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Invited review

Immune evasion genes from filarial nematodes

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Abstract

Helminth parasites have large genomes (~10^8 bp) which are likely to encode a spectrum of products able to block or divert the host immune response. We have employed three parallel approaches to identify the first generation of 'immune evasion genes' from parasites such as the filarial nematode *Brugia malayi*. The first strategy is a conventional route to characterise prominent surface or secreted antigens. In this way we have identified a 15-kDa protein, which is located on the surface of both L3 and adult *B. malayi*, and secreted by these parasites in vitro, as a member of the cystatin (cysteine protease inhibitor) family. This product, *Bm*-CPI-2, blocks conventional cysteine proteases such as papain, but also the aspariginyl endopeptidase involved in the Class II antigen processing pathway in human B cells. In parallel, we identified the major T cell-stimulating antigen from the microfilarial stage as a serpin (serine protease inhibitor), *Bm*-SPN-2. Microfilariae secrete this product which blocks two key proteases of the neutrophil, a key mediator of inflammation and innate immunity. The second route involves a priori hypotheses that helminth parasites encode homologues of mammalian cytokines such as TGF-β which are members of broad, ancient metazoan gene families. We have identified two TGF-β homologues in *B. malayi*, and shown that one form (*Bm*-TGH-2) is both secreted by adult parasites in vitro and able to bind to host TGF-β receptors. Likewise, *B. malayi* expresses homologues of mammalian MIF, which are remarkably similar in both structure and function to the host protein, even though amino acid identity is only 28%. Finally, we deployed a third method of selecting critical genes, using an expression-based criterion to select abundant mRNAs taken from key points in parasite life histories. By this means, we have shown that the major transcript present in mosquito-borne infective larvae, *Bm*-ALT, is a credible vaccine candidate for use against lymphatic filariasis, while a second abundantly-expressed gene, *Bm*-VAL-1, is similar to a likely vaccine antigen being developed against hookworm parasites.

Keywords: Helminth; Cytokine; Cystatin; Serpin; Vaccine

1. Introduction

Long-lived helminth parasites are highly accomplished practitioners of immune evasion and manipulation, using strategies honed during their long co-evolutionary interaction with the mammalian immune system (Behnke et al., 1992; Maizels et al., 1993; Allen and Maizels, 1996). Multicellular helminths present very different infection dynamics from micro-parasites (viruses, bacteria and protozoa) as helminth organisms do not replicate themselves, and parasite loads increase only as a result of accumulated exposure and infection. Thus, as argued elsewhere, helminths are not equipped to outpace the immune system by faster cell division or rapid antigenic variation (Maizels et al., 1993). Rather, their strategy appears to be assimilation, defusing aggressive immune reactions and inducing forms of immunological tolerance to permit their long-term survival (Maizels and Lawrence, 1991). This feat requires helminth organisms to override the normal rules of the immune system, which is capable of rejection of tissue expressing even single-amino acid changes in antigenic profile.

Worldwide infections with helminth parasites such as the gastrointestinal nematodes *Ascaris*, *Trichuris* and *Necator*, the vector borne filarial nematodes (e.g. *Brugia*), and the blood flukes (trematodes) *Schistosoma* afflict over 2 billion people (Crompton, 1999). The enormous burden this parasitic load places on the human population, particularly in economically disadvantaged communities, has yet to be matched by new advances in drug and vaccine development. Moreover, it is likely that by understanding the mechanisms constructed by parasites to elude immune rejection, we can design new techniques for managing the human immune

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response in many other crucial areas such as transplantation, allergy, autoimmunity and tumour immunology.

Within the large and diverse group of helminths, we will focus in this review on immune evasion by filarial nematodes, which infest the lymphatic system of mammals. The filarial worms live in a milieu bathed in immune effector cells, antibodies and other immune molecules, and yet can survive for many years. As well as being artful immunologists, these parasites also infect more than 100 million people in tropical countries today (Michael et al., 1996). Direct fatality is uncommon, but the scale of these infections combined with the widespread morbidity and incapacitating disease cannot be underestimated (Rajan and Gundlapalli, 1997).

A consistent picture of immune down-regulation has now been established in human filariasis (Maizels and Lawrence, 1991; King et al., 1992; Ottesen, 1992; Maizels et al., 1999, 2000). Peripheral blood T cell populations show severely impaired antigen-specific proliferative responses, as well as reduced production of the type-1 cytokine IFN-γ (Piessens et al., 1980; Hitch et al., 1991; Yazdanbakhsh et al., 1993). The depression in human filariasis cannot be interpreted as a simple Th2 bias, because Th2-like T cells produce IL-4 but not IL-5, and are also subject to proliferative block (Sartono et al., 1997). The humoral immune response is similarly skewed, rather than ablated in its entirety. Most antibody is found in the IgG4 isotype, normally less than 5% of serum immunoglobulin (Hussain et al., 1987; Maizels et al., 1995). This pattern of immune deviation or selective suppression can also be found in animal models: for example, *Brugia* infected dogs show local suppression in the infected lymph nodes (Schreuer and Hammerberg 1993), while mice transplanted with *Brugia* adults generate a suppressive macrophage population which both blocks proliferation (Allen et al., 1996; Allen and MacDonald 1998; MacDonald et al., 1998; Loke et al., 2000a,b) and favours the expression of a Th2 phenotype (Loke et al., 2000a,b).

