

Differential Antibody Isotype Reactivity to Specific Antigens in Human Lymphatic Filariasis: gp15/400 Preferentially Induces Immunoglobulin E (IgE), IgG4, and IgG2

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Lymphatic filarial infection in humans is associated with a strong skewing of the immune response towards the TH2 arm, with prominent interleukin 4-producing cells and elevated levels of immunoglobulin G4 (IgG4) and IgE antibodies in peripheral blood. To determine how such a generalized TH2 imbalance governs responses to individual parasite antigens, the profiles of isotypes of antibodies to two recombinant proteins of *Brugia* spp. were studied. One molecule was the C-terminal portion of the filarial heat shock protein 70 (Bpa-26), representative of a cytoplasmic protein, and the second antigen was a single unit of the tandem repeats of a *Brugia* polypeptide (BpL-4), a secreted product which is prominently exposed to the immune system. Serum samples from 146 individuals resident in areas in which brugian filariasis is endemic were used, and it was found that whereas the levels of IgG1 and IgG3 responses to both Bpa-26 and BpL-4 were high, IgG4 and IgE antibodies to only BpL-4, not to Bpa-26, were prominent. Thus, an antigen which is chronically exposed to the immune system elicited a TH2-dependent isotype switch, as manifested by increased IgG4 and IgE responses. Moreover, IgG4 and IgE responses to BpL-4 showed a strong negative association, suggesting that mediators other than interleukin 4 must be responsible for such differential regulation of these two isotypes. When the data were analyzed as a function of clinical status, a striking association between elevated levels of IgG3 antibodies to Bpa-26 and manifestation of chronic obstructive disease was found; elephantiasis patients showed significantly higher levels of IgG3 antibodies to Bpa-26 than microfilaremics and asymptomatic amicrofilaremics. This indicates that an imbalance of isotypes of antibodies to particular filarial antigens might play a role in the pathogenesis of chronic disease.

Lymphatic filariasis, a major tropical disease caused by nematode parasites of *Wuchereria* and *Brugia* species, is characterized by expansion of TH2 T-cell subsets and striking elevation of levels of immunoglobulin G4 (IgG4) and IgE antibodies in peripheral circulation (15, 19, 24). In long-lived parasitic infections, an additional aspect is that the parasites modulate host immune reactions to allow their persistence within immunocompetent hosts. Filariasis is a prototype of such a situation. Individuals harboring high levels of parasites in their circulation who show no symptoms of disease have specific T-cell hyporesponsiveness in terms of proliferation and gamma interferon release (14, 21, 29, 42) but exhibit strong TH2-cell expansion following polyclonal T-cell stimulation (19, 43). Total IgE levels in these patients are high, while antigen-specific antibody responses are low, with the exception that levels of IgG4 to filarial antigens are extremely high (15).

Patients with elephantiasis, who develop chronic obstructive disease, show high-level antigen-specific antibody responses of isotypes IgG1, IgG2, IgG3, and IgE, while IgG4 levels are dichotomous, high in individuals harboring active infection and low in those who seem to have eliminated their worm burden

(15, 16, 18). T-cell proliferative responses are strong in elephantiasis patients free of active infection (this group constitutes the majority of the patients), while those with elevated levels of antifilarial IgG4 show reduced T-cell proliferation, analogous to the situation seen in microfilaremics (42). The so-called asymptomatic amicrofilaremic population consists of individuals who harbor no apparent infection and are free of clinical symptoms. This group is the most difficult to define with precision (15, 23). Immunologically, a proportion of asymptomatic amicrofilaremics appears infected as determined by elevated levels of antifilarial IgG4, while others show low antifilarial IgG4 antibody levels (20).

Exactly how antibody isotypes and the TH1-TH2 balance are involved in immunity to various stages of the filarial life cycle or pathogenesis of elephantiasis is not clear. In animal models of schistosomiasis, the TH2-type responses, associated with elevated IgE levels and eosinophilia, are involved in establishment of chronic infection, while TH1-mediated responses confer resistance (28, 41). On the other hand, in *Trichuris muris* infections, gamma interferon mediates establishment of chronic infection, while interleukin 4 (IL-4) seems to promote worm expulsion (7). In any case, it is clear that the TH1-TH2 balance *in vivo* is a critical determinant of the outcome of numerous infections.

A key question to be answered is what is responsible for the polarization of the T-cell subsets. Among the possible factors are the primary structure of the antigen, the route of entry and

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TABLE 1. Description of the study population

Clinical condition	No. of subjects			Age (yr)	
	Total	Male	Female	Mean	Range
Elephantiasis	51	42	9	46	15–75
Microfilaremia	42	39	3	38	15–64
Asymptomatic amicrofilaremia	40	24	16	32	15–60
Symptomatic amicrofilaremia	13	8	5	42	19–56

presentation, and the genetic makeup of the host (31). The route of entry can influence presentation in multiple ways, including selecting which population of cells may serve to present antigen or affecting the persistence of antigen and the kinetics of antigen uptake. Dissection of immune responses to individual molecules derived from the infectious agent may help to determine how polarization of the immune response is initiated. Filarial worms are complex multicellular organisms, and crude extracts from parasites have been utilized to study the immunological responses of parasitized individuals in a number of areas of endemicity (9, 15, 25). With the increasing availability of recombinant antigens, it is now possible to dissect and examine molecular immunological responses to defined antigens (11, 37, 40). In this study, we have used two recombinant molecules which differ in terms of exposure to the immune system and have analyzed the antibody isotypes generated by a large number of individuals resident in areas where filariae are endemic who exhibit different clinical manifestations. BpL-4 corresponds to one repeat unit from gp15/400 (38). This glycoprotein has a repetitive structure, is expressed in all life cycle stages, and is secreted *in vitro* (35) and would therefore be expected to be exposed continuously to the immune system. The second antigen studied is Bpa-26, which corresponds to the immunodominant C-terminal portion of a constitutively expressed heat shock protein 70 (hsp70) (34, 44). This cytoplasmic protein should be exposed to the immune system only upon the death and degeneration of parasites. The data obtained indicate that different isotypes of antibodies to the two antigens are produced during infection. Also highlighted is an inverse association between IgG4 and IgE isotype responses to one of the recombinant antigens (BpL-4) and the presence of elevated levels of IgG3 antibodies to Bpa-26 in patients with chronic pathology.

