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## Differential recognition of two cloned *Brugia malayi* antigens by antibody class

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The humoral and cellular immune response to filarial parasites is complex. Numerous studies have shown that antibodies to a large number of protein and non-protein antigens may be produced over the course of infection and that immune recognition of any given antigen may vary by disease manifestation and by immunoglobulin class. We have used the techniques of molecular cloning to attempt to dissect this complex interaction, and describe here two clones, isolated from an expression library constructed from *Brugia malayi* genomic DNA, whose products are recognized by distinct immunoglobulin classes. A  $\lambda$ gt11 fusion protein containing part of the *B. malayi* myosin tail region is recognized by antibodies of the IgG class from a high percentage of bancroftian filariasis patients. A fusion protein containing a collagen-like sequence is less frequently and weakly recognized under the same experimental conditions, but is almost universally recognized when the developing reagent is specific for IgE. We thus identify specific filarial proteins against which the infected human host responds preferentially with antibodies of a specific immunoglobulin class.

**Key words:** Lymphatic filariasis; *Brugia malayi*;  $\lambda$ gt11 fusion protein expression; IgG; IgE; Collagen; Myosin

### Introduction

Human lymphatic filariasis is a major public health problem in many developing nations, where an estimated 91 million persons [1] are reported to suffer from the disease. Affected persons frequently manifest acute symptoms of filarial fever, lymphadenitis, and lymphangitis. In some of these individuals the disease progresses

to chronic pathology characterized by lymphedema and elephantiasis resulting from lymphatic blockage. A small percentage of individuals exhibit acute hypersensitivity reactions following infection, produce elevated levels of both total and parasite-specific IgE [2] and present asthmatic symptoms, a syndrome designated tropical pulmonary eosinophilia (TPE). The majority of people living in endemic areas however, exhibit no symptoms of disease. Ten to twenty percent of these have demonstrable microfilariae in their blood (asymptomatic microfilaremic) while, despite almost certain exposure to the bites of infected mosquitos, 60–70% exhibit no overt signs of infections (endemic normal) [3], although an undefined fraction of these may harbor occult or subclinical infections. A number of workers, most notably Hussain and Ottesen, have conducted systematic analyses of the recognition of filarial antigens using the Western blotting technique, and have demonstrated qualitative and quantita-

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*Note:* Nucleotide sequence data reported in this paper have been submitted to the GenBank™ Data Bank with the accession numbers J04644 and J04645.

*Abbreviations:* SDS, Sodium Dodecyl Sulfate; PMSF, Phenylmethylsulphonyl fluoride; TBS, Tris-buffered saline; MF, Microfilariae; TPE, tropical pulmonary eosinophilia; Ig, immunoglobulin; HRP, horseradish peroxidase; IPTG, isopropyl- $\beta$ -D-thiogalactopyranoside.

tive differences in the immune recognition of antigens of different molecular weight both by disease manifestation and by antibody subclass [4–7]. The identity of most of these antigens remains to be determined.

Through the use of recombinant DNA technology, notably the construction of expression libraries which can encode filarial protein antigens as bacterial fusion proteins, it is possible to dissect the host parasite interaction, and identify some of these filarial proteins. In another study, we have performed differential screening of a number of filarial expression libraries to demonstrate that individuals living within an endemic zone, but presenting with distinct clinical symptoms, differ with respect to the antigens they recognize [8]. At the same time, we sought to determine if any of these antigens are differentially recognized by the various classes of human antibodies. In this study, we demonstrate two filarial antigens which evoke different classes of antibody in patients with bancroftian filariasis.