The life cycle of filarial parasites encompasses five major stages, delineated by four complete moults of the cuticle (Scott, 2000). Two of these moults occur in the arthropod vector, which takes up blood microfilariae (or L1) and permits rapid development of parasites to the L3 or infective larval stage. Once L3s gain access to the mammalian host, they continue developing over many weeks and through two further moults into dioecious adult worms. After mating, the females release large numbers of microfilariae which continue the cycle when ingested in the vector blood meal. Each of the major life cycle stages (microfilariae, L3 and adults) has unique immunological characteristics. For example, chronic pathology in lymphatic filariasis is associated with heightened immune reactivity to adult stages. For this reason, a new vaccine should present antigens not expressed by adult worms, thereby excluding any exacerbation of disease by vaccine-induced immune responses (Maizels et al., 1999, 2000).

2. Identification of immune evasion genes

We have developed three parallel approaches to identify novel 'immune evasion genes' from filarial nematodes (Table 1). Firstly, we employ a conventional, protein-led route of characterising major surface or secreted antigens using biochemical or immunological approaches but greatly facilitated by advances in filarial genomics. Secondly, we use a priori hypotheses that nematode parasites encode homologues of critical immune system genes, in a genome-led approach involving database searching and PCR. Finally, our third method is essentially mRNA-led, selecting genes on an expression-based criterion to represent the most abundant mRNAs at key points in parasite life histories.

The first focus for protein-led molecular studies on filarial parasites was the suite of molecules exposed on the surface of adult worms and microfilariae, where antigens might act as targets for host immunity and where parasites may express proteins essential for their survival in the host. Of course, not all such surface proteins act to promote immune evasion, but one important group which may fall into this category are the anti-oxidant products glutathione peroxidase and superoxide dismutase. *Bm*-GPX-1 is the major 29 kDa surface glycoprotein of adult *Brugia* (Maizels et al., 1989; Cookson et al., 1992), which is believed to act as a lipid hydroperoxidase, protecting parasite membranes from peroxidation caused by free oxygen radicals (Tang et al., 1996). A minor surface-associated protein of similar mol.wt. is a superoxide dismutase, allowing the parasite to detoxify superoxide radicals (Tang et al., 1994). In addition, a 15-kDa protein linked to the surface of both L3 and adult worms has more recently been identified as a cystatin, *Bm*-CPI-2, as described below.

Many other surface-associated molecules may contribute to immune escape in a less obvious manner. For example, the polyprotein antigen (variously named gp15/400 or *Bm*-NPA-1) has a very high affinity for fatty acids (Kennedy et al., 1995) which could sequester substrate required for host leukotriene synthesis. Non-protein filarial products are also

Table 1
Three approaches for discovery of potential immune evasion genes from filarial nematodes

Approach	Method	Examples
Immunological profile	Purification of surface/secreted proteins and other prominent antigens	*Bm*-CPI-1 and -2 *Bm*-SPN-2
Homology	PCR and database searching	*Bm*-MIF-1 and -2 *Bm*-TGH-1 and -2
Abundance	Select genes highly expressed, particularly if absent from *C.elegans*	*Bm*-ALT-1 and -2 *Bm*-VAL-1

likely to play a significant role: a novel lipid found in the cuticle of *B. malayi* acts as a sink absorbing oxidative attack, perhaps protecting essential membrane lipids and proteins from degradation (Smith et al., 1998). A further component prominently expressed by *B. malayi* is phosphorylcholine (Maizels et al., 1987). Not only has this been suggested as an immunosuppressive moiety in lymphatic filariasis (Lal et al., 1990), but it has been possible to demonstrate direct down-regulation of both B (Deehan et al., 1998) and T cell (Harnett et al., 1998) function by a PC-bearing protein secreted from the filarial parasite *Acanthocheilonema viteae*. Thus, this article summarises our knowledge of only the first filarial products to be implicated in immune modulation, and these will surely be followed by an increasingly diverse and fascinating spectrum of molecules.

2.1. Cystatins

Cystatins are a widely-distributed family of cysteine protease inhibitors which play essential roles in a spectrum of physiological processes (Barrett, 1987). A number of filarial cystatins have now been characterised, as summarised in Fig. 1. Most detail is available on two homologues from *B. malayi* (Gregory and Maizels, unpublished observations). One, *Bm*-CPI-1, is selectively expressed by late L2 and L3 in the mosquito vector, but is lost within 2 days' infection of the mammalian host. The second, *Bm*-CPI-2, is constitutively expressed around the parasite life cycle (Gregory and Maizels, unpublished observations). Both are located on the surface of the *B. malayi* L3, while *Bm*-CPI-2 is also a significant adult surface antigen, as demonstrated by surface labelling techniques. Moreover, *Bm*-CPI-2 is secreted by both L3 and adult stages of the parasite.