MATERIALS AND METHODS

Study population. The study population has been described in detail elsewhere (15, 42). Briefly, serum specimens from 146 individuals resident in an area in Sumatra, Indonesia, in which brugian filariasis is endemic who were classified as asymptomatic amicrofilaremics (individuals free of clinical symptoms and negative for circulating microfilariae as determined by filtration of 1 ml of nocturnally obtained blood), microfilaremics (persons positive for circulating microfilariae and clinically asymptomatic), symptomatic amicrofilaremics (patients with clinical symptoms attributable to filarial infection but free of microfilariae), and elephantiasis patients (patients with chronic irreversible edema of the extremities) were used in this study to examine the isotypes of antibodies mounted in response to the recombinant proteins (Table 1). When the antibody isotype responses of the different clinical categories were compared, the group of symptomatic amicrofilaremics was left out because of the small number of individuals within this category.

Preparation of recombinant antigens. The Bpa-26 cDNA, which encodes the C-terminal domain of filarial hsp70, was cloned from a *Brugia pahangi* cDNA library in lambda gt11 as described earlier (34). The repeating unit (BpL-4) for gp15/400 was amplified from genomic DNA of *Brugia malayi* by PCR using primers derived from the sequence of the *B. pahangi* cDNA, cloned from the lambda gt11 library (38). Both Bpa-26 and BpL-4 were subcloned into PDS56/RBS11 for expression (27). The expressed proteins contained six histidine residues at their N termini, which allowed their purification over a nickel column as described previously (27).

Enzyme-linked immunosorbent assay for IgG antibodies to recombinant proteins. Maxisorp plates were coated overnight at 4°C with 100 µl of phosphate-buffered saline containing recombinant antigens at an optimal concentration of 2 µg/ml. Sera (in duplicate samples) were added at a dilution of 1/40, and the plates were incubated for 1.5 h at room temperature with continuous shaking. After a washing, isotype-specific monoclonal antibodies (anti-IgG1 clone HP-6001, anti-IgG2 clone HP-6002, anti-IgG3 clone HP-6050, and anti-IgG4 clone HP-6025 [Sigma]) were added at a 1/2,000 dilution, and the mixtures were incubated for 1 h at room temperature with shaking. Following two washing steps, the wells were incubated with a 1/2,000 dilution of peroxidase-conjugated rabbit anti-mouse immunoglobulin antibodies (P260; Dako) for 1 h at room temperature with shaking. The plates were washed, and color was developed with the substrate 3,5,3',5'-tetramethylbenzidine. The color reaction was stopped with H₂SO₄, and readings were taken at 450 nm. On each plate, a reference serum sample from a patient with high-level anti-recombinant protein reactivity was used to construct a standard curve with which all individual serum specimens were compared. Data are expressed as arbitrary units per milliliter as derived from the standard curve. The background was calculated from the reactivities of 12 serum samples obtained from European control subjects in areas of nonendemicity.

SPRIA for IgE. Both recombinant BpL-4 and recombinant Bpa-26 were coupled to cyanogen bromide-activated Sepharose beads (Pharmacia) for a solid-phase radioimmunoassay (SPRIA). The optimal amount of antigen for coupling to 10 mg of Sepharose was found to be 30 µg for both recombinant proteins. Plasma samples were depleted of IgG subclasses prior to the IgE assay by overnight incubation with protein G-Sepharose as described in detail previously (15). The assay for measuring IgE antibodies to BpL-4 and Bpa-26 was similar to the assay described for determination of IgE antibodies to adult *Brugia* antigen (15). Briefly, 0.5 mg of antigen-coupled Sepharose was incubated with 50 µl of 1/2-diluted plasma overnight at room temperature with continuous shaking. After a washing, 20,000 cpm of ¹²⁵I-labeled polyclonal sheep antibody against IgE (Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands; 19-F1) was added to the test samples and they were incubated overnight at room temperature. Following wash steps, the radioactivity of the samples was determined with a gamma counter and expressed as percent binding of the added label and then plotted against a nonlinear regression curve of a reference serum. The reference curve was constructed by incubating Sepharose-coupled sheep anti-IgE (CLB; 19-F2) with serial dilutions of a reference serum under conditions identical to those for the test samples. The background values were calculated from incubation of samples from nonfilaria allergic patients.

SPRIA for IgG4. In order to be able to compare IgE and IgG4 antibody responses directly, anti-BpL-4 and anti-Bpa-26 IgG4 levels were determined in an SPRIA. One hundred plasma samples were randomly selected and used in this assay. The test was identical to that for IgE except for the following differences: (i) 10 µl of plasma was added instead of 50 and (ii) ¹²⁵I-labeled monoclonal antiserum against IgG4 (CLB; M6H64/1) was used instead of anti-IgE.

Statistical analysis. For comparison of isotype responses to the two recombinant proteins, the Wilcoxon test was applied. Spearman's rank correlation was utilized for correlation of IgG4 and IgE responses to BpL-4. When anti-recombinant protein isotype responses of different clinical groups were compared, the Mann-Whitney U test was utilized. The *P* values were multiplied by a factor of 3 to correct for the comparisons made between the three clinical categories of asymptomatic amicrofilaremics, microfilaremics, and elephantiasis patients.

RESULTS

Antibody isotype reactivity to Bpa-26 and BpL-4 in total study population. To compare antibody isotype recognition of BpL-4 and Bpa-26, we tested IgG1, IgG2, IgG3, IgG4, and IgE reactivities to the two recombinant proteins. In Fig. 1, the reactivity of each individual with BpL-4 and Bpa-26 is charted, demonstrating antigen-specific IgG1 and IgG3 in equivalent numbers of individuals. The levels of IgG1 antibodies to BpL-4 were higher, with a mean of 48.3 U/ml (range, 0 to 8,831.7 U/ml), than those for Bpa-26, with a mean of 16.4 U/ml (range, 0 to 2,112.7 U/ml). The extent of recognition of these two recombinant antigens by IgG2, IgG4, and IgE was considerably more diverse. Seventy-three percent of individuals mounted an IgG2 response to BpL-4 and 35% mounted an IgG2 response to Bpa-26, while these values for IgG4 and IgE recognition were 81 versus 12% for BpL-4 and 55 versus 30% for Bpa-26, respectively. These differences are reflected in the levels of antibody to the recombinant proteins, which were significantly higher for BpL-4 than for Bpa-26 (*P* < 0.0001 for IgG2, *P* < 0.0001 for IgG4, and *P* < 0.0001 for IgE, by Wilcoxon test).

