INFECTION AND IMMUNITY, Feb. 1999, p. 484–489 Vol. 67, No. 2
0019-9567/99/$04.00+0
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# Natural Immunity to *Ascaris lumbricoides* Associated with Immunoglobulin E Antibody to ABA-1 Allergen and Inflammation Indicators in Children

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Received 10 August 1998/Returned for modification 1 October 1998/Accepted 11 November 1998

**Children putatively immune to the large roundworm *Ascaris lumbricoides* were identified in an area of Nigeria where infection is hyperendemic. Immunity was associated with higher levels of serum ferritin, C-reactive protein, and eosinophil cationic protein, indicating ongoing acute phase or inflammatory processes. In contrast, children who were susceptible to the infection had little serological evidence of inflammation despite their high parasite burdens. Immunoglobulin G (IgG) antibody activity in all subclasses was present in high titer in most children but appeared to have no protective function. Despite exceptionally high total IgE levels, there was no evidence that atopic responses to local common allergens was associated with natural immunity to *Ascaris*. Among those individuals who produced IgG antibody to recombinant ABA-1 allergen of *Ascaris*, the naturally immune group had significantly more IgE antibody to the allergen than did those susceptible to the infection. IgE antibody responses in conjunction with innate inflammatory processes therefore appear to associate with natural immunity to ascariasis.**

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Helminth parasites are renowned for inducing elevated levels of serum immunoglobulin E (IgE) (20, 33), but the protective role of the antibody component of this response remains debatable (1, 3, 28, 30, 31, 36). There is, however, epidemiological evidence for an association between IgE antibody levels and the development of resistance to reinfection with the blood flukes *Schistosoma haematobium* and *Schistosoma mansoni* (6, 13, 38). IgE is also associated with pathology, and IgG4 antibody is thought to act as a blocking antibody in competition with IgE in a trade-off between protection and pathology in certain helminth infections (1, 13).

The large roundworm of humans, *Ascaris lumbricoides*, inhabits the intestine, but juvenile-stage worms undergo a tissue-migratory phase involving the liver and lungs before returning to the intestine, where they mature to large adult worms. The pulmonary phase can cause potentially lethal hypersensitivity responses in infected individuals, particularly children, and worm material is notorious for the allergic reactions that it provokes in laboratory workers (33). *A. lumbricoides* infects a quarter of humanity and people can remain infected for much of their lives, although at the population level, intensity of infection decreases with age after a peak within the first decade of life in high-intensity areas (15, 18). At the level of the individual, however, there is strong evidence of predisposition to high- or low-level infections which persists over several rounds of drug cure and natural reinfection (15, 18). This effect provides an opportunity to compare immune responses in individuals who fall into the two extremes in order to investigate the immune mechanisms potentially involved in protection.

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We have examined a range of serum factors in African children living in an area highly endemic for *A. lumbricoides*, using the number of worms developing to maturity as a measure of immunity status. Quantifying worm burden is superior to using the number of eggs released, because egg production is a poor indicator of the number of adult worms present (15, 18) and may miss low-level infections. The children were examined for infection on two separate occasions, and those either consistently infected or putatively immune were identified.

Neither the mechanisms by which immunity to *A. lumbricoides* operates nor the site within the body at which it is manifest is known. Therefore, in addition to measuring of antibody in the different isotypes, we examined a range of serological markers for inflammatory responses to provide an indication of the pathological processes which might accompany immune killing of the parasites. We find that natural immunity to *Ascaris* is associated with IgE antibody to a major allergen of the parasite and a serum protein profile consistent with ongoing inflammatory processes.

## MATERIALS AND METHODS

**Study population.** The study site was in an area of Nigeria (Ile-Ife) in which more than 80% of the school children (5 to 15 years old) were infected with intestinal nematodes, particularly *A. lumbricoides* (for full details, see reference 18). A group of children were treated for their intestinal nematode infections, and their worm burdens were collected and counted over a 48-h period after anthelminthic treatment (phase 1). The anthelminthic used was Ketrax (levamisole; ICI Pharmaceuticals, Macclesfield, United Kingdom), and children were given the appropriate dosage according to the manufacturer's instructions. The exercise was repeated 6 months later (phase 2), at which time blood samples were collected from 92 of the children. The children were classified as follows: category 1, those with no worms on either of the two occasions (putatively immune); category 2, those with consistently light infections (1 to 24 worms in phase 1 and 1 to 8 worms in phase 2); or category 3, those who were consistently heavily infected or susceptible, i.e., had more than the population mean plus 1 standard deviation worm burden on both occasions. The means ± standard deviations of the worm burdens in phases 1 and 2 were 11.02 ± 13.7 and 3.5 ± 5.6, respectively. Category 3 comprised children with worm counts of ≥25 after the first treatment and ≥9 after the second treatment. There were 22, 47, and 23 children in categories 1, 2, and 3, respectively. None of the children showed overt signs of

