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# Immunological genomics of *Brugia malayi*: filarial genes implicated in immune evasion and protective immunity

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## SUMMARY

*Filarial nematodes are metazoan parasites with genome sizes of > 100 million base pairs, probably encoding 15 000–20 000 genes. Within this considerable gene complement, it seems likely that filariae have evolved a spectrum of immune evasion products which underpin their ability to live for many years within the human host. Moreover, no suitable vaccine currently exists for human filarial diseases, and few markers have yet been established for diagnostic use. In this review, we bring together biochemical and immunological data on prominent filarial proteins with the exciting new information provided by the Filarial Genome Project's expressed sequence tag (EST) database. In this discussion, we focus on those genes with the highest immunological profile, such as inhibitors of host enzymes, cytokine homologues and stage-specific surface proteins, as well as products associated with the mosquito-borne infective larva which offer the best opportunity for an anti-filarial vaccine. These gene products provide a fascinating glimpse of the molecular repertoire which helminth parasites have evolved to manipulate and evade the mammalian immune response.*

**Keywords** *filariasis, antigens, inhibitors, cytokine homologues, vaccine*

## INTRODUCTION

Filarial nematodes enjoy one of the longest lifespans of any human pathogen. Individual adult worms may live for 7 years or more (1), and their microfilarial offspring can remain in the bloodstream for at least 1 year (2). Their success implies highly effective immune evasion strategies (3). With immunological pathways becoming increasingly well defined, and with the progress of the filarial genome project (4), it is now possible to search for and characterize individual molecules from these parasites which encode proteins involved in neutralizing or evading the host immune response. These may also serve as ideal vaccine or drug targets.

One paradigm for immune evasion by pathogens has been provided by work on viruses (5,6). Here, despite the constraints of small genome size (< 200 kb) and limited gene number (< 200), there are a remarkable series of products which interfere with major histocompatibility complex (MHC) Class I antigen processing, or which mimic host cytokines or cytokine receptors. Nematode parasites may adopt similar strategies, with two interesting complexities. First, rather than capture immunomodulatory genes from their hosts, nematodes may use the genetic heritage they share with mammals, and adapt conserved regulatory molecules for immune evasion (7,8). As nematode genomes are relatively large (100 million bp or more) and encode approximately 20 000 proteins, the scope for expressing direct immune modulation products is immense. Second, nematode parasites such as the filariae can induce host cells to adopt a suppressive function (9), perhaps providing the long-term immunological tolerance thought to prevail in infection (10,11).

Different approaches can be taken to identify immune evasion genes. The classical path has been to characterize major products found at the host–parasite interface,

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such as surface and secreted molecules, which led to the identification of antioxidant proteins on the filarial cuticle (12). Second, we can probe for predicted gene families by polymerase chain reaction (PCR), as used to isolate a homologue of transforming growth factor (TGF)- $\beta$  (13). With the database of expressed sequence tag (EST) sequences now exceeding 22,000, similar searches can be done *in silico* and, from this route, homologues of mammalian macrophage migration inhibitory factor (MIF) have been discovered (7,14). Third, we can use abundance alone as a measure of importance leading us to discover novel genes with no precedent in other infectious organisms (15,16). The completion of the *Caenorhabditis elegans* genome (17) adds a comparative dimension to these studies because immune evasion genes would be expected to have diverged sharply from homologues expressed in *C. elegans* or even represent proteins altogether absent from the free-living organism.

The rate of gene discovery in large parasite genomes is rapidly accelerating, due to the success of EST projects which perform large-scale sequencing of cDNAs from multiple life cycle stages (18). For example, it is estimated that approximately one-third of the predicted 20 000 genes in the filarial nematode *Brugia malayi* are represented in at least one EST sequence (4,19). It is essential that stock is taken regularly of the advances offered by genome-based research, and that new information is effectively integrated with both existing and new protein-based analyses. The purpose of this

review is to summarize the most prominent and promising filarial gene products in the realm of immunology, focusing on those proteins most likely to play a role in immune evasion and modulation on behalf of the parasite, and/or most implicated as targets of the immune response including those with potential for new vaccines.

Filarial nematodes belong to the family *Onchocercidae* (Table 1). Of several human pathogens, only *B. malayi* can be readily maintained in laboratory animals, and this species has consequently been studied in greatest detail. Where comparisons can be made between homologous genes from different species, high levels of sequence conservation are generally seen (13,20,21). It is valuable therefore to integrate all available information from related species to establish the function of novel proteins from these parasites.

Table 2 lists a selection of *B. malayi* genes for which full cDNA sequence is available and which are implicated as important components that influence the host immunological profile. Together with essential information on each protein, we have also conducted a virtual expression profile (4), enumerating the frequency with which each transcript is represented in EST sequences derived from each stage of the life cycle. Thus, both stage-specific and constitutive genes can be identified, and particular features from this analysis are referred to under the respective gene below. Table 3 summarizes the similarities of

**Table 1** Gene and protein sequences from filarial nematodes in public databases (as of 8 January 2001)

	Nucleotide sequences	Protein sequences	Protein structures	ESTs
Human pathogens				
<i>Brugia malayi</i>	517	153	3	22392
<i>Onchocerca volvulus</i>	581	256	0	13806
<i>Wuchereria bancrofti</i>	35	30	0	131
Animal models				
<i>Acanthocheilonema viteae</i> (jird)	18	23	0	0
<i>Brugia pahangi</i> (jird/cat)	57	79	0	28
<i>Dirofilaria immitis</i> (dog)	61	64	0	0
<i>Litomosoides sigmodontis</i> (mouse)	14	15	0	198
<i>Onchocerca ochengi</i> (cow)	6	5	0	60
Other human parasites				
<i>Brugia timori</i>	1	0	0	0
<i>Loa loa</i>	8	5	0	0
<i>Mansonella perstans</i>	1	0	0	0
Total (including additional minor datasets)	1329	641	3	36615

homologues from other filarial species, and from *C. elegans*, where these are known. The main part of this review discusses the categories of filarial proteins thought to be important in the immunological interaction between host and parasite. These genes are summarized in Table 2.

## ADULT SURFACE-ASSOCIATED PROTEINS.

### Gp29 or *Bm*-GPX-1 (glutathione peroxidase)

The major surface-associated proteins of adult *B. malayi* have been identified by molecular cloning. The dominant noncollagenous component is a 29 kDa glycoprotein designated gp29 (22) and subsequently identified as a glutathione peroxidase, *Bm*-GPX-1 (12). This enzyme has activity against lipid peroxides in preference to hydroperoxide (23), and thus may protect the lipid phase of the epicuticle from immune reactive-oxygen intermediate attack. It may also have a role in modifying or removing host immunomodulatory lipids (24). Thus, *Bm*-GPX-1 was the first filarial product for which an explicit role was proposed in defending parasites against host immunity. However, there is no evidence as yet that host responses against *Bm*-GPX-1 can protect against infection because antibodies to this protein are present in most infected individuals.