Reciprocal relationship between IgG4 and IgE. In order to

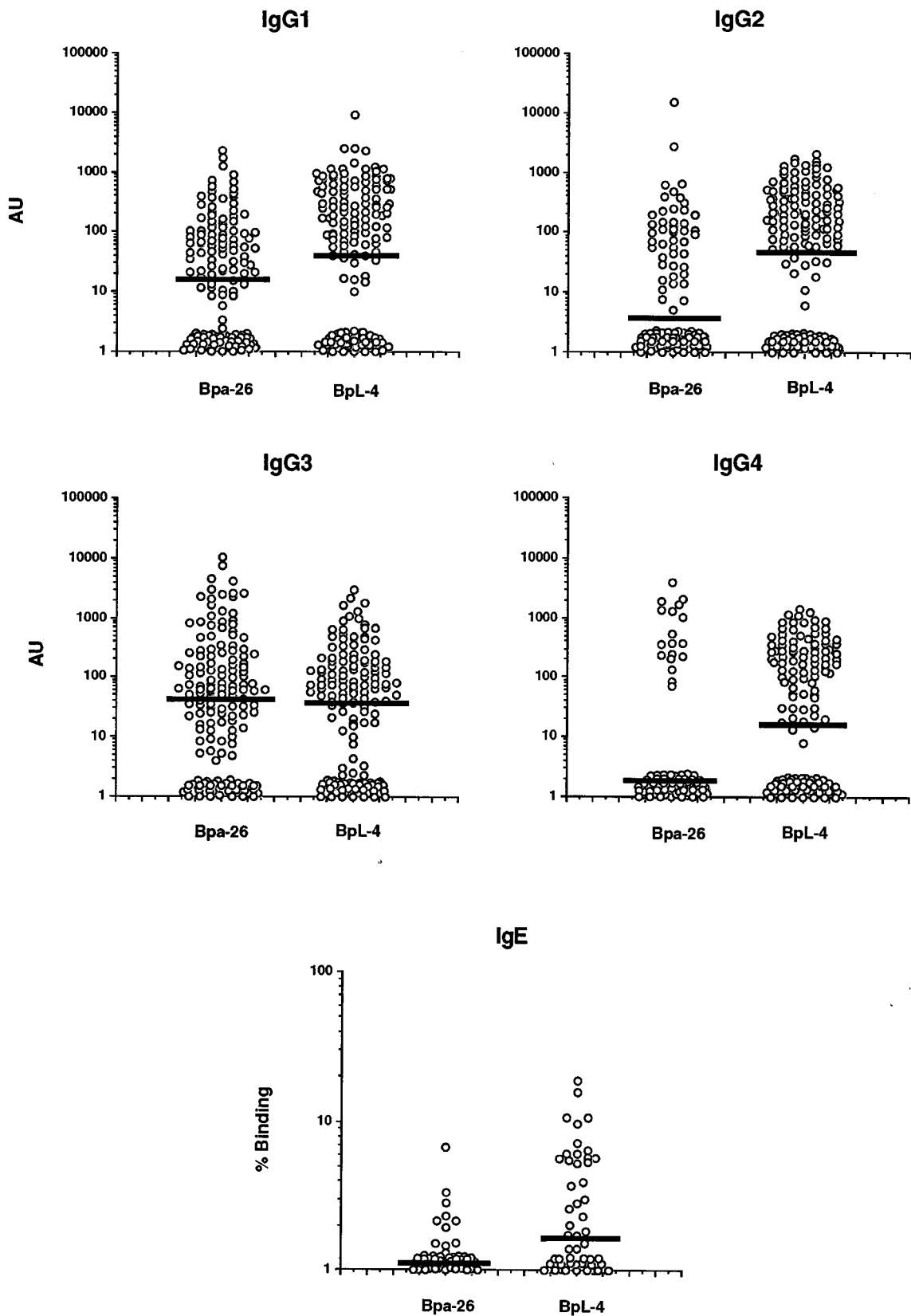


FIG. 1. Isotypes of antibodies to Bpa-26 and BpL-4. Each point represents an individual patient. Values for IgG1 to IgG4 are expressed as arbitrary units (AU), and those for IgE are expressed as percent binding (see Materials and Methods). Horizontal bars represent the geometric mean for each group. Background values have been subtracted from all datum points. The background values (means + 2 standard deviations) were determined by measuring the responses of 12 control subjects from areas of nonendemicity. The values for Bpa-26 are as follows: IgG1, 46.6; IgG2, 180.5; IgG3, 40.1; and IgG4, 880.2. The values for BpL-4 are as follows: IgG1, 248.6; IgG2, 309.8; IgG3, 120.6; and IgG4, 140.1. The background values (mean + 2 standard deviations) for IgE were determined by measuring the responses of 10 control subjects from regions of nonendemicity who had a food or cat allergy and exhibited elevated total IgE levels (values, 1.4 and 1.3% for Bpa-26 and BpL-4, respectively). IgG1, IgG2, IgG4, and IgE responses to BpL-4 were significantly higher than the responses to Bpa-26 (Wilcoxon test, $P < 0.0001$).