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                   1                                                   50
A. lumbricoides    HHFTLESSLD THLKWLSQEQ KDELLKMKKD GKTKKDLQAK ILYYYDELEG
Consensus          |||||||||| |||||||||| |||||||||| || ||:|:|| || |||||||
A. suum            HHFTLESSLD THLKWLSQEQ KDELLKMKKD GKAKKELEAK ILHYYDELEG

                   51                                                 100
A. lumbricoides    DAKKEATEHL KDGCREILKH VVGEEKEAEL KKLKDSGASK EEVKAKVEEA
Consensus          |||||||||| | |||||||| |||||| ||| | |||||||| ||:|||||||
A. suum            DAKKEATEHL KGGCREILKH VVGEEKAAEL KNLKDSGASK EELKAKVEEA

                   101                             130
A.lumbricoides     LHAVTDEEKK QYIADFGPAC KKIFAAAHTSRRRR
Consensus          |||||||||| |||||||||| |||:    ||||||
A. suum            LHAVTDEEKK QYIADFGPAC KKIYGV.HTSRRRR
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FIG. 1. Amino acid sequence comparison of the previously described ABA-1 allergen of *A. suum* (infecting pigs) and the homologue from *A. lumbricoides* (infecting humans) used in this study. The alignment was carried out with the MultAlin program (4) set for the Dayhoff comparison matrix (5). In the consensus lines, positions with identical amino acids (|) and those with conservative substitutions as defined by Dayhoff (:) are indicated. ., gap in the sequence.

any disease at the time of sampling. Informed consent was obtained from all subjects and their parents, the procedures were explained in the local language, and ethical approval was obtained from the University of Glasgow and the appropriate local authorities in Nigeria.

The intensity of infection with whipworm (*Trichuris trichiura*) was high, with an overall mean of 454 ± 579 (range, 0 to 3,047, prevalence 88.3%) (category 1, 328 ± 260 [range 0 to 771]; category 2, 261 ± 387 [range, 0 to 1983]; category 3, 899 ± 798 [range, 43 to 3,047]), and all subjects in category 3 were infected with *T. trichiura*. There was a positive association between infection with *A. lumbricoides* and *T. trichiura* (18). Infection with hookworm (*Necator americanus*) was low (18).

**Antigens.** Three different sources of *Ascaris* antigen were used. First, *Ascaris* body/pseudocoelomic fluid (ABF) was obtained from *A. suum* as previously described (24). Second, commercially prepared crude allergen extract from the porcine roundworm *A. suum* (*Ascaris* p1) was used for one of the *Ascaris* IgE assays (see below). Third, *Ascaris* ABA-1 allergen was used. Recombinant ABA-1 (rABA-1) was produced as follows. DNA encoding the ABA-1 allergen of *A. lumbricoides* was amplified by PCR from genomic DNA of parasites obtained by anthelminthic expulsion from humans in Guatemala (courtesy of T. J. C. Anderson, University of Oxford). Oligonucleotide primers were based on the sequence of the ABA-1 allergen of *A. suum* (43); primer sequences were 5′-ggaattcCATCATTTCACCCTTG-3′ (forward) and 5′-ggaattcCCTCCTTCGT CGCGAAG-3′ (reverse) (lowercase denotes *Bam*HI restriction sites added to permit insertion into the vector). A sequence comparison of the ABA-1 from *A. suum* and the ABA-1 homologue of *A. lumbricoides* used in this study is given in Fig. 1. Identical and slightly variant sequences were also been found in *A. lumbricoides* from China by using the above methods. The DNA was inserted into the pET-15b expression vector (Novagen, Abingdon, United Kingdom), using the *Bam*HI restriction sites encoded in the oligonucleotide primers. The ABA-1 allergen protein was expressed in the transformed BL21 strain of *Escherichia coli* (to yield clone PAL2) with 1 mM isopropyl-β-D-thiogalactopyranoside, and the fusion protein bearing a six-histidine tag was purified on a nickel ion affinity column as recommended by the manufacturer (Novagen). The $His_6$ tag was cleaved from the recombinant ABA-1 with thrombin (0.5 U of thrombin per mg of rABA-1) according to the manufacturer's instructions, and the protein solution was then dialyzed against phosphate-buffered saline and stored at −70°C before use. The concentration of recombinant protein was estimated by absorbance at 280 nm, using a theoretical molar extinction coefficient of 10,810 $cm^{-1}$ $M^{-1}$ calculated from its amino acid composition (11).