## Materials and Methods

**Isolation of parasite nucleic acid.** Jirds (*Meriones unguiculatus*) infected with *B. malayi* were obtained from Dr. J.W. McCall, University of Georgia, through the U.S./Japan collaborative program in filariasis. Microfilariae were harvested by peritoneal lavage and washed with phosphate-buffered saline. Following separation from white blood cells by Ficoll-Paque gradient centrifugation and lysis of red blood cells with 0.17 M  $\text{NH}_4\text{Cl}$ , the microfilariae were resuspended in 100 mM Tris/50 mM EDTA, pH 8.5 with 0.5% 2-mercaptoethanol, 1% SDS, and 1 mg  $\text{ml}^{-1}$  heparin. The reaction mixture was inverted and heated to 65°C. NaCl was added to 200 mM, and Proteinase K (proteinase type XI, Sigma) to 100  $\mu\text{g ml}^{-1}$ . The reaction mixture was again mixed, and reincubated at 65°C for 15–20 min, followed by two extractions with phenol chloroform isoamyl alcohol (25:25:1) and two extractions with 10 mM Tris (pH 8.0), 5 mM EDTA-saturated diethyl ether. Sodium acetate was added to 0.3 M and 3 vol. of ice-cold ethanol carefully added. The nucleic acid that precipitated was spooled out with a Pasteur pipette and transferred to another tube.

Approximately 80% of the DNA was found in the spooled portion, while about 90% of the RNA remained in the original tube, and was allowed to precipitate overnight and then pelleted by centrifugation.

Nucleic acid from the pellet was washed with 70% ethanol, resuspended in 10 mM Tris 5 mM EDTA 0.1% SDS, transferred to an eppendorf tube, reprecipitated with ethanol, resuspended in a high salt buffer and treated with RNase-free DNase (Worthington, Freehold, NJ), followed by reextraction as above. This yielded relatively pure non-degraded RNA for the construction of cDNA libraries.

Spooled DNA was washed with 70% ethanol and rehydrated in 10 mM Tris (pH 7.5), 5 mM EDTA. When the DNA was in solution, ribonuclease previously boiled to destroy any contaminating DNase was added to a final concentration of 20  $\mu\text{g}$  per ml. The reaction mixture was again incubated with proteinase K as described above, and extracted as above.

**Source of patient sera.** The serum used in the initial screen of the expression library was from a patient with TPE. For differential screening, peripheral blood was obtained from villagers residing in a well characterized endemic area for *Wuchereria bancrofti* filariasis in Andhra Pradesh, India [9]. Samples were obtained from endemic normals with negative blood smears for microfilariae and without history of clinical filariasis, microfilaremic individuals who were without clinical signs or symptoms, and chronic clinical filariasis patients with lymphadenitis, lymphedema, hydrocoele or elephantiasis and without microfilaremia. The age range was 12–60 years with female to male ratio of 1:1.28. Twenty additional sera, representing 10 TPE patients and 10 endemic normal individuals were obtained from individuals residing in Madras, India. Sera were stored at –20°C until used. Prior to screening, sera were diluted 1:100 in a 1% gelatin solution in TBS, and stored at 4°C.

**Construction of expression libraries.** Double-stranded cDNA was synthesized from *B. malayi* RNA by the method of Gubler and Hoffman [10]. Following synthesis, *EcoRI* linkers were ligated

to the blunt-ended cDNA, followed by *EcoRI* digestion and separation of free linkers from cDNA on a Biogel AcA-34 column. The cDNA was ligated to *EcoRI*-digested phosphatase-treated arms of bacteriophage  $\lambda$ gt11 (Promega, Madison, WI) and in-vitro packaged using the Packagene™ system (Promega). Approximately 10 000 recombinant phage were obtained.

A genomic expression library was constructed in the same manner, except that *B. malayi* DNA digested with *AluI*, which generates blunt-ended fragments, was used. This library resulted in approximately 100 000 recombinants.

