

BIOCHEMICAL AND IMMUNOLOGIC CHARACTERIZATION OF A MAJOR IgE-INDUCING FILARIAL ANTIGEN OF *Brugia malayi* AND IMPLICATIONS FOR THE PATHOGENESIS OF TROPICAL PULMONARY EOSINOPHILIA

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A major allergen of the human filarial parasite *Brugia malayi* has been identified by two-dimensional immunoblot analysis using a serum pool from patients with tropical pulmonary eosinophilia. The allergen is composed of two Ag with Mr 23 and Mr 25 and acidic isoelectric point (Bm23–25). Immunoblots using affinity-purified IgE antibodies to Bm23–25 indicated that Bm23–25 is expressed mainly in the microfilarial stage. Digestion of the allergen with endoglycosidases indicates that it has N-linked oligosaccharide chains. Analysis of the reactivity of T cells derived from patients with lymphatic filariasis revealed that the Bm23–25 allergen was capable of stimulating T cell proliferation; Bm23–25 was also shown to induce IgE production in vitro from PBMC derived from patients with either TPE or other filarial symptoms. Bronchoalveolar lavage fluid of patients with TPE contained IgE antibodies that recognized Bm23–25 strongly, an observation suggesting that the microfilarial allergen might be involved in the pathogenesis of the TPE syndrome.

Three parasitic filarial nematodes—*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*—are the causative agents of lymphatic filariasis, a disease of appreciable morbidity prevalent in many tropical and subtropical countries. Approximately 90 million people are infected, and an additional 800 million are at risk for infection with filarial parasites (1). The clinical manifestations of lymphatic filariasis are varied and range from an asymptomatic state, with or without circulating MF,³ to the more classical clinical syndromes of filarial fevers, acute lymphangitis, lymphadenitis, elephantiasis, and hydrocele—to the immunologic hyperresponsive syndrome of TPE (2). TPE is an unusual manifestation of filarial infection (<1% of those infected) characterized acutely by cough, dyspnea, nocturnal wheezing, fever, weight loss,

and restrictive defects of pulmonary function (3–7). It is accompanied by high levels of IgE, marked elevations in antifilarial antibodies (IgG and IgE) (4, 5, 8), extreme eosinophilia, and a favorable response to diethylcarbamazine (5, 9, 10).

High serum levels of IgE antibodies are a characteristic finding in helminth infections, both in experimental animals (11, 12) and in humans (13, 14), a finding suggesting profound dysregulation in the otherwise tightly regulated IgE antibody response. Although most of the IgE antibodies reflect nonspecific polyclonal activation of IgE-producing B cells (15), parasite-specific IgE antibodies are markedly elevated, and they almost certainly play an important role in the pathogenesis of the TPE syndrome (2, 7, 15). In fact, analysis of antifilarial antibody localized to the lung in TPE (collected by BAL) demonstrated striking elevations of both total IgE and parasite-specific IgE (16); however, the lack of defined allergens and the absence of information about the nature of the IgE-inducing filarial Ag other than their m.w. (as defined by immunoblot analysis) (15–17) have been obstacles to the study of parasite-specific immediate hypersensitivity responses. Furthermore, because characterization of the allergenic signals is of paramount importance for the study of the mechanisms underlying the regulation of allergic responses, in the present study we sought to identify and characterize biochemically and immunologically major IgE-inducing Ag of the human filarial parasite *B. malayi*.

MATERIALS AND METHODS

Ag. B. malayi filarial parasites, maintained in jirds (*Meriones unguiculatus*), were the source of *Ag. B. malayi* adult worms were removed from the peritoneal cavities of infected jirds, and a soluble antigenic extract was prepared from adult worms as described (18). The infective larvae (L₃) were obtained by dissection of *Aedes aegypti* 12 to 14 days after the mosquitoes had been fed on microfilaremic jirds. MF were harvested from the peritoneal cavities of infected jirds, and contaminating host cells were removed by differential centrifugation on Ficoll-Hypaque (19, 20).

Patient population. All individuals (*n* = 8) with acute TPE were residents either of Madras, India, as described in detail previously (6, 21), or of Recife, Brazil. All individuals met the criteria for having TPE (3). North Americans with no relevant travel history or known exposure to human filarial parasites were used as controls.

