LONGITUDINAL ANALYSIS OF THE DEVELOPMENT OF FILARIAL INFECTION AND ANTIFILARIAL IMMUNITY IN A COHORT OF HAITIAN CHILDREN

PATRICK J. LAMMIE, MERRYL D. REISS, KATHLEEN A. DIMOCK, THOMAS G. STREIT, JACQUELINE M. ROBERTS, AND MARK L. EBERHARD

Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; and Department of Cell Biology, University of Georgia, Athens, Georgia

Abstract. Longitudinal studies are being conducted in Leogane, Haiti, to investigate the relationship between acquisition of filarial infection and development of antifilarial immunity as well as the impact of maternal infection on this relationship. Children (0–24 months of age) residing in Leogane were enrolled and were examined periodically to monitor parasitologic status and to collect serum for antigen and antifilarial antibody determinations. To examine the development of filarial antigenemia and antifilarial antibody responses in this cohort, serum samples were selected from a cross section of the population at two (n = 82) and four years of age (n = 76). Antigen prevalence increased from 6% among two-year-olds to more than 30% among four-year-olds, but in only one four-year-old child were microfilaria detected in a 20-μl smear. The proportion of antigen-positive children born to antigen-positive mothers was higher than the proportion of antigen-positive children born to antigen-negative mothers (9.8% versus 0% for two-year-olds; P = 0.15; and 39.6% versus 22.7% for four-year-olds; P = 0.18). Antifilarial IgG4 levels were significantly higher among antigen-positive children at both two and four years of age (P < 0.001). In analyses of paired samples, antifilarial IgG4 responses increased significantly more among children who acquired infection by four years of age than among children who remained antigen negative, whereas antifilarial IgG1 and IgG2 responses changed equally for antigen-positive and -negative children. Antifilarial antibody levels were not influenced by maternal infection status, but were significantly influenced by age, antigen status, and the neighborhood within the community. These results provide evidence that children acquire infection early in life and suggest that antifilarial antibody responses may peak in early childhood.

Although antifilarial immune responsiveness is correlated with filarial infection status in humans, it is unclear whether antifilarial immunity directly influences parasitologic or clinical status or merely reflects circulating antigen levels. Evidence that suggests that immunity does play a pivotal role in determining infection outcome has come from studies of the relationship between filarial infections in mothers and their offspring. Epidemiologic evidence indicates that children of infected mothers are more likely to develop infection than children born to uninfected mothers. This increased susceptibility seems to be associated with a persistent change in antifilarial immunity. We reasoned that longitudinal studies of the development of antifilarial immunity in a population of children would provide an opportunity to investigate the relationship between antifilarial immunity and acquisition of filarial infection as well as the impact of maternal infection on this relationship. We began studies in Haiti to determine whether the incidence of filarial infection would be higher in children of infected than uninfected mothers and whether antifilarial immune responses would develop differently as a consequence of in utero exposure to filarial antigens. Although Wuchereria bancrofti antigen prevalence exceeds 50% in adult residents of Leogane, our knowledge of the dynamics of filarial infection and of the host response to infection in children is extremely limited. In the present study, as a first order approach, we monitored the development of filarial antigenemia and antifilarial antibody responses in children enrolled in our longitudinal study at two and four years of age.

MATERIALS AND METHODS

Study site. This study was performed in Leogane, Haiti, a coastal community located approximately 30 km from Port au Prince with a microfilaria prevalence that increases to approximately 30% in adults. Based on surveys performed in 1990 and 1991, we selected neighborhoods as the focus of the study that had a microfilaria prevalence of greater than 20% and that were relatively homogeneous with respect to type of housing construction. Most homes had no electricity; none had running water. After the purposes of the study were explained in Creole, mothers of children less than 24 months of age were asked to participate in the study. Mothers provided informed consent for themselves and their children to participate in the study. Recruitment of new mothers and children continued on an ongoing basis. In many cases, pregnant women were enrolled before the birth of the child to permit collection of maternal and cord blood. The protocol for this study was reviewed and approved by the Centers for Disease Control and Prevention Institutional Review Board and the Ethical Committee of Ste. Croix Hospital (Leogane, Haiti).

