Protective Immunity to Amebiasis: New Insights and New Challenges

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Amebiasis, infection with the protozoan parasite Entamoeba histolytica, is the third leading cause of death from parasitic diseases. Despite its importance, we know little about protective immunity to amebiasis. Now, studies from a cohort of children in Bangladesh have provided some critical insights into immunity to intestinal amebiasis. Children with mucosal IgA antibodies to the amebic adherence lectin were found to be resistant to reinfection with E. histolytica. However, immunity was short-lived, and ~20% of children in the cohort had a second episode of E. histolytica infection during the study period. Theses findings indicate that immunity to amebiasis can develop in some children after intestinal infection, but protective immunity may be transient, and its importance in preventing disease remains to be established.

Amebiasis remains a threat to public health in much of the world. The protozoan parasite Entamoeba histolytica causes amebic colitis and amebic liver abscess, diseases that afflict millions of individuals in developing countries. In recent years, molecular genetic techniques and new models of disease have taught us much about amebic pathogenesis [1], but fundamental questions about this disease persist. One reason for this is that many of the careful epidemiologic and clinical studies of amebiasis done over the past century were fundamentally flawed, because they failed to distinguish between infection with E. histolytica and the microscopically identical but genetically distinct nonpathogenic E. dispar. Only the pioneering studies that cultured trophozoites from stool and used isoenzyme analysis to distinguish between E. histolytica and E. dispar withstand this scrutiny, and those studies are the basis of our current concepts of the epidemiology of amebic disease [2].

One of the most important clinical questions has been whether there is protective immunity to amebiasis. Data from human studies on protective immunity are limited and, in many cases, are difficult to interpret because of the aforementioned problems in distinguishing between E. histolytica and E. dispar infection. Anecdotal reports indicate that individuals can have a second episode of either amebic colitis or amebic liver abscess, but whether these represent exceptions or the norm has been unclear.

The recent study by Haque et al. [3] sheds some welcome light on this critical question [3]. The authors previously had performed a cross-sectional analysis of E. histolytica infection in a population of 1164 asymptomatic children in an area of Dhaka with poor sanitation [4]. Stool samples were examined for E. histolytica infection, using an antigen-detection test that recognizes the E. histolytica GalNAc lectin and reportedly can distinguish between E. histolytica and E. dispar infection. The authors found that 50 (4.3%) of the 1164 asymptomatic children were stool antigen positive, a finding that is consistent with current E. histolytica infection. Serum IgG antilectin antibodies, which are consistent with a prior or current episode of E. histolytica infection, were found in 170 (~15%) of the 1164 children [4]. The prevalence of E. histolytica infection in this population was impressive, which makes it an excellent site for further epidemiologic studies. However, because asymptomatic children were screened, this initial study did not address the role of amebiasis in diarrheal disease in this population.

The recent study by Haque et al. [3] started with the same cohort of 1164 children but included the measurement of stool IgA antilectin antibodies in a group of 310 of the children (consisting of 146 children who had serum IgG antibodies to the lectin and 164 children who did not). Sixty-four (~20%) of the 310 children had stool IgA antibodies to the lectin and 164 children who did not). Sixty-four (~20%) of the 310 children had stool IgA antibodies to the lectin and 164 children who did not have stool IgA antibodies. The striking finding came when one looked at E. histolytica colonization in this group of children: one of the 64 children with stool IgA antilectin antibodies had a positive antigen detection test for E. histolytica infection, whereas 33 of 246 children who were stool IgA lectin antibody negative had a positive antigen detection test. These were the first data linking the presence of stool IgA antilectin antibodies with resistance to the acquisition of E. histolytica infection.

Stool culture, an independent measure of E. histolytica infection, gave a similar result: no positive cultures for E. histolytica were found among the children with stool IgA antilectin antibodies, whereas 16 of the 246 children who lacked antilectin IgA antibodies in their stool had positive cultures. This is an important control, since one possible explanation for the initial
findings is that the presence of stool IgA antilectin antibodies decreases the sensitivity of the lectin antigen-detection test. The authors report that this does not occur, and the finding that the 64 children who had stool IgA lectin antibodies also were culture negative is reassuring. It is worth noting that the prevalence of *E. histolytica* infection in the cohort of 310 children is ∼3 times higher than that in the initial group of 1164 children, as 33 of the 50 patients who were positive in the initial screening were included in the cohort. This reflects the high number of infected children (26 of 146) in the serum IgG antilectin antibody-positive group. In fact, if one looks separately at the IgG antibodies, but the antibody response was relatively short-lived, with detectable levels found for a mean of only 17 days. In contrast to the protective association seen for stool IgA lectin, those children who had serum IgG antilectin antibodies at the beginning of the prospective study had a higher risk for the acquisition of a new *E. histolytica* infection.