*Bp*-GPX-1 from *Brugia pahangi* shows an identical amino acid sequence (25) while that from *Wuchereria bancrofti* diverges by only 3% (20) (Table 3). However, there are a number of synonymous nucleotide substitutions, with introns diverging by 10% (*B. malayi*/*B. pahangi*) and 28% (*Brugia*/*Wuchereria*). Curiously, no homologue has been found in *Onchocerca* parasites. A three-dimensional model is available in which the *Brugia* GPX-1 structure has been predicted according to the bovine homologue (26). The residues differing between the filarial genera map to the exterior of this model, consistent with either neutral drift of structurally unconstrained amino acids, or variation at antigenically significant positions.

### Gp15/400 or NPA-1 (nematode polyprotein allergen)

Among the most intriguing components of the adult nematode surface is gp15/400, a repeat-motif protein with variable numbers of 15-kDa subunits, giving a molecular weight range from 15 kDa to approximately 400 kDa (22,27). This polyprotein is related to antigens from many different nematodes, including the *Ascaris* body fluid allergen ABA-1 (28–30). The

gene family has thus been designated *npa* (nematode polyprotein allergen/antigen) (31). *Bm*-NPA-1 is synthesized as a 400 kDa precursor, containing regularly spaced 132-amino acid subunits separated by tetrabasic motifs. Incomplete cleavage of the primary translation product at these tetrabasic sites is thought to yield the characteristic 'ladder' observed on SDS-PAGE gels (28,32). All known nematode genes for NPA proteins contain 10 or more degenerate tandem repeats, although the degree to which sibling repeats vary within a single gene differs greatly according to species (21).

*Bm*-NPA-1 is secreted by adult parasites (28,29), and is a major target for immunoglobulin (Ig)E antibody responses (33), indicating a prominent immunogenicity *in vivo*. Filarial and other nematode NPA bind retinol and fatty acids with high affinity. NPA-1 could be considered to act as a 'worm albumin', perhaps sequestering host signalling lipids (34). The *Dirofilaria immitis* protein has also been reported to act as a neutrophil chemotactic factor (35), indicating some form of recognition by the innate immune system of a protein which may perhaps be essential for worm survival in the host.

### *Bm*15 or *Bm*-CPI-2 (cysteine protease inhibitor-2)

Second in prominence to gp29 on the surface of adult *Brugia* is a 15 kDa nonglycosylated surface protein, *Bm*15 (36,37). This protein is also evident in parasite secretions (37), and is present on the L3 surface. Once cloned, its sequence revealed similarity to vertebrate cysteine protease inhibitors or cystatins, and it has been designated *Bm*-CPI-2 (cysteine protease inhibitor-2), in distinction to the L3-specific cystatin *Bm*-CPI-1 described below (38). Close homologues of *Bm*-CPI-2 have been reported from *Onchocerca volvulus* (39,40) and *Acanthocheilonema viteae* (41), and the latter molecule directly inhibits T cell proliferation and induces interleukin (IL)-10 release from murine macrophages (41).

The CPI-2 homologues from these three species contain an intriguing 23-amino acid *N*-terminal extension absent from all other members of the family. This flanking sequence has been suggested to fulfil another function, such as targetting for entry into host cells. If so, *Bm*-CPI-2 would be a novel example in the evolution of cystatins. A further point of interest is that EST sequences for *Bm*-cpi-2 so far deposited reveal approximately equal numbers of two variants differing by a single amino acid (Asn or Lys at residue 90). Such

**Table 2** *B. malayi* genes for which full cDNA sequence is available

Gene name	aka	BMC cluster number	Identification	Homologues in other species	cDNA accession number	Protein length (aa)	Native MW (Western) (kDa)	EST abundance by stage (no. of sequences)										Reference
								Mf	L2	vL3	L3d6	L3d9	L4	YA	AdF	AdM	Total	
<b>Adult surface-associated proteins</b>																		
<i>api-1</i>	Bm-33	04136	Aspartyl protease inhibitor	Av, Ce, Di, Ov	L11001	233	33	4	0	7	0	0	0	0	1	8	20	(51)
<i>cpi-2</i>	Cystatin	01649	Cysteine protease inhibitor-2	Av, Ce, Ov	AF015263	161	15/16	0	0	14	2	0	0	0	2	2	20	(38)
<i>gpx-1</i>	gp29	02584	Glutathione peroxidase-1	Bp, Ce, Di, Wb	X69127	223	29	2	0	0	0	0	7	1	2	14	26	(20)
<i>far-1</i>	Bm20	04112	Fatty acid and retinol-binding protein-1	Ce, Ov	U69169	179	20	0	2	3	0	0	1	1	0	4	11	(46)
<i>nlt-1</i>	Bm-24; Di22U-like	03432	Secreted protein	Bp, Ce, Di, Ov	AF072679	211	24	0	0	31	1	0	0	3	0	4	39	(49)
<i>npa-1</i>	gp15/400 ladder pRUNG	04904	Nematode polyprotein allergen-1	Bp, Di	S76365	> 1000	> 200	0	0	1	0	0	0	0	0	0	1	(29)
<i>sod-2</i>	ecSOD	00677	Superoxide Dismutase (Cu/Zn)	Bp, Ov	X76283	199	24	1	1	2	0	0	2	0	2	1	9	(55)
<i>tpx-1</i>		00122	Thioredoxin peroxidase-1	Ce, Di, Ls, Ov	U34251	229	25/45	1	0	3	0	1	0	1	0	1	7	(60)
<i>tpx-2</i>		00211	Thioredoxin peroxidase-2	Ce, Di, Ov	U47100	199	22	1	1	57	0	0	10	0	13	1	83	Scott <i>et al.</i> *
<b>Microfilarial surface and secreted proteins</b>																		
<i>chi-1</i>	Mf-1	00298	Chitinase	Bp, Av, Ov	M73689	504	70	8	1	0	0	0	0	0	1	1	11	(64)
<i>chi-2</i>	Mf-1	00762	Chitinase	Bp, Av, Ov	U59688	518	75	2	1	0	0	0	0	0	0	1	4	(64)
<i>shp-1</i>	Mf22	01695	Mf sheath protein-1	Bp, Ls	U43568	200	22	0	0	0	0	0	0	0	26	1	27	Hirzmann <i>et al.</i> *
<i>shp-2</i>		01663	Mf sheath protein-2	Bp, Ls	Z35444	237		0	0	0	0	0	0	0	2	0	2	Hirzmann <i>et al.</i> *
<i>shp-3</i>		00617	Mf sheath protein-3	Bp, Ls	AF030944	222		5	0	0	0	0	0	0	2	1	8	Hirzmann <i>et al.</i> *
<i>shp-3a</i>		00617	Mf sheath protein-3a	Bp, Ls	AF030944	140		0	0	0	0	0	0	0	1	0	1	Hirzmann <i>et al.</i> *
<i>spn-2</i>	Serpin	00328	Serpin-2	Ce	AF009825	428	47.5	50	0	0	0	0	0	0	1	0	51	(78,79)
<b>Larval proteins</b>																		
<i>alt-1</i>		00123	Abundant larval transcript-1	Di, Ov, Wb	U57547	125	20	0	0	35	0	0	0	0	0	0	35	(15)