**Antibody and total immunoglobulin assays.** IgG, IgM, IgA, and IgG subclass antibodies against parasite antigens ABF and rABA-1 were measured by indirect enzyme immunoassay (EIA). Briefly, each antigen was incubated at 10 μg/ml in bicarbonate coupling buffer (pH 9.5), 100 μl per well in microtiter EIA plates (Dynatech, Guernsey, United Kingdom) overnight at 4°C. The plates were washed three times by shaking out and immersion into wash buffer (0.02 M [pH 7.4] phosphate-buffered saline containing 0.05% Tween 20). Patient and control sera were diluted 1:50 (dilution optimized for this assay by checkerboard analysis) with wash buffer and added at 100 μl per well. After 2 h of incubation, the plates were washed as before, and bound antibody with alkaline phosphatase-conjugated, isotype-specific anti-human IgG, IgA, and IgM (Sigma, Poole, Dorset, United Kingdom) or IgG subclasses (antibodies from The Binding Site Ltd., Birmingham, United Kingdom, and CLB Dutch Red Cross antibodies from Eurogenetics UK Ltd., Hampton, United Kingdom), diluted with wash buffer according to the manufacturers' recommendations, was added at 100 μl per well for 2 h at room temperature. After washing, the enzyme activity of bound conjugate was detected by addition of *p*-nitrophenyl phosphate (Sigma), 100 μl per well, at 1 mg/ml in 10% diethanolamine in water (pH 10.5). The reaction was stopped after 30 min by the addition of 3 N NaOH, and the reaction product was measured by spectrophotometry (Dynatech MR 600) at 405 nm; antibody activity was expressed as arbitrary optical density units. The specificity of the EIA was verified by the reproducibility of the test measurements (coefficient of variation less than 15%), by the dose responsiveness of the optical density measurements with increasing dilution of a high-titer serum, and by specific inhibition of the antibody activity by incubating test serum with excess free antigen overnight prior to assay. The assays were carried out at antigen excess in order to avoid competitive exclusion of minor isotype antibodies.

Total IgG, IgM, IgA, and IgG subclasses were measured by radial immunodiffusion (The Binding Site); IgE was measured by EIA (Pharmacia, Milton Keynes, United Kingdom). The summed concentration of the four IgG subclasses was consistent with the measured total IgG concentration ($r = 0.64$, $P < 0.001$).

IgE antibodies against *Ascaris* (p1), house dust mite (d1), grass pollen (g6), peanut (f13), rice (f9), egg (f1), and milk (f2) allergens were all measured by radioimmunoassay (Pharmacia). The intervals (classes) of IgE antibody units per milliliter (as defined by the manufacturer and a widely recognized classification) were as follows: class 0, <0.35; class 1, 0.35 to 0.74; class 2, 0.75 to 3.49; class 3, 3.5 to 17.49; and class 4, >17.5. The environmental and food allergens used are all relevant to the local conditions in Nigeria (35a). IgE antibody against the ABF or rABA-1 allergen was measured similarly using cyanogen bromide-activated discs to which the allergens were bound, and values in units per milliliter above the normal level were considered significant. The quantities of plasma available from the children were too limited to permit absorption of competing IgG from plasma tested for IgE antibodies.

The IgG antibody repertoire was analyzed by protein A-based radioimmunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and autoradiography as previously described (25).

Humans or experimental animals infected with hookworms or *Trichuris* do not produce antibodies to *Ascaris* ABA-1 (unpublished data).