Sequence analysis reveals both common and unexpected features of the filarial cystatins. Compared with established families of cystatins, which contain either zero or four cysteine residues (Abrahamson, 1994), the nematode proteins represent an intermediate, two-cysteine form. Like all cystatins, there is a consensus motif (QxVaG, as in QVVAG, 93–97 of the *Bm*-CPI-2 sequence) associated with binding to papain-like enzymes (Fig. 1), and in all except *Bm*-CPI-1 a conserved glycine residue near the N-terminus (49 in *Bm*-CPI-2). Since both *Bm*-CPI-1 and –2 have been confirmed as functional inhibitors of papain, this demonstrates that the N-terminal glycine is not an essential requirement for activity.

Bm-CPI-2 also possesses a novel motif (SND, amino acids 76–78) previously found only in certain mammalian cystatins. In these mammalian cystatins, the SND motif is required to inhibit a distinct class of protease, legumain, or asparaginyl endopeptidase, which cleaves at asparaginyl residues (Alvarez-Fernandez et al., 1999). Significantly, we found that *Bm*-CPI-2 can inhibit both papain-like and legumain-like enzymes; (Gregory and Maizels, unpublished observations; Manoury et al., 2001). Similar studies have yet to be conducted with *C. elegans* cystatins, which do not contain the precise SND sequence.

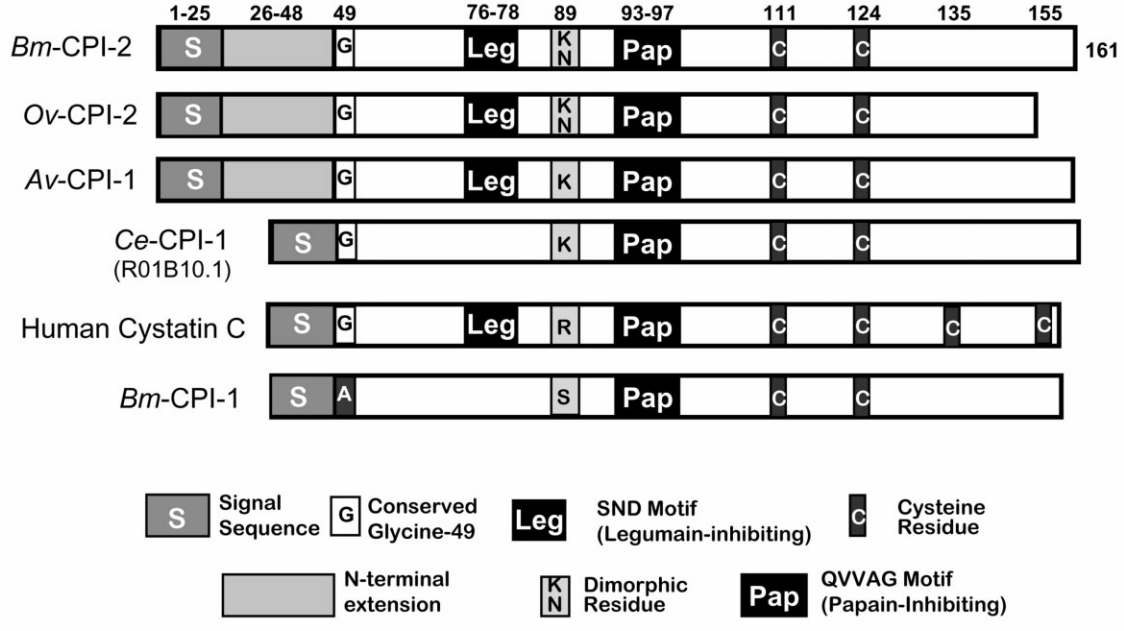

Fig. 1. Schematic of the Cystatin gene family in filarial parasites. Cystatin sequences are shown, numbered according to the *Bm*-CPI-2 sequence. Signal sequences are shown in shaded boxes, and the N-terminal extension (amino acids 26–48) present in *Bm*-CPI-2 and its orthologues is shown in hatched boxes. The legumain (Leg) and papain (Pap) inhibition sites are solid boxed where they are found. Accession Numbers are: *Bm*-CPI-1, U80972; *Bm*-CPI-2 (AF015263); *Ov*-CPI-2/onchocystatin (P22085), *Av*-CPI-1/Av-17, L43053; *C. elegans*. R01B10.1, AF068718. The site of the lysine/asparagine dimorphism at residue 89 is depicted (K/N) in the two species for which this has been observed. The positions of the two cysteine residues in all filarial cystatins are shown, as are the four cysteines in mammalian cystatin C.

Legumain-like asparaginyl endopeptidases are known to be important in intracellular processing of antigens for presentation with MHC class II (Manoury et al., 1998). We therefore tested whether *Bm*-CPI-2 can block class II-mediated antigen processing, using a model system in which human B cells process and present peptides from tetanus toxin, to defined T cell clones. We found complete inhibition of presentation of some epitopes, while others remained unaffected by the presence of the filarial cystatin, demonstrating that *Bm*-CPI-2 targets one of a set of alternative processing enzymes in the human lymphocyte (Manoury et al., 2001).

