presence of stool anti–lectin IgA. However, to make the study as rigorous as possible, we performed antigen detection and culture on every stool specimen, to provide independent determination of E. histolytica infection.

Incidence of amebiasis in the cohort of children. New E. histolytica infections were detected in 55% (127/230) of the children during 2 years of observation. More children were infected for the first time in year 1 than in year 2 (90/230 vs. 37/230, respectively; P < .001). Of the 127 children with new E. histolytica infections, 80% (101/127) were asymptomatic. Of children with asymptomatic E. histolytica infection, 12% (12/101) went on to develop diarrhea within 2 months of onset of asymptomatic infection. Overall, 20% (26/127) of the E. histolytica–infected children had diarrhea, and 8% (10/127) had amebic colitis. More than one new E. histolytica infection was seen in 17% (40/230) of the study children. We concluded that E. histolytica infection was common and that the lower rate of new infection in year 2 was consistent with a subpopulation of the children being immune.

Consecutive infection of children by different strains of E. histolytica. Since many of the children were infected more than once, we wondered whether these infections were due to different strains of the parasite. The development of PCR techniques for the identification of genetic polymorphisms in the gene encoding serine-rich E. histolytica protein (SREHP) enabled us to identify or “type” different strains of E. histolytica from infected children in Mirpur [27]. We succeeded in amplifying the SREHP gene in ≥2 stool samples collected monthly, from the same child, in a subset of 39 children over a period of 2 years of observation. These samples gave a total of 119 amplification products and 80 pairs of comparison samples. There were 62 different SREHP polymorphisms observed among the 119 samples (figure 1). Differences in the SREHP gene were most frequently observed between 2 E. histolytica infections in the same child that were separated in time by ≥2 months of no infection. For example, 88% (30/34) of strains were different when they were separated in time by ≥2 months of no infection, compared with 22% (6/27) of strains being different when there was no interval without E. histolytica infection. This result served as independent verification of our definition of “new infection,” which required that a minimum of 2 monthly surveillance stool samples be negative for E. his-

![Figure 1](image1.png)

Figure 1. Reinfection with genetically distinct strains of Entamoeba histolytica. The serine-rich E. histolytica protein (SREHP) gene was amplified, by polymerase chain reaction (PCR), from stool samples from 2 children infected twice with E. histolytica during the study period. Child A (A and a) was infected at months 8 and 13 of the study, and child B (B and b) was infected at months 10 and 19 of the study. The amplified SREHP gene products are shown undigested (A and B) and Akl digested (a and b). Note that the sizes of the PCR products are different with each infection in the same child. The molecular-mass marker is a 50-bp ladder.
Resistance to Amebiasis

Figure 2. Association between stool anti–carbohydrate-recognition domain (CRD) IgA lectin antibodies and incidence of Entamoeba histolytica infection. A. The presence of stool anti–CRD IgA lectin antibodies at study enrollment was associated with a lower incidence of new E. histolytica infections. Children from Mirpur with (+ n = 12) or without (− n = 218) stool anti–CRD IgA at the time of enrollment were monitored for >2 years, for new E. histolytica infections, as detected by antigen detection in monthly stool samples. The 2 groups were statistically significantly different (P < .001; figure 2A). There was also a lower (but statistically insignificant) incidence of amebic diarrhea and colitis in children with stool anti–CRD IgA: 1 of 29 children with diarrhea and 1 of 10 children with dysentery associated with E. histolytica infection had stool anti–CRD IgA at entry into the study (P = .1 and P = .44, respectively, compared with the frequency of E. histolytica–associated diarrhea or dysentery in children without anti–CRD IgA).

Stool anti–CRD IgA was detected in the monthly surveillance stool samples of 81 children in the first year of the observational study. Stool anti–CRD IgA was detected within 1 month of resolution of infection in 69% (11/16) of children who were monitored with weekly stool samples. Children identified as stool anti–CRD IgA+ in year 1 were monitored for new E. histolytica infection, for an additional year. They too had a significantly lower incidence of new E. histolytica infection (5.9% of IgA+ children vs. 42% of IgA− children; P < .001;

We concluded that many of the new E. histolytica infections in Mirpur were caused by genetically distinct strains of E. histolytica.

Mucosal IgA anti–Gal/GalNAc-lectin CRD antibodies and protection from amebiasis. We have reported elsewhere [4] that the children who had stool lectin IgA–specific antibodies at entry into the cohort had 64% fewer new E. histolytica infections at month 5 of follow-up. In the present study, we aimed to refine this analysis by examining the association between protection from infection and IgA antibody responses against the active site of the lectin (i.e., the CRD). The CRD is located within the cysteine-rich region of the Hgl heavy subunit of the lectin [22]. The CRD region was expressed in E. coli, purified, and used to coat microtiter plates, to detect IgA antibodies.