**Serum markers of inflammation.** Ferritin levels were measured by enzyme immunoassay (Cambridge Life Sciences, Ely, United Kingdom), albumin and C-reactive protein (CRP) were measured by radial immunodiffusion (The Binding Site), and eosinophil cationic protein (ECP) was measured by radioimmunoassay (Pharmacia), as specified by the manufacturers. Serum IgG anti-human hsp27, hsp60, and hsp90 were measured by EIA (2) using recombinant proteins (Bioquote, York, United Kingdom).

**Statistical analysis.** Data were stored on Minitab and Excel spreadsheets, and analysis of variance by the Kruskal-Wallis rank and Fisher's exact tests were used to test for the effect of patient categorization on the various measurements. The Mann-Whitney *U* test was also used to test for differences between medians, and Spearman's rank correlation was used to test for correlations between variables where appropriate. Since the data were not normally distributed, nonparametric tests were used throughout.

**Nucleotide sequence accession numbers.** The *A. lumbricoides* sequence has been submitted to GenBank (accession no. U86091). Identical and slightly variant sequences found in *A. lumbricoides* from China are entered in GenBank under accession no. U86091 to U86099.

## RESULTS

**Isotype-specific antibody responses.** Antibody activity against *Ascaris* ABF antigen showed strong responses in IgG, IgM, and IgA (Table 1), the main component of the IgG response being in IgG1. There were no statistically significant associations between antibody levels in any isotype and infection category. Similar results were obtained when the target antigen was rABA-1 (Fig. 1), with a similar lack of association between antibody level and infection category (not shown). ABA-1 is an approximately 14.4-kDa, helix-rich protein which has lipid-binding activity and is abundantly produced by *Ascaris* (22).

Of the 92 children, 19 had significant IgE antibody levels

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TABLE 1. Isotype-specific antibody responses to *Ascaris* ABF antigen[a]

| Isotype | Median antibody response (interquartile range) | | |
|---|---|---|---|
| | Category 1 | Category 2 | Category 3 |
| IgG | 2.00 (1.29–2.25) | 1.95 (1.55–2.59) | 2.07 (1.58–2.38) |
| IgG1 | 2.10 (1.70–2.89) | 1.85 (1.47–2.40) | 2.27 (1.43–2.80) |
| IgG2 | 0.29 (0.18–0.49) | 0.32 (0.21–0.59) | 0.30 (0.23–0.79) |
| IgG3 | 0.20 (0.16–0.32) | 0.23 (0.18–0.33) | 0.21 (0.19–0.46) |
| IgG4 | 0.13 (0.08–0.74) | 0.12 (0.07–0.24) | 0.14 (0.07–0.36) |
| IgM | 0.91 (0.77–1.08) | 0.82 (0.64–0.99) | 0.88 (0.72–0.99) |
| IgA | 0.94 (0.65–1.35) | 0.97 (0.62–1.30) | 0.88 (0.65–1.30) |
| IgE | 0.2 (0.1–0.3) | 0.2 (0.1–0.3) | 0.1 (0.1–0.2) |

[a] Class- and subclass-specific antibody responses to *Ascaris* ABF antigen were measured as described in Materials and Methods; results are expressed in arbitrary optical density units (IgG, IgM, IgA, and IgG subclasses) or in arbitrary units (IgE). The IgG subclass measurements were carried out with two different sources of detection antibody; the results were similar, and only one of the data sets is shown. While some trends are apparent, Kruskal-Wallis analysis revealed no significant association between any of the antibody responses and infection category.

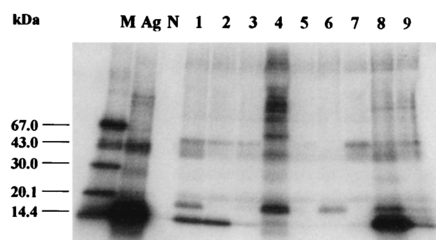


FIG. 2. Typical patterns of heterogeneity in the specificity of the IgG antibody responses to *A. lumbricoides*. ABF was labeled with $^{125}$I and immunoprecipitated with serum from an uninfected European (track N) or from individual children from the study population (numbered tracks). The immunoprecipitates were analyzed by gradient SDS-PAGE, along with a sample of the iodinated antigen (track Ag), and autoradiographed. Track M was loaded with iodinated standard marker proteins, molecular masses of which are indicated on the left. ABA-1 is the prominent band comigrating with the 14.4-kDa marker. Of the samples illustrated, only children 1, 2, 8, and 9 would be selected for the analysis illustrated in Fig. 3.

against *Ascaris* ABF antigen, 25 had IgE antibody against rABA-1, and 61 had IgE antibody against the *Ascaris* p1 antigen (Table 2), but there were no significant differences between the IgE antibody levels in the three infection categories.