Collection of BAL fluid. Bronchoscopy with BAL was performed under local anesthesia, without complications, in the outpatient facility of the Tuberculosis Research Center, Madras, India, as previously described (6, 22). Lavage fluid was collected and separated from the cellular components by centrifugation at 400 × *g* and analyzed as described previously (23, 24).

Measurement of total and filarial-specific and IgE antibodies. Patient sera were preadsorbed with protein G coupled to Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) and used at a final

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³ Abbreviations used in this paper: MF, microfilariae; TPE, tropical pulmonary eosinophilia; BAL, bronchoalveolar lavage; NCP, nitrocellulose paper; 2-DGE, two-dimensional gel electrophoresis; pI, isoelectric point.

dilution of 1/5. Total and specific antifilarial IgE were measured by ELISA as already described (25).

Qualitative analysis of *B. malayi* proteins by SDS-PAGE and immunoblotting. Solubilized *B. malayi* Ag were separated by SDS-PAGE using a method modified from Laemmli (26), in 12.5% or 13.5% homogeneous gels or in 10 to 20% gradient mini-gels (Daiichi Pure Chemical, Tokyo, Japan). Ag were transferred to NCP as described by Towbin (27) using a Trans-blot cell (Bio-Rad Laboratories, Richmond, CA) at 150 mAmp for 16 h at 4°C, and processed (14, 15).

Immunoblot analysis of SDS-PAGE or 2-DGE was performed using a pool of serum from five patients with TPE that had been individually preadsorbed with protein G (28). The blotted *B. malayi* Ag were incubated with a 1/60 dilution of the TPE serum pool diluted in a buffer containing 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.3% Tween 20, 0.05% Triton X-100, and 0.5% BSA. After an overnight incubation, the blots were washed and incubated for 1 h at room temperature with a anti-human IgE mAb (7.12; kindly provided by Dr. Andrew Saxon, UCLA, Los Angeles, CA). The NCP was again washed, incubated for an additional hour in alkaline phosphatase-conjugated goat anti-mouse IgG (Jackson Immunoresearch, Avondale, PA), and developed using a bromochloroindophenyl/nitroblue tetrazolium substrate (Kirkegaard and Perry, Gaithersburg, MD).

Before study of the IgE antibodies in the BAL fluid, the fluid was concentrated 100× and IgG antibodies were adsorbed by incubation with protein G (28). This concentrated and adsorbed BAL fluid was then used in Western blots to detect IgE antibodies as described above.

Immunoblots were scanned with a 600ZS scanner (Molecular Dynamics, CA) calibrated with a densitometric step tablet (Kodak, Rochester, NY) and analyzed using Image (version 1.43).

2-DGE. IEF separation was performed in the model 175 chamber (Bio-Rad); the Protean II chamber (Bio-Rad) was employed for SDS-PAGE. Power was supplied with a model 3000/300xi (Bio-Rad) for IEF separation.

Reagents were from the following sources: acrylamide methylene-bisacrylamide, tetramethylethylenediamine, ammonium persulfate, and SDS from Bio-Rad; Tris-HCl and glycine from ICN Biomedicals, Cleveland, OH; urea and dithiothreitol from BRL, Bethesda, MD; cholamide propyldimethylhydroxy protansulfonate from Calbiochem, La Jolla, CA; ampholytes 3.5–10, 5–8, and 9–11 from Pharmacia-LKB, Bromma, Sweden.

2-DGE was performed as described by O'Farrell (29) and modified by Duncan and Hershey (30) and Hoffstrasser et al. (31) using gels with 12.5% acrylamide. Soluble PBS-extracts of adult *B. malayi* parasites were treated with 20 µg/ml DNase I and 20 µg/ml pancreatic RNase (Sigma) for 1 h at rt, lyophilized, and mixed with lysis buffer as described (29). Prefocusing was carried out by increasing the voltage every 15 min typically starting at 80 V (20 V increase) up to 220 V. IEF gels were run at a constant 700 V for a total of 12,000 Vh. The cathodic buffer was 50 mM NaOH; the anodic buffer, 25 mM H₃PO₄. Isoelectric gels were stored at –70°C or loaded after equilibration onto SDS-PAGE gels.