<i>alt-2</i>		00213	Abundant larval transcript-2	Bp, Ce, Di, Ls, Ov, Wb	U84723	128	20	0	0	80	7	0	0	0	0	0	87	(15)	
<i>cpi-1</i>	Cystatin	00178	Cysteine protease inhibitor-1	Av, Ce, Ov	U80972	127	14	0	0	8	0	0	0	0	0	1	9	(15)	
<i>glt-3</i>	alt-3	00136	Glycine /tyrosine-rich transcript-3	Ov	U80974	55	6	0	0	16	0	0	0	0	0	0	16	(15)	
<i>glt-4</i>	alt-4	00148	Glycine /tyrosine-rich transcript-4		R47625	73		0	0	10	0	0	0	0	0	1	11	(15)	
<i>lid-1</i>	Av-L3 homologue	00031	LIM domain	Av, Ov	AA454417	566		2	0	15	5	1	1	0	2	0	26	Murray <i>et al.</i> * (49)	
<i>nlt-1</i>	Bm-24; Di22U-like	03432	Novel larval transcript	Bp, Di, Ov, Ce	AF072679	211	24	0	0	31	1	0	0	3	0	4	39		
<i>spn-1</i>	Serpin	00322	Serpin-1	Ce	U04206	392		1	0	26	1	0	0	0	0	0	28	(81)	
<i>sxp-1</i>	BmM14	05831	Related to RAL-2 and BmM5	Av, Bp, Ce, Ls, Ov, Wb	M98813, M95546	162	13	2	0	28	3	1	1	4	3	5	47	(98,99)	
<i>val-1</i>	Asp	00351	Venom allergen like-1	Ce, Di, Ov, Wb	AF042088	220	45	3	0	72	4	1	0	0	0	2	82	McCarthy & Hopkins* (95)	
<b>Cytokine/cytokine receptor homologues</b>																			
<i>mif-1</i>		00238	MIF homologue-1	Bp, Ce, Di, Ov, Wb	U88035	115	12.3	0	4	3	0	0	18	1	8	7	41	(7)	
<i>mif-2</i>		02968	MIF homologue-2	Ce, Ov	AY004865	120	12.8	1	0	0	1	0	0	0	1	0	3	(14)	
<i>tgh-1</i>		11366	TGF- $\beta$ homologue-1	Bp, Ce	AF012878	428		0	0	0	0	0	0	1	0	0	1	(13)	
<i>tgh-2</i>		01513	TGF- $\beta$ homologue-2	Ce	AF104016	349	12	4	0	0	0	0	2	3	4	5	18	(8)	
<i>tph-1</i>	TCTP	00169	Tumour protein homologue-1	Ce	U80971	181	24	15	2	5	1	0	11	0	8	22	64	(15)	
<i>trk-1</i>			TGF- $\beta$ receptor (type 1)-1	Bp, Ce	AF013991	645		0	0	0	0	0	0	0	0	0	0	(107)	
Total ESTs deposited in dbEST								2977	608	3249	1330	196	1529	1041	3335	4289	18743	(4)	

\*Unpublished data.

**Table 3** Amino acid identities between key filarial proteins from different species

<i>B. malayi</i>			<i>C. elegans</i>						
Gene	Protein	Length (amino acids)	<i>B. pahangi</i>	<i>W. bancrofti</i>	<i>L. sigmodontis</i>	<i>A. viteae</i>	<i>D. immitis</i>	<i>O. volvulus</i>	Presumed orthologue
<i>alt-2</i>	ALT-2	128	96.9%	87.0%	64.8%	64.8%	57.8%	46.6%	19.0% CO8A9
<i>api-1</i>	API-1	233				76.1%	75.2%	72.2%	41.5% F35A5.4
<i>cpi-2</i>	CPI-2	161				66.0%		65.0%	26.7% R01B10.1
<i>far-1</i>	FAR-1	178						83.8%	34.1% F02A9.2
<i>gpx-1</i>	GPX-1	223	100.0%	96.9%			74.0%		52.9% C11E4.1
<i>mif-1</i>	MIF-1	115	86.0%	93.0%			84.0%	85.0%	35.0% Y53A3A.3
<i>mif-2</i>	MIF-2	115						75.0%	38.0% C52E4.2
<i>shp-1</i>	SHP-1	200	90.0%		45.8%				
<i>sxp-1</i>	SXP-1	153	30.0%	82.5%	43.0%	32.0%		58.9%	27.2% F57H12.2
<i>tgh-1</i>	TGH-1	428	99.1%						42.4% <i>dbl-1</i> (T25F10)
<i>tpx-1</i>	TPX-1	229	49.0%		63.0%		60.0%	62.0%	65.0% R07E5.2
<i>val-1</i>	VAL-1	220		90.9%			63.3%	55.0%	17.8% T05A10.4

Percentage identities are calculated as exact matches between the *B. malayi* deduced protein sequence and that of the closest known homologue from other filarial species. It is possible that some comparisons are not between true orthologues. Slightly different calculations can be reached depending on the numbers of gaps permitted in alignments.

evidence for coding polymorphisms remains rare among filarial proteins.

The functions of *Bm*-CPI-2 present an instructive example of how filarial proteins may interfere with the host immune response. Sequence analysis reveals two potential active sites, the universal papain-inhibiting motif common to *Bm*-CPI-1 and -2, and a second SND-containing site, found in *Bm*-CPI-2, which blocks enzymes of the legumain or asparaginyl endopeptidase (AEP) clade (42). Interestingly, an AEP is involved in the MHC class II antigen processing pathway (43), and recent work has established that purified recombinant *Bm*-CPI-2 can specifically inhibit AEP activity and block the class II-dependent presentation of exogenous antigen by human B cells (44).

#### **Bm20 or *Bm*-FAR-1 (fatty acid and retinol binding protein)**

Surface labelling of adult *B.malayi* gives weaker and variable iodination of a 20 kDa protein related to Ov20, a much more prominent surface antigen on adult *O. volvulus* (45). Including the conserved signal sequence, both *Bm*-FAR-1 and Ov20, are 178 amino acids in length, but the proteins differ in 26/160 amino acids (84% identity) over the mature polypeptide (46). Three of these substitutions replace asparagine residues found in Ov20, nullifying potential glycosylation sites, and although one new site is

observed in *Bm*-FAR-1, this is thought not be used in the native product.