We investigated further this apparent lack of correlation between antibody level and infection category by accounting for individual differences in antibody repertoires. In assays using a heterogeneous antigen such as ABF, considerable individuality in antibody repertoires (both IgG and IgE) has been observed in ascariasis (10, 25); this individual difference is probably under genetic control (23, 44). The fact that some individuals will respond to a particular set of antigens of the parasite and others will respond to a different set serves to confuse the analysis and obscure an association between antibody responses and relative resistance or susceptibility. We therefore examined the antibody activity in a subgroup with a more defined response repertoire, selecting only those who responded to the ABA-1 allergen. To this end, the samples were screened in a protein A-based (IgG-specific) radioimmunoprecipitation and SDS-PAGE assay using $^{125}$I-labeled ABF, typical results of which are illustrated in Fig. 2. We have demonstrated the stability of this system in six individuals for whom serum samples were obtained more than 3 years apart, over which time there was no change in *Ascaris* antigen recognition patterns (data not shown). Those individuals with detectable IgG antibody to the ABA-1 protein within the unfractionated ABF preparation were selected for analysis of the other antibody isotype responses to this protein alone, using rABA-1. This selection reduced the number of subjects to 38: 12 in the putatively immune category 1, 16 in the moderately infected category 2, and 10 in the highly infected category 3.

With rABA-1 as the target antigen, there were no significant differences between IgG, IgM, and IgA antibody levels and the infection categories (data not shown). There was, however, a significant association between a reduced rABA-1-specific IgE antibody titer with increasing parasite load; when the subjects in each of the three groups were further subdivided according to high or low levels of IgE antibody, using a threshold at the median value of the data (0.2 IU/ml), a distinct pattern emerged (Fig. 3). The putatively immune individuals tended to have higher levels of rABA-1-specific IgE and the susceptible group had low levels, with the intermediate group having sim-

TABLE 2. Distribution of IgE antibody activity against *Ascaris* and common inhalant and food allergens

| Allergen | Response for IgE antibody class[a]: | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| *Ascaris*: | | | | | |
| ABF | 73 | 9 | 8 | 2 | 0 |
| rABA-1 | 67 | 18 | 6 | 1 | 0 |
| p1 | 31 | 27 | 19 | 13 | 2 |
| Common: | | | | | |
| Mites | 79 | 13 | 0 | 0 | 0 |
| Pollen | 81 | 8 | 3 | 0 | 0 |
| Milk | 37 | 55 | 0 | 0 | 0 |
| Rice | 72 | 11 | 9 | 0 | 0 |
| Egg | 31 | 37 | 24 | 0 | 0 |
| Peanut | 76 | 11 | 3 | 2 | 0 |

[a] IgE antibodies to the allergen preparations were measured, and the individual IgE responses were placed into class intervals of standard antibody units as defined in Materials and Methods.

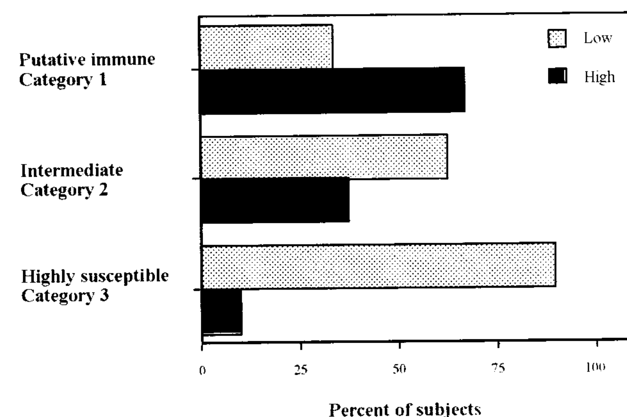


FIG. 3. Putative immune subjects have higher IgE antibody levels against rABA-1 allergen of *Ascaris*. Subjects were categorized as positive for IgG antibody to ABA-1 in the ABF antigen preparation by radioimmunoassay (Fig. 2) and then subdivided into those responding with high or low levels of IgE antibody to rABA-1 (see text for the criteria used for subselection). The proportion of individuals with the higher levels of anti-rABA IgE within the three infection classes showed pronounced differences (66.7% in category 1, 37.5% in category 2, and only 10% in category 3), with more individuals in the putatively immune group having higher levels. Fisher's exact and Mann-Whitney $U$ tests give $P$ values of 0.0063 and 0.0144 ($Z$ = 2.447), respectively.