Two other important features of *Bm*-CPI-2 should be noted. First, there is a 23-amino acid insertion at the N-terminus of the protein, absent from mammalian cystatins and also not found in homologues from the free-living nematode *C. elegans*. While this insert has no known function, it is tempting to suggest that it contains a motif facilitating entry into mammalian antigen-presenting cells, as would be necessary if it is to fufill a role of interfering with the class II pathway. Secondly, *Bm*-CPI-2 presents one of the first coding polymorphisms reported for a filarial protein: a lysine/asparagine dimorphism at residue 89. The same dimorphism can be found in ESTs of *Ov*-CPI-2 (onchocystatin), indicating a variation that may be retained for functional reasons.

2.2. The microfilarial serpin (SPN-2)

One of the major antigens from the microfilarial stage was identified on the basis of strong immunological stimulation of murine T cells. Intrigued by the ability of microfilariae, unlike nearly every other helminth organism, to drive Th1 responses (Lawrence et al., 1994), we set out to purify the most active T cell-stimulating antigens from this stage of *B. malayi*. The most stimulatory fraction, taken from preparative SDS-PAGE, was used to raise polyclonal antiserum, in order to screen a microfilariae cDNA library (Zang et al., 1999; Zang et al., 2000). The clone isolated, later assigned as *Bm-spn-2*, was used to express protein which mimicked whole microfilariae extract in eliciting Th1-type recall responses from microfilariae-primed mouse T cells (Zang et al., 2000).

Our information on the *Brugia* serpins is summarised in Fig. 2. Several independent indications exist that *Bm-spn-2* is strictly microfilaria-specific in its expression. First, PCR from six different cDNA libraries with *Bm-spn-2*-specific primers demonstrated that the message for this gene was present only in the microfilariae. Second, the *Bm*-SPN-2 protein was detected only in microfilariae extracts. Third, a survey of some 18 000 ESTs deposited from *B. malayi*cDNA libraries showed that every *Bm-spn-2* clone is derived from the microfilariae stage, bar one from an adult female library which includes cDNA from developing microfilariae *in utero*.

The serpins are an extensive gene family, with no fewer than eight members in *C. elegans* and at least two in *B. malayi* (Pastrana et al., 1998; Whisstock et al., 1999; Zang and Maizels, 2001). Interestingly, none of the *C. elegans* serpins are predicted to possess a signal sequence, indicating that the parasitic species has adapted these genes to fulfill an extracellular function. The two *B. malayi* serpins share only 27.8% identity (110/395) at the amino acid level, although both serpin 'motif' and 'signature' sequences are conserved. These sequences flank the functional inhibitory loop which binds to target serine proteases, and this active domain is extremely variable across the whole gene family, presumably reflecting a diversity of complementary serine proteases (Zang and Maizels, 2001). In contrast to the microfilaria-specific expression of *Bm-spn-2*, *Bm-spn-1* is preferentially produced by the L3 stage (Pastrana et al., 1998), and indeed is one of the most abundant mRNA species present in *B. malayi* L3 (Allen et al., 2000).

The *Bm*-SPN-2 was tested against a panel of serine proteases, with no observed reactivity against well-known enzymes such as trypsin, chymotyrpsin or pancreatic elastase. However, the parasite protein is a potent inhibitor of two neutrophil enzymes, cathepsin G and neutrophil elastase. There is an intriguing correlation between exclusive expression of *Bm*-SPN-2 by the only blood-dwelling filarial stage, and the ability of *Bm*-SPN-2 to block products of the most abundant nucleated cell type in the blood, the neutrophil (Zang et al., 1999; Zang and Maizels, 2001).

2.3. Cytokine homologues

Two sets of cytokine homologues from *B. malayi* are related to the Transforming Growth Factor-β (TGF-β) and the macrophage migration inhibitory factor (MIF) gene families. The *Brugia* TGF-β (*Bm*-TGH-1 and -2) and MIF (*Bm*-MIF-1 and -2) homologues are members of ancient gene families conserved across metazoan organisms, bearing 28–42% amino acid identity between filarial and human proteins.

The TGF-β gene superfamily encompasses many proteins conducting both developmental and immunological processes (Massagué et al., 1994). In the context of a helminth infection, the most intriguing role for a parasite TGF-β would be the down-regulation of host inflammatory responses, such as ablation of nitric oxide synthesis. To accomplish this, a parasite TGF-β homologue would need to ligate host TGF-β receptors. At a sequence level, *Bm*-TGH-1 is more closely related to tissue differentiation-inducing molecules, while *Bm*-TGH-2 is more similar to *C. elegans daf-7* and to the mammalian TGF-β cytokines (Fig. 3). Moreover, *Bm*-TGH-2 is expressed by arrested microfilariae as well as adult *Brugia*, is secreted in vitro (Fig. 4), and binds to host TGF-β receptors. In addition, the TGF-β receptor (TGF-βR) family, containing type I and II receptors, is also shared between mammals and

