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Review

Helminth immunoregulation: The role of parasite secreted proteins in modulating host immunity

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ABSTRACT

Helminths are masterful immunoregulators. A characteristic feature of helminth infection is a Th2-dominated immune response, but stimulation of immunoregulatory cell populations, such as regulatory T cells and alternatively activated macrophages, is equally common. Typically, Th1/17 immunity is blocked and productive effector responses are muted, allowing survival of the parasite in a "modified Th2" environment. Drug treatment to clear the worms reverses the immunoregulatory effects, indicating that a state of active suppression is maintained by the parasite. Hence, research has focussed on "excretory–secretory" products released by live parasites, which can interfere with every aspect of host immunity from initial recognition to end-stage effector mechanisms. In this review, we survey our knowledge of helminth secreted molecules, and summarise current understanding of the growing number of individual helminth mediators that have been shown to target key receptors or pathways in the mammalian immune system.

Contents

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1. Immune modulation during helminth infection

The capacity of helminth parasites to modulate the immune system underpins their longevity in the mammalian host [1,2]. There is consequently intense interest in understanding the molecular basis of helminth immunomodulation [3,4]. The remarkable range of parasite life histories, transmission strategies, and physiological niches, is reflected in the variety of immunomodulatory activities observed across the three taxonomic categories (nematodes, cestodes, and trematodes) that comprise the helminth grouping [5–9]. However, general patterns have emerged, revealing the ways in which helminths can dampen host immunity, and how immunopathology may result from a dysregulated response to infection [10]. For instance, both schistosome (for example, *Schistosoma mansoni*) and filarial (e.g. *Brugia malayi*) infections result in antigen-specific unresponsiveness in the peripheral T cell populations of heavily infected patients [11–13]. Moreover, helminth infection is associated with diminished reactivity to bystander allergens and autoantigens, both in model systems [8,14] and in human studies [15,16].

A key feature is that helminth immune suppression is dependent on live parasites, as shown *in vivo* by the recovery of responsiveness following curative chemotherapy [17], as well as by the regulatory effects of live parasites *in vitro* [18]. Hence, there is a particular focus on mediators released by live parasites and the analysis of how these products, in total and as individual components, may be responsible for the noted ability of helminths to redirect the host immune system.

2. Helminth secreted products: the rationale

Mechanistically, parasite modulation of the immune system is most likely to be effected through the release of soluble mediators which ligate, degrade or otherwise interact with host immune cells and molecules [19]. Modulation may also occur through the release (and death of some proportion) of transmission stages such as the eggs of schistosomes or the newborn microfilarial larvae of filarial parasites. In tissue-dwelling parasites, important engagements also occur at the surface of the helminth itself. Much of the earlier literature on immunological effects of helminth products depended on crude extracts (such as SEA schistosome egg antigen), although the degree to which the host is exposed to constituent molecules was uncertain. While both somatically derived and secreted products are known to have immunological activity [4], the secreted helminth modulators are those most likely to be physiological actors at the interface between live parasites and the host, and these are the subject of this review.

"Excretory/secretory" (ES) is inevitably a working definition, with an imprecise line between products actively exported through secretory pathways and those which may diffuse or leak from the parasite soma. *In vivo*, "secreted" antigens will include digestive enzymes emanating from the intestine of adult worms, as well as uterine contents which female worms release along with transmission stage eggs or larvae. However, parasites may well have adapted such "secretions" to fulfill a new role in the host, once they are released from their primary locale within the worm. Hence, it is rational to analyse all ES products without prejudice as to their physiological origin, and subject them to a full range of biochemical, immunological and proteomic analyses.

Biochemical analyses have primarily concerned enzymatic activities in helminth ES, such as the proteases ranging in activity from parasite invasion [20] to degradation of host chemokines [21]. Where enzymes (also including antioxidants, acetylcholinesterases and platelet activating factor hydrolase) act in an immunological context, these are detailed further in Section 4.7 below. Immunological assays of ES have included the induction of Th2 responsiveness, leading in the case of *S. mansoni* to the products described in Section 4.1. An alternative, transcriptomic-based, avenue led to identifying ES products which are encoded by abundant mRNA species (e.g. filarial ALT proteins [22], see Section 4.9 below). More recently, with the development of helminth genomics, systematic proteomic analyses of many major helminth ES products have become possible (Table 1). These studies revealed a common set of proteins secreted by helminths, including proteases, protease inhibitors, venom allergen homologues, glycolytic enzymes and lectins. However, the relative abundance of each of these varied between different parasites and individual life cycle stage, reflecting the range of sites of parasitism.