Parasitologic determinations. Baseline filaria infection status of mothers and children was determined by nocturnal blood exam (Giemsa-stained 20 μl-thick films). Every 6–9 months, mothers and children were re-examined to monitor microfilaremia, again by thick film, and to collect serum (100 μl) for antigen and antifilarial antibody determinations. Stool examinations were also performed at regular intervals to monitor intestinal parasite burdens in the children. Stools were preserved in 10% formalin and examined for ova and parasites following concentration by the formalin/ethyl acetate technique. When infections were detected, microfilaremic persons were provided treatment with diethylcarbamazine and those with Ascaris, Trichuris, and hookworm infections were provided treatment with mebendazole. Infections with Ascaris, Trichuris and hookworm were common in this setting by 18 months of age and reinfection was common following treatment.
TABLE 1
Characteristics of the study populations

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>(Male/</th>
<th>Mean age</th>
<th>Age range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>female)</td>
<td></td>
<td>(months)</td>
</tr>
<tr>
<td>2-year olds</td>
<td>82</td>
<td>47/35</td>
<td>24.4</td>
<td>15.5–33.1</td>
</tr>
<tr>
<td>4-year olds</td>
<td>76</td>
<td>44/32</td>
<td>50.4</td>
<td>40.8–65.2</td>
</tr>
<tr>
<td>Paired set</td>
<td>39</td>
<td>23/16</td>
<td>(24.5)*</td>
<td></td>
</tr>
</tbody>
</table>

* Mean difference in months between the two time points.

Antigen assays. Filarial antigen status was determined by the Og4C3 ELISA. Serum samples were diluted 1:10 in sample buffer and then assayed in duplicate according to the manufacturer’s instructions (TropBio, Townsville, Australia). Samples with an optical density greater than the mean plus three standard deviations of the optical density of serum samples from negative controls were considered antigen positive. At a 1:10 serum dilution, all serum samples from persons with microfilariae detectable by examination of a single 20 μl-thick film are antigen-positive. The antigen status of microfilariaemic mothers was determined, in most cases, by testing serum collected at or before the birth of the child. In some cases, the antigen status of the mother at the time of the birth of the child was inferred from her antigen status when the child was 1–2 years of age. Mothers defined as antigen-negative were microfilaria- and antigen-negative on repeated follow-up.

Antibody assays. Isotype-specific antifilarial antibody responses were measured by an ELISA with adult Brugia pahangi antigen as previously described. Thresholds for positive responses were defined based on the mean plus two standard deviations of the isotype-specific response of serum samples from nonendemic persons. A reference serum, generously provided by Dr. Eric Ottesen (National Institutes of Health, Bethesda, MD) was used to standardize antifilarial antibody levels.

Statistical analysis. Differences in antigen prevalence by age or maternal infection status were analyzed by the chi-square or Fisher’s exact test. Differences in antibody level were compared with the Kruskal-Wallis test. Multivariate regression was used to investigate the effect of age, antigen status, maternal infection status, residence, and intestinal infections on log-transformed antifilarial antibody responses.

RESULTS

Serum samples were selected to represent a cross section of the cohort of children enrolled in our longitudinal study as they reached two and four years of age. Although a range of ages was obtained in this selection, the mean age did approximate two and four years, respectively, for the two groups (Table 1). From these two sets, paired specimens were available from 39 children to assess changes in infection and immune status with age.

Of the two-year-old children, five (6%) of 82 were antigen-positive by the Og4C3 ELISA (Figure 1); none were microfilaria-positive. Antigen prevalence increased significantly among four-year-old children to more than 30% (*P*, 0.001), but only a single four-year-old child had microfilaria detectable in a 20 μl-thick smear. Although the geometric mean antigen level for four-year-old children was higher than that for two-year-old children, the difference was not statistically significant.
than that of two-year-old children, this difference was not statistically significant.

The proportion of antigen-positive children born to infected (either microfilaremic or antigen-positive) mothers was higher than the proportion of antigen-positive children born to antigen-negative mothers (9.8% versus 0% for two-year-old children; \( P = 0.15 \), and 39.6% versus 22.7% for four-year-old children, \( P = 0.18 \)); however, this trend did not achieve statistical significance for this sample of either two- or four-year-old children (Table 2).

To analyze the prevalence of antibody reactivity, antibody responses of children were considered positive if the antibody level exceeded the threshold defined by negative controls. By these criteria, 70 (85.4%) of 82 two-year-old children mounted antifilarial IgG1 responses and half mounted antifilarial IgG4 responses. Less than 40% of the two-year-old children mounted antifilarial IgG2 and IgG3 responses. By four years of age, all children mounted positive antifilarial IgG1 responses, and all but one had antifilarial IgG4 responses.