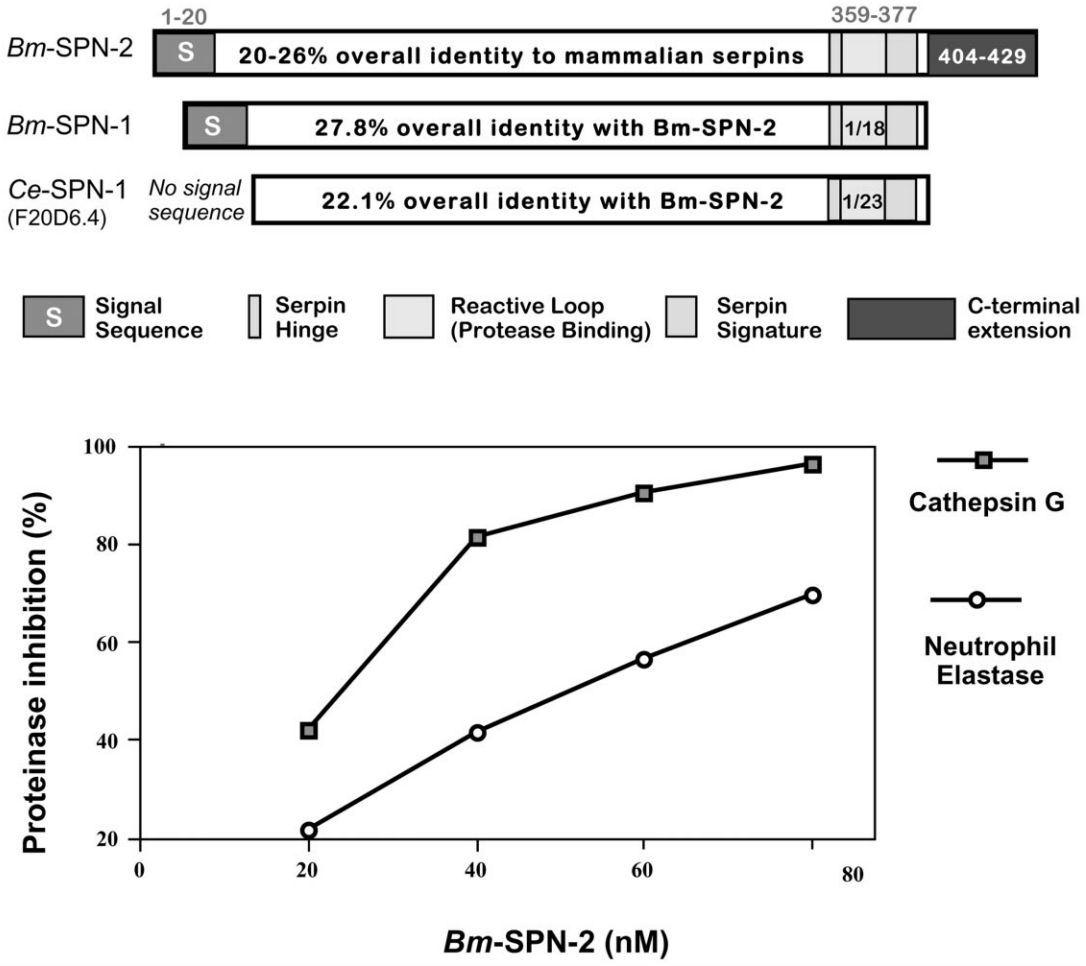

Fig. 2. Schematic of *Brugia* serpins and comparison with predicted sequence of a *C. elegans* serpin. Upper Panel: Serpin sequences are numbered according to the *Bm*-SPN-2 sequence. Signal sequences are shown in shaded boxes at the N-terminus. The conserved serpin hinge and signature regions are dark shaded, eithe side of a highly variable reactive loop (amino acids 359–377). Amino acid identites with *Bm*-SPN-2 are shown for this loop. The C-terminal extension unique to *Bm*-SPN-2 is shown in a black box. Lower Panel: Inhibition of elastase and cathepsin G by recombinant *Bm*-SPN-2 protein (from Zang et al., 1999).

helminths (Gomez-Escobar et al., 1997; Davies et al., 1998). Although only type I receptors have been isolated from *Brugia* and *Schistosoma*, the presence of type II receptors in *C. elegans* (e.g. *daf-4*) suggests that helminth parasites will also express two-component TGF-β receptors.

The MIF gene family is likewise derived from an ancient metazoan ancestor, but in mammalian organisms these genes are associated with a largely pro-inflammatory cytokine. Mammalian MIF has many enigmatic features, including secretion in the absence of a signal sequence, the lack of any obvious receptor for this cytokine, and an unusual enzymatic activity dependent upon a proline at residue 2.

Two distinct MIF homologues have been found for *B. malayi* (Pastrana et al., 1998; Zang, Scott and Maizels, unpublished observations). The two *Brugia* MIF molecules are only 27% identical to each other, but both 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) previously described for human MIF. We have also determined the crystal structure of *Bm*-MIF-2, which despite being only 28% identical to human MIF, presents a highly similar 3-dimensional structure including trimerisation around an intriguing central channel.