While Ov20 is recognized by serum antibodies from a high proportion of onchocerciasis patients (47), the *B. malayi* homologue has a lower immunological profile, and only marginal serological responses are detected in lymphatic filariasis patients (46). However, both *Onchocerca* (48) and *Brugia* (Allen & Kennedy, personal communication) proteins show functional fatty acid and retinol binding, and the gene has accordingly been re-designated *far-1*. Because no NPA-1 homologue has yet been found in *O. volvulus*, it may be that Ov-20 is upregulated to provide a compensatory level of retinol binding in the *Onchocerca* species.

#### **Bm-24 or *Bm*-NLT-1 (novel larval transcript; *D. immitis*-P22U-like)**

In *D.immitis*, a secreted 22 kDa protein (Di-P22U) has been isolated, antibodies to which react with a 24 kDa surface-labelled protein from the L3 and L4 of *B. malayi*; the same antigen is also weakly represented on the adult parasite (49). Electron microscopy locates Di-P22U to the cuticle and hypodermis of adult *D. immitis*, but the protein is evidently obscured on the surface because no binding is detected by immunofluorescence assays on intact worms. Cloning of the *B. malayi* homologue reveals a 211-amino acid protein

with a typical signal peptide (49). This sequence matches a highly expressed vector-derived L3 transcript in the *B. malayi* EST database which has been designated *Bm-nlt-1* or novel larval transcript-1 (50). So far, no insight has been gained into the biological function of this product, and there are as yet no data on immunological recognition by infected or immune hosts.

### Bm33 or *Bm-API-1* (aspartyl protease inhibitor)

A minor protein observed by surface labelling adult *B. malayi* is Bm-33, a 33 kDa protein with similarity to the aspartyl protease inhibitor gene family (51). The best characterized homologue is in *O. volvulus*, in which the immunodominant Ov-33 antigen elicits strong antibody responses in 96% of patients (52); similarly, a high proportion of *B. malayi*-infected patients express antibodies to *Bm-API-1*, particularly of IgG1 and IgG4 isotypes (16). Functionally, Ov-33 expressed in yeast shows inhibition of the aspartyl protease pepsin (53), and the presence of a 17-amino acid putative signal peptide at the *N*-terminus of Ov-33 and Bm-33 indicates that the product may be secreted by the filariae. Filarial aspartyl protease inhibitors show between 20% and 25% sequence identities to known inhibitors from nonfilarial nematodes, such as *Ascaris* PI-3 (51) which have been shown to interfere with a murine cathepsin E involved in antigen processing (54). No immunological role for the filarial aspartyl protease inhibitors has yet been established, although it is plausible that a similar set of host aspartyl proteases are physiological target of these inhibitors.

### Bm-SODs (superoxide dismutases)

Biochemical analysis of the antioxyradical defences of *B. malayi* indicated the presence of superoxide dismutase, a key enzyme in detoxification of reactive oxygen intermediates produced by host granulocytes. Two distinct *Brugia* SOD genes have been cloned (55). One encodes an intracellular (cytoplasmic) SOD, while the second is a secreted extracellular isoform. The secreted SOD is found at the surface of adult nematodes, and is identified as a low abundance 22 kDa product accessible to iodine labelling (56). Together with GPX, SOD probably provides the parasite with essential defence against reactive oxygen species. Notably, adult *Brugia* are relatively resistant to killing by superoxide compared to the microfilariae, which do not express either GPX-1, nor the SOD genes (57).

### *Bm*-TPXs (thioredoxin peroxidases)

Thioredoxin peroxidases (TPXs) are a large family of antioxidant proteins produced by organisms from all kingdoms. The members of the TPX family have various assignments such as thiol-specific antioxidants (TSA) (58), peroxidoxins (PXN) (59), and natural killer cell enhancing factor (NKEFA) (59). The antioxidant activity of TPX is based on its ability to efficiently degrade H<sub>2</sub>O<sub>2</sub> and alkyl hydroperoxides, thus preventing the production of toxic hydroxyl radicals that damage proteins, lipids and DNA (59).

The *Brugia* EST database has revealed that, similar to a majority of eukaryotic organisms, *Brugia* transcribes two distinct classes of *tpx* genes. One class includes both *Bm-TPX-1* (25 kDa) and *Bm-TPX-2* (22 kDa). Although only 55% identical in amino acid sequence, both proteins contain two highly conserved cysteine residues that correspond to Cys-47 and Cys-170 of the prototype TPX from yeast (58). The second class of *tpx* gene encodes *Bm-TPX-3* (26 kDa) which is approximately 20% identical in amino acid sequence to *Bm-TPX-1* and *Bm-TPX-2* and lacks the conserved Cys-170. While it has been demonstrated that a recombinant form of *Bm-TPX-1* is capable of protecting DNA from oxygen-radical-induced damage (60), *Bm-TPX-2* and *Bm-TPX-3* have not been tested for biological activity. However, the orthologues of *Bm-TPX-2* and *Bm-TPX-3* from *O. volvulus* have been shown to have antioxidant activity (61,62). *Bm-tpx1* and *Bm-tpx-2* have similar patterns of expression with peaks of transcription in the vector-stage L3s and in adult females (Table 2). *Bm-tpx-3* appears to have a much lower level of transcription with 3, 2 and 1 ESTs from the microfilarial, male and female libraries, respectively (data not shown).

The roles that the *Brugia*-derived TPXs play in the immunobiology of infection have not been fully defined. *Bm-TPX-1*, which is not secreted, contains a mitochondrial localization signal. Presumably, this TPX functions to protect cells from the endogenously produced toxic byproducts of aerobic metabolism (60). Studies to define the localization and function of *Bm-TPX-2* and *Bm-TPX-3* have not been carried out. However, *Ov-TPX-2* is associated with the surface of microfilariae where it may serve to protect the parasite from host-derived oxidative damage (62).

### MICROFILARIAL SURFACE AND SECRETED ANTIGENS

Microfilariae (mf) of lymphatic filarial nematodes (*Brugia*,



*Wuchereria*) and certain other species (e.g. *Litomosoides sigmodontis*) are enveloped in a sheath, separate from the cuticle, which is derived from the eggshell *in utero*. The mf are thought to retain their sheath within the mammalian host, and to exsheath in the vector blood meal. Strictly, surface antigens of the mf relate to the sheath components, but surface-iodination reagents penetrate the sheath and label cuticle proteins, some of which are also described below.