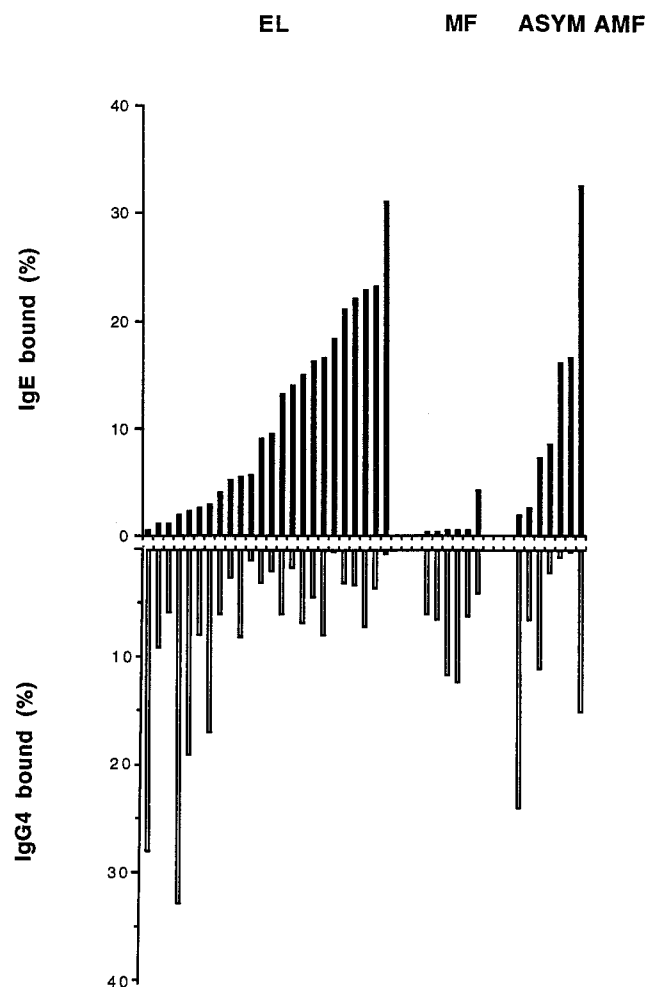


FIG. 2. Anti-BpL-4 IgG4 (downward bars) and IgE (upward bars) levels in responsive individuals belonging to the clinical categories of elephantiasis patients (EL), microfilaremics (MF), and asymptomatic amicrofilaremics (ASYM AMF) as measured in an SPRIA (see Materials and Methods). Background has already been subtracted and was 1.3% for both IgE and IgG4.

directly compare specific IgE and IgG4 antibodies to BpL-4 and Bpa-26, we tested for IgG4 antibody levels in an SPRIA (i.e., in an assay identical to that for IgE) and all responders were compared (individuals with neither an IgG4 nor an IgE response to the recombinants were excluded). Because the IgG4 and IgE binding to Bpa-26 was extremely weak, the comparison was restricted to BpL-4. As shown in Fig. 2, there was an inverse association between IgG4 and IgE responses ($\rho = -0.430$, $P < 0.01$, Spearman's rank correlation). For IgE determination, IgG in plasma samples was preabsorbed with protein G-Sepharose, excluding any competition by IgG4 in this assay.

Antibody isotype reactivity as a function of clinical status. Human populations exposed to filarial transmission may be classified in three well-defined groups, elephantiasis patients, microfilaremics, and asymptomatic amicrofilaremics, on the basis of clinical and parasitological examination (23). Figure 3 details the antibody responses to BpL-4 and Bpa-26 mounted by individuals in each of these clinical categories. It is clear that despite variability and overlap in the antibody responses, some interesting trends emerge. Generally, elephantiasis patients showed greater reactivity to both Bpa-26 and BpL-4 than did

microfilaremics and asymptomatic amicrofilaremics. These differences reached statistical significance in the following measurements. Elephantiasis patients showed significantly higher levels of IgG1 reactivity to both recombinant antigens than did asymptomatic amicrofilaremics. The levels of IgG2 antibody to BpL-4 in microfilaremics were significantly lower than those in elephantiasis patients and asymptomatic amicrofilaremics, reflecting the general hyporesponsiveness of microfilaremics to crude parasite extract observed previously (15, 42). Interesting differences in the levels of IgG3 antibody to Bpa-26 were noted: elephantiasis patients had significantly higher levels of these antibodies in their circulation than did microfilaremics or asymptomatic amicrofilaremics. Examination of the anti-BpL-4 reactivity revealed that levels of IgG3 antibodies to this recombinant in asymptomatic amicrofilaremics were significantly lower than those in elephantiasis patients and microfilaremics. The IgG4 and IgE responses to Bpa-26 were too weak to reveal any differences between the three clinical groups, but significant differences in these isotypes were observed with BpL-4 as the target antigen. Of the three groups, microfilaremics showed the highest levels of IgG4 to BpL-4. In contrast, IgE responses to BpL-4 in microfilaremics were significantly weaker than those in elephantiasis patients and asymptomatic amicrofilaremics. This pattern of IgG4 and IgE antibody isotypes in the clinical groups reflects the results obtained with total parasite extract used as an antigen.

DISCUSSION

Since the demonstration of T helper cell subsets in humans, there has been increasing interest in the contrasting functions of these cells and their products in immunity, inflammation, and pathological responses. Generally, bacterial and protozoal infections stimulate TH1-cell responses while helminthic infections stimulate the TH2 arm of the immune response (10, 26, 33). How these skewed T-cell manifestations might be responsible for immunity or pathology has not been delineated, but understanding the basis and consequences of differential helper cell induction is likely to be critical for appropriate immunological intervention.

Expansion of the TH2-cell subset in the peripheral circulation is a prominent feature of lymphatic filariasis (14, 19, 42). Filarial infections are correspondingly associated with elevated polyclonal IgE levels and eosinophilia, although antigen-specific antibody profiles vary according to the clinical status of the affected individual (22). For example, elephantiasis patients and asymptomatic amicrofilaremics ("endemic normals") have higher levels of antigen-specific IgE than microfilaremics, but the levels of antifilarial IgG4 antibodies are highest in microfilaremics (5). Such profiles are obtained by measuring antibodies to total parasite antigen; the question we have addressed in this study is whether the high levels of antiparasite IgE seen in many individuals are mounted against each one of the spectrum of parasite antigens, irrespective of differences in antigenic structure or modes of presentation. To study this, we compared two recombinant antigens with distinct structural characteristics and patterns of expression.