Available parasitic helminth genomes encode >10,000 genes [23], a figure supported by independent transcriptomic analyses [24,25]. Bioinformatic approaches to predict secreted proteins on the basis of signal peptide sequences [26,27] have some merit, but in a metazoan not all secretory proteins will be exported from the organism, and proteomic data show a surprisingly large proportion of ES proteins are not encoded with a signal peptide [28–30]; hence empirical proteomic studies remain essential. Although ES products will only represent a fraction of the full genomic complement, determining the function of several hundred secreted proteins is a formidable task involving cloning and recombinant expression, as well as the production of neutralising antibodies.

Several other caveats about our current technologies should be borne in mind. While proteomic analysis can reveal the composition of helminth secretions and the relative abundance of each protein, it gives no information on the non-protein components (e.g. carbohydrates [31,32]), and post-translational modifications are not easily ascertained. Secondly, not all secreted products are macromolecules: filarial parasites secrete prostacyclin and prostaglandin for example [33], and schistosome eggs release free glycans [34]. Thirdly, while proteomic techniques allow unbiased identification of the more abundant ES proteins (Fig. 1), they may still miss those expressed at low, but bioactive, levels [29,30,35]. Even with these reservations in mind, however, it is clear that a rich and fascinating set of parasite modulators have already been discovered.

In the following sections, we briefly summarise in Section 3 the molecular and immunological information available on the secreted products from each major helminth species, before discussing in Section 4 the key individual molecular mediators now identified from the ES products of these parasites.

3. Functional and molecular analyses of helminth products

3.1. Trematodes: S. mansoni and Fasciola hepatica

Schistosome infections commence when cercariae of this trematode penetrate the vertebrate skin, transforming into schistosomula larvae in the process. Schistosomulae migrate to the lung, mature as adults in the vasculature, and produce eggs which exit through the intestine. Each of these stages is implicated in immune modulation. Larval secretions are also highly immunogenic vaccine targets as passive immunisation with antisera to ES confers around 50% protection against challenge infection [36]. The same skin-stage schistosome ES directs DCs to drive Th2 responses *in vivo* [37]. This ES contains abundant proteases, including several elastases that facilitate parasite skin penetration [38], and can cleave host IgE antibodies [39]. The presence of multiple isoforms of cercarial elastase and a metalloprotease was confirmed by proteomics of cultured parasites [40,41], and by proteomic analysis of human skin traversed by invading cercariae [42]. Additionally, skin-stage parasites were shown to secrete a number of glycolytic enzymes, such as triose phosphate isomerase, GADPH, aldolase and enolase, as well as several homologues of the venom allergen-like (VAL) family, as discussed in Section 4.8. Cercarial ES also contains the

Table 1
Proteomic analyses of helminth secretions.

Species	Stage/niche	Proteins identified	Prominent proteins	Reference	Notes
Ancylostoma caninum	Adult/duodenum	105	ASPs (VALs)	[70]	Over 30 different VAL homologues present
			C-type lectins and galectins, proteases		
Brugia malayi	Adult, male and female/lymphatics	80	Triose phosphate isomerase	[29,30]	GlcNAcT, but not LAP, bears PC
		193	Galectin, GlcNAcT LAP, NPA, MIF-1		
	Microfilaria/blood	76	Serpin-2 PEBP, Bm-R1	[30]	
Haemonchus contortus	Adult/abomosum	107	VALs, proteases, gut proteins	[78]	Multiple VALs
Heligmosomoides polygyrus	Adult/duodenum	44	VALs, proteases, NPA, acetylcholinesterase	Harcus unpublished[a]	Multiple VALs
Nippostrongylus brasiliensis	Adult/duodenum	3	VALs, globin	Harcus unpublished[a]	
Ostertagia ostertagi	Adult/abomosum	2	VALs	[80]	
Schistosoma mansoni	Larva (schistosomula)/skin and lung	16	Cercarial elastase	[40-42]	
		82	Metalloproteinase VALs, Sm16		
	Adult, gut contents/blood	8	Antioxidants, cystatin FABP, immunophilin	[167]	Gut contents likely to be released as "ES"
	Egg/GI tract	188	IPSE (alpha-1), omega-1 VALs, aldolase, enolase	[28]	
Teladorsagia circumcincta	Larva (L3/L4) and adult/abomosum	15 larval	VALs, proteases, TPX	[81]	
		13 adult		[168]	
Toxocara canis	Larva (L2)/tissues	8	Mucins, C-type lectins, PEBP	[88]	
				Harcus unpublished[a]	
Trichinella spiralis	Muscle-stage (L1) larva	43	Cystatin, 5′ nucleotidase Galectin, proteases	[97]	
Stages or species not parasitic to vertebrates					
Fasciola hepatica	Mollusc-dwelling larva	8	Antioxidants (SOD, TRX)	[47]	
Meloidogyne incognita	Plant parasitic	486	Heat shock proteins	[51]	Interesting overlap with *B. malayi* ES
			Glycolytic enzymes		
Schistosoma mansoni	Sporocyst (snail dwelling)	7	Antioxidants (SOD, GST)	[169]	
			Glycolytic enzymes (aldolase, enolase, triose phosphate isomerase)		