Because anti–lectin IgA is detectable in stool samples for, on average, only 31 days (range 8–112 days) after a new E. histolytica infection, very few (n = 12) of the children had detectable stool anti–CRD IgA at entry into the study. The 12 children who had stool anti–CRD IgA at entry into the study had a 17% incidence of new E. histolytica infection at 2 years, compared with 57% in children without this response (P < .001; figure 2A). There was also a lower (but statistically insignificant) incidence of amebic diarrhea and colitis in children with stool anti–CRD IgA: 1 of 29 children with diarrhea and 1 of 10 children with dysentery associated with E. histolytica infection had stool anti–CRD IgA at entry into the study (P = .1 and P = .44, respectively, compared with the frequency of E. histolytica–associated diarrhea or dysentery in children without anti–CRD IgA).

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The serum anti–trophozoite IgG antibody response associated with susceptibility to amebiasis runs in families. The failure of most children who lacked serum anti–trophozoite IgG to develop anti–trophozoite IgG along with a new E. histolytica infection was consistent with a genetic basis for this trait. Familial aggregation of serum anti–trophozoite IgG would also be expected if the trait is genetic, but it could be seen if the trait is due to environmental spread within a household. We examined family members of the index children, for serum trophozoite–specific IgG: 217 and 211 family members of 65 anti–trophozoite IgG–negative (IgG–) and anti–trophozoite IgG–positive (IgG+) children; 217 and 211 family members of 65 anti–trophozoite IgG–negative (IgG–) and anti–trophozoite IgG–positive (IgG+) children.

Table 2. Serum anti–trophozoite IgG status of family members of children from the Mirpur study.

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a +, Positive; −, negative.
b Ratio is no. of siblings with no. of siblings without anti–trophozoite serum IgG.
A new *Entamoeba histolytica* with genetically diverse strains of infection was seen despite the fact that children were infected against the CRD of the Gal/GalNAc-lectin. Resistance to acquired immunity was linked to a mucosal immune response was associated with the lack of serum anti–parasite IgG. Acquisition of innate and acquired immunity to amebiasis. Innate immunity

**Discussion**

The most important finding of this study is the contribution of innate and acquired immunity to amebiasis. Innate immunity was associated with the lack of serum anti–parasite IgG. Acquired immunity was linked to a mucosal immune response against the CRD of the Gal/GalNAc-lectin. Resistance to infection was seen despite the fact that children were infected with genetically diverse strains of *E. histolytica*. Although, in an observational study, it is not possible to prove causation, one can speculate that the intestinal anti–CRD IgA response was at least partially responsible for the observed immunity. The CRD region is antigenically conserved, and antibodies against it neutralize amebic adherence and passively protect against amebiasis in an animal model [22]. These studies provide, at the very least, a surrogate marker for vaccine-induced protection against amebiasis.

Although it may seem paradoxical that children who lacked serum antibodies against *E. histolytica* were at decreased risk for future infection, there is precedent for this in other infectious diseases. Antibodies against *Leishmania* species are observed in highest titers in patients with the highest parasite burdens [29–30]. Likewise, serum anti–*Mycobacterium leprae* antibodies are present in 95% of patients with lepromatous leprosy (the form of leprosy with the highest concentration of bacteria) but in only 50% of patients with tuberculoid leprosy [31]. It is of note that, in all 3 diseases (amebiasis, leishmaniasis, and leprosy), a proinflammatory Th1-type cytokine response is implicated in protective immunity [29–36].

One could postulate that the association between serum anti–trypozoite IgG antibodies and more-frequent and -severe disease is a reflection of a Th2-type immune response that does not protect against colonic invasion. In this light, it is interesting that IgG4-isotype antibodies (induced in humans by the Th2 cytokine interleukin-4) were present in the serum anti–trypozoite IgG response associated with susceptibility to amebiasis but were lacking from the serum anti–CRD IgG response not associated with higher susceptibility. The presence of serum anti–trypozoite IgG antibodies may, therefore, be a marker in children who have suffered an invasive *E. histolytica* infection due to inadequate local innate or adaptive immune responses. For example, serum anti–trypozoite IgG antibodies are produced in response to infection by the invasive parasite *E. histolytica* but not with noninvasive infection by the closely related parasite *Entamoeba dispar* [1, 2].

Two pieces of evidence are consistent with the notion that the anti–trypozoite IgG trait is inherited. First, family aggregation studies demonstrated that siblings of children with anti–trypozoite IgG have 4.8-times-higher odds of having anti–trypozoite IgG themselves. Second, prospective study showed that most children who are anti–trypozoite IgG− do not become anti–trypozoite IgG+ after new infection. The finding that absence of the trait is not due to absence of exposure to *E. histolytica* and the finding of aggregation in families are good initial indicators of genetic involvement and highlight the need for further genetic studies. It is often difficult to disentangle the genetic and environmental factors in complex or multifactorial disorders, however. To focus the scope of further genetic studies, we will, in the future, examine a number of biologically plausible candidate genes, for association, linkage, and linkage disequilibrium with this trait. Identification of genetic polymorphism associated with the susceptibility trait (anti–trypozoite IgG antibodies) may prove to be enlightening as to the contributions of different facets of the immune system to protection from amebiasis.

Finally, the high frequency of amebiasis in this community deserves comment. Half the children were infected during the 2 years of observation, and 4% had amebic colitis. It is perhaps appropriate that, as we begin to understand the contribution that genetics and acquired immune responses make to susceptibility, the true burden of disease due to amebiasis is comprehended.

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References