Protective Immunity in Human Filariasis: A Role for Parasite-Specific IgA Responses

Bikash R. Sahu,1,a Madhu C. Mohanty,1,a Prakash K. Sahoo,1 Ashok K. Satapathy,1 and Balachandran Ravindran1,2

Background. Filaria-specific antibodies of immunoglobulin (Ig) G, IgE, and IgM isotypes have been correlated with acquired immunity in the literature, but the status of filaria-specific IgA and its role in human filariasis has not been addressed. The present study attempts to fill this lacuna.

Methods. Both total and filaria-specific IgA to different developmental stages of filarial parasites were quantified by solid-phase immunoassays in 412 clinically and parasitologically defined cases occurring in an area endemic for human bancroftian filariasis in Orissa, India.

Results. Compared with other clinical categories, microfilariae carriers were deficient in total as well as filaria-specific IgA. More crucially, significantly high levels were observed in putatively immune control subjects from areas of endemicity. These associations were also related to sex; female subjects in each category displayed higher levels of filaria-specific IgA than did male subjects.

Conclusion. The study demonstrates, for the first time, a positive correlation between protective immunity and increased levels of filaria-specific IgA in human bancroftian filariasis. Furthermore, filaria-specific IgA appears to be an immunological window for the sex-related differences in susceptibility to infection observed in human filariasis.

Lymphatic filariasis, a chronic and debilitating tropical disease that often leads to clinical manifestations (such as lymphedema, elephantiasis, and/or hydrocele), is caused by vectorborne nematode parasites (e.g., Wuchereria bancrofti, Brugia malayi, and Brugia timori) [1]. In the absence of any preventive vaccine and effective chemotherapy to kill lymphatic-dwelling adult-stage parasites (which persist in infected hosts for several years), ~120 million people suffer from at least one form of the disease, and ~1.3 billion are at risk of infection [2].

The existence of acquired immunity in human filariasis has been a subject of intense debate, because the nature of protective immunity, if any, is yet to be unambiguously demonstrated [3]. Antibody and T cell responses in human filarial infections have been analyzed with a view to understanding their relevance to the development of protective immunity [4]. The role played by filaria-specific antibodies in preventing the persistence of infection or the development of chronic disease manifestations in lymphatic filariasis is still not understood. Early work in this area focused on antibody responses in microfilaremic individuals compared with subjects with chronic lymphatic pathology or subjects from areas of endemicity who have been exposed to infective bites but do not display any patent infection (hereafter, “endemic controls”), and findings suggested qualitative and quantitative differences in the production of filaria-specific antibody responses [5–7]. Individuals with active filarial infection display higher levels of filaria-specific IgG4 but lower levels of IgG3 and IgE than do subjects free of established infection, and raised levels of filaria-specific IgG1 and IgG2 have been correlated with the presence of microfilariae (Mf), irrespective of the presence or absence of adult worms [8]. Negative associations have been reported between (1) IgG1, IgG2, and IgE antibodies and Mf status and (2) IgG3 and filarial antigenemia, and positive correlations between IgG4 levels and active filarial infection have also been reported [9]. These and other investigators have quan-
tified the status of IgG, IgM, and IgE responses to different filarial antigens, but, curiously, there have been no reports in the literature on the status of IgA responses in human bancroftian filariasis. Investigations of other helminthic infections, such as infections with *Schistosoma mansoni*, *Taenia taeniformis*, and *Trichinella spiralis*, have indicated a protective role for parasite-specific IgA [10–12], and a negative correlation has been observed between parasite-specific IgA levels and egg counts in subjects with *Schistosoma* infections, indicating a role for IgA in restricting the intensity of infections [13]. In this communication, we report the results of our investigations into the role played by IgA antibodies in human bancroftian filariasis. We considered the absence of demonstrable patent infection and disease manifestations as indicators of protective immunity. We report for the first time a protective role for IgA antibodies in human filariasis: putatively immune subjects from areas of endemicity; GMI, geometric mean index for positive subjects; Mf, microfilariae.

**SUBJECTS, MATERIALS, AND METHODS**

**Parasites.** Male and female adult filarial parasites (*Setaria digitata*) were collected from the peritonea of slaughtered cattle and added to sterile Hank’s balanced salt solution containing antibiotics [14]. Lyophilized third-stage larvae and adult worms of *Brugia pahangi* were gifts from E. Devaney, University of Glasgow.