These findings raise 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 densensitization or by stimulating macrophages beyond the short-term acute time period which has generally been examined. 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 (Allen et al., 1996; MacDonald et al., 1998; Loke et al., 2000a,b)

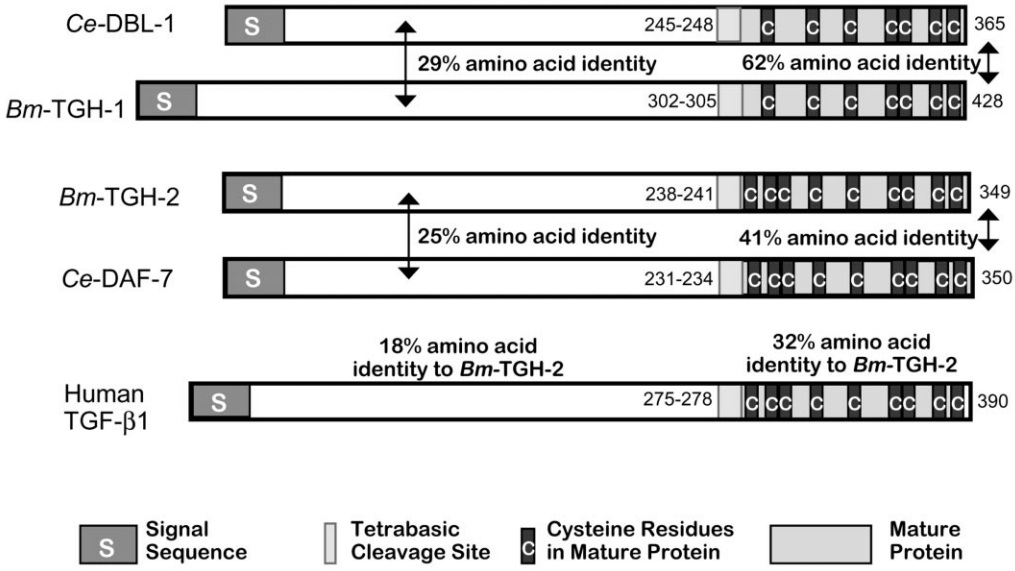

Fig. 3. Schematic of *Brugia* and *C. elegans* transforming growth factor β–homologues. All members of the TGF-β family are produced as secreted pre-proteins with a pro-segment of 250–300 amino acids and a mature C-terminal domain of 100–110 residues. This schematic shows the relationships between *Bm*-TGH-1, TGH-2 and their closest relatives from *C. elegans*, *ceg-1* and *daf-7* respectively, as well as the human homologue, TGF-β1. In each case, signal sequences are shown in dark shaded boxes, pro-peptides in open boxes, and the mature polypeptides in light shaded boxes. The tetrabasic cleavage site (typically KKRK) is numbered for each protein and depicted by horizontal stripes. The positions of the seven or nine cysteine residues in the mature protein are also shown: note that *Bm*-TGH-2, *Ce*-DAF-7 and human TGF–β1 share an additional two cysteine residues compared with other members of the superfamily.

2.4. Abundantly expressed genes: the filarial alt (abundant larval transcript) family

An alternative strategy to identify gene products critical to parasite establishment in the host is to focus on the most-highly expressed genes within the life cycle stages associated with primary invasion, the L3 or infective larvae (Gregory et al., 1997; Tetteh et al., 1999). Our original method was to amplify mRNA from *B. malayi* L3 with the 5′ spliced leader sequence found on a proportion of mRNAs across the nematode phylum (Gems et al., 1995; Blaxter and Liu, 1996). With the advent of the Filarial Genome Project, a more comprehensive approach became possible, ranking ESTs by their frequency of appearance in a randomly selected set of clones, based on a large dataset (1000–2000 per stage) and independent of the SL sequence (Allen et al., 2000).

The SL-directed technique highlighted a set of genes now designated *alt* (abundant larval transcript), and this assignation has been supported by an analysis of filarial ESTs in which ALT-1 and ALT-2 together represent some 5% of the L3 cDNA (Allen et al., 2000). Even if this figure is discounted to allow for bias towards shorter sequences, and acknowledging that post-transcriptional events may control translation, there can be little doubt that the ALT products are the most conspicuous of all L3 proteins.

Structurally, *Bm*-ALT-1 and -2 are very similar small polypeptides of 125–128 amino acids, with 79% overall identity to each other (Fig. 5). Amino acid differences are concentrated in one 25–28 amino acid acidic tract near the N-terminus (Gregory et al., 2000). Interest in the ALT

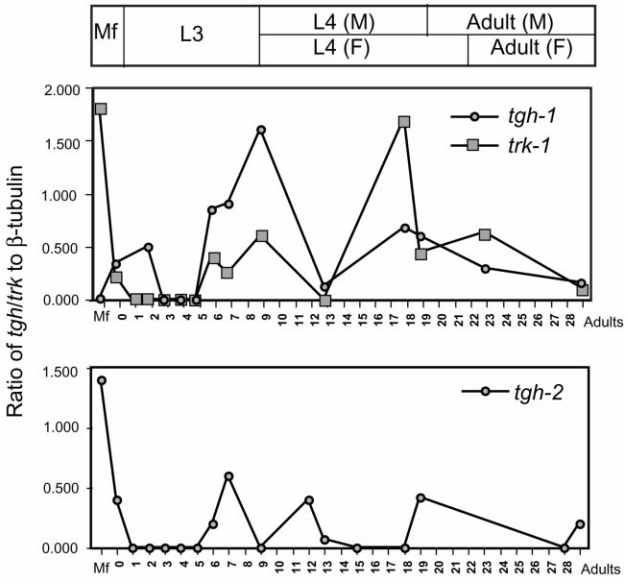

Fig. 4. Expression of *Brugia* tgh genes through the parasite life cycle. First-strand cDNA was prepared from *B. malayi* parasites recovered from jirds at intervals following infection with mosquito-borne L3 from day 1 to 23, as well as from microfilariae, L3 direct from mosquitoes, and mature adult worms. PCR reactions were set up to measure the ratio of specific transcript to that of the constitutively expressed β-tubulin. Both tgh genes (*Bm-tgh-1* and *tgh-2*) were analysed, as well as the gene for a TGF-β receptor homologue, *Bm-trk-1*. This figure is redrawn from data presented previously (Gomez-Escobar et al., 1997; Gomez-Escobar et al., 1998; Gomez-Escobar et al., 2000).