#### *Screening expression libraries with patient sera.*

Screening was performed as described by Huyhn, Young, and Davis [11]. In summary, phage were plated on a lawn of *Escherichia coli* K-12 strain Y1090, and grown at 42°C. Upon the appearance of visible plaques, the plates were overlaid with nitrocellulose filter papers soaked in 10 mM IPTG. After 1–2 h of induction, the nitrocellulose filters were removed, and unfilled binding sites were saturated by soaking the filters in a 3% gelatin solution in TBS. Sera, generally diluted 100-fold in 1% gelatin/TBS, were then added for overnight incubation with gentle rocking. After extensive washing with TBS, the filters were reacted with an anti-immunoglobulin (rabbit or human, as appropriate) antibody conjugated to horseradish peroxidase (BioRad, Richmond, CA; Cappel, West Chester, PA). After 3 h the filters were again washed extensively and treated with substrate. Positive clones were picked on the basis of a higher than background color reaction.

*B. malayi* libraries were initially screened with a human serum from a patient with TPE. Positive plaques were identified, purified, and rescreened to insure plaque purity. Pure clones were rescreened with individual sera.

For later screenings, including those in which the second antibody was specific for IgE, a variation of the above was used. Lysogens of the  $\lambda$ gt11 recombinant phage were made in *E. coli* strain Y1089. These lysogens were grown at 30°C, heat shocked for several minutes at 42°C, grown at 37°C for 20 min to allow phage DNA replication, and then induced with 10 mM IPTG for 30–60 min to allow fusion protein expression.

Cells were spun down, resuspended in 100 mM NaCl, 10 mM Tris, pH 7.5, 5 mM EDTA with 200  $\mu$ g ml<sup>-1</sup> ml PMSF added as a protease inhibitor, and then lysed by immersion in liquid nitrogen. These freeze-thaw lysates were then spotted onto nitrocellulose and treated as above.

Soluble extracts of *B. malayi* microfilariae were produced by resuspending purified microfilariae in TBS, subjecting them to several pulses of sonication, followed by high-speed centrifugation. The supernatant was collected and stored at –20°C.

*Subcloning and sequencing.* Although the inserts were inserted into the *EcoRI* site of  $\lambda$ gt11, we initially subcloned the inserts into pUC18 with a double digestion of *KpnI* and *SacI*, which cut  $\lambda$ gt11 approximately 1 kb on either side of the *EcoRI* site. This facilitated dideoxynucleotide (Sanger-Coulson) sequencing [12] using oligonucleotides specific for the  $\lambda$ gt11 flanking sequence (New England Biolabs, Beverly, MA), thus allowing us to directly determine the orientation and reading frame of the inserts. Double-stranded sequencing [13] was performed using either the *E. coli* Polymerase I Klenow fragment, or the Sequenase<sup>R</sup> kit (U.S. Biochemical Co., Cleveland, OH).

## Results

*Library screening.* By screening the *B. malayi* *AluI* genomic expression library with serum from a patient with TPE we obtained two positive clones: a weakly reacting clone, AP2; and a strongly reacting clone, AP18. Another weakly reacting clone, 3P7, was obtained from the cDNA library. This is shown in Fig. 1. A Western blot was performed on a lysogen of AP18 to confirm that the immunoreactivity was due to the induced fusion protein. This is shown in Fig. 2. Partial sequencing showed that AP2 and 3P7 were essentially the same gene, and so further analysis was limited to the genomic clones AP2 and AP18. We rescreened these clones with all the sera we had available to us, since we wished to see whether the difference in signal was an intrinsic property of the antigen, or represented the idiosyncratic response of a single, or at best a few, patients.