Electrophoresis and electroelution of Bm23–25 fraction. *B. malayi* filarial Ag was separated on a preparative 12.5% SDS-PAGE gel under reducing conditions. A lane containing prestained m.w. markers was used as a reference to localize the appropriate fraction on an unstained gel. The region of interest was cut and neutralized in Tris-buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.1% NaN₃) until a pH of 7.5 was reached and electroeluted using an inner chamber buffer of 10 mM Tris-acetate, pH 8.6, 1 mM EDTA, and 0.1% SDS, and an outer chamber buffer of 40 mM Tris-acetate, pH 8.6. Electroelution was at 4°C for 4 to 5 h at a power setting of 3 W. The effluents were pooled from three consecutive elutions and dialyzed overnight in Tris buffer, pH 7.6, using dialysis tubing with a 10-kDa exclusion.

Estimation of protein concentrations. Protein concentrations were determined using a commercially available kit employing bicinchoninic acid (Pierce Chemical Co., Rockford, IL).

Anion exchange chromatography. An amberlite CG-400, (Sigma Chemical Co., St. Louis, MO) anion-exchange resin (100 to 200 mesh), obtained in the chloride form, was regenerated by sequential washing with 7 N NaOH, water, 1 N HCl, water; it was equilibrated with 50 mM Tris-HCl, pH 7.6, containing 150 mM NaCl 0.1% NaN₃. One milliliter of resin was poured into each of several mini-columns, and the electroeluted Bm23–25 fraction was mixed with 1 ml of Tris buffer, run over the column and fractions collected. The fractions were concentrated using a Centricon filter (Amicon), and the protein concentration was measured.

Endoglycosidase digestion of Bm23–25. The enzyme N-glycanase (Genzyme, Boston, MA) was used at concentrations ranging from 1 to 10 U/ml after the protein samples were preheated for 3 min at 100°C in 1% SDS and 1% 2-ME. The samples were diluted 1/4 with

digestion buffer (200 mM sodium phosphate, pH 8.6, 10 mM 1, 10 phenanthroline (Sigma) and 1.25% Nonidet P-40 (Sigma)) and digested for 24 h at 37°C. They were then concentrated and loaded on a 10 to 20% SDS-PAGE gel.

Affinity purification of anti-Bm23–25 IgE antibodies. One hundred micrograms of the electroeluted filarial proteins were dialyzed against 0.1 M morpholinopropanesulfonic acid, pH 7.5, 80 mM CaCl₂ and coupled to 0.5 ml of Affi-Gel 10 (Bio-Rad), following the manufacturer's instructions. The efficiency of coupling was found to be 89%. The Affi-Gel 10 containing the electroeluted protein was incubated with a serum pool previously depleted of IgG antibodies (protein G, Pharmacia) from patients with TPE. After several washes in PBS, anti-23–25 antibody (including IgE antibodies) were eluted by the addition of 0.1 M glycine-HCl, pH 2.8, 150 mM NaCl and neutralized with 0.1 N NaOH. The fractions were pooled, and BSA was added to make up 0.5% final concentrations.

T cell Western blot with the isolated Bm 23–25. PBMC were collected from heparinized blood by sedimentation on a Ficoll-diatroazate gradient (Organon Technika, Durham, NC) at 400 × g for 40 min at 20°C. Cells at the interface were collected and washed three times in RPMI 1640 (Biofluids, Rockville, MD), and viable cells were counted by trypan blue exclusion. The Bm23–25 allergens were localized after immunoblotting and Ponceau S staining. The NCP-Bm23–25 strips (4 mm × 2 mm) and NCP (with no Ag) were incubated in duplicate with 1 × 10⁵ cells in a microtiter plate in RPMI 1640 supplemented with gentamicin 80 µg/ml, 25 mM HEPES, and 10% AB-serum (C-RPMI). The cells were incubated for 5 days and then pulsed with 1 µCi of [³H]TdR for 6 h and harvested as described (21).