**Collection of human blood samples for serum.** Blood samples were drawn from endemic subjects in the state of Orissa, India. Clinical examination and nocturnal blood survey were conducted in 6 villages in Orissa: Rathipur, Ranapur, Siruli, Beldal, Beleswarpatna, and Bhimpur. The individuals were examined clinically for the presentation of acute or/and chronic disease manifestations of lymphatic filariasis by means of a proforma. The criteria for inclusion or exclusion and for precise classification of clinical groups—namely, subjects with chronic filarial disease, Mf carriers, subjects with cryptic infections, and endemic controls—have been elaborately described by us elsewhere [15]. Approximately 5 mL of blood was collected from person in each of the above categories, but only from those who volunteered to give blood samples. Serum samples were separated and frozen at −20°C. Approval for the study was obtained from the institutional ethical committee of the Regional Medical Research Center (Indian Council of Medical Research), Bhubaneswar, Orissa, and informed consent was given by the study subjects for the collection of blood samples. Serum samples from blood bank donors residing in filaria-nonendemic areas were gifts from A. Hoerauf, Bonn University.

**Preparation of soluble filarial antigens.** PBS-solubilized extracts from adult-stage *S. digitata* parasites (male and female worms separately) and soluble extract of embryogenic stages of female adult *S. digitata* were prepared as described by us elsewhere [14], and the antigens were designated as *Sd*-M-Ag, *Sd*-F-Ag, and *Sd*-IUS-Ag for this study. Similarly, extracts of adult *B. pahangi* worms were prepared and labeled as *Bp*-F-Ag. Protein and carbohydrate fractions of *Sd*-F-Ag were prepared as described elsewhere [16]. For preparation of L3 extracts of *B. pahangi*, lyophilized larvae were ultrasonicated in PBS and labeled as *Bp*-L3-Ag. All of the solubilized antigen preparations were frozen at −20°C until further use.

**Preparation of excretory-secretory antigen of female *S. digitata.** Excretory-secretory antigens from adult female *S. digitata* worms were prepared by a procedure described by us elsewhere [14]. The product was labeled *Sd*-F-ES-Ag and was stored at −20°C until further use.

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**Table 1. Details of the study population in different areas of Orissa endemic for bancroftian filariasis.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>EC (n = 113)</th>
<th>CH (n = 98)</th>
<th>AS (n = 100)</th>
<th>CR (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, no. male/no. female</td>
<td>60/53</td>
<td>81/17</td>
<td>70/30</td>
<td>69/32</td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>20 (5–75)</td>
<td>36 (12–70)</td>
<td>27 (9–75)</td>
<td>26 (8–75)</td>
</tr>
<tr>
<td>Mf status, %</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Mf density, GMI</td>
<td>0</td>
<td>0</td>
<td>5.92</td>
<td>0</td>
</tr>
<tr>
<td>CFA status, %</td>
<td>0</td>
<td>14.28</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CFA density, GMI</td>
<td>0</td>
<td>1311</td>
<td>13,118</td>
<td>1411</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

**NOTE.** Villages in Orissa included Rathipur, Ranapur, Bhimpur, Beldal, Siruli, and Beleswarpatna. Samples from Rathipur and Ranapur were used for the data shown in figure 1; samples from Bhimpur were used for the data shown in figures 2, 5, and 6; and samples from Beldal, Siruli, and Beleswarpatna were used for the data shown in figures 3 and 4. AS, asymptomatic microfilariae carriers; CFA, circulating filarial antigen; CH, subjects with chronic disease; CR, subjects with cryptic infection; EC, endemic controls (putatively immune subjects from areas of endemicity); GMI, geometric mean index for positive subjects; Mf, microfilariae.
Carbohydrate and protein estimation. The protein content in all parasite antigen preparations was estimated using Folin–Ciocalteu’s phenol reagent, and the carbohydrate content was quantified using phenol sulfuric acid reagent, as described elsewhere [17].

W. bancrofti antigen assay (Og4C3). Circulating filarial antigen (CFA) levels in serum samples were measured as described by us elsewhere [15], using a TropBio ELISA Kit (Tropical Biotechnology) according to the manufacturer’s protocol. Levels are expressed as arbitrary antigen units, determined according to internal standards provided in the kit.