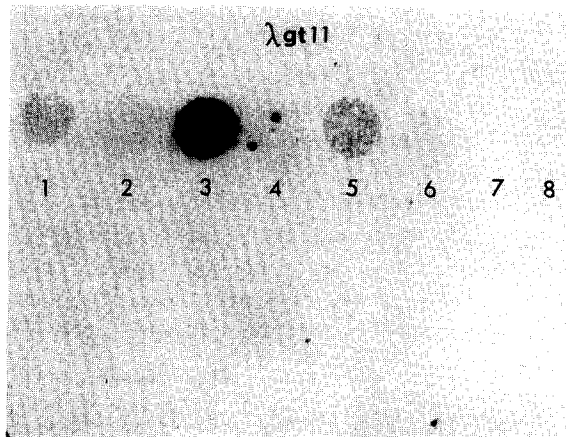


Fig. 1. Titrated phage stocks were diluted so that a 5  $\mu$ l spot would yield approximately 100 plaques, contiguous but not confluent. Spots were placed on a lawn of *E. coli* Y1090, and allowed to grow up at 42°C until visible. The plate was then overlaid with nitrocellulose, incubated with the TPE serum used to originally isolate the clones, and developed as described for the original screen. Spot 1 (AP2) and spot 5 (3P7) display a signal significantly above that of wild-type  $\lambda$ gt11, but weak when compared to that of spot 3 (AP18). The remaining labeled spots, as well as an unlabeled second row of eight additional clones containing irrelevant *B. malayi* inserts, display signals which do not differ significantly from that of  $\lambda$ gt11 on this filter. The two dark plaques in position 4 resulted from splattering of the AP18 spot at position 3 during pipetting.

**Differential screening.** Nitrocellulose strips were spotted with soluble extracts of *B. malayi* microfilariae (MF), and of *E. coli* infected with wild-type  $\lambda$ gt11, AP2, and AP18. Sera were considered uninformative if either they failed to react with any sample or if the reactivity to *E. coli* was so high that it obscured potentially meaningful differences in signal. The results are presented in Table I.

Assay development with a reagent recognizing predominantly IgG showed that clone AP18 was recognized above background by 32/55 sera, strongly by 19 of these. Under the same conditions, clone AP2 was recognized by 20/55 sera, but strongly by only two of these, one from a TPE patient (TPE1 in Table I) and another from a patient with asymptomatic microfilaremia (Hf7 in Table I). There was no clear correlation between clinical state and intensity of reactivity to the antigen. This is demonstrated by the fact that of the 19 that had high titres against AP18, 6 were TPE

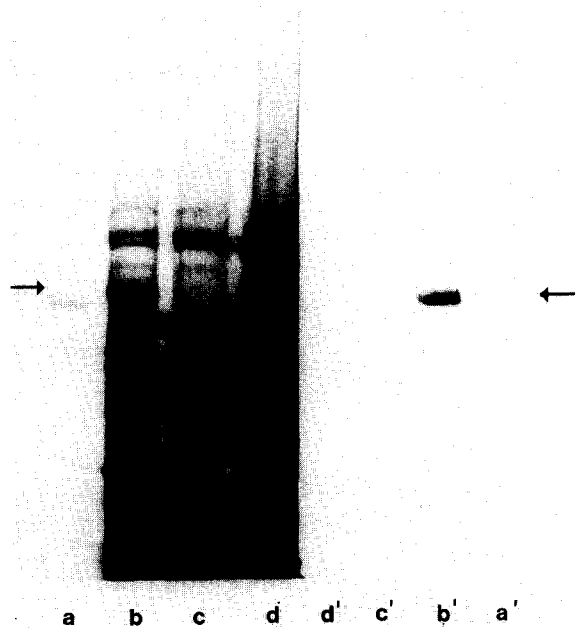


Fig. 2. Duplicate sets of the following samples were subjected to electrophoresis on the same gel: a/a', purified  $\beta$ -galactosidase; b/b', AP18 lysogen of Y1089 induced with IPTG; c/c', non-induced AP18 lysogen; d/d', wild-type induced  $\lambda$ gt11 lysogen. The gel was then cut in half. The left half was stained with Coomassie Brilliant Blue. The right half was transferred to nitrocellulose, and subjected to a Western blot. As can be readily seen, the de-novo band seen induced in lane b (arrow), which migrates slightly slower than wild-type  $\beta$ -galactosidase, corresponds to the lone visible band seen in lane b'.

patients, 7 endemic normals, 3 asymptomatic microfilaremics and 3 with chronic symptoms; whereas the 13 that recognized the same antigen weakly fell into these classes in approximately the same distribution – 3 TPE, 6 endemic normal, 2 asymptomatic and 2 chronic. In addition, the strength of reactivity to AP18 was independent of reactivity towards soluble MF protein or other cloned proteins (data not shown). To reiterate, while the IgG response to AP18 was frequent and strong, that to AP2 was not that frequent, and almost always weak.