It is clear from the data obtained that antibody isotype responses to the two antigens are differentially regulated. Although both recombinant proteins are immunogenic, as seen from elevated levels of IgG1 and IgG3 to both BpL-4 and Bpa-26, the IgG2, IgG4, and IgE responses to the two recombinant antigens differ. IgG2 antibodies are commonly directed towards T-cell-independent antigens, such as carbohydrates and proteins with repetitive structures (36). BpL-4 is a subunit of a highly repetitive glycoprotein, and this could explain the

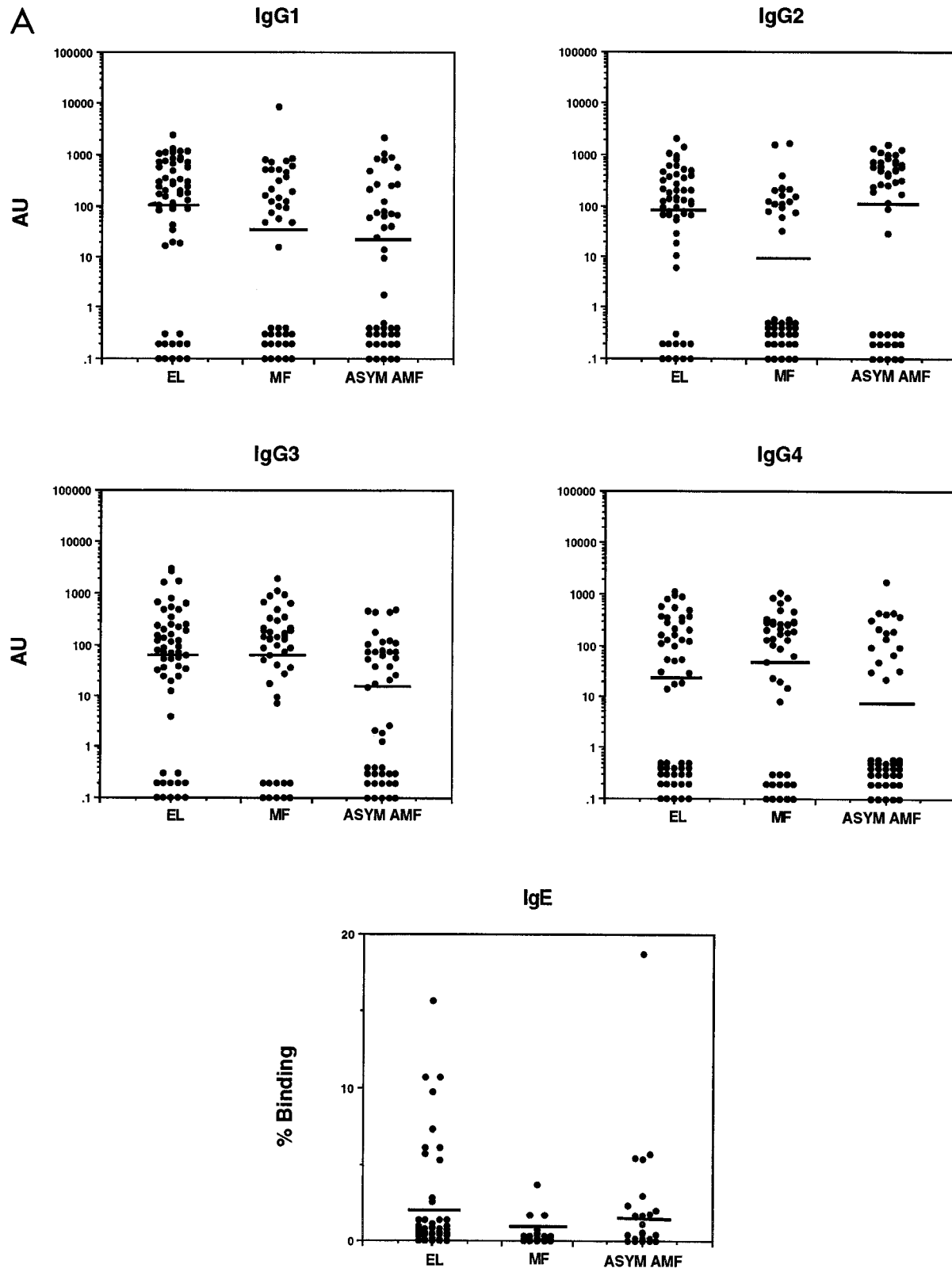


FIG. 3. Antibody isotype responses to BpL-4 (A) and Bpa-26 (B) by elephantiasis patients (EL) ($n = 51$), microfilaremics (MF) ($n = 42$), and asymptomatic amicrofilaremics (ASYM AMF) ($n = 40$). Horizontal bars represent the geometric mean for each group. Background values have already been subtracted and are as given in Fig. 1. Corrected P values for significant differences (Mann-Whitney U test) in isotype responses mounted by distinct clinical groups are as follows: IgG1 to BpL-4 and to Bpa-26 (EL versus EN), <0.03 and <0.015 , respectively; IgG2 to BpL-4, <0.003 for EL versus MF and <0.0003 for EN versus MF; IgG3 to BpL-4 (MF versus EN), <0.024 ; IgG3 to Bpa-26, <0.006 for EL versus MF and $P < 0.0003$ for EL versus EN; and IgE to BpL-4 and to Bpa-26 (EL versus MF), <0.0003 and <0.012 , respectively.