Culture conditions for in vitro antibody production. PBMC were resuspended in complete Iscove's modified Dulbecco's medium supplemented with human transferrin, bovine insulin, BSA, palmitic acid, and linoleic acid (UBI, Lake Placid, NY) and FCS. PBMC were placed in flat bottomed wells at a concentration of 1 × 10⁶/ml. To these cultures were added media alone, cycloheximide (100 µg/ml), a fraction electroeluted from a SDS-PAGE with no protein, *B. malayi* total adult extract, and a fraction containing the electroeluted Bm23–25 fraction. All cultures were incubated at 37°C in 5% CO₂ for 14 days after which the supernatants were harvested and assayed.

RESULTS

Identification of the major IgE-inducing Ag from *B. malayi* recognized by patients with TPE. 2-DGE was used to analyze the complex *B. malayi*-soluble protein extract. The optimized 2-DGE for *B. malayi* adult extract is shown in Figure 1A. Two dimensional gels of *B. malayi* proteins demonstrated 150 to 250 spots ranging in molecular mass from 200 to 10 kDa, and ranging in pI from 4.05 to 7.20. Western blot analysis of 2-DGE using a pool of sera from five patients with TPE revealed more than 60 Ag that bound IgE. Upon visual comparison, an Ag of approximately 23 to 25 kDa and with an estimated pI of 5.50 to 5.75 was especially prominent in its IgE-binding capacity (Fig. 1B). Western blot analysis of individual sera from TPE patients revealed that also all (8 of 8) recognized this allergen (data not shown). Sera pooled from normal individuals with no known history of parasitic or lung diseases showed no recognition by IgE of any of the filarial parasite proteins (Fig. 1C).

There were several proteins with similar pI in the area of intense IgE-binding of approximately 23 to 25 kDa. To identify more precisely the molecules responsible for this allergenic activity, SDS-PAGE gels of different polyacrylamide concentration were run. After transfer of the proteins to NCP, parallel Ponceau S staining to localize the proteins and immunolocalization of the allergen were performed. Using a 14% homogeneous gel, the 23- to 25-kDa *B. malayi* allergen was identified as two discrete Ag (Fig. 2) with minimally different *M_r* as assessed by densitometry (Bm23: 3060 ± 105 U; Bm25: 2132 ± 5 U). Of interest is the finding that Bm23–25 appears to be only

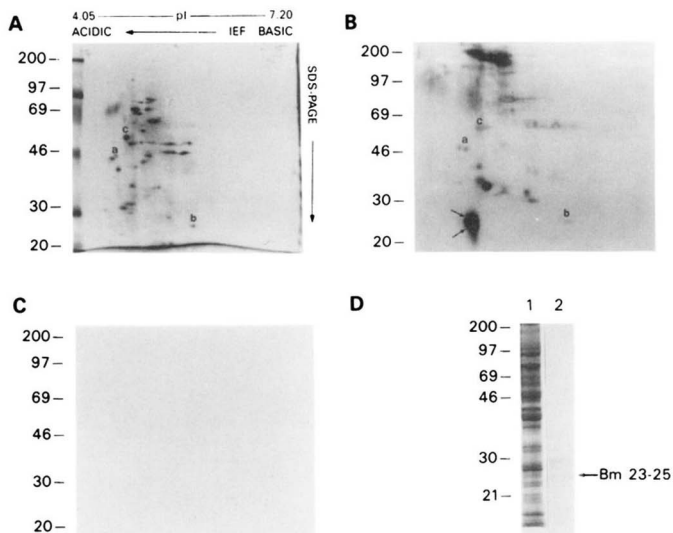


Figure 1. A, Coomassie blue stained 2-DGE of *B. malayi* adult worm extract. The filarial proteins were treated with 20 μ g/ml DNaseI, and 20 μ g/ml pancreatic RNase. Samples containing 190 μ g protein were lyophilized and were made up to 9.5 M urea, 2% NP-40, 2% ampholines and 5% β -mercaptoethanol and analyzed by 2-DGE. B, 2-DGE immunoblot analysis of IgE-binding *B. malayi* Ag recognized by a serum pool of patients with TPE. Arrows indicate the position of the main low m.w. allergen Bm23-25. C, negative control 2-DGE immunoblot using a serum pool from normal individuals. D, Coomassie blue staining of SDS-PAGE-separated *B. malayi* total proteins, and the electroeluted fraction containing Bm23-25. Thirty micrograms of total filarial extract (lane 1) and 3 μ g of the electroeluted fraction Bm23-25 (lane 2) were loaded on a 10 to 20% mini-gel. Numbers at the left of the gel indicate m.w. standards. Letters on A and B indicate antigenic identity.