Abbreviations: ASP, ancylostoma secreted protein; FABP, fatty acid binding protein; GlcNAcT, *N*-acetylglucosaminyltransferas; GST, glutathione-*S*-transferase; IPSE, IL-4 inducing principle of schistosome eggs; LAP, leucyl aminopeptidase; MIF, macrophage migration inhibitory factor homologue; NPA, nematode polyprotein allergen; PC, phosphorylcholine; PEBP, phosphatidylethanolamine binding protein; SOD, superoxide dismutase; TPX, thioredoxin peroxidase; TRX, thioredoxin; VAL, venom allergen/Ancylostoma secreted protein-like proteins; BmR1 and Sm16 are non-acronymic designations.

[a] Harcus, Y., Hewitson, J., Curwen, R., Dowie, A., Ashton, P., Wilson, R.A. and Maizels, R.M., manuscript in preparation.

immunomodulator Sm16 that can inhibit toll-like receptor signalling in monocytes [43].

Completion of the schistosome life cycle requires that eggs transit from the mesenteric veins, through the intestinal mucosa, into the lumen of the intestine, in a manner dependent on the inflammatory response of the host. Proteomic analysis of egg ES reveals two abundant proteins, alpha-1 (since renamed IPSE, IL-4-inducing principle of schistosome eggs) and a ribonuclease omega-1 [28,44] (see Section 4.1). Glycolytic enzymes (particularly aldolase and enolase) are again well represented in the secretions, as are VAL homologues.

The trematode liver fluke *F. hepatica* releases an extensive series of cathepsin L thiol proteases, which can induce significant protection in vaccine form [45]. Adult flukes also secrete thioredoxin peroxidase, which stimulates the alternative activation of macrophages both *in vitro* and *in vivo* [46]. A recent proteomic analysis of larval *F. hepatica* has identified additional antioxidant enzymes as prominent ES products [47].

3.2. Filarial nematodes: B. malayi and Acanthocheilonema viteae

The immunomodulatory potential of secretions of adult *Brugia* (BES) were noted some years ago, when BES treatment of infected dogs resulted in the loss of antigen-driven lymphocyte proliferation [48]. Further, in mice, BES injection generated suppressive alternatively activated macrophages [49]. Together these studies show that *Brugia* secretions mimic at least some of the immunomodulatory effects of actual infection.

The secretomes of adult and microfilarial stages of *B. malayi* have recently been analysed [29,30], matching data