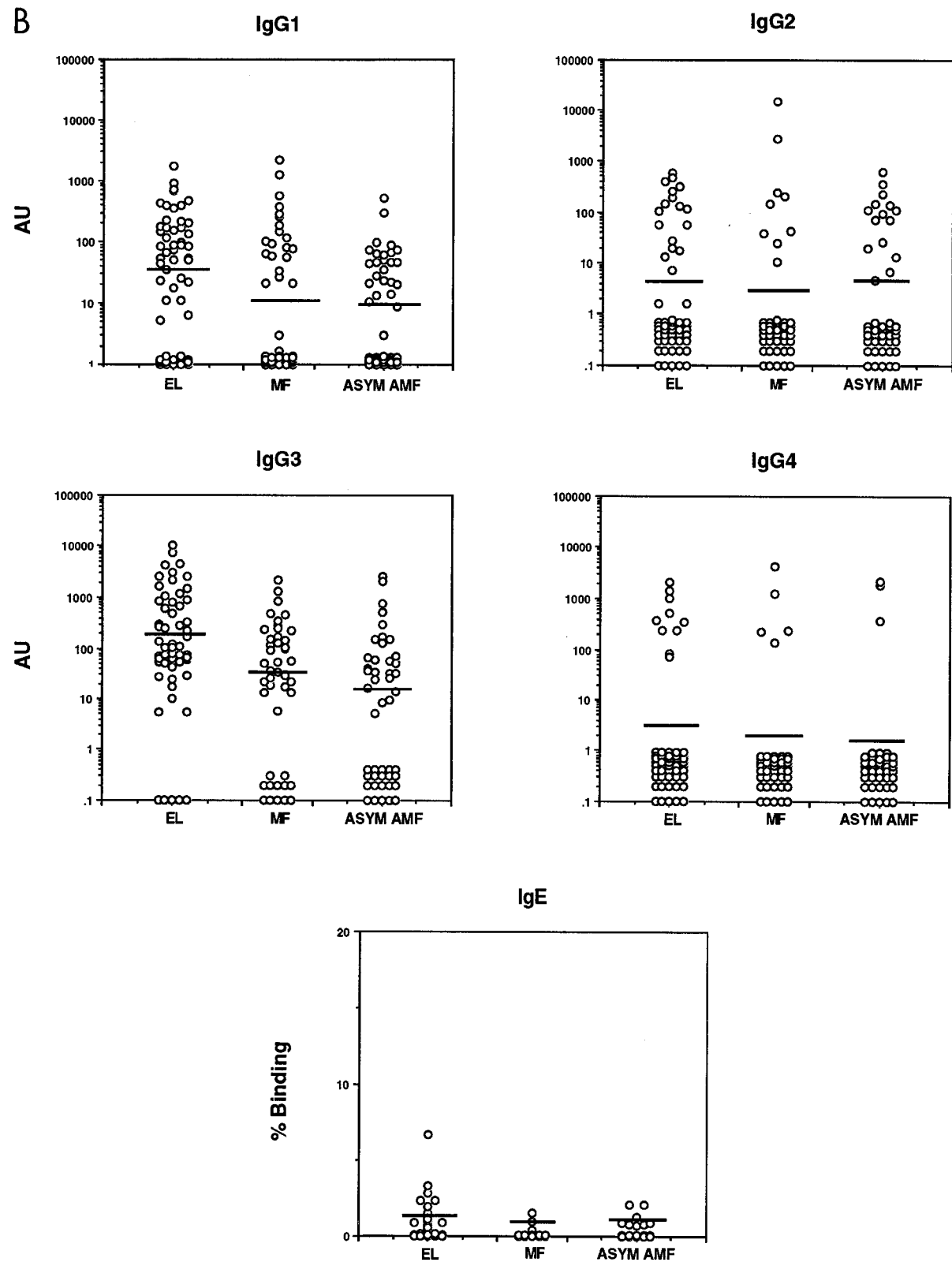


FIG. 3—Continued.

strong IgG2 responses to this recombinant protein. In addition, BpL-4 evokes TH2-dependent antibody responses; the levels of both IgG4 and IgE antibodies to BpL-4 are markedly higher than those for Bpa-26. In other words, individuals infected with filarial parasites can mount an IgE or IgG4 response to one filarial antigen and an IgG1 or IgG3 response to another. Interestingly, a T-cell clone generated against BpL-4 released substantial amounts of IL-4 and limited amounts of gamma interferon, a cytokine profile that supports the existence of TH2-type cells reactive to BpL-4 in filarial patients (unpublished results). It is noteworthy that homologs of BpL-4 have been isolated from several nematode species (30), and one of the major protein allergens of *Ascaris* spp. has been identified as a member of this family (4). The differential stimulation of T-cell subsets by distinct antigens has been recently demonstrated with a murine model of schistosomiasis; from lymph nodes of infected animals that exhibit strong TH2 responses to total parasite antigens, TH1 clones against a recombinant triose phosphate isomerase were generated (32).

The selective stimulation of TH2-dependent antibody isotypes may be due in part to the dynamics of antigen release in a continuous infection and in part to the nature of extracellular parasitism and antigen presentation. Chronic antigenic challenge has been shown to result in an increase in CD27⁺ T cells with IL-4-releasing capacity in the peripheral circulation (42). In a noninfectious setting, beekeepers exposed to bee venom show initial IgG1 antibodies to PLA2 (the major bee venom allergen) but elevated levels of IgG4 upon chronic exposure (1). However, since the high turnover of microfilariae would result in continuous exposure of hsp70 to the immune system, chronic stimulation in itself is insufficient to account for the TH2 bias in filariasis. One additional aspect to be considered is that gp15/400 is a glycosylated protein, while hsp70 is not. Recent work on murine schistosomiasis has indicated that specific oligosaccharides can drive B cells to produce IL-10 and prostaglandin E₂ (39), both of which can act on antigen-presenting cells. The differential roles of antigen-presenting cells in driving TH1 versus TH2 responses has been demonstrated previously (3).

Not every individual in this study developed an antibody response to these antigens; 5 and 12 individuals were totally unresponsive to BpL-4 and Bpa-26, respectively, by any antibody isotype assayed. As these individuals showed heightened immune responses to total parasite extract (not shown), their unresponsiveness might be attributed to their inability to process or present these particular proteins in the context of their major histocompatibility complexes. Genetic restriction of the response to a number of filarial excreted and secreted antigens has been reported for mice (17). Interestingly, in both BpL-4 and Bpa-26-unresponsive individuals, there was a considerably lower frequency of the DR2 allele than in the total population (the frequency of DR2 in the total population is 59%, and those in BpL-4 and Bpa-26 nonresponders are 20 and 42%, respectively [unpublished results]).

In the majority of the individuals responding to BpL-4 in terms of IgG4 or IgE, a skewing of the response towards either IgE or IgG4, as opposed to a balanced 1:1 ratio of these isotypes, is observed. While production of both IgG4 and IgE is known to be induced by IL-4 (8), there are examples of the regulation of IgG4 being uncoupled from IgE (12, 13). In our previous studies, we observed differential levels of IgE and IgG4 responses to total parasite extract (15). Thus, microfilaremic individuals show high-level antifilarial IgG4 responses but generally display little specific IgE antibody. On the other hand, in most patients with elephantiasis, the IgE levels are high while the IgG4 levels are low. In this study, we have

pursued this issue at the single-antigen level for BpL-4 (IgG4 and IgE responses were too weak to assess Bpa-26). We find that, as with total parasite extract, individuals generally mount either an IgG4 or an IgE response to BpL-4 and that the IgG4 and IgE levels are negatively associated. Thus, in lymphatic filariasis, it appears that the IL-4-dependent switch to IgG4 and IgE production is under further control by as yet undefined modulators.