To analyze the relationship between antigen status and levels of antifilarial antibody, responses of antigen-positive and antigen-negative children were compared. Among both two- and four-year-old children, only antifilarial IgG4 responses were significantly different between antigen-negative and antigen-positive children (\( P < 0.001 \); Figure 2). Antifilarial IgG4 responses were significantly higher among antigen-positive children.

Paired data allowed us to examine changes in antibody level over time and to determine whether the changes in antibody responses that were observed were associated with changes in antigen status. The cohort of children from whom paired samples were available was similar to that of the two- and four-year-old cohorts in terms of gender and antigen prevalence at both ages. Antifilarial IgG4 levels increased significantly more among children who acquired infection by four years of age than among children who remained antigen negative (\( P < 0.003 \); Figure 3). Antifilarial IgG1 and IgG2 responses changed equally for antigen-positive and -negative children.

To further analyze antifilarial antibody responses, the influence of age, antigen status, neighborhood, intestinal infections (including Ascaris, Trichuris, hookworm, and Giardia), and maternal infection status on antibody responses was investigated by multivariate regression. Among the

### Table 2

<table>
<thead>
<tr>
<th>Mothers’ infection status</th>
<th>Children’s antigen status at ages two and four</th>
<th>Ag+</th>
<th>Ag−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two years of age:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mf+</td>
<td></td>
<td>4 (15.4)</td>
<td>22 (84.6)</td>
</tr>
<tr>
<td>Ag+</td>
<td></td>
<td>1 (4.0)</td>
<td>24 (96.0)</td>
</tr>
<tr>
<td>Ag−</td>
<td></td>
<td>0 (0)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Four years of age:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mf+</td>
<td></td>
<td>8 (34.8)</td>
<td>15 (65.2)</td>
</tr>
<tr>
<td>Ag+</td>
<td></td>
<td>11 (44.0)</td>
<td>14 (56.0)</td>
</tr>
<tr>
<td>Ag−</td>
<td></td>
<td>5 (22.0)</td>
<td>17 (77.3)</td>
</tr>
</tbody>
</table>

*Ag+ = antigen positive; Ag− = antigen negative; Mf+ = microfilaremic.

![Figure 2](image)

**Figure 2.** Mean isotype-specific antifilarial antibody responses of A, two-year-old (n = 82) and B, four-year-old (n = 76) children. Antifilarial IgG4 responses of antigen filarial antigen-positive children (solid bars) were significantly higher than those of antigen-negative children (cross-hatched bars) for both age groups.

![Figure 3](image)

**Figure 3.** Changes in antifilarial IgG level as a function of antigen status. The difference in isotype-specific antifilarial antibody level was determined for individual pairs of serum samples collected at two years and at four years (n = 39 pairs). The mean change in response is shown for children who were antigen negative at two years of age, but antigen positive at four years of age (solid bars) and for children who were antigen negative at both time points (cross-hatched bars). Decreases in mean antifilarial IgG3 levels are shown as negative numbers.
Immunoblot responses also showed no consistent differences that related to maternal infection status.

**DISCUSSION**

Analysis of circulating filarial antigen levels in young children in Leogane demonstrated that filarial infections are acquired early in life. Microfilaria prevalence among four-year-old children was less than 2%, yet antigen prevalence was greater than 30%. These findings suggest either that initial microfilaraemias are low and require some time to build up to levels detectable in 20-μL blood films or that most early infections are characterized by the presence of few unmated adult or a single sex of worms. These two possibilities are not mutually exclusive.

Based on these data, transmission of filarial infection in Leogane is relatively intense; the incidence of infection, as defined by antigenemia, is greater than 10% per year in young children. Clearly, acquisition of infection at this level is not maintained in older age groups. Antigen prevalence continues to increase in adults in Leogane, but at a much slower rate.\(^9\) In addition, the absence of changes in antigen intensity with age in adults indicates that adult worm burdens are not cumulative.\(^7\) These findings argue that some process(es), either host- or parasite-mediated, regulates parasite density in older persons and are consistent with results obtained by Day and others, who reported that increases in microfilarial densities are not cumulative.\(^9\) These findings argue that some process(es), either host- or parasite-mediated, regulates parasite density in older persons and are consistent with results obtained by Day and others, who reported that increases in microfilarial densities are not cumulative.

Although the differences were not significant for either two- or four-year-old children, the number of infected children born to infected mothers was greater than the number of infected children born to antigen-negative mothers. Our power to address the hypothesis that children born to infected mothers are more susceptible to infection was limited by the relatively small sample size and the focused nature of the present study, but the trend we observed is consistent with the hypothesis. Continued follow-up of the full cohort will provide us with a more rigorous test of this trend’s significance.