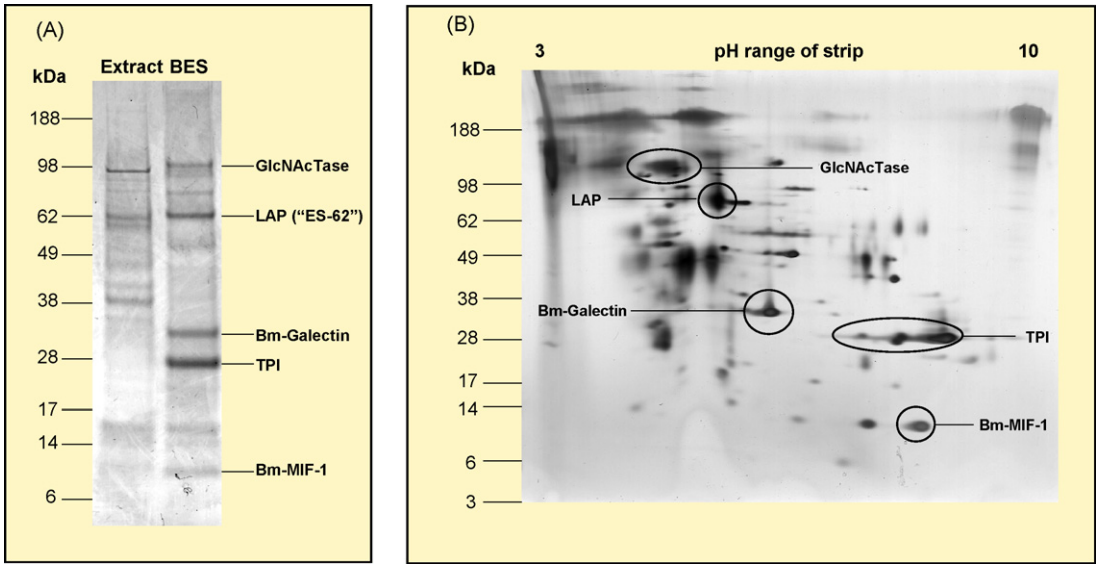

Fig. 1. Helminth ES proteins: an example of the complexity of secreted proteins, from adult *B. malayi* [29], highlighting products discussed in the text. (A) One-dimensional gel, Coomassie Blue stained, showing selective secretion compared to whole somatic extract, indicating the migration of *N*-acetylglucosaminyltransferase (GlcNaTase), leucyl aminopeptidase (LAP, the homologue of ES-62), galectin, triose phosphate isomerase (TPI) and *B. malayi* homologue of macrophage migration inhibitory factor-1 (Bm-MIF-1). (B) Two-dimensional, silver stained gel, with the positions of the same proteins indicated.

to the recently published genome [23]. Abundant proteins secreted by adult parasites include the cytokine homologue Bm-MIF-1 [50], a leucyl aminopeptidase, the PC-bearing protein *N*-acetylglucosaminyltransferase, and a *Brugia* galectin *Bm*-GAL-1 [29]. Surprisingly, the most abundant protein released by adult parasites, highly enriched compared to worm homogenate, was the glycolytic enzyme triose phosphate isomerase (TPI). TPI is also preferentially secreted by the plant nematode *Meloidogyne incognita* [51], and its role may not therefore be specific to the mammalian immune system. Experimental testing of TPI and the other major ES products are now under way in our laboratory.

B. malayi microfilariae secrete qualitatively and quantitatively different proteins to adult parasites, likely reflecting their different location within the host [30]. Abundant proteins include the diagnostic antigen R1 [52], and a serpin (serine protease inhibitor, SPN-2; [53]). Both adults and microfilariae release phosphatidylethanolamine binding protein (homologous to *Onchocerca volvulus* Ov-16 and *Toxocara canis* secreted TES-26 [54]). Secretions from the mosquito-borne infective larval (L3) stage are more difficult to analyse due to limitations on material, although it is known from biochemical studies that a novel protein family (abundant novel transcript, ALT) is released from glandular stockpiles, while other products include cysteine protease inhibitors and a homologue of VAL (B Gregory and J Murray, unpublished observations).

Rodent models for filariasis include *A. viteae*, in which adult worms can be recovered from the peritoneal cavity of gerbils. Adults secrete a single predominant molecule, ES-62, a leucyl aminopeptidase carrying multiple phosphorylcholine (PC) sidechains [55], as discussed in Section 4.2 below.

3.3. Rodent intestinal nematodes: Nippostrongylus brasiliensis and Heligmosomoides polygyrus

N. brasiliensis is a widely used model of nematode infection of rodents characterised by robust Th2 differentiation and parasite clearance within a week [56]. *In vivo* administration of *N. brasiliensis* adult ES (NES), directly [57] or through NES-pulsed dendritic cells (DCs) [58], results in strong Th2 responses. NES also induces alternative activation of macrophages [49]. Notably, NES results in strong IL-4 production, even in the presence of Th1/Th17-inducing complete Freund's adjuvant, indicating a dominant Th2-inducing component which is heat- and protease-labile [57,58], but is not itself a protease. As well as driving Th2 responses *in vivo*, NES can also regulate pro-inflammatory Th1 responses, inhibiting both mitogen-dependent interferon-γ production by naïve mesenteric lymph node cells [59] and LPS-induced IL-12p70 production by DC [58]. Notably, NES under the same conditions does not reduce IL-6 production, and heat-inactivated NES has no inhibitory properties, indicating that a selective and heat-sensitive pathway is in play. Blocking IL-12p70 responsiveness is a common property of many helminth ES products, and may represent a shared strategy to forestall Th1 responses [10].