### Chitinases, MF-1, *Bm*-CHI-1/2

Surface iodination of *B. pahangi* mf labels a triplet of proteins of approximately 70 kDa (36), while an mf-reactive monoclonal antibody, MF-1, binds two bands of this size in *B. malayi* mf (63). The smaller of the *B. malayi* proteins, p70, was cloned first (64), and proved to be a functional chitin-degrading enzyme with a preference for internal cleavage of chitin polymers. *N*-terminal sequencing confirms that both p70 and the larger p75 from *B. malayi*, and all three isoforms (p69, p73, p76) in *B. pahangi* are closely related (65). The structure of p70 (*Bm*-CHI-1) reveals a large catalytic domain with close homology to eukaryotic and bacterial chitinases, a mucin-like domain with three 14-amino acid repeats rich in serine, threonine and acidic residues, and a *C*-terminal 57-amino acid 6-cysteine domain. Southern blot analysis later indicated that there are at least two chitinase genes in *B. malayi* (66). A larger transcript, containing an additional 14-amino acid repeat in the serine/threonine-rich tract, was then isolated which may encode p75 (65).

The catalytic domain is the only one with homology to chitinases from bacteria, yeast and other metazoa. However, many other chitinases include adjacent mucin-like and cysteine-rich domains. For example, insect chitinases contain *C*-terminal regions with six cysteine residues which align closely to the *Brugia* sequence. This arrangement, termed NC6/2, is also found in other nematodes including *C. elegans* (67). Cysteine-rich domains from other chitinases function to recruit and/or hold chitin substrate to maximize activity (68). Such a role is supported by findings that chitin binding by recombinant *Bm*-CHI-1 is diminished when the *C*-terminal noncatalytic segments are removed from the protein (69).

The expression of *Bm*-CHI-1/2 shows an interesting pattern. It is absent from adult worms and newborn mf, but synthesis is initiated from 2 days after birth, and reaches a plateau 2 weeks later (63,70). Because 'immature' mf are known to be incompetent at infection of mosquitoes (63), the chitinases may play their role in

effecting transmission through the vector. Three possible mechanisms have been suggested (70): degradation of the mf sheath, a chitin-rich structure from which mf must escape in the blood meal; breakdown of a chitin-containing barrier such as the peritrophic membrane in the mosquito vector; or enzymatic release of chitobiose sugars in the mosquito, blocking vector agglutinins from attacking the parasite. As yet, none of these alternatives can be excluded.

An immunological role for chitinases in the mammalian host may also be important. Although in *Brugia*, chitinase expression is mf-specific, the homologous protein is prominent in *A. viteae* infective larvae (71,72). *Av*-CHI-1 is strongly recognized by serum antibodies from jirds vaccinated with irradiated L3 of this species, and is not even expressed by mf (71). Moreover, a homologue is also expressed in L3 of *O. volvulus*, a species in which mf do not carry a sheath (72).

The filarial chitinases are only distantly related to a 43 kDa chitinase reported to be recognized by humans immune to *W. bancrofti* infection (73); subsequent cloning (74) revealed a sequence most similar to the Rickettsial organism *Serratia*, indicating that the L3-chitinase in *W. bancrofti* is encoded by the endosymbiotic *Wolbachia* (see below). It is interesting to consider that chitinase expression may be essential for filarial L3 stages, but that in *W. bancrofti* this requirement is fulfilled by the endosymbiotic bacteria rather than by a filarial-derived gene.

### Major sheath proteins: *Bm*-SHP-1 or Mf-22 and *Bm*-SHP-2

Following the identification of a major sheath protein, Mf22, by Selkirk *et al.* (75), the Giessen group performed a comprehensive analysis of filarial sheath proteins, renaming Mf22 as SHP-1. Using *L. sigmodontis* as their model, four further SHP proteins have been defined (76). One of these additional products (*Bm*-SHP-2) has been fully characterized from *B. malayi* (77), and SHP-3 homologues are present in the EST database. In general, the mRNAs for these products are expressed only in the female uterus.

One striking feature of SHP-1 is the extent of diversity between the two *Brugia* species. While GPX-1 shows a complete identity of amino acid sequence between these two species (Table 3), *Bm*- and *Bp*-SHP-1 display over 10% variation in amino acid sequence, including two indels (*Bp*-SHP-1 has a GGA insertion at nt 79–81, the ATG codon being 1–3; there follows a 42-nt tract almost identical between the two species, and then a GGA

insertion only in *Bm-SHP-1*. Thus the two sequences are of identical length). Remarkably, analysis of the EST data indicate that some of the *B. malayi* entries possess the *B. pahangi* indel pattern. Although it is conceivable that these represent two distinct genes, it remains an attractive possibility that MF-22 displays a degree of polymorphism or variation.

*Bm-SHP-2* is a 236-amino acid protein containing both proline-alanine tracts and a segment rich in glutamines and tyrosines, the latter being suitable substrates for cuticlin-like oxidative cross-linking (76). In *L. sigmodontis*, three further sheath proteins have been identified (SHP-3, -4 and -5), including components exposed on the outermost layer of the mf sheath (76). Homologues for SHP-4 and -5 have yet to be found in *B. malayi*.

#### Serpin: serine protease inhibitors (*Bm-SPN-1* and -2)

The most abundant microfilarial-specific transcript from *B. malayi* encodes a serine protease inhibitor (serpin), a 428-amino acid 47.5 kDa protein found only in this stage of the parasite. *Bm-SPN-2* is homologous to mammalian protease inhibitors such as  $\alpha$ -1-antitrypsin, but has no inhibitory activity against common serine proteases such as trypsin, pancreatic elastase, or the thrombin-cleaving enzymes (78). However, *Bm-SPN-2* is able to inhibit the activity of neutrophil elastase and cathepsin G, two major proteases secreted by the most abundant cell type in the blood, the habitat of the microfilaria itself.

*Bm-SPN-2* is the only antigen so far to have been selected on the basis of strong stimulation of host T cells (79), in distinction to others isolated by antibody reactivity or mRNA abundance. Moreover, *Bm-SPN-2* drives a response rarely seen in helminth infections, dominated by a type 1 (Th1) phenotype. It is known that live mf induce a relatively strong Th1-type response in mice, in contrast to the adult and larval stages which drive overwhelmingly Th2-like reactions (80). *Bm-SPN-2* is able to recall pure Th1 responses in mf-primed cells, whereas even mf extract develops a balanced response with both subsets represented (79). The molecular basis for the Th1-bias in *Bm-SPN-2* remains to be investigated.

In contrast, *Bm-SPN-1* is expressed at a high level in mosquito-derived larvae, but minimally by any stages in the mammalian host (81). Moreover, at a sequence level, *Bm-SPN-1* represents a very distinct branch of the family not closely related to *Bm-SPN-2* (82).