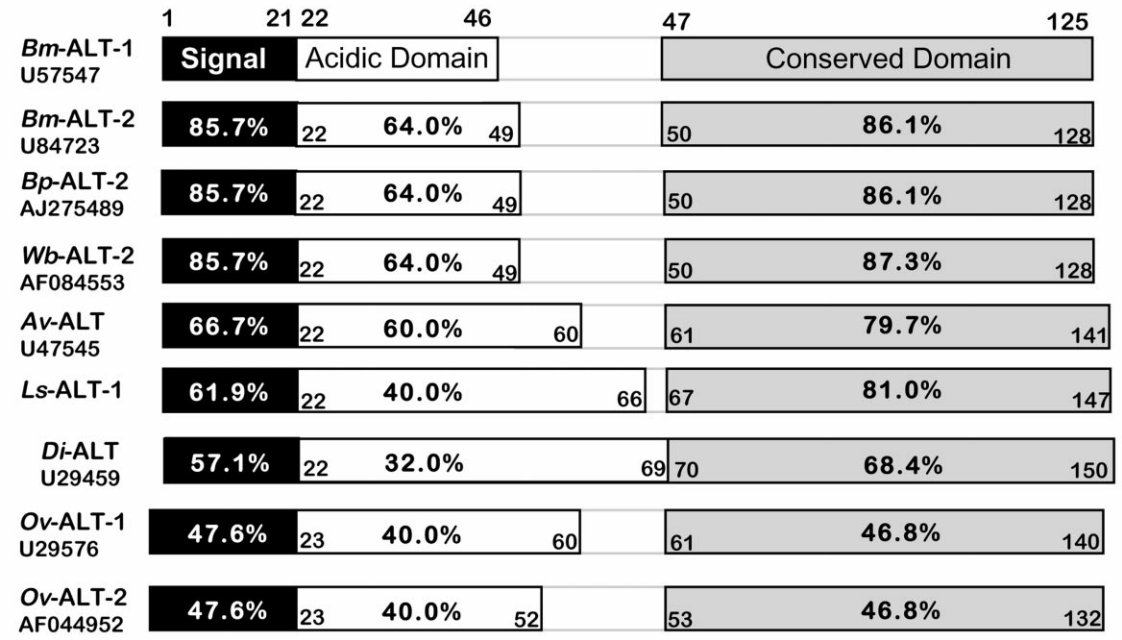

Fig. 5. Schematic of filarial ALT genes. A schematic of the filarial ANT protein family. Percentage identity to *Bm*-ALT-1 is given for each domain. For the variable acidic domain, different values for identity can be calculated depending on the number of gaps allowed, and on the length of the reference sequence. Homologues with similar levels of identity to *Bm*-ALT-1 are not necessarily identical to each other (e.g. *Bm*-ALT-2 and *Bp*-ALT-2 or *Ov*-ALT-1 and *Ov*-ALT-2). Accession numbers are for the first deposited sequence, although database entries may not be identified as ALT-encoding genes (e.g. Av-ALT, *Di*-ALT). The only ALT sequence from *W. bancrofti* is more similar to *Bm*-ALT-2 than *Bm*-ALT-1 and hence has been named *Wb*-ALT-2. The sequence for *Lc*-ALT-1 is from Allen et al. (2000). The predicted signal cleavage site is between residues 18/19, but for clarity the signal peptide is depicted as extending to residue 21.

products is intensified by parallel findings across a range of filarial parasites with different host specificities. In fact, the first members of this gene family (Di20/22L, referred to here as *Di*-ALT) were discovered in the dog filarial parasite *Dirofilaria immitis*, through analysis of L3 ES and reactivity with immune dog sera (Frank and Grieve, 1991; Frank and Grieve, 1995; Frank et al., 1995). *Di*-ALT is found in *D. immitis* L3 ES, and released during the L3/L4 moult. Homologues are also known in *Onchocerca volvulus* in which no less than 4.6% of *O. volvulus* L3 mRNA corresponds to *alt* transcripts, and the protein is partially protective in mouse chamber experiments (Joseph et al., 1998). Again, the divergence between ALT homologues within the filariae is surprising (Fig. 5): *Ov*-ALT-1 (Joseph et al., 1998) shows only 46% identity to *Bm*-ALT-1 and 45% to *Bm*-ALT-2 over the mature polypeptide sequence. Additional *alt* homologues have also been cloned from other filarial species. The ALT protein from *W. bancrofti* differs at 13% of amino acids from *B. malayi* ALT-2, the highest divergence yet observed for a pair of *Brugia/Wuchereria* homologues. Members of the same gene family have also been reported from *A. viteae* (Av18, *Av*-ALT) and *Litomosoides sigmodontis* (Allen et al., 2000). There is a distantly related gene in *C. elegans*, although the sequence similarity is very low, and it is not expressed at a particularly high level (Gregory et al., 2000).