Surprisingly, despite acting as a Th2-inducing adjuvant, NES can also inhibit Th2-mediated pathology. Both *N. brasiliensis* infection [60] and NES alone can inhibit allergen-induced lung inflammation [61]. *In vivo* studies showed that ES from *N. brasiliensis* L3 larvae (L-NES) inhibited LPS-dependent neutrophil recruitment to the lungs [62]. Despite the protective effects of NES against lung inflammation, L-NES is intrinsically allergenic [63], suggesting that different components may be acting in opposing manners over the longer term. Currently, few individual components of NES have been identified (for example, at least two VAL homologues, Table 1), but as the genome sequencing of this parasite is undertaken, this deficiency should soon be addressed.

H. polygyrus is closely related to *N. brasiliensis* but is able to establish chronic infections in mice. Immunosuppressive properties of *H. polygyrus* ES (HES) were first shown by Pritchard and colleagues on KLH-specific bystander responses *in vitro* [64]. More recently, a single HES fraction was reported to inhibit T cell proliferation and macrophage nitric oxide production [65]. HES treatment of DCs ablates IL-12p70 responsiveness to TLR agonists such as LPS [66]. Furthermore, HES-exposed DCs can induce differentiation of IL-10-producing $CD4^+$ Tregs, which suppress bystander T cell proliferation [66]. One candidate immunomodulator is calreticulin, secreted by tissue-phase intestinal larvae, which can induce Th2 differentiation [67]. We have also established that at least six homologues of VAL are secreted by the adult worm (Table 1), as well as a TGF-β-like ligand which induces functional, suppressive Tregs from naive precursors (see Section 4.4 below).

3.4. Human and canine hookworms: Ancylostoma caninum and Necator americanus

Hookworm research has focussed on both the infective L3 stage, as a vaccine target, and on the blood-feeding adult worms. *A. caninum* L3 release the VAL homologue *Ancylostoma* secreted protein (ASP) [68], and a similar antigen from the human hookworm *N. americanus* is now in a vaccine trial [69]. ASPs are also abundant in adult *A. caninum* ES [70], together with proteases which play a role as anti-coagulants and in digestion of blood contents [71]. *A. caninum* adult-secreted mediators include a fatty acid/retinol binding protein [72], and a tissue inhibitor of metalloprotease [73] while an adult *N. americanus* protein binds to human NK cells, resulting in IFN-γ production [74]. Finally, *A. caninum* ES can reduce TNBS-induced intestinal inflammation, demonstrating its immunomodulatory potential [75].

3.5. Trichostrongyles of ruminants: Haemonchus contortus and related species

H. contortus is a trichostrongyle nematode and one of the most prevalent helminth parasites, distributed in ruminant livestock worldwide. Vaccination of sheep with *H. contortus* adult ES proteins induces significant protection (>70%) against challenge [76]; the major antigens are Hc15, and Hc24, the latter being a VAL homologue [77]. Proteomic analysis of the ES [78] indicates both Hc24 and a further VAL homologue Hc40 are expressed as numerous isoforms; galectins (GALs) are also prominent. Other intestinal nematodes of livestock, very closely related to *H. contortus,* secrete a similar GAL/VAL-dominated suite of ES proteins including *Cooperia* spp. [79], *Ostertagia ostertagi* [80], and *Teladorsagia circumcincta* [81].

3.6. Toxocara canis and Trichinella spiralis

T. canis is a parasite which, in its larval form, can infect a wide variety of hosts, causing visceral larva migrans in humans. Larval TES is type-2 stimulating [82] and comprises a relatively simple set of glycoproteins which is dominated by three gene families [83]. Most ES proteins match a small transcriptomic dataset [84], reflecting the secretion of a small number of relatively abundant proteins, including two C-type lectins [85,86] and three mucins [87,88]. The latter carry abundant *O*-linked glycans, similar in structure to mammalian blood group H [89], which are the target of dominant IgM antibodies in infected hosts [90].