When antibody isotype responses to BpL-4 and Bpa-26 were analyzed as a function of clinical status, it was observed that there was an increased IgG3 response to Bpa-26 in elephantiasis patients. The IgG3 responses to BpL-4 in elephantiasis and microfilaremic patients were similar, both being greater than those in asymptomatic amicrofilaremics, suggesting that exposure to or the existence of large parasite loads drives IgG3 responses to BpL-4. As the levels of IgG3 antibodies to Bpa-26 were significantly elevated only in elephantiasis patients compared with those in disease-free microfilaremics and asymptomatic amicrofilaremics, it is possible that this particular response is associated with pathology. Increased IgG3 responses to a number of other internal antigens have been observed in filarial disease. In patients with onchocerciasis, the subpopulation with severe skin lesions had a particularly pronounced IgG3 response to paramyosin (37). In addition, patients with bancroftian filariasis showed elevated levels of IgG3 antibody to aspartyl tRNA synthetases (11), although no individuals with chronic obstructive disease were tested in that study. The measured IgG3 in patients with Sowda manifestations of onchocerciasis (2) and the fact that a number of autoimmune conditions, such as lupus erythematosus, cause elevated levels of IgG3 antibodies (6) might be suggestive of the involvement of IgG3 in tissue damage. In our study, the elevation of the IgG3 level, although statistically significant, is not dramatic (factor of 1.5). With the increasing availability of recombinant antigens, a definitive role for IgG3 in the etiology of pathology may yet be determined. Future studies will aim to enlarge the scope of filarial antigen types to be analyzed in this regard, with the goal of determining markers and candidate mechanisms for immunity and disease.

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REFERENCES

1. Aalberse, R. C., R. van der Gaag, and J. van Leeuwen. 1983. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J. Immunol.* **130**:722-726.
2. Cabrera, Z., D. W. Buttner, and R. M. E. Parkhouse. 1988. Unique recognition of a low molecular weight *Onchocerca volvulus* antigen by IgG3 antibodies in chronic hyperreactive oncho-dermatitis (Sowda). *Clin. Exp. Immunol.* **74**:223-229.
3. Chang, T. L., C. M. Shea, S. Urioste, R. C. Thompson, W. H. Boom, and A. K. Abbas. 1990. Heterogeneity of helper/inducer T lymphocytes. III. Responses of IL-2- and IL-4-producing (Th1 and Th2) clones to antigens presented by different accessory cells. *J. Immunol.* **145**:2803-2808.
4. Christie, J. F., B. Dunbar, I. Davidson, and M. W. Kennedy. 1990. N-terminal amino acid sequence identity between a major allergen of *Ascaris lumbricoides* and *Ascaris suum*, and MHC-restricted IgE responses to it. *Immunology* **69**:596-602.
5. Day, K. P. 1991. The endemic normal in filariasis: a static concept. *Parasitol. Today* **7**:341-343.
6. Eisenberg, R. A., K. Dyer, S. Y. Craven, R. Fuller, and W. J. Yount. 1985. Subclass restriction and polyclonality of the systemic lupus erythematosus marker antibody anti-Sm. *J. Clin. Invest.* **75**:1270-1277.
7. Else, K. J., F. D. Finkelman, C. R. Maliszewski, and R. K. Grencis. 1994. Cytokine-mediated regulation of chronic intestinal helminth infection. *J. Exp. Med.* **179**:347-351.