#### THIRD STAGE LARVAL ANTIGENS

Although surface-labelling data exist for *Brugia* infective larvae (36,83), the surface antigens from this stage have not been fully identified. Both cystatin proteins (*Bm-CPI-1* and *CPI-2*) can be iodinated by surface-directed methods, and are secreted by L3 maintained *in vitro*, while *Bm-24* can also be identified in surface-iodinated L3 preparations (49). A molecular approach to identify major L3 proteins by mRNA or EST abundance indicates which proteins may be required in quantity for invading parasites. Most highly expressed overall are genes for *Bm-ALT-2*, *Bm-VAL-1* and *Bm-ALT-1* (50); and among other prominent transcripts are *Bm-nlt-1*, *Bm-tpx-2*, *Bm-cpi-1* and *Bm-spn-1*. Of these only *Bm-alt-1/-2*, *Bm-cpi-1* and *Bm-spn-1* are *trans*-spliced with the conserved nematode 22-nt leader sequence (15,81).

#### *Bm-CPI-1* (cysteine protease inhibitor-1)

The first cysteine protease inhibitor to be identified in *Brugia* was isolated from an analysis of highly expressed *trans*-spliced mRNAs from the L3 stage (15). *Bm-cpi-1* expression, as revealed by a fine analysis with mRNA taken at daily intervals throughout the life cycle, is initiated in late L2 and L3 in the mosquito vector, but terminates within 2 days of infection of the mammalian host. In contrast, *Bm-cpi-2* is constitutively expressed at all stages of the parasite life cycle. *Bm-CPI-1* can be surface-labelled on the mosquito L3 and is found in secretions. *Bm-CPI-1* (similar to *Bm-CPI-2*) is a good inhibitor of papain and cathepsin B, but the physiological target of this product (whether in mosquito, mammal or within the nematode itself) has yet to be defined.

#### Abundant larval transcripts (*Bm-alt-1*, -2)

In a strategy to identify parasite gene products required for invasion and establishment in the mammalian host, we set out to characterize those mRNAs from *B. malayi* L3 which are highly expressed in mosquito-borne infective larvae (15). The 5' spliced leader sequence found on a proportion of nematode mRNAs (84) was combined with an oligo-dT primer to PCR amplify all *trans*-spliced transcripts (81, 85). Two highly expressed genes were designated *alt* (abundant larval transcript) -1 and -2 (15). The corresponding proteins are closely related (79% amino acid identity) small proteins of 125–128 amino acids. An acidic tract near the N-terminus contains most of the variation between the two polypeptide sequences. Significantly,

*alt* mRNA expression is essentially limited to the larval stages, and is not detectable in Mf or adult worms (16).

The Filarial Genome Project provides an independent assessment of the abundance of these two products (18,19). *alt-2* represents > 3% of all L3 ESTs, and *alt-1* approximately 1-5% (50). The ALT-1/-2 proteins are stored in the oesophageal gland of *B. malayi* L3 (Wu *et al.*, personal communication), and are secreted by larvae when cultured following recovery from mosquitoes (Murray and Maizels, unpublished data). Such prominent expression implies a critical role in parasite entry into the host. We therefore tested *Bm*-ALT-1 for its ability to vaccinate jirds (*Meriones unguiculatus*) against *B. malayi* L3 challenge. Immunization resulted in a 76% reduction in worm load, the highest yet reported for a recombinant filarial antigen (16).

The *alt* gene family is also conspicuous in other filarial nematode species (50). In *D. immitis*, the Di20/22L protein is found in the L3 ES and is strongly recognized by sera from immune dogs (86). In *O. volvulus*, *alt* transcripts comprise 4-6% of L3 mRNA, anti-ALT-1 antibodies stain the glandular oesophagus, and *Ov*-ALT elicits partial protection in mice (87). *alt-1* also ranks highest in abundance among transcripts from L3 of *L. sigmodontis* (50), and an *A. viteae* homologue (Av18) figures strongly in material released by moulting L3s (88). The *Brugia* EST dataset includes a number of other ALT-related gene transcripts suggesting that the gene family may be significantly larger in some filaria (Gregory *et al.*, unpublished). Thus, across the range of species, ALT proteins are important candidates for a specific vaccine against filarial infection (16).

#### Glycine-tyrosine rich abundant transcripts (*Bm*-*glt-3*, -4)

Additional highly expressed *trans*-spliced transcripts from L3 are two genes originally assigned *Bm*-*alt-3* and -4 (15). Because their sequence is not related to the *alt-1/-2* family, these genes have been redesignated *Bm*-*glt-3* and -4. Detailed expression profiling of *Bm*-*glt-3* around the filarial life cycle shows it to be initiated midway through the mosquito phase, during L2, and terminated immediately following infection; there is no expression by mature mammalian stages (Gregory and Maizels, unpublished data). Similar to the ALT proteins, there is no indication of the functions of the putative GLT proteins. However, 11 homologous genes exist in *C. elegans*, in two arrays each containing five copies, plus a single additional copy, all on chromosome 5 (Gregory, unpublished data). This evidence for gene multiplication implies an important functional

role, possibly conserved between free-living and filarial nematodes.

#### *Bm*-LID-1 (Ov-L3 homologue)

An interesting antigen was isolated from the L3 of *O. volvulus* by screening a cDNA library with serum from a human volunteer vaccinated with irradiated L3 larvae (89). This clone, initially termed Ov-L3-1, encodes a protein with a LIM domain, a motif identified in a variety of vertebrate genes, including those encoding certain adhesion proteins and transcription factors. The full-length *Brugia* homologue, *Bm*-LID-1, contains 588 amino acids with six potential *N*-linked glycosylation sites and multiple CxxC motifs (Murray & Maizels, unpublished data). There is no typical signal sequence and, in *O. volvulus* at least, the mature protein is relatively insoluble, arguing that LID-1 is not a secreted protein. However, *Bm*-*lid-1* is relatively highly transcribed by *B. malayi* L3, and this elevated expression may account for the strength of the immune response.

#### Venom allergen-like protein, *Bm*-VAL-1 (also known as *Ancylostoma* secreted protein homologue)

A prominent transcript among ESTs from *B. malayi* L3 (18) is a member of an expanding gene family for which the prototype is *Ancylostoma* secreted protein (ASP), first identified in the hookworm *Ancylostoma caninum* (90). The *Ancylostoma* ASP is a front-running vaccine candidate against canine hookworm. Subsequently, related genes have been identified in a range of other nematode species, including products which block neutrophil activation (91) and induce angiogenesis in mice (92).

Members of this family have either single (approximately 200 amino acids) or double (approximately 400 amino acids) homology units, preceded by signal sequences. In *C. elegans* there are at least 20 homologous genes, only one of which has a double-domain structure (93,94). Because the *asp* gene name in *C. elegans* is assigned to an aspartic protease, the filarial gene is designated *Bm*-*val-1*.