ELISA. A lectin-based method was used to quantify total IgA1 in serum samples, as described elsewhere [18]. First, 96-well polystyrene plates (MaxiSorp; Nunc) were coated with Ja-calin lectin (Artocarpus integrifolia) (L-3515; Sigma) at a concentration of 10 µg/well in 0.15 mol/L carbonate buffer (pH 9.6). After wells were blocked with 1% skim milk in PBS, 200-fold-diluted human filarial serum was incubated in PBS with 0.1% skim milk and Tween 20. Bound IgA was detected by using 1000-fold-diluted peroxidase-labeled anti–human IgA (P0216; Dako) in the same buffer. The enzyme activity was measured using orthophenylenediamine (P1526; Sigma). Absorbance was read at 492 nm, and the results are expressed as arbitrary ELISA units, determined using internal laboratory standard serum. Levels of IgA to different filarial extracts in human filarial serum samples were quantified by the method described above, with the following modifications. Plates were coated with 1 µg/well Sd-F-Ag, Sd-M-Ag, Bp-F-Ag, Sd-F-ES-Ag, Sd-F-pro (protein), Sd-IUS-Ag, or Bp-L3-Ag in PBS. Before being coated with Sd-F-cho (carbohydrate), plates were pretreated with poly-L-lysine hydrochloride (P1274; Sigma), as described by us elsewhere [16]. First, human filarial serum was diluted serially in PBS-Tween with 0.1% skim milk (25–25,600-fold dilutions) and were tested for levels of filaria-specific IgA to Sd-F-Ag. A serum dilution of 1:100 was considered optimum for quantifying serum IgA.

Immunoperoxidase assay. We used an immunoperoxidase assay to detect IgA to the surface (epicuticle or cuticle) of adult female worms, as described by us elsewhere for anti-sheath antibodies [14], with the following modifications. Human serum, diluted 10-fold in PBS containing 0.1% bovine serum albumin, was applied to microscopic slides with fixed Mf and then incubated for 2.5 h in humid chambers. The slides were washed in
PBS treated with 100-fold-diluted peroxidase-conjugated anti-human IgA (P0216; Dako) in the same buffer. Enzyme reactivity was studied using diaminobenzidine (D5637; Sigma) at a concentration of 0.5 mg/mL in Tris-HCl (pH 8.6) per 1/9262 H2O2.

Data analysis. Student’s t test or the χ2 test was applied to determine the statistical significance of differences between or within groups, and the relationship between variables was evaluated by Pearson’s coefficient of correlation. All statistical tests were performed using GraphPad Prism software (version 4.0). Differences were considered significant at P < .05.

RESULTS

Based on the status of microfilaremia, antigenemia, and chronic disease manifestations, the study subjects were classified into 4 categories, as described in Subjects, Materials, and Methods. Table 1 shows the number of subjects, sex distribution, median age, Mf status, Mf density, CFA status, and CFA density for the different categories.

Total serum IgA1 levels in the clinical spectrum of human filariasis. Because IgA1 constitutes ~90% of total plasma [19], the lectin-based solid-phase assay, which quantifies IgA1, was taken as a measure of total IgA in this study. IgA1 levels in Mf carriers were significantly lower than those in the other 3 groups (figure 1A). The negative correlation observed between the density of filarial infection (CFA levels) and total serum IgA1 levels suggests that the intensity of infection with adult worms could influence total IgA1 levels in human filariasis (figure 1B). Such an association, however, was not found between Mf density and IgA1 levels (figure 1C).

Comparison of B. pahangi and S. digitata antigens for IgA quantification. Parasite extracts prepared from B. pahangi and S. digitata were compared for their sensitivity and specificity, because many investigators use extracts of adult Brugia organisms for immunological assays. The IgA levels measured using the 2 parasite preparations were comparable (figure 2A), indicating the applicability of S. digitata parasites for immunological studies of human filariasis. This was further substantiated when a panel of serum samples (from 5 clinical groups) was tested against Sd-F-Ag and Bp-F-Ag, with comparable results; significantly lower levels of IgA were observed in Mf carriers, and, more interestingly, the IgA levels were significantly higher in putatively immune subjects (the endemic controls) than in all
other groups. These observations were consistent regardless of the parasite species used in the assays, whether *S. digitata* or *B. pahangi* (figure 2B and 2C). The geometric mean index (GMI) of IgA to *Sd*-F-Ag in Mf carriers was 24.68% of that in endemic controls, whereas the GMI of IgA to *Sd*-F-cho in asymptomatic subjects was 41.32% of that in endemic controls, whereas the GMI of IgA to *Sd*-F-pro in Mf carriers was 54.82% of that in putatively immune subjects.