Analysis of antibody responses supported the conclusion that exposure occurs early in life, although the range of responses suggests that either the level of exposure or the host response to exposure is not uniform. In earlier studies, we also detected antibody responses to larval antigens in most children less than five years of age.\(^15\) These results emphasize that exposure to infection is effectively universal in young children living in Leogane, but that not all exposures result in infection, as defined by detection of antigenemia or microfilaraemia.

The pattern of antifilarial antibody responses in these children in the present study conformed, in general, to patterns observed in previous studies of older children and adults.\(^13, 16-18\) Specifically, antifilarial IgG4 responses were higher among antigen-positive children than antigen-negative children and increases in antifilarial IgG4 were associated with acquisition of infection, as defined by a shift in antigen status. In contrast, antifilarial IgG1 and IgG2 increased to the same extent among children who acquired infection and among those who remained antigen negative. This observation is consistent with the conclusion that IgG1 and IgG2 responses are driven by exposure to filarial larvae rather than infection. In addition, antifilarial IgG2 responses did not differ significantly as a function of antigen status. In adults, antifilarial IgG2 responses of antigen-negative persons also were similar to those of antigen-positive (microfilaria-negative) persons, but were significantly higher than those of microfilaria-positive individuals.\(^3, 19\) The reason for decreased antifilarial IgG2 responses in microfilaremic persons is not clear, but may relate to binding of IgG2 to the surface of microfilariae (Simonsen PE, unpublished data) or to shifts in cytokine production.\(^20\)

Although this study was not designed to compare antibody levels in children and adults, levels of antifilarial antibody in children were relatively high compared to previous studies of adult residents of Leogane of similar antigen status.\(^13\) We and others have previously reported that antifilarial antibody levels in children were elevated compared with adults.\(^16, 21\) Our present results support these findings and imply that antifilarial antibody responses are stable, or perhaps, down-regulated in adults.

Using multivariate analyses, antifilarial antibody levels were influenced by age, infection status, and neighborhood, but not intestinal helminth infections or maternal infection status. It is important to emphasize, however, that none of the regression models accounted for more than 50% of the observed variation in antibody responses. Thus, other factors, including person-to-person variation in exposure as well as host genetics, must contribute significantly to antibody levels.

One possible explanation for the absence of a detectable influence of maternal infection on antifilarial antibody responses in children is that the impact of *in utero* exposure to filarial antigens is restricted to parasite-specific cytokine responses.\(^8\) In contrast, Malhotra and others found that the cytokine profile of cord blood mononuclear cells from children born to infected mothers was similar to that of the peripheral blood mononuclear cells from the mothers, which argues that *in utero* exposure to parasite antigens does not lead to tolerance or skew the immune reactivity of children.\(^22\) We are now analyzing cytokine production of filarial-antigen-stimulated peripheral blood mononuclear cells collected from small quantities of peripheral blood by fingerstick from the children in this cohort and should have the opportunity to address these issues in the context of the study.

In summary, our results emphasize the importance of monitoring children to understand immunologic events associated with acquisition of filarial infection. Further studies also are needed to monitor the variation in the exposure of individual children to infective larvae and to evaluate the clinical consequences of infection in children to determine how these events contribute to the pathogenesis of lymphatic disease.

Acknowledgments: We express our gratitude to the staff of Hopital Ste. Croix and the members of the filariasis research team, including David Addiss, Michael Beach, Wendy Hitch, Njeri Wamae, Essie Walker, Rosette Prosperie, Jacky Louis Charles, Christian Registre, Jean Marc Brissau, and Dardith Desire. The editorial suggestions of David Addiss, in particular, are sincerely appreciated.

Financial support: This work was supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases grants # 920528 and # 940441. Thomas G. Streit was sup-
ported by the Molecular Parasitology and Vector Biology Training Grant awarded to the University of Georgia.

Authors' addresses: Patrick J. Lammie, Meryl D. Reiss, Kathleen A. Dimock, Jacqueline M. Roberts, and Mark L. Eberhard, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Mailstop F-13, 4770 Buford Highway NE, Chamblee, GA 30341-3724. Thomas G. Streit, Department of Cell Biology, University of Georgia, Athens, GA, 30602 (present address: Department of Biology, Notre Dame University, Notre Dame, IN 46556).

REFERENCES