T. spiralis (the pork worm) can also infect a broad host range, and ES antigens from this parasite were among the first to be characterised by biosynthetic labelling [91]. An intriguing set of functional properties have been discovered in ES, including the only known secreted protein kinase [92], a 5′-nucleotidase [93], macrophage migration inhibitory factor [94], and a prosaposin [95]. A *T. spiralis* nucleoside diphosphate kinase is secreted [96] and a similar product reported in ES from other nematodes [78,81]. Detailed proteomic analyses of the muscle-stage (infective) larvae have been undertaken [97,98].

3.7. Taenia and Echinococcus

Larval forms of cestode Taeniid tapeworms cause cysticercosis in humans; a model of this disease is *T. crassiceps* in mice, in which larval parasites in the peritoneal cavity can multiply asexually, accompanied by suppression of Th1 responses [99]. Larval ES products suppress *in vitro* T cell responses [100], although individual components of the secreted material were not identified. Additionally, larval ES contains a functional mimic of host IFN-γ, but the role of this protein in immunoregulation is unclear [101]. Hydatid cysts, surrounding metacestodes of *Echinococcus granulosus*, are considered to comprise both host proteins and parasite secretions: prominent among the latter are the antigen B family which is implicated in Th2 induction and is reported to inhibit neutrophil migration [102].

4. Immunomodulatory molecules from helminths

4.1. Alpha to omega of schistosome Th2 induction

The schistosome-secreted proteins alpha-1 and omega-1 promote Th2 differentiation. Alpha-1, released by schistosome eggs [28], induces IL-4 release and degranulation by human and mouse basophils, thereby initiating a Th2 environment [103,104]. Also named IL-4-inducing principle of schistosome eggs (IPSE), alpha-1 is a dimer that binds and cross-links surface IgE on basophils, in an antigen-independent manner. IPSE has also been shown to function as a chemokine binding protein, which by sequestering ligands, can prevent chemokine-mediated recruitment of inflammatory cells such as neutrophils [105]. Neutralisation of IPSE, using polyclonal sera, leads to increased egg-induced inflammation, directly implicating IPSE in the modulation of egg granulomatous responses. Omega-1 is a ribonuclease abundantly secreted by eggs [106] which is hypothesised to stimulate the immune response necessary for egg transit across host tissues, allowing excretion. Supporting this, recent evidence indicates omega-1 can directly induce Th2 responses (M. Mohrs, M. Yazdanbakhsh and G. Schramm personal communication).

4.2. ES-62 and phosphorylcholine inhibition of immune cell signalling

Phosphorycholine is a small hapten-like moiety present in secretions of many helminths. ES-62 is the leucine aminopeptidase secreted by *A. viteae*, which is heavily conjugated with phosphorylcholine and represents the dominant ES product of adult worms of this species [107]. Through PC modifications, ES-62 can inhibit the proliferation of $CD4^+$ T cells and conventional B2 cells *in vivo*, and reduces $CD4^+$ cell IL-4 and IFN-γ production [108,109]. Conversely, ES-62 promotes proliferation and IL-10 production by peritoneal B1 cells [110]. Antigen-presenting cells are also targeted, as ES-62 pulsed bone marrow-derived DCs drive Th2 differentiation *in vitro* [111], and pre-treatment of DC and macrophages with ES-62 inhibits their ability to produce IL-12p70 in response to LPS [112]. Inhibition of pro-inflammatory Th1 responses occurs as ES-62 interacts with toll-like receptor (TLR) 4 through its PC residues [113], and in mast cells TLR4 binding results in the sequestration and degradation of intracellular PKCa, thereby inhibiting degranulation and release of inflammatory mediators [114]. ES-62 also protects mice against collagen-induced arthritis [115].

Notably, in *B. malayi* PC is not found on the ES-62 homologue (LAP), but on another secretory protein, *N*-acetylglucosaminyltransferase [29]. In the rodent filarial parasite *Litomosoides sigmodontis*, the major ES product is modified with DMAE (dimethylaminoethanol) [116], which contains one less methyl group than PC, giving rise to suggestions that DMAE may function immunologically in a manner similar to PC [117].