**Association between decreased filaria-specific IgA levels and active infection.** When the serum samples were reclassified according to infection status without regard for presentation of symptoms, subjects with current infection (as shown by the presence of CFA [figure 4A] and/or Mf [figure 4B]) were found to have significantly less filaria-specific IgA than subjects who were free of active infection, suggesting that increased levels of IgA could contribute to the absence of active infection.

**Influence of sex on levels of filaria-specific IgA.** A significant difference in filaria-specific IgA was observed when IgA levels were analyzed according to sex; the antibody levels were significantly higher in female subjects than in male subjects (figure 4C). This association was consistent when the samples were classified on the basis of the presence or absence of filarial infection in male and female subjects (figure 4D).

**Age-specific distribution of IgA antibodies.** In filariasis-endemic areas, the population is subjected to continuous exposure to infective larvae, and filaria-specific IgA levels were studied in an age-stratified population. Antibody levels reached their peak by age 20 years and remained stable in older age groups (figure 4F), suggesting that continued exposure beyond 20 years does not significantly alter filaria-specific IgA responses. All of the serum samples for the age group <20 years were from subjects aged 10–20 years, and there was no significant correlation between age and acquisition of filaria-specific IgA (figure 4E).

**IgA to developmental stages of filarial parasites.** The above-described investigations were performed with antigen extracts prepared from adult female-stage parasites (*Sd*-F-Ag). Because the antibody levels were significantly higher in putatively immune subjects, it was of interest to study levels of IgA to other developmental stages to which the human immune system is exposed during natural infection. The following preparations were tested: (1) adult male-stage parasites of *S. digitata* (*Sd*-M-Ag), (2) intrauterine stages of adult female parasites of *S. digitata* (*Sd*-IUS-Ag), (3) excretory-secretory antigens of adult female worms of *S. digitata* (*Sd*-F-ES-Ag), and (4) infective larval (L3) extract of *B. pahangi* (*Bp*-L3-Ag). Detailed results are shown in figure 5. First, profiles of filaria-specific IgA to male worms (*Sd*-M-Ag) were very similar to those observed earlier for IgA to female worms (*Sd*-F-Ag); that is, putatively immune subjects displayed significantly higher levels than did subjects in other categories (figure 5A). Second, levels of IgA to *Sd*-IUS-Ag were comparable in all the clinical groups (figure 5B). Because intrauterine stages are constitutively part of adult female worms, the results shown in figures 2B and 5B suggest that protective IgA to
adult worms are directed toward filarial antigens other than those present in intrauterine embryogenic stages in female worms. On the other hand, levels of IgA to Sd-F-ES-Ag were significantly higher in infection-free individuals (endemic controls and subjects with chronic disease) than in infected subjects (asymptomatic subjects and those with cryptic infection) (figure 5C).

The presence of similar antigenic epitopes in Sd-F-Ag and Bp-F-Ag prompted us to study IgA to larval extract of B. pahangi (Bp-L3-Ag) in different clinical categories. The levels were significantly higher in subjects with chronic disease than in endemic controls and Mf carriers. IgA levels were also significantly higher in subjects with cryptic infections than in Mf carriers (figure 5D).
All of the solid-phase immunoassays described above were performed by using soluble products or solubilized extracts of the parasites. Reactivity in such assays does not indicate the anatomical location of the epitopes in parasites reacting to filaria-specific IgA. Because the surfaces of adult-stage pathogenic systemic nematodes are constantly exposed to the host immune system, an immunoperoxidase assay was performed using cross-sections of adult female worms of *S. digitata* to score for reactivity of filaria-specific IgA to the surface (epicuticle or cuticle) (*Sd*-F-S) of parasites in different clinical groups; the results are shown in figure 6. IgA antibodies to *Sd*-F-S were demonstrated in 80% of endemic controls, 48% of subjects with chronic disease, and 48% of Mf carriers (figure 6C).