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TABLE 3. Total (nonspecific) immunoglobulin isotype and subclass leels

| Isotype | Median concn[a] (interquartile range) | | | Kruskal-Wallis P value |
|---|---|---|---|---|
| | Category 1 | Category 2 | Category 3 | |
| IgE | **1,239** (525–1,800) | **1,036** (544–1,892) | **681** (456–1,237) | **0.06** |
| IgG | **21.4** (19.0–22.1) | **22.1** (18.4–24.6) | **23.9** (20.5–25.4) | **0.09** |
| IgM | **1.57** (0.92–3.31) | **1.57** (1.15–2.54) | **2.38** (1.55–2.92) | **NS**[b] |
| IgA | **1.66** (1.22–2.42) | **1.55** (1.16–2.03) | **1.72** (1.21–2.06) | **NS** |
| IgG1 | **18.0** (14.6–19.7) | **19.6** (17.0–25.0) | **19.1** (16.4–22.9) | **NS** |
| IgG2 | **1.73** (1.12–2.46) | **1.81** (1.47–2.66) | **1.63** (0.84–2.50) | **NS** |
| IgG3 | 2.06 (1.0–3.41) | **2.36** (1.46–4.41) | **3.65** (1.63–6.48) | **NS** |
| IgG4 | **0.47** (0.20–0.80) | **0.25** (0.12–0.75) | **0.28** (0.17–0.45) | **NS** |

[a] Measured as described in Materials and Methods. Concentrations are expressed in IU (IgE) or milligrams (other isotypes) per milliliter. For comparison, the normal ranges found for healthy unselected Europeans 5 to 15 years old are as follows: IgG, 5.4 to 16.1; IgM, 0.5 to 1.9; IgA, 0.5 to 2.8; IgG1, 3.6 to 7.7; IgG2, 1.4 to 4.6; IgG3, 0.3 to 1.2; and IgG4, <1.3 (47). Total IgG correlated with the sum of the individual IgG subclasses (Spearman's rho, $r = 0.64$, $P < 0.001$. Significantly higher levels of IgG and IgG3 and significantly lower levels of IgE occurred in category 3 subjects compared with those in category 1 ($P = 0.02$, 0.04, and 0.05, respectively).

[b] NS, not significant.

ilar numbers of low and high responders. Fisher's exact test and Mann-Whitney analysis showed that these differences were highly significant ($P = 0.0063$ and 0.014, respectively).

**Innate immunity and inflammation indicators.** The levels of total serum immunoglobulins in the Nigerian children were considerably higher than in European children (47), and this applied to all isotypes measured (Table 3). There were significantly higher levels of IgG and IgG3, and significantly lower levels of IgE, in category 3 than in category 1 ($P = 0.02$, 0.04, and 0.05, respectively). When the data were analyzed on an individual basis, total IgG levels correlated significantly with worm number ($r = 0.31$, $P < 0.01$).

While the total serum IgE levels were substantially greater than those of U.K. Caucasian children [95th centile = 63 IU/ml [47]), there were significantly lower total IgE levels in the heavily infected category than in the noninfected category ($P = 0.05$). The prevalence of atopy (as defined by the presence of IgE antibody to common allergens, in this case house dust mite, grass pollen, and food allergens) was lower than for unselected U.K. Caucasians (Table 2). A highly significant correlation was apparent between total IgE and IgE antibody to rABA-1 ($r = 0.441$, $P < 0.001$) and to the *Ascaris* p1 antigen ($r = 0.342$, $P < 0.001$), but no significant correlations appeared between total IgE and IgE antibody to *Ascaris* ABF antigen or with IgE antibody levels to any of the common allergens. This finding suggested that the high total IgE levels in these children were driven by the specific *Ascaris* response, and it is notable that the magnitude of the IgE response could not be accounted for by antibody activity to any of the preparations used as target allergens here.