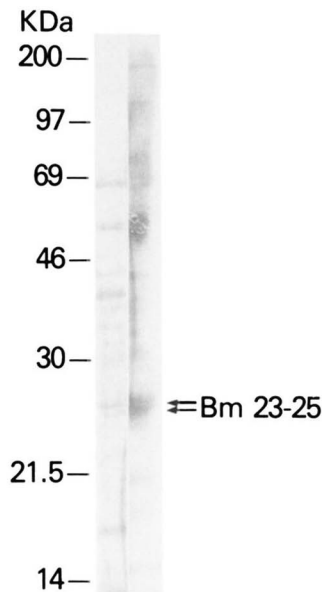


Figure 2. Identification of Bm23-25 as a doublet. *B. malayi* proteins separated on a 14% SDS-PAGE were transferred to nitrocellulose and then stained with Ponceau S (left lane) or incubated with a serum pool from patients with TPE (right lane). Arrows indicate the position of Bm23-25.

a minor component of the total *B. malayi* protein extract (compare Fig. 2 left lane with right lane).

Affinity-purification of anti-Bm23-25 IgE antibodies and stage-specificity of its expression. Because of the very limited amounts of Bm23-25 allergen present in the total parasite extract, selective enrichment (from 480 μ g of total parasite extract) was performed by electroelution of proteins from gel slices containing Bm23-25 (Fig. 1D, lane 2). The Bm23-25 fraction was freed of SDS by

anion-exchange chromatography and was subsequently coupled to Affi-Gel 10. The coupled Bm23-25 was used to affinity-purify IgE antibodies from a serum pool taken from patients with TPE. To identify the parasite stage specificity of the Bm23-25 allergens, protein extracts from the different stages in the life cycle of the filarial parasite were analyzed using the previously affinity-purified anti-Bm23-25 IgE antibodies (Fig. 3). As seen, Bm23-25 affinity-purified IgE antibodies reacted with Ag in the extracts of adult worms (lane 5), male worms (lane 6), and predominantly the microfilarial extract (lane 7). In addition, an allergen with an apparent M_r of 47 was uniquely recognized by the IgE antibodies in the adult extract and not in the other stages (lane 5).

Biochemical evidence of glycosylation of the Bm23-25 allergen. To examine the possibility that the Bm23-25 IgE-inducing Ag of *B. malayi* might be glycosylated and, at the same time, to evaluate the contribution of the carbohydrate moieties to the allergenicity of the molecule, enzymatic deglycosylation using the enzyme peptide *N*-glycosidase F and subsequent immunoblot analysis was performed using affinity-purified IgE antibodies. Figure 3B shows that *N*-glycanase cleaves oligosaccharides from the 23- to 25-kDa *B. malayi* allergen leaving a peptide backbone of 22-kDa (lanes 3 and 4). No m.w. shift was observed in the control (lane 2). Moreover, the ability of IgE to bind to both the glycosylated and deglycosylated forms of Bm23-25 indicates that the IgE-inducing epitopes of these Ag reside in the protein domain, although O-linked carbohydrates have not been formally evaluated.