Specific IgE responses were also measured. In contrast to the observation with the IgG response, only AP2 was recognized strongly and consistently when the developing reagent was an anti-IgE antibody conjugated to HRP (Table I). TPE patients (8/8) were particularly notable in

TABLE I

Immunoreactivity of sera against antigens AP18 and AP2 using anti IgG or IgE developing antisera

	IgG		IgE			IgG		IgE	
	AP18	AP2	AP18	AP2		AP18	AP2	AP18	AP2
<b>Tropical pulmonary eosinophilia</b>					<b>Asymptomatic microfilaremic</b>				
TPE 1	+/-	+	-	+	Hf 7	+	+	-	+
TPE 2	+	+/-	-	+/-	Hf 11	+/-	+/-	-	+
TPE 3	+/-	-	-	+	Hf 12	*	*	-	+/-
TPE 4	+	-	-	+/-	Hf 18	-	+/-	+/-	+
TPE 5	+	+/-	*	*	Hf 20	?	-	*	*
TPE 21	+	-	-	+/-	Hf 21	?	-	-	+/-
TPE 22	+	-	-	+/-	Hf 22	+/-	+/-	+/-	+/-
TPE 23	+/-	+/-	*	*	Hf 23	-	-	-	+/-
TPE 24	+	-	-	+	Hf 25	*	*	-	+
<b>Endemic normal</b>					<b>Chronic pathology</b>				
EN 1	+	+/-	-	+	Hf 1	-	-	*	*
EN 2	*	*	+/-	+	Hf 4	-	-	-	+
EN 3	+/-	+/-	-	+/-	Hf 5	+/-	+/-	-	+/-
EN 4	*	*	-	+	Hf 6	-	+/-	-	+
EN 5	**	**	-	-	Hf 10	-	-	-	+/-
EN 6	+	-	+/-	+	Hf 15	-	-	+	-
EN 7	+/-	-	-	+/-	Hf 17	-	-	*	*
EN 8	-	-	-	+/-	Hf 31	-	-	+/-	+
EN 9	*	*	ND		Hf 32	+	-	-	-
EN 10	+	+/-	-	+	Hf 38	-	+/-	ND	
Hf 2	+/-	+/-	-	+/-	Hf 42	?	+/-	ND	
Hf 3	**	**	-	+/-	Hf 43	*	*	ND	
Hf 8	*	*	**	**	Hf 44	+	+/-	ND	
Hf 9	-	-	-	+/-	Hf 45	-	-	ND	
Hf 13	-	-	-	+/-	Hf 46	+/-	-	ND	
Hf 16	+	+/-	+/-	+/-	Hf 47	+	-	ND	
Hf 19	+	-	*	*					
Hf 24	*	*	*	*					
Hf 33	+/-	-	ND						
Hf 34	-	-	ND						
Hf 35	-	+/-	ND						
Hf 36	+/-	-	ND						
Hf 37	+	-	ND						
Hf 39	-	+/-	ND						
Hf 40	+	-	ND						
Hf 41	+/-	-	ND						