There were significantly higher levels of the inflammatory indicator proteins ferritin, ECP, and CRP in the putatively immune category 1 subjects, whereas there were no differences in the serum albumin levels between the groups (Table 4). The anti-heat shock protein antibodies increased in titer with greater infection, but this was significant only for hsp27 ($P = 0.04$) and was borderline for hsp90 ($P = 0.077$). Assays were also carried out for the presence of interleukin-4 (IL-4), soluble CD23, and mast cell tryptase, but neither of the former two were detectable and only one of the children had significant mast cell tryptase in circulation (data not shown).

## DISCUSSION

Experimental work has indicated a crucial role for T helper type 2 (Th2) cell responses in immune elimination of gastrointestinal nematodes (40). Th2 cells control the cytokines IL-3, IL-4, and IL-5, which regulate the characteristic mastocytosis, IgE, and eosinophilia, respectively, of nematode infections (7, 12, 40, 46), although which Th2-controlled effector mechanisms are responsible for parasite loss remains a matter of conjecture. There is a relative paucity of field-based observations on the immunology of human infections with these parasites, and we report a statistical association between natural immunity to *A. lumbricoides*, IgE antibody to the parasite, and ongoing inflammatory processes.

IgE antibody has variously been reported to be associated with protection against intestinal nematodes or to be irrelevant, and there are contradictory reports on IgE and ascariasis (14, 32, 34, 37). The particular advantages of the present study were that the prevalence and intensity of the infection were very high in the population, there was no previous drug intervention, and parasite burdens (rather than the less predictive parasite egg output) were recorded, all of which permitted the identification of putatively immune individuals with confidence. Nevertheless, confining attention to a defined antigen or allergen of the parasite was required in order to demonstrate an association between IgE antibody and susceptibility or resistance. This principle will presumably apply for other infections. The effect limits the usefulness of such assays for identification of susceptible or resistant individuals but indicates that allergens may be of particular value, and a balanced mixture of allergens may be generally useful. It is noteworthy that an antigen homologous to the ABA-1 allergen used here is preferentially subject to IgE antibody responses in filariasis in humans (48).

An issue which has occupied a great deal of thought is whether helminth infections predispose to, or protect against, atopic reactions to environmental and food allergens, but no consensus has emerged. It has been argued that helminth infection potentiates IgE responses causing increased atopic reactions (28), supportive of which is a study in which anthelminthic treatment protects against asthma (29). But with high-level infections, mast cells are postulated to become blockaded with irrelevant IgE, thereby dampening atopic responses (28). High levels of irrelevant IgE, however, do not protect against anaphylaxis in an experimental system (19). In general, atopy

TABLE 4. Distribution of levels of protein markers of inflammation according to category of infection

| Marker | Median concn[a] (interquartile range) | | | Kruskal-Wallis P value |
|---|---|---|---|---|
| | Category 1 | Category 2 | Category 3 | |
| Albumin (mg/ml) | **50.0** (45.0–59.2) | **50.0** (38.6–60.9) | 52.6 (45.0–63.0) | **NS**[b] |
| CRP (μg/ml) | **7.9** (4.0–21.8) | **4.0** (0.0–9.5) | **0.0** (0.0–7.2) | **0.05** |
| Ferritin (ng/ml) | **121.2** (68.2–158.7) | **87.4** (35.7–147.5) | **43.6** (29.1–103.1) | **0.004** |
| ECP (ng/ml) | **75** (48–150) | **50** (36.5–70) | **59** (49.5–75.7) | **0.028** |
| hsp27 | **0.175** (0.11–0.34) | **0.215** (0.09–0.42) | **0.48** (0.13–0.76) | **0.04** |
| hsp60 | **0.596** (0.37–1.34) | **0.782** (0.32–1.67) | **1.127** (0.54–1.45) | **NS** |
| hsp90 | **0.501** (0.37–0.79) | **0.575** (0.43–0.75) | **0.636** (0.49–1.03) | **0.077** |

[a] Measured as described in Materials and Methods. Normal values for Caucasian children: albumin, 37 to 56 mg/ml; CRP, <10 μg/ml; ferritin, 37 to 142 ng/ml; ECP, 2.3 to 16 ng/ml (reference 47 and Yorkhill Children's Hospital, Glasgow, biochemistry values). The anti-heat shock protein responses are expressed in arbitrary optical density units.

[b] NS, not significant.