What do these results tell us? First, they show that some children who were infected previously with *E. histolytica* and who developed stool IgA antilectin antibodies displayed resistance to the acquisition of a new *E. histolytica* infection. This is definitive evidence that naturally acquired immunity to amebiasis can occur in this population. As noted above, this has been an elusive concept, and thus the current findings are of significant interest to the field. It should be noted that these results are consistent with unpublished data from an ongoing prospective study looking at the acquisition of both *E. histolytica* and *E. dispar* infection in South African patients who presented with amebic liver abscess: among those patients, the presence of stool IgA antilectin antibodies was associated with reduced acquisition of *E. dispar* infection (J. I. Ravdin, University of Minnesota Medical School, Minneapolis, personal communication).

Both of these studies suggest that immunity to reinfection is mediated by gut IgA antilectin antibody. In their study, Haque et al. [3] further bolstered this concept by finding that IgA antibody isolated from individuals who were stool IgA antilectin antibody positive could inhibit amebic adherence to mammalian cells in vitro. However, the linkage of protection to antibodies directed against the lectin is complicated by the serologic finding that individuals with serum IgG antibodies to the same molecule had a higher risk for developing *E. histolytica* infection. As noted by the authors, this may reflect complex immune responses to the lectin. In the initial studies of immunization with the native lectin in animals, most vaccinated animals were protected against liver abscess, but those that developed abscesses had significantly larger abscesses than unvaccinated animals [6]. Subsequent studies using recombinantly derived peptides mapped both the protective and disease-enhancing regions of the molecule and demonstrated that antibody to certain epitopes mediated protection, whereas antibody to a different epitope was associated with increased susceptibility to disease and increased abscess size [7]. Thus, it is possible that the predominant epitopes recognized by the mucosal IgA and serum IgG antibodies differ and have resulting differences in antibody effects. Alternatively, possession of serum IgG antilectin antibodies may simply identify a group that was at greater risk for the acquisition of disease (e.g., increased exposure) and may not be a causal factor. Last, naturally acquired immunity could be mediated by antibodies to a different amebic antigen(s), with the IgA antilectin antibodies representing a surrogate marker for the presence of mucosal immunity to that antigen. All these possibilities merit further investigation.

How do these findings impact work toward development of a vaccine for amebiasis? Importantly, they establish that acquired immunity to amebiasis exists and indicate that vaccine
strategies should incorporate methods to induce IgA mucosal antiamebic antibodies. In fact, this has been the working hypothesis for a number of investigators, and vaccine preparations capable of inducing mucosal IgA antiamebic antibodies in animal models have been developed already [8]. On the other hand, the findings by Haque et al. [3] also sound a cautionary note by demonstrating significant limitations in the naturally acquired immunity to *E. histolytica* infection. Acquired immunity in this population appeared to be short lived, was not completely effective, and did not develop in all individuals who were infected with *E. histolytica*. It was striking that ~20% of the children who acquired *E. histolytica* infection had a second episode within the study period. Although it is far too early to be conclusive, the current study and an ongoing epidemiologic study of amebic liver abscess in Vietnam (where second episodes of amebic liver abscess appear frequently; E. Tannich, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, personal communication) raise questions about the efficacy of naturally acquired immunity to amebiasis. This is relevant to vaccine development, because it suggests that a vaccine against amebiasis may have the daunting task of improving upon the immunity conferred by natural infection. The experience with attempting to develop vaccines for other parasitic diseases for which the immunity conferred by infection is incomplete (e.g., malaria) indicates that this could be a significant challenge.

In summary, we now know that naturally acquired immunity to *E. histolytica* infection can be seen in humans. How effective it is in the population as a whole and whether we can induce it and, if necessary, improve upon it are the next critical questions.

References