The full sequence of the ASP-like *B. malayi* homologue contains a single consensus domain (95). *Bm*-*val-1* transcripts are the second most-abundant among ESTs from the vector-borne L3 stage, but are poorly represented in cDNA libraries from other points in the life cycle (50). However, a finer analysis using first-strand cDNA taken at daily intervals revealed intermittent expression by mature mammalian stages, including adult parasites (95), so that *Bm*-*val-1* is not stringently stage-specific in its expression. Perhaps reflecting this, human antibody responses to

Bm-VAL-1 noted in most infected patients include a substantial IgG4 component in addition to IgG1 and IgG3 (95).

### ***Bm-SXP-1***

*Bm-SXP-1* (the gene name is not an acronym) is not larval-specific as it was first isolated as an immunodominant clone from a male cDNA library. SXP-1 was subsequently shown to be useful as a serodiagnostic reagent for patients with patent infections with lymphatic filariasis, including infections with *W. bancrofti* (96,97). *Bm-SXP-1* is a member of a nematode-specific family of proteins that are 12–14 kDa in mass and are distinguished by a highly conserved motif structure (98). There is a high level of serological reactivity to *Bm-SXP-1* by lymphatic filariasis patients (99), predominantly of the IgG4 isotype, and a percentage of patients with patent *O. volvulus* and *Loa loa* infections also recognize *Bm-SXP-1* (96,97). Based on the utility of *Bm-SXP-1* to diagnose patent infections, it would be predicted that the expression profile would show enhanced transcription at the microfilarial stage. The EST database suggests that, while it is transcribed by all of the stages present in the vertebrate host, the highest levels of transcription may be by the L3s (Table 2). Interestingly, gerbils immunized with *Bm-SXP-1* showed substantial reduction (up to 97%) in microfilarial numbers, as well as a partial reduction (approximately 35%) in adult numbers following L3 challenge (100). Although the lower adult worm burden did not reach statistical significance, this outcome suggests that *Bm-SXP-1* may be a suitable target for immunoprophylaxis against multiple stages of the filarial parasite.

### CYTOKINE AND CYTOKINE RECEPTOR-LIKE PROTEINS

Three gene families have been discovered in filarial nematodes with similarities to mammalian cytokines, two of which (MIF and TGF- $\beta$ ) are likely to be of major importance in the host–parasite relationship, while the third [tumour protein homologue (TPH) or translationally controlled tumour protein (TCTP)] is of less certain significance. In the case of both MIF and TGF- $\beta$  families, two homologues from *B. malayi* have been isolated. However, the same gene families are represented in *C. elegans* (although with different expression patterns), and the question is being addressed of whether the filarial homologues fulfil physiological roles for the nematode or immunological roles for the parasitic mode of life.

### MIF: *Bm-MIF-1* and -2

The macrophage migration inhibitory factor (MIF) gene family, derived from an ancient metazoan ancestor, encodes small (approximately 115-amino acid) proteins with many surprising features (101). For example, these proteins are secreted despite lacking a signal sequence, activate target cells through no known receptor, and display an unusual enzymatic activity dependent upon a proline at residue 2. Within the mammalian immune system, MIF has generally been considered a pro-inflammatory cytokine, but very recent work indicates that, dependent upon dose, MIF can equally exert a counter-inflammatory effect (102).

The EST database for *B. malayi* contains two distinct MIF homologues which encode proteins secreted by parasite (7,14,18). At the amino acid level, *Bm-MIF-1* is 40% identical to human MIF and only 27% identical to *Bm-MIF-2*; the latter shares 28% of residues with the human homologue. Despite these differences, the three molecules show very similar cytokine-like activity (e.g. macrophage kinesis, monocyte activation and induction of endogenous cytokine secretion) as well as the enzyme activity (dopachrome tautomerase). We have also determined the crystal structure of *Bm-MIF-2*, which despite being only 28% identical to human MIF, presents a highly similar trimeric three-dimensional structure (14). The active site combines residues from adjacent monomers, so that trimerization is essential for biological function.

The function of parasite-derived MIF molecules is indeed a controversial question. In most in-vitro assays, *Bm-MIFs* replicate the activity of human MIF, presenting a conundrum: why should a filarial parasite, intent on avoiding an inflammatory tissue reaction, secrete MIF homologues which are thought to amplify the inflammatory pathway? Currently, we favour the hypothesis that continuous secretion of MIF molecules by parasites may induce a counter-inflammatory phenotype, either by desensitization or by exposing host macrophages to a dose or duration of MIF not reproduced *in vitro*. Such a hypothesis may also explain the generation of ‘alternatively activated macrophages’ in filarial infections, which exert counter-inflammatory effects such as suppression of lymphocyte proliferation (9,103,104). Recent results in mice repeatedly exposed to recombinant *Bm-MIF* do indeed indicate that macrophages are induced to differentiate into a novel phenotype by the parasite molecule (Falcone *et al.*, unpublished). If so, then the *Bm-MIFs* represent gene products which modulate the host immune system by recruiting and driving host cells into a more ‘suppressive’ mode.

### TGF- $\beta$ family members (*Bm-TGH-1* and *-2*)

The transforming growth factor- $\beta$  superfamily includes a diverse set of intercellular signalling molecules controlling embryonic patterning and tissue differentiation, as well as regulation of immune responses. The morphogens are represented by *Drosophila decapentaplegic* (DPP) and mammalian bone morphogenetic proteins (BMPs), while the cytokine-like TGF- $\beta$ s constitute a distinct subfamily with an additional conserved cysteine in the mature protein (105). In the mid-1990s, it was established that TGF- $\beta$  family members were involved in *C. elegans* development (106). This prompted us to predict that *Brugia* would express similar proteins, and to hypothesize that at least one form in the parasitic nematode would be secreted to down-regulate host immune cell responses.

Searching first by degenerate polymerase chain reaction, and then from *Brugia* EST sequences as they became available, two distinct TGF- $\beta$  homologues have been characterized. Almost identical *tgh-1* genes were isolated from *B. malayi* and *B. pahangi*, which fall into the DPP/BMP subfamily by sequence similarity. The 428-amino acid protein contains a C-terminal homology domain corresponding to the mature 102-amino acid active product. This domain shows 62% amino acid identity to *C. elegans* DBL-1, 54% to DPP and 45% to mouse *Nodal*, a differentiation-inducing protein. Transcription is relatively low but peaks at periods surrounding growth in the mammalian host. However, expression is absent from the arrested microfilarial stage (13). This expression pattern, and the sequence analysis, suggested that *Bm-TGH-1* plays a role in the maturational physiology and growth of the filarial nematode.

A second TGF- $\beta$  homologue, designated TGH-2, has since been isolated from the EST dataset (8). The active domain contains an additional cysteine, as found in mammalian TGF- $\beta$ s as well as other members of this subfamily (which include myostatin, a muscle-inducer in vertebrates as well as the *C. elegans* dauer-controlling product DAF-7). *Bm-TGH-2* has properties which are more likely to represent a parasite immunomodulator: it is expressed by all stages resident in the mammal, especially by the microfilariae. Because this stage is devoid of developmental activity, such a pattern is inconsistent with a morphogen. Moreover, it is secreted by cultured adult and microfilariae of *B. malayi*, suggesting a role in the interaction with the host (8). Preliminary assays show a low but consistent level of binding to host TGF- $\beta$  receptors.