Western blot analysis of the Bm23-25 fraction recognized by antibodies in the BAL fluid. To examine the presence of IgE antibody directed against Bm23-25 localized in the lung in patients with TPE, the electroeluted filarial Ag fraction containing Bm23-25 was resolved using SDS-PAGE, transferred to NCP, and probed with BAL fluid from three patients with acute TPE and three normal control individuals. In each of the three patients with acute TPE, the immunoblot analysis revealed the

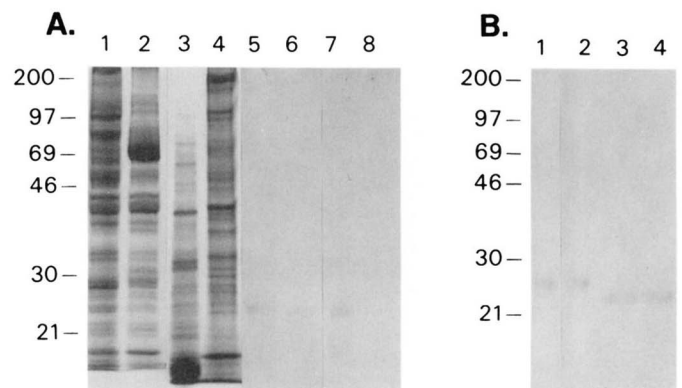


Figure 3. A, stage-specific expression of Bm23-25. Resolved by SDS-PAGE on a 10 to 20% mini-gel stained with Coomassie blue are protein extracts from 1) adult male and MF-producing female worms (30 μ g), 2) male worms (30 μ g), 3) MF 20 (μ g), and 4) L3 (30 μ g). Lanes 5 to 8 show Western blot analyses of a similar gel using affinity-purified IgE antibodies and demonstrate the expression of Bm23-25 in the total worm extract (lane 5), in the male (lane 6), and in the MF extracts (lane 7). B, analysis of the cleavage of oligosaccharide side chains from the isolated Bm23-25 by peptide *N*-glycosidase F (*N*-glycanase); track 1, 3 μ g isolated Bm23-25; track 2, no enzyme; track 3, 1 mU ml^{-1} ; track 4, 10 mU ml^{-1} . The digestion products were separated on a 10% to 20% mini-gel, transferred to nitrocellulose, and probed with affinity-purified anti-Bm23-25 IgE antibodies.

presence of IgE antibodies directed against the filarial Bm23-25 Ag (Fig. 4, lanes 1, 2, and 3). Lung lavage fluid from three control individuals had no antibodies directed against the isolated filarial Ag (lanes 4, 5, and 6).

Proliferative responses of PBMC to electrophoretically separated, NC-bound Bm23-25 Ag. The lymphocyte-proliferative responses of PBMC from two patients to NCP-bound Bm23-25 are shown in Figure 5. Lymphocytes from the patients had a strong proliferative response to NCP-bound Bm23-25 Ag. Lymphocytes from normal donors showed no significant response to the NCP-bound Bm23-25 Ag.

In vitro IgE induction by the isolated Bm23-25 Ag. To analyze the capacity of the isolated Bm23-25 allergen to induce IgE production, in vitro responses of PBMC from two representative patients and from two normal controls (Table I) to a wide concentration range of Bm23-25 were assessed. As seen, the purified Bm23-25 induced IgE production in vitro in both of the patients, but in neither of the controls. Maximal Ag-specific IgE induction was observed at 0.1 μ g/ml of Bm23-25 for both patients. Interestingly, the amount of IgE induced by Bm23-25 exceeded that induced by soluble crude parasite extract.

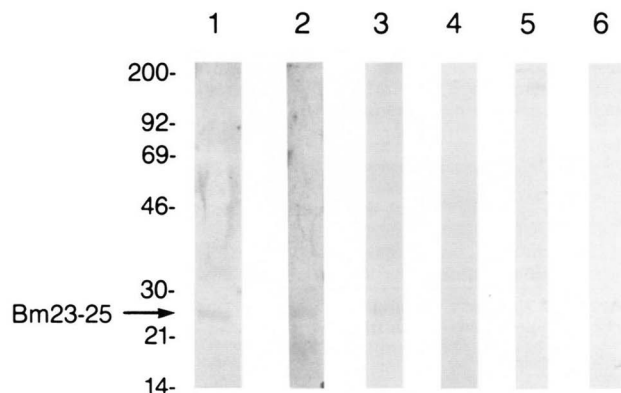


Figure 4. Recognition of Bm23-25 by IgE antibodies in the BAL fluid. Immunoblot analysis using BAL fluid from three patients with acute TPE (lanes 1, 2, and 3) and three normal individuals (lanes 4, 5, and 6). Arrow indicates the detection of the main *B. malayi* allergen Bm23-25 by antibodies present in the lung epithelial lining fluid.