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appears to be of reduced frequency in areas endemic for the major communicable diseases, and it has been suggested that powerful Th1-inducing infections such as tuberculosis counterbalance Th2 responses induced by helminths (42).

While our study collected no information on allergy among the study population, it is clear that their IgE responses are quite different from those of inhabitants of areas with less exposure to helminth infections. The Nigerian children had extremely high levels of IgE in their sera, but specific IgE against a selection of environmental and food allergens was lower than for European or North American atopic individuals. Also, there was a statistical association between high total IgE immunoglobulin levels and anti-*Ascaris* IgE, which would be consistent with the specific antiparasite response driving the total IgE response, although without disproportionate responses to individual allergens. If the amount of measurable IgE against an allergen can be taken as being indicative of potential atopy, then helminth infection appears to be counteratopic in this population. Certainly, we find that levels of specific and total IgE seem to be related more to protection than to exposure to the infection, which would argue against hyperinduction of nonspecific IgE by the parasites serving to block a protective function for specific IgE (36) and may indicate that intrinsically high IgE responders are more resistant. A point to stress is that there is no evidence here that IgE antibody is directly protective since IgE is one of a set of mechanisms driven in parallel by Th2 responses (7, 12, 40, 46).

IgG4 has been postulated to be a blocking antibody in schistosomiasis, acting to impair IgE-based protection in younger age classes, and it has been proposed that as the IgE response strengthens with age, so does its protective effect (13). In filariasis, the ratio between IgG4 and IgE antibody may determine the clinical outcome of the infection in that high IgE-low IgG4 correlates with relative resistance to infection but also with pathology (1). Our finding that IgG4 antibody responses are minimal in ascariasis and that there is no relationship between IgG4 and IgE antibody, or with infection levels, was therefore unexpected. But the possibility remains that a relationship will emerge in a longitudinal or age cross-sectional study, as was the case in schistosomiasis (14).

Indices of inflammation (increased CRP, ferritin, and ECP) suggest that there are ongoing inflammatory responses in the putatively immune children compared with the infected group, in which a more florid response might have been expected. Although inflammatory responses may have several different causes in this population, it is conceivable that the antiparasite effector mechanism is itself an inflammatory process. Alternatively, ascariasis may involve parasite-derived inflammatory suppressive factors which have reduced influence in immune subjects, and/or the putatively immune subjects may have a greater innate tendency to inflammatory responses. The increased ECP levels in putatively immune subjects is of potential importance given the association between IgE and eosinophilia in other helminth infections (30), although experimental studies differ in their demonstration of protective activities of eosinophils in vivo (17, 26, 27, 35, 41). The meaning of the occurrence of increased levels of antibodies to hsp27 (particularly), hsp60, and hsp90 in the *Ascaris*-susceptible children is unclear but it is notable that a similar pattern is also found in inflammatory diseases such as arthritis (2). Whether this is due to cross-reactive antibodies elicited by increased exposure to heat shock proteins from nematodes, self, or other pathogens remains to be seen (2, 21, 39), but the effect may reflect increased antigenic exposure in these children.

To conclude, IgE-mediated or associated mechanisms may be involved in resistance to ascariasis, and inflammatory reactions coordinate with, or are caused by, the protective mechanisms. Whether parasite attrition occurs during the tissue migratory phase of infection (as considered to be the case for *A. suum* in pigs [8, 9, 16, 45]) or in the intestine remains to be established, although the elevated inflammatory indicators in putative immune subjects might argue for the former. The findings also reinforce the idea that identification of immune or susceptible individuals by serology will require consideration of a range of factors and that defined allergens will be useful in this regard.

## ACKNOWLEDGMENTS

This work was supported by grants from the Wellcome Trust (grant 048488), the UK Medical Research Council (G9001232PB), and the Robertson Trust (University of Glasgow) to M.W.K. and The Higher Education Development Organisation (Ireland) to C.V.H. We are indebted to Samuel Asaolu and D. W. T. Crompton for assistance in provision of the plasmas used in this study and for the former's efforts in developing a working relationship with the study community, to Kirsten McLeod for help with some of the immunological assays, to Tim Anderson for the *A. lumbricoides* parasite samples for DNA, and to Joyce Moore and Heather Spence for their contribution to the cloning of DNA encoding ABA-1 from human *Ascaris*.

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*Editor:* J. M. Mansfield

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