TGF- $\beta$  acts on cells through a heterodimeric receptor. In vertebrates and insects, a type II receptor chain ligates TGF- $\beta$ , and phosphorylates the type I receptor, leading to a signal transduction cascade. The two receptors are related

but quite distinct in their phosphorylation sites, and because the type I receptor may signal independently in some instances, it is thought to be the ancestral gene. The *Brugia* type I receptor has been cloned by PCR (107) but, despite repeated efforts, no type II receptor has been identified. A type II receptor, with recognizable homology to mammalian type II receptors, exists in *C. elegans* (DAF-4). Thus, the question remains open as to whether *Brugia* uses classical TGF- $\beta$  signalling pathways or has reverted to a simplified, one-subunit receptor system.

### *Bm-TPH-1* (tumour protein homologue/histamine releasing factor)

One of the abundant transcripts found in infective larvae is homologous to a proposed mammalian cytokine, histamine releasing factor (15). However, the identical sequence has been recorded as TCTP. In *Brugia*, this gene is transcribed throughout the life cycle (38). It is also secreted by the adult parasite, indicating that it may be available to play a role in the host-parasite interaction. Experiments to ascertain whether *Bm-TPH-1* can stimulate histamine release from human mast cells have so far given negative results (Falcone, personal communication).

## A BROADER IMMUNOLOGICAL PROFILE OF FILARIAE

### The full genetic complement?

The approximately 22 000 ESTs from *B. malayi* represent about 8800 different genes. Of these, approximately 55% have no significant similarity (a BLASTX score probability greater than  $e^{-10}$ ) to either *C. elegans* proteins, other known nematode proteins, or to any other sequences from other organisms represented in GenBank. This dataset is sufficiently extensive that comment can be passed on those gene families which are conspicuous by their absence. For example, catalase is not represented in *B. malayi*, although a catalase-like activity has been shown to protect adult and mf from hydrogen peroxide-induced damage (57). Interestingly, this enzyme is encoded in *O. volvulus* by the endosymbiotic *Wolbachia* (108). A similar contrast is with infective larvae of *Toxocara canis*, in which 6% of cDNAs encode a secreted C-type lectin (109), and yet not one member of this major gene family appears in the 20 000-strong *B. malayi* EST dataset.

### ***Wolbachia* antigens and lipopolysaccharides (LPS)**

The filarial nematodes harbour an intracellular rickettsia-like bacterium related to the *Wolbachia* reproductive parasites of arthropods. The *Wolbachia* endosymbionts from *B. malayi* and *O. volvulus* have been selected for genome sequencing, and whole-genome clone maps have been built for *B. malayi*. The genomes are approximately 1 Mb in size, and are likely to encode 2–3000 protein products. While many may be involved in the bacterium–nematode interaction, some rickettsial proteins could exert effects at the nematode–mammal interface. Such products would be excellent candidates for new drug targets. Tetracycline has a significant effect on both *B. malayi* and *L. sigmodontis* in animal infections, resulting in reduced nematode survival and abrogation of fertility, while doxycycline has been trialled in human onchocerciasis with significant effect (110). Importantly, antibiotics known not to affect rickettsiae have no effect on worm survival, and nematode species that do not harbour bacteria are not affected by antibiotic treatment. The early IL-1 response of immune cells to crude antigens prepared from *B. pahang* has the hallmarks of an antilipopolysaccharide response, and can be abrogated by pretreatment with polymyxinB, an anti-LPS agent. LPS is a cell wall product unique to bacteria, and thus the bacterial genome may be driving some of the response to the nematode (19,111–114).

### **Non-peptide antigens**

Attention has increasingly focused on protein molecules because of the wealth of sequence information and the technical ease of expression and analysis of peptide products. However, it would be a mistake to ignore nonpeptide antigens. An important nonprotein component expressed by filarial nematodes is phosphorylcholine (115–117), which is secreted by adult filariae (116) and forms the basis for the first circulating antigen test for bancroftian filariasis.

Phosphorylcholine may play a significant immunomodulatory role. First reports that phosphorylcholine-conjugates from *B. malayi* cause reduced T cell proliferation (118), have been followed by detailed studies on ES-62, a secreted phosphorylcholine-containing antigen from *A. viteae* which downregulates signalling in both T cells (119) and B cells (120) and induces dendritic cells towards a type 2 phenotype (121). ES-62 contains phosphorylcholine linked through a novel N-linked glycan (122,123), while in *B. malayi* phosphorylcholine is O-linked (116).

Other protein modifications, especially glycosylation, are likely to have a major bearing on the immunological response to filarial antigens. For example, certain strains of mice are unable to recognize *Bm*-NPA-1 (gp15/400) or *Bm*-GPX-1 (gp29) in their native, glycosylated state (124); however, immunization with nonglycosylated bacterially expressed forms of these antigens restores responsiveness to ‘nonresponder’ strains (30,125). Although glycosylation *per se* was not shown to be responsible for these observations, this work illustrated that post-translational modifications of products may profoundly influence the immune response to individual filarial antigens.

### **CONCLUSIONS**

Parasite immunology has been transformed by the molecular insights and precise tools offered from genome-led research. The filarial genes we discuss here include some of the most important players in successful parasite establishment in the immunocompetent host, but there are likely to be many more products to be discovered. Although the first generation of these filarial proteins is dominated by homologues of known gene families, for which gene function can quickly be deduced, there is bound to be an increasing emphasis on genes of unknown function, such as the filarial *alts*, particularly where they have diverged greatly from sequences in the free-living comparator, *C. elegans*. A further dimension which now needs to be assessed is how variable are different populations and strains of the filarial parasites, a question which can be asked with precision at the level of individual defined genes.

The new filarial molecular biology is likely to have a sweeping impact on research into and management of filarial disease. For example, the SPN-2 and SXP-1 proteins may provide excellent serodiagnostic tools, and additional recombinant antigens are now available to probe the pathways by which the immune response causes chronic disease in infection. For each functional protein which is characterized, a potential new drug target emerges, and with the application of structural biology to filarial proteins (14,126), the design of new drugs on a rational basis will become possible. Several of the new proteins discussed here have been suggested as candidate vaccines for filariasis. Perhaps the prospects for the ALT antigens are most promising, as their larval-specific expression is unlikely to mean that immunized patients will develop exacerbated pathogenic responses to adult worms. In addition, the ALT proteins have the advantage of being filarial-specific, reducing the risk of damaging cross-reactivity

to host or environmental antigens (127). Filariasis immunology is thus well-poised to integrate genome-led data into innovative research into one of the world's foremost parasitic diseases.

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