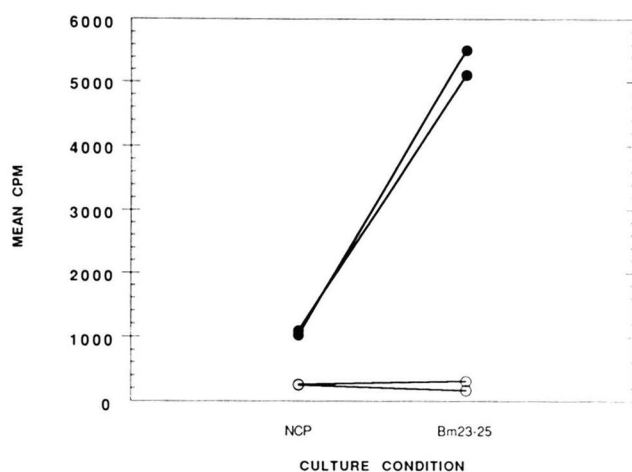


Figure 5. Analysis of allergen-specific T cell-proliferative responses by NCP-immobilized Bm23-25 of PBMC from individuals with lymphatic filariasis (closed circles) or normal individuals (open circles).

TABLE I
Bm23-25 specifically induces IgE synthesis in vitro^a

Diagnosis	IgE Production (pg/ml) in Vitro in Response to			
	Media + control ^b electroeluted Ag	Cycloheximide (100 μ g/ml)	BmA (0.1 ng/ml)	Bm23-25 (0.1 μ g/ml)
Normal	<50	<50	<50	<50
Normal	<50	<50	<50	<50
TPE	2800	<50	3096	3229
Lymphatic Filariasis	721	<50	947	1172

^a PBMC were cultured for 14 days at 1×10^6 /ml in the presence of media (not shown), media + control-electroeluted Ag, cycloheximide, BmA, and electroeluted Bm23-25.

^b Did not differ from media alone.

DISCUSSION

Although enhanced IgE antibody production is a characteristic feature of helminth infections, the exact role of this IgE in the host response to these parasites remains uncertain (32). Some studies have implicated IgE as a mediator of protective immunity to helminth infections (33-37) (particularly nematode parasites of the gut) (33-35), but others have implicated it in a more pathogenic role (33). Whatever its role, the particular nature of the parasitic Ag that induce IgE responses is also largely undefined, despite the extensive work of several groups to characterize and partially purify a number of nematode allergens. Among the most well characterized of these allergens are those from *Ascaris* spp. (reviewed in References 39 and 40), especially the 25-kDa allergen (ABA-1) from *Ascaris suum* and *Ascaris lumbricoides* (41, 42); the 12- to 17-kDa adult worm glycoprotein and the excretory/secretory products from *Nippostrongylus brasiliensis* (43-45); and the egg and adult worm allergens from *Schistosoma mansoni* (46, 47).

Lymphatic filariasis offers a convenient model for studying the nature of nematode allergens because many of the basic features of the human IgE response to these parasites have already been defined. For example, quantitative and qualitative analyses of IgE responses in patients with both lymphatic filariasis and TPE have been previously detailed, (48, 49) and frequency profile analysis of the Ag recognized by IgE antibodies have identified the proteins of low molecular mass as being the major IgE-inducing Ag (49, 50) (although these same Ag may also induce a "parallel" IgG4 response) (50).

In the present study, we have extended these analyses by examining the IgE responses of patients with TPE to filarial *B. malayi* proteins separated by 2-DGE and identified an Ag with acidic pI of 5.50 to 5.75 and with an apparent M_r of 23 to 25 kDa (Bm23-25) as being one of the most prominent components.