**DISCUSSION**

The existence of acquired protective immunity in lymphatic filariasis has been a subject of intense debate [3]. The antigen-detection assays for bancroftian filariasis that we used in the present study have allowed us to identify infected subjects precisely, addressing the issue of protective immunity. Generally, the absence of infection or a low infection load is considered a parameter of protective immunity in experimental filariasis (as in many other models of pathogens) [14, 20, 21]. In human filariasis, however, genuine protective immunity should be a state associated with the absence of infection as well as disease because a large proportion of infected subjects are free of clinical disease and most patients with acute or chronic disease are free of demonstrable current infection [3]. Because endemic controls are free of demonstrable infection as well as disease, we and others have considered this group to represent putatively immune individuals in filariasis-endemic areas [3, 22–24]. Several studies have been conducted of IgG (isotypes IgG1, IgG2, IgG3, and IgG4), IgM, and IgE antibody responses to different filarial antigens in the context of protective immunity in human and experimental filariasis [25–27]. Although serum IgA constitutes nearly 15%–20% of the total immunoglobulins in humans [28], there has been no attempt to date to investigate the role played by the IgA antibody response in the clinical spectrum of bancroft-
ian filariasis, and the present study is an attempt to fill this lacuna.

Access to male and female adult *S. digitata* worms free of host tissue or cells, intrauterine embryogenic stages, and excretory-secretory antigens of this parasite offered opportunities to investigate the role played by filaria-specific IgA in bancroftian filariasis. The utility of *S. digitata* for immunological, chemotherapeutic, and other biological studies has been shown by us and others [29–31]. The current investigations have revealed several hitherto unknown aspects of the immunology of human filariasis. Subjects with circulating Mf were not only deficient in filaria-specific IgA, compared with other clinical groups, but they also had significantly lower levels of total serum IgA1. IgA deficiency in mice has been reported to be associated with decreased Th1 responses, characterized by low interferon-γ production by T cells [32]. Humans with microfilaremia display a phenotype characteristic of lower Th1 responses [33], and our observation of decreased levels of IgA fits into this paradigm. We had expected, however, that this could have been compensated for by dominant Th3/Tr1-type filaria-specific responses (associated with increased production of transforming growth factor–β and/or interleukin-10), which are observed in helminth infections in general [34] and filariasis in particular [35, 36]. Such compensatory responses do not seem to be operational, because Mf-infected subjects displayed significantly lower total and filaria-specific serum IgA levels. Recently, it has been reported that inducible nitric oxide synthase (iNOS) could very significantly influence a class switch from μ-chain to α-chain [37], and our observation of decreased IgA levels could be related to the status of iNOS induction in the Mf-infected subjects. Although a role for nitric oxide in the experimental murine filariasis model has been reported by us and others [38–40], it is not clear whether such a scenario is responsible for the low IgA levels in Mf carriers that we observed in this study.

Regardless of the factors that influence the production of IgA during the immune response, this study has indicated a protective role for IgA in human filariasis. We have adopted a rigorous and robust definition of protective immunity, because infection and disease often do not coexist in human filariasis [41, 42]. Filaria-specific IgA levels were significantly higher in endemic controls than in subjects with chronic filarial (CH) disease and asymptomatic microfilaria carriers (AS) (n = 15 in each group).
further light on the precise role played by IgA antibodies in fi-
tations using IgA-deficient mice [47] can be expected to throw
nity in experimental models of filariasis [46]. Similar investiga-
been used recently to demonstrate their role in antilarval immu-
human filariasis. Mouse strains deficient in secretory IgM have
none of the other immunological parameters reported in the
literature account for sex-related differences in the susceptibility
to filarial infection in human communities. Ongoing studies in
our laboratory are now directed toward identification of mole-
cules or epitopes that are recognized by protective filarial IgA in
human filariasis. Mouse strains deficient in secretory IgM have
been used recently to demonstrate their role in antilarval immu-
ity in experimental models of filariasis [46]. Similar investiga-
tions using IgA-deficient mice [47] can be expected to throw
further light on the precise role played by IgA antibodies in fi-
larial immunology.

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