The ALT protein may be stockpiled by infective larvae for release in the mammalian host. This supposition is supported by immunoelectron microscopy in which anti-ALT-1 antibodies stain the glandular oesophagus in both *Onchocerca* (Joseph et al., 1998) and *Brugia* (A.E. Bianco, unpublished) L3, and by in vitro culture experiments showing a high level of secretion of *Bm*-ALT-1 by *B. malayi* L3 (J. Murray, unpublished). Significantly, by day-1 p.i., this staining pattern is lost and the granules have dispersed (Joseph et al., 1998).

In view of these data indicating unusually high expression in preparation for entry into the mammalian host, we tested *Bm*-ALT-1 in a jird (*Meriones unguiculatus*) model of vaccination against *B. malayi* L3 challenge. We found that immunisation with *Bm*-ALT-1 engendered 76% reduction in worm load, a higher level than recorded with any other filarial recombinant antigen to date (Gregory et al., 2000). The prospects for an ALT-based vaccine against filariasis are further encouraged by the larval-specific expression of this antigen, indicating that heightened reactivity to ALT is unlikely to exacerbate pathogenic responses to adult worms that might initiate lymphatic damage. Moreover, the filarial-specific nature of the structure renders any undesirable cross-reactivity with host or environmental antigens quite unlikely.

2.5. The venom allergen Ancylostoma secreted protein-like (VAL) family

Another of the most abundant transcripts from *B. malayi*

L3 is related to a gene family first identified in the hookworm *Ancylostoma caninum* (Hawdon et al., 1996), coding for the *Ancylostoma* secreted protein (ASP) antigen. This protein, now named ASP-1, is secreted by *Ancylostoma* larvae when stimulated to commence invasion of the host. Because of its prominence in infection, ASP-1 is now being tested as vaccine antigens against canine hookworm (Ghosh et al., 1996; Sen et al., 2000).

Homologous genes have now been reported not only in other hookworm species (Bin et al., 1999; Blaxter, 2000), but in strongylid (Schallig et al., 1997; Rehman and Jasmer, 1998) and ascarid (Tetteh et al., 1999) parasites. All known members of the gene family have signal sequences, and several have been confirmed as encoding secreted proteins. Smaller (short-form) homologues represent a single conserved domain, and large form relatives contain a duplication of the ASP domain. Some species, including *A. caninum*, have both forms encoded in separate genes (Hawdon et al., 1999). No fewer than 17 homologues exist in the *C. elegans* genome, all but one of which are single-domain sequences (Hawdon et al., 1999; Daub et al., 2000).

We have cloned the *B. malayi* homologue, which encodes a single ASP homology domain. A near-identical sequence has been deposited as venom allergen homologue, and as the *asp* gene name in *C. elegans* is assigned to an aspartic protease (Tcherepanova et al., 2000), we refer to the filarial gene as *Bm-val-1*.

The biological function of the whole ASP/VAL gene family remains enigmatic, but there are hints of activity at the level of mammalian cell activation. A distant relative (NIF), also from *A. caninum*, has been found to inhibit neutrophil activation (Moyle et al., 1994), and two forms from *O. volvulus* induce angiogenesis in mice (Tawe et al., 2000). If parasite VAL proteins have evolved to act on mammalian ligands, it would be expected that *C. elegans* homologues do not show similar properties. Whether the filarial VAL family prove to be active 'immune evasion' products remains to be determined, while parallel studies are under way to test their effectiveness as vaccine antigens in eliciting protective immunity.

3. Conclusion

The first steps have been taken to understanding the molecular basis of immune evasion by filarial nematodes. The sophistication of mammalian innate and adaptive immune systems, and the long co-evolutionary relationship between host and parasite, both imply that considerable number of molecular interactions are in play. Thus, *Bm*-CPI-2 appears to target a single specialised enzyme within the MHC class II processing pathway, thereby ablating some but not all class-II-dependent antigen presentation. This example illustrates the precision with which each parasite product is likely to selectively block a particular component of host immunity.

Can we generalise from such examples, and define a common evasion strategy for parasitic helminths? Probably not, because specific ploys may have evolved uniquely in separate parasite lineages, with each successful pathogen expressing its own formula for circumventing host immunity. For example, the ascarid nematode *Toxocara canis* secretes large quantities of a C-type lectin thought to compete with host innate immune system receptors (Loukas et al., 1999); yet not a single member of this gene family can be found in >20 000 *B. malayi* ESTs (Loukas and Maizels, 2000). Conversely, the cysteine and serine protease inhibitors which potently inhibit host immune system enzymes appear, on an admittedly smaller sample, to be absent outside the filarial parasite group. In this context, the rapidly growing information on immune evasion genes from all categories of pathogen will be increasingly intriguing and instructive.

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