4.3. Glycans and lipid molecules—connecting with DCs?

Helminth ES preparations are generally rich in glycoproteins and lipids, leading to many potential interactions with innate pattern-recognition receptors, such as TLRs and C-type lectins on host DCs. Blood group-like glycans from *T. canis* bind the lectin DC-SIGN, hypothesised to favour immune regulation [90]. Schistosome glycoproteins show extensive glycosylation [32], including $Lewis^X$ motifs that trigger Th2 responses *in vivo* through TLR4 ligation [118]. The

consequences of glycan-dependent stimulation include granuloma development *in vivo* [119]. Additionally, macrophage stimulation by schistosome larval secretions is dependent on carbohydrates [120]. Helminth lipids have also been implicated in immune modulation; schistosome phosphatidylserine (PS) induces DCs to polarise IL-4/IL-10-producing T cells. In contrast, schistosome lyso-PS, containing only a single acyl chain, conditions DCs to induce IL-10 secreting regulatory T cells, thus swaying the immune system away from a protective Th2 response [121].

4.4. Cytokine homologues—on the host's home turf

It is now clear that certain highly conserved cytokine gene families are present in helminths, and that their products can ligate receptors on mammalian immune cells. For example, *B. malayi* and *A. ceylanicum* express homologues of the mammalian cytokine macrophage migration inhibitory factor (MIF) [122]. Mammalian MIF is considered to be pro-inflammatory, playing a key role for example in septic shock. Perhaps surprisingly, nematode MIF homologues mimic host MIF by induction of pro-inflammatory cytokines [50,123,124]. However, we have recently found that *Brugia* MIF synergises with IL-4 to induce the development of fully suppressive alternatively activated macrophages *in vitro* [125], to a level beyond that observed for IL-4 alone [126]. One pathway for this effect may be through the induction by MIF of IL-4R expression on macrophages [125], thereby amplifying the potency of IL-4 itself. Thus, in a Th2 environment, MIF may prevent the classical, pro-inflammatory, activation of macrophages.

Worms also express members of the TGF-β and TGF-β receptor superfamilies. *B. malayi* adults secrete TGH-2, a homologue of host TGF-β and of the *C. elegans* developmental protein, DAF-7 [35]. Recombinant TGH-2 can bind to the mammalian TGF-β receptor, suggesting it may promote the generation of regulatory T cells [127], as has been found for mammalian TGF-β. However, TGH-2 is secreted at very low levels, below the limit of detection for proteomics, and it is unclear whether this is sufficient for bioactivity [29,30]. In contrast, a *H. polygyrus* TGF-β mimic is able to directly induce Foxp3^{+} expression in activated T cells, implying a key role in parasite immune avoidance (Grainger et al., submitted for publication). Parasite TGF-β homologues also have non-immune roles, and one such *S. mansoni* protein is involved in egg development [128].

4.5. C-type lectins and galectins—targetting mammalian glycans?

Lectins are carbohydrate binding proteins, and host C-type lectins and galectins are involved in a variety of immune processes, such as antigen uptake and presentation, cell adhesion, apoptosis and T cell polarisation [129]. C-type lectins (C-TLs) are particularly abundant in the secretions of *T. canis* [85,86] and those of hookworms [70]. The biological roles of parasite C-TLs are unclear, but two *T. canis* C-TLs (TES-32 and TES-70) show greater homology to mammalian proteins such as CD23 (low affinity IgE receptor) and macrophage mannose receptor, than to any *C. elegans* protein [86]. Furthermore, TES-70 is able to bind mammalian carbohydrates in a calcium-dependent manner [85] suggesting a role in immune evasion by e.g. inhibiting the migration of host cells. Alternatively, parasite C-TLs may bind to and mask worm carbohydrates from host immune cells. Additionally, nematode C-TLs have roles unconnected with immune evasion. The acquisition of symbiotic bacteria by the marine nematode *Laxus oneistus* requires its secretion of a C-TL [130], while a non-secretory C-TL from *A. ceylanicum*, specifically expressed by sperm cells, has a putative role in nematode reproduction [131]. Secreted galectins are more apparent in other species such as *H. contortus* [132] and particularly *B. malayi* [29]. A recombinant *Brugia* galectin, *Bm*-GAL-1, is able to bind to host immune cells in a carbohydrate dependent manner (J.P.H. unpublished observations), but does not share the eosinophil chemoattractant properties reported for a *H. contortus* galectin [133].

4.6. Protease inhibitors—blocking innate cell functions

Two highly expressed sets of protease inhibitors are the cystatins and the serpins, each with proposed immunomodulatory roles. Cystatins (cysteine protease inhibitors) from *A. viteae*, *B. malayi*, *O. volvulus* and *N. brasiliensis* act as immunomodulators, through at least two mechanisms [134,135]. Firstly, they inhibit cysteine proteases (cathepsins and aspartyl endopeptidase) required for host APC antigen processing and presentation, so leading