8. Gascan, H., J.-F. Gauchat, M.-G. Roncarolo, H. Yssel, H. Spits, and J. E. de Vries. 1991. Human B cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by CD4⁺ T cell clones. *J. Exp. Med.* **173**:747–750.
9. Hitch, W. L., P. J. Lammie, E. M. Walker, A. W. Hightower, and M. L. Eberhard. 1991. Anti-filarial cellular responses detected in a Haitian pediatric population by use of a microblastogenesis assay. *J. Infect. Dis.* **164**:811–813.
10. Hsieh, C.-Y., S. E. Macatonia, C. S. Tripp, S. F. Solf, A. O'Garra, and K. M. Murphy. 1993. Development of T_H1 CD4⁺ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* **260**:547–549.
11. Kazura, J. W., F. E. Hazlett, Jr., E. Pearlman, K. Day, A. El-Zeiny, T. W. Nielsen, and M. P. Alpers. 1992. Antigenicity of a protective recombinant filarial protein in human bancroftian filariasis. *J. Infect. Dis.* **166**:1453–1457.
12. Kimata, H., A. Yoshida, C. Ishioka, T. Kusunoki, S. Hosoi, and H. Mikawa. 1991. Nerve growth factor specifically induces human IgG4 production. *Eur. J. Immunol.* **21**:137–141.
13. Kimata, H., A. Yoshida, C. Ishioka, and H. Mikawa. 1991. Disodium cromoglycate (DSCG) selectively inhibits IgE production and enhances IgG4 production by human B cells *in vitro*. *Clin. Exp. Immunol.* **84**:395–399.
14. King, C. L., S. Mahanty, V. Kumaraswami, J. S. Abrams, J. Regunathan, K. Jayaraman, E. A. Ottesen, and T. B. Nutman. 1993. Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. *J. Clin. Invest.* **92**:1667–1673.
15. Kurniawan, A., M. Yazdanbakhsh, R. van Ree, R. Aalberse, M. E. Selkirk, F. Partono, and R. M. Maizels. 1993. Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. *J. Immunol.* **150**:3941–3950.
16. Kwan-Lim, G.-E., K. P. Forsyth, and R. M. Maizels. 1990. Filarial-specific IgG4 response correlates with active *Wuchereria bancrofti* infection. *J. Immunol.* **145**:4298–4305.
17. Kwan-Lim, G.-E., and R. M. Maizels. 1990. MHC and non-MHC restricted recognition of filarial surface antigens in mice transplanted with adult *Brugia malayi* parasites. *J. Immunol.* **145**:1912–1920.
18. Lammie, P. J., D. G. Addiss, G. Leonard, A. W. Hightower, and M. L. Eberhard. 1993. Heterogeneity in filarial-specific immune responsiveness among patients with lymphatic obstruction. *J. Infect. Dis.* **167**:1178–1183.
19. Mahanty, S., J. S. Abrams, C. L. King, A. P. Limaye, and T. B. Nutman. 1992. Parallel regulation of IL-4 and IL-5 in human helminth infections. *J. Immunol.* **148**:3567–3571.
20. Maizels, R. M., A. Kurniawan, M. E. Selkirk, and M. Yazdanbakhsh. 1991. Immune responses to filarial parasites. *Immunol. Lett.* **30**:249–254.
21. Nutman, T. B., V. Kumaraswami, and E. A. Ottesen. 1987. Parasite-specific anergy in human filariasis. Insights after analysis of parasite antigen-driven lymphokine production. *J. Clin. Invest.* **79**:1516–1523.
22. Ottesen, E. A. 1984. Immunological aspects of lymphatic filariasis and onchocerciasis in man. *Trans. R. Soc. Trop. Med. Hyg.* **78**(Suppl.):9–18.
23. Ottesen, E. A. 1992. Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* **104**:S71–S79.
24. Ottesen, E. A., F. Skvaril, S. R. Tripathy, R. W. Poindexter, and R. Hussain. 1985. Prominence of IgG4 in the IgG antibody response to human filariasis. *J. Immunol.* **134**:2707–2712.
25. Ottesen, E. A., P. F. Weller, and L. Heck. 1977. Specific cellular immune unresponsiveness in human filariasis. *Immunology* **33**:413–421.
26. Paul, W. E., and R. A. Seder. 1993. Lymphocyte responses and cytokines. *Cell* **76**:241–251.
27. Paxton, W. A., M. Yazdanbakhsh, A. Kurniawan, F. Partono, R. M. Maizels, and M. E. Selkirk. 1993. Primary structure of and immunoglobulin E response to the repeat subunit of gp15/400 from human lymphatic filarial parasites. *Infect. Immun.* **61**:2827–2833.
28. Pearce, E. J., P. Caspar, J.-M. Grzych, F. A. Lewis, and A. Sher. 1991. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med.* **173**:159–166.
29. Piessens, W. F., P. B. McGreevy, P. W. Piessens, M. McGreevy, I. Koiman, J. S. Saroso, and D. T. Dennis. 1980. Immune responses in human infections with *Brugia malayi*. Specific cellular unresponsiveness to filarial antigens. *J. Clin. Invest.* **65**:172–179.
30. Poole, C. B., A. G. I. Granda, C. V. Maina, R. E. Jenkins, M. E. Selkirk, and L. A. McReynolds. 1992. Cloning of a cuticular antigen that contains multiple tandem repeats from the filarial parasite *Dirofilaria immitis*. *Proc. Natl. Acad. Sci. USA* **89**:5986–5990.
31. Reiner, S. L., and R. M. Locksley. 1993. The worm and the protozoa: stereotyped responses or distinct antigens? *Parasitol. Today* **9**:258–260.
32. Reynolds, S. R., C. E. Dahl, and D. A. Harn. 1994. T-epitope and B-epitope determination and analysis of multiple antigenic peptides for the *Schistosoma mansoni* experimental vaccine triose-phosphate isomerase. *J. Immunol.* **152**:193–200.
33. Scott, P., and H. E. Kaufmann. 1991. The role of T-cell subsets and cytokines in the regulation of infection. *Immunol. Today* **12**:346–348.
34. Selkirk, M. E., D. A. Denham, F. Partono, and R. M. Maizels. 1989. Heat shock cognate 70 is a prominent immunogen in Brugian filariasis. *J. Immunol.* **143**:299–308.
35. Selkirk, M. E., W. F. Gregory, R. E. Jenkins, and R. M. Maizels. 1993. Localisation, turnover and conservation of gp15/400 in different stages of *Brugia malayi*. *Parasitology* **107**:449–457.
36. Siber, G. R., P. H. Schur, A. C. Aisenberg, S. A. Weitzman, and G. Schiffman. 1980. Correlation between serum IgG-2 concentrations and the antibody response to bacterial polysaccharide antigens. *N. Engl. J. Med.* **303**:178–182.
37. Steel, C., L. A. Limberger, L. A. McReynolds, E. A. Ottesen, and T. B. Nutman. 1990. B cell responses to paramyosin. Isotypic and epitope mapping of filarial paramyosin in patients with onchocerciasis. *J. Immunol.* **145**:3917–3923.
38. Tweedie, S., W. A. Paxton, L. Ingram, R. M. Maizels, L. A. McReynolds, and M. E. Selkirk. 1993. *Brugia pahangi* and *Brugia malayi*: a surface-associated glycoprotein (gp15/400) is composed of multiple tandemly repeated units and processed from a 400-kDa precursor. *Exp. Parasitol.* **76**:156–164.
39. Velupillai, P., and D. A. Harn. 1994. Oligosaccharide-specific induction of interleukin-10 production by B220⁺ cells from schistosome infected mice: a mechanism for regulation of CD4⁺ T-cell subsets. *Proc. Natl. Acad. Sci. USA* **91**:18–22.
40. Werner, C., G. I. Higashi, J. A. Yates, and T. V. Rajan. 1989. Differential recognition of two cloned *Brugia malayi* antigens by antibody class. *Mol. Biochem. Parasitol.* **35**:209–218.
41. Wilson, R. A. 1993. Immunity and immunoregulation in helminth infections. *Curr. Opin. Immunol.* **5**:538–547.
42. Yazdanbakhsh, M., W. A. Paxton, Y. C. M. Kruize, E. Sartono, A. Kurniawan, A. van het Wout, M. E. Selkirk, F. Partono, and R. M. Maizels. 1993. T cell responsiveness correlates differentially with antibody isotype levels in clinical and asymptomatic filariasis. *J. Infect. Dis.* **167**:925–931.
43. Yazdanbakhsh, M., E. Sartono, Y. Kruize, T. van der Pouw-Kraan, P. H. van der Meide, M. E. Selkirk, R. Q. Hintzen, R. A. W. van Lie, and R. M. Maizels. 1993. Elevated levels of T cell activation antigen CD27 and increased interleukin-4 production in human lymphatic filariasis. *Eur. J. Immunol.* **23**:3312–3317.
44. Young, D. B. 1992. Heat shock proteins: immunity and autoimmunity. *Curr. Opin. Immunol.* **4**:396–400.