The stage specificity of the Bm23-25 allergen is important because the pathologic consequences of filarial infection (lymphatic filariasis) may derive either from immune responses to the adult worm Ag or from immune responses directed against the MF (51) as proposed for TPE. Indeed, affinity-purified anti-Bm23-25 IgE antibodies revealed that Bm23-25 was detected especially prominently in the MF (Fig. 3), with comparatively low levels of reactivity being found in extracts from both the mixed adult worms (which also contained MF in the fecund females) and the male worms. The finding, however, that this Ag of *B. malayi* was so prominently expressed in MF is important because TPE is characterized by immunologic hyperresponsiveness to this stage of the parasite.

Furthermore, because patients with TPE essentially never have MF in the peripheral blood, this syndrome likely reflects first pass microfilarial clearance in the lung. Although the clearance could be mediated by IgG antibodies, the asthma-like symptoms seen in TPE suggest that specific anti-microfilarial IgE antibodies are responsible for mediating the mast cell activation known to occur in this syndrome (7).

Stage-specific expression of carbohydrate moieties on the sheathed surface of MF has previously been reported (52), and these carbohydrate side chains are thought to contribute to the parasite's overall antigenicity (53). The Bm23-25 allergen was shown to be glycosylated, and glycosylation may very well account for the heterogeneity in the observed m.w. More important, however, is the finding that the deglycosylated peptide backbone of Bm23-25 retained the IgE-binding capacity, i.e., the allergenicity of the molecule was protein, not carbohydrate, based.

The Bm23-25 was shown to cause IgE production in vitro by PBMC obtained from patients both with TPE and those with other forms of lymphatic filariasis and elevated IgE. Peak induction increased over the spontaneous in vitro IgE production at Bm23-25 concentration of 0.1 pg/ml, a concentration 1000-fold lower than that necessary to induce IgE using crude parasite extract. This finding suggests that, despite the marked complexity of the crude parasite extract, only a limited number of Ag are actually required for IgE induction.

The potentiating effect of helminth infection for the IgE isotype has been shown to be T cell-dependent both in animal model systems (54) and in humans (55). Furthermore, extensive studies have recently demonstrated that the cytokine IL-4 is a critical stimulus for the development of both human and animal IgE responses (56-58). That Bm23-25-induced specific lymphocyte proliferation indicates that T cells can be sensitized to these Ag, but perhaps even more importantly, preliminary analysis of cytokine production in vitro indicates the generation of IL-4 after Bm23-25 stimulation (data not shown). These results are in agreement with the previous demonstration that parasite-specific Ag-stimulated IgE production in patients with filariasis is mediated by IL-4 and down-regulated by IFN- γ (59).

Not only do the Bm23-25 allergens act systemically on PBMC, but it is clear that they also are involved in local immune responses in the lungs of patients with TPE. Indeed, anti-Bm23-25 antibodies of the IgE isotype were readily identified in BAL fluid from such patients. These results corroborate previous findings indicating that BAL fluid antibodies recognize distinct subsets of filarial Ag (16). Furthermore, the identification of Bm23-25 as an Ag predominantly in MF lends support to the hypothesis that there is compartmentalization of such antibodies at sites where pathology is occurring.

The identification of parasite-specific IgE in the lung may also have implications for understanding the pathology of the TPE syndrome. IgE is known to mediate immediate hypersensitivity reactions through its interaction with the high affinity receptor on mast cells (60). Furthermore, the release of preformed or newly synthesized mediators by activated cells causes the recruitment and activation of inflammatory cells such as eosinophils (61, 62) that may further enhance the effector function

of such cells (63). The release of vasoactive mediators from these cells is believed to cause some of the changes seen in TPE. As there is a massive recruitment of eosinophils to the lung in TPE, much of the short and long term damage observed in the lower respiratory tract is felt to be related as eosinophil-dependant inflammatory processes (64, 65).

Although the present study was limited by the finding that a major IgE-inducing Ag of *B. malayi* in patients with TPE is a minor component of total filarial parasite extract, we have clearly identified and characterized a filarial allergen capable of not only binding IgE but also of potentiating in vitro IgE responses. Molecular cloning of Bm23-25 should provide sufficient material to study further its role in MF-induced pulmonary eosinophilia both in the TPE syndrome and in the previously described relevant animal models (66, 67); similarly, with increased supplies of the Ag, its potential role in IgE-mediated protective immune response can also be explored.

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