Protein extracts were spotted onto nitrocellulose prior to incubation in the test sera. The sera are classified by patient symptomatology. All patients are presumptively infected with *W. bancrofti*. Sera designated Hf were collected in Andhra Pradesh, as described in Methods, while those designated TPE or EN were collected in Madras. + Signifies a strong reactivity; +/- signifies a weak reactivity; - signifies no specific reactivity. \* Indicates a serum whose reactivity to *E. coli* was so high that it obscured any specific signal; \*\* indicates a serum that failed to react with either test protein, soluble MF extract, and several control proteins, and so was considered uninformative; ? indicates ambiguous or uninterpretable results; ND, not done.

that their serum IgE reacted only with AP2 and MF extract, even though most had shown strong IgG-specific reactivity against AP18. Among the other sera for which a detectable IgE signal could be obtained, 31/35 showed strong reactivity

against AP2. Only 4 sera did not contain measurable anti-IgE activity against this antigen. As with the IgG assays above, the intensity of reactivity to the antigen did not demonstrate any correlation with disease state. AP18 was recognized

by the IgE of only 8/35 sera, and even for these eight, the reactivity was weak, with one exception – a serum designated Hf26, from a patient with asymptomatic microfilaremia, that demonstrated strong IgE reactivity towards AP18.

Of the four sera that failed to recognize AP2, two were from endemic normals, and two were from patients with chronic symptoms. Notably, these four sera showed some reactivity to MF soluble extract, implying that the lack of reactivity to AP2 was not due to a total absence of IgE in these sera. In fact, one of these sera, designated EN5, from an endemic normal individual, had the strongest IgE anti-MF extract reactivity of all sera tested.

**Identification of clones of interest.** Clone AP2 contained an insert of just over 200 bp. The sequence of clone AP2 and its deduced translation product are shown in Fig. 3. The sequence is noteworthy for its regular Gly-X-Y pattern, where X and/or Y are frequently proline. This pattern is typical of a collagen [14].

Clone AP18 contains an insert of almost 600 bp. The open reading frame occupies the 5' one-third of the insert. The remaining two-thirds, beginning at an internal *AluI* site, shows near identity to a previously described *B. malayi* repeat family [15,16], although further analysis has proved this association to be an artefact of cloning (data not shown). A computer search of the GenBank [17] nucleic acid database using the DFASTN [18] program reveals that the gene is homologous to that encoding body wall myosin, *unc-54*, of the free-living nematode *Caenorhabditis elegans* [19]. The conservation is not particularly dramatic. In contrast, when the nucleic acid sequence is trans-

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Gly Tyr Pro Gly Leu Leu Gly Pro Gln Gly Glu Arg Gly Leu Pro
GGT TAT CCG GGC TTG CTA GGA CCA CAA GGT GAA AGG GGA TTA CCA

Gly Val Pro Gly Pro Gln Gly Pro Pro Gly Asp Asp Gly Met Pro
GGA GTG CCA GGT CCT CAG GGT CCC CCT GGA GAT GAT GGA ATG CCT

Gly Ala Pro Gly Arg Pro Gly Pro Pro Gly Pro Pro Gly Lys Asp
GGT GCG CCA GGA AGA CCT GGA CCA CCG GGA CCA CCT GGA AAA GAC

Gly Ile Pro Gly Leu Pro Gly Gln Lys Gly Glu Pro Thr
GGC ATT CCC GGT TTA CCA GGA CAG AAG GGT GAA CCA ACA

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Fig. 3. Nucleotide and deduced protein sequence of AP2. Glycine residues have been highlighted. As is typical for collagen, they regularly occupy every third position with only a single absence. In addition, proline makes up nearly 30% of the protein, also a characteristic of collagen.

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AP18          RLKSEQDEVLETIEGLRRENKELAQEIKDLTDQLG
unc-54 1495   KAKNAQEELAEVVEGLRRENKSLSQEIKDLTDQLG

AP18          EGGRSVFEMQKIIRRKEVEKDELQHALDEAEA
unc-54 1530   EGGRSVHEMQKIIRRKEIEKEELQHALDEAEA

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Fig. 4. Alignment of the translation product of AP18 (top) with *C. elegans* body wall myosin (bottom). Two dots represents an identity between the two species, a single dot represents a conserved substitution, as determined by the DFASTP computer program. The number to the left of the *unc-54* sequence is the amino acid position within the *C. elegans* protein (1495–1560). This corresponds to a short region in the middle of the light meromyosin (LMM)  $\alpha$ -helical tail. In this 66-amino-acid stretch, the two proteins are 80% identical, and 96% conserved.

lated into amino acid sequence, the match with the corresponding portion of *C. elegans* body wall myosin, as shown in Fig. 4, is almost complete (96% conservation, with 80% identity). Comparison of the translation product with the NBRF Protein Identification Resource [20] using the DFASTP program results in significant matches not only with *C. elegans* myosin, but also several other sequenced myosins.

The discrepancy between amino acid and nucleic acid conservation can be explained by the severely biased codon usage employed by *B. malayi*. The nucleic acid alignment is shown in Fig. 5. The bases corresponding to identical amino acids have been marked by stars. Inspection of these residues shows a distinct pattern of divergence at the third position of each codon. The third codon usage for these residues is compiled in Fig. 6. Compared to *C. elegans*, *B. malayi* has a preference for A or T at the expense of G or C.

## Discussion

The immune response of the infected individual to a complex organism such as the filarial parasite is likely to be multi-faceted. In a parallel study [8], we have analyzed the differential recognition of cloned filarial protein antigens by individuals from an endemic area exhibiting varied clinical symptomatology. Having demonstrated differential recognition based on clinical classification, we sought to determine whether there was any difference in the recognition profile with respect to antibody class. Isotype limited recogni-



is a major antigen with regard to recognition by IgG. This differential recognition by antibody class at first appears to contradict the results of Hussain and Ottesen [5], who found parallel antigen recognition by antibodies of the IgE and IgG4 isotypes. However, the developing reagent we used was IgG-class specific rather than isotype-specific for the four human IgG subclasses and, since IgG4 is a minor component of serum immunoglobulin (4% of serum immunoglobulin), it is likely that our second antibody has limited reactivity towards that subclass, recognizing instead the more abundantly represented IgG subclasses. Given that consideration, our results are consistent with those of Hussain and Ottesen, since they have shown that IgG subclasses other than IgG4 recognize a different subset of antigens than the IgE isotype.

It is formally possible that the strong IgG response to AP18 has masked the detection of an IgE response. While this possibility cannot be excluded in the absence of IgG depletion data, the pattern of reactivity does not support this explanation. IgG reactivity did not preclude IgE reactivity for the small minority of sera that recognized AP18, nor did IgG reactivity interfere with IgE activity for AP2. However, even if the differential recognition represents relative rather than absolute antibody titre and/or avidity, the dichotomy of reaction between the two assays, especially in the case of AP2, is quite noteworthy.

Another striking feature that has emerged from our sequence analysis of filarial antigens is that similarity searches at the nucleic acid level reveal a lack of long stretches of identity, even when the translated amino acid sequences themselves are identical. This is clearly because of the strong bias of the filarial genome, which has one of the highest percentages of A/T content of all multicellular organisms [25], for the use of A or T in the third base position of each codon. When aligned with the homologous gene, a striking regular '2 and 1' pattern occurs: the first two bases of each codon are conserved, as they generally must be to conserve the amino acid, and the third base, in most cases redundant, diverges. In the few cases where the third base is conserved between *C. elegans* and *B. malayi*, it is generally because the third base

in the *C. elegans* codon is already an A or T. This biased codon usage is of considerable experimental importance. An inspection of the sequence alignment shows that there is no oligonucleotide that could be constructed that would cross-hybridize between the *B. malayi* and *C. elegans* gene. Experiments in our laboratory have shown that a cross-species probe for histones, genes known to be strongly conserved throughout evolution, does not detectably hybridize at a variety of stringencies to *B. malayi* DNA (M. Cameron, personal communication). Hence, this codon bias has important implications for cloning strategies when working with filarial organisms.

As noted earlier, in a parallel study we have analyzed the differential recognition of cloned filarial antigens by symptomatic groups, using *Onchocerca volvulus* libraries [8]. There are some interesting differences and similarities between the two studies. One similarity is the use of sera from patients infected with one species of filarial worm (*W. bancrofti*, in this case) on libraries constructed from nucleic acids from a different filarial nematode. We feel that this lack of identity should have no effect on the antigens detected, since our sequence analyses so far have highlighted remarkable sequence conservation among the nematodes. In terms of the differences, one that is remarkable is that while we isolated over a hundred clones from the *O. volvulus* libraries, only a small number were recognized by more than a few of the sera tested. In the case of the *B. malayi* *AluI* genomic expression library, we isolated only two independent clones, but these two are recognized by most of the sera tested. What is most provocative is that of the few clones from the *O. volvulus* library that were recognized by many patients, one designated K11, cross-hybridizes with AP18, implying that it too represents myosin. Partial sequencing of K11 has reinforced this conclusion (data not shown). Thus, myosin from two independent sources is a major antigen recognized by filarial patients. (We have identified AP18 as myosin as opposed to paramyosin on the basis of its sequence analysis as well as the failure of a rabbit anti-paramyosin antiserum to react with AP18). Donelson also has reported the isolation of an immunodominant protein with similarity to myosin/paramyosin from *O.*



*volvulus* [26] which by restriction map is distinct from K11.

It is worth noting a methodological difference between our earlier study [8] and the present one. In that study, we used plaques directly for analysis; in this study we have used lysates from induced lysogens. That study demonstrated a restricted recognition of the *O. volvulus* clone K11 primarily by asymptomatic microfilaremic patients. In this study, we find a much more promiscuous recognition of the homologous *B. malayi* clone AP18. Thus, the limited and restricted recognition of this recombinant fusion protein revealed by screening phage plaques is blurred when one uses a higher concentration of antigen, as is the case when one uses lysogen extracts. This phenomenon has also been reported by Selkirk et al. [27] in a similar study using *B. pahangi* expression libraries. However, we must qualify any conclusions by emphasizing the differences between the two clones, in that K11 represents a significantly larger insert than AP18 and is from *O. volvulus* rather than *B. malayi*.

An intriguing question concerns the mode of presentation of these antigens to the immune system. Collagen is known to be a major constituent of nematode cuticle, and therefore is a logical immune target. Myosin, on the other hand, is usually contained within muscle fibers. It is not known whether it is presented to the immune system upon the death and breakdown of the worm (population turnover), or whether the protein itself is broken down and excreted during normal functioning of the microfilariae (physiological turnover). There is also a third possible means of myosin presentation to the immune system. The myosin tail forms an  $\alpha$ -helix which interacts with another myosin tail in a helical arrangement, a so-called coiled coil. This motif is shared by several proteins, including fibrinogen [28], and the M protein of *Streptococcus pyogenes* [29,30]. This

latter protein is used by the *Streptococcus* to escape immune recognition. The coiled coil of M protein binds serum Factor H [31], which inhibits the alternate pathway of complement activation. It is tempting to postulate that the physiological turnover of myosin may have evolved a secondary role as part of the parasite's evasion of immune recognition, whereby fragments of the myosin tail are secreted and displayed on the parasite surface performing a function similar to that of streptococcal M protein.

In conclusion, we have identified collagen and myosin as filarial products which are recognized antigenically by the infected human host. The most striking recognition difference between these two proteins is the class of antibody which they elicit. Myosin is recognized primarily by antibodies of the IgG class, collagen primarily by antibodies of the IgE class. Sequencing of these clones has revealed an unusual codon usage which must be taken into account for cloning filarial genes in general. The functional relevance of such isotype dominant antigens in the pathogenesis of filarial disease remains to be determined.

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