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Letter to the Editor

Serological Responses to *Cryptosporidium* Infection

Dann et al. (1) tested immune responses of volunteers exposed to *Cryptosporidium* oocysts using an enzyme-linked immunosorbent assay (ELISA). They found fecal immunoglobulin A (IgA) but no serum IgG2 response to infection. They concluded that in the naive individual, the parasite elicits a strong secretory antibody response but fails to provide adequate immune stimulation for serum antibody detectable by ELISA.

Since prior research indicates the ELISA, using unpurified antigen, may lack the sensitivity to detect a serological response to infection (2–4), we believe this conclusion is misleading. The implied conclusion that these infected volunteers did not respond contradicts findings of another study of the same volunteer group (5). Moss et al., using a Western blot assay, found a characteristic antibody response to either 15/17- or 27-kDa markers following infection in 10 of the 11 volunteers who experienced symptomatic illness (5). The Moss study also found serological response to *Cryptosporidium* antigens at day 0 in 21 of 29 volunteers (5), contradicting the assumptions of the Dann study that volunteers were immunologically naive at baseline. Volunteers immunologically naive by Western blotting excreted a higher number of oocysts following infection (5).

We tracked serological responses to infection, using a previously described Western blot assay (3), for one laboratory worker accidentally infected with *C. parvum*. During the 1.5 years prior to the accidental oocyst exposure, the worker had no IgA or IgM serological response to either the 15/17- or 27-kDa marker and no IgG response to the 15/17-kDa marker. He had a very weak IgG response to the 27-kDa marker that was only periodically detectable. He would, therefore, be classified as immunologically naive by Western blotting (5). Classical cryptosporidiosis developed 10 days following the exposure and 4 weeks later he developed an intense IgA, IgM, and IgG response to both the 15/17- and 27-kDa markers. The IgG and IgM responses peaked at 4 weeks postinfection and declined rapidly. IgM reached low levels by 22 weeks postinfection while IgA remained significantly elevated at 42 weeks postinfection. The IgG response peaked at 14 weeks postinfection. Response to the 15/17-kDa marker declined to near background by 42 weeks postinfection whereas response to the 27-kDa marker remained elevated at 71 weeks postinfection. If infection in immunologically naive individuals fails to provide adequate immune stimulation for serum antibody to develop, as suggested by Dann et al. (1), we should not have observed these classical serum antibody responses to infection.

Because of the large number of antigens contained in an oocyst, any assay based on aggregate serological responses to all of these antigens may be less sensitive than an assay, such as Western blotting, that examines responses to *Cryptosporidium*-specific antigens (2–4). Given published concerns with the ELISA, the failure of the authors to cite Western blot results for the same volunteers is puzzling. Their ELISA was clearly better able to detect secretory than serum responses to infection. This may have occurred either because the response was stronger or because of less cross-reacting secretory antibody.

This issue should not detract from the important and other supported findings of the study. Improved methods to detect infection may help better understand the epidemiology of *Cryptosporidium* infection.

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Authors' Reply

Muller et al. have suggested that the serum enzyme-linked immunosorbent assay (ELISA) results referred to in our recent publications are misleading and may have failed to detect an antibody response that can only be revealed with the immunoblot assay (2). We have responded to this issue previously and have attempted to clarify our position (5).

The ELISA method used in our experiments was adopted since it was (and continues to be) a standard by which *Crypto-*

sporidium prevalence is measured. The prevalence results of our screening assays (i.e., prior to challenge) are consistent with serological assays done in the United States (3, 7) and other countries (4, 6), suggesting that our technique is no less sensitive. Perhaps more importantly, we have shown that prechallenge ELISA results correlate well with the relative resistance of individuals to subsequent challenge (1). Lastly, the ELISA method in our hands is sufficiently sensitive to identify immunoglobulin A (IgA) reactivity in samples containing as little as 2 ng of total IgA.

The sensitivity and specificity of any assay system are complicated, especially in those instances where antigen preparations are complex (and potentially cross-reactive) and where there is no defined negative population. Both of these problems face those working with *Cryptosporidium* serological studies, regardless of the assay format (immunoblot or ELISA) in use. To further complicate the picture, *Cryptosporidium* oocysts are purified from fecal material. Thus, the degree of purity can affect results since bacteria and yeast antigens may be included to various extents in the assay and complicate interpretation. Further, there may be lot-to-lot variability in the antigen preparation, especially if the antigen preparations are obtained from different facilities or stored under various conditions. These problems will be lessened when a defined, purified antigen, such as a recombinant or peptide fragment, can be identified. However, this uniform preparation will have to exhibit the desired sensitivity and specificity before it can be generally adopted for serological studies. Further, such an antigen must represent a protein that is immunogenic and found in all *Cryptosporidium* species that are capable of infecting humans. When such a system is developed and adopted as the standard, direct comparison of results from different studies will be possible.

These and other issues surrounding the accurate measurement of the antibody response will be illuminated by careful, scientific studies using defined assay systems. We are currently examining the fecal IgA response to recombinant antigens of sporozoite surface proteins and immunogenic components of the oocyst. Further, immunoblot assays of fecal IgA are in progress and will provide evidence for the specific antigens recognized at the mucosal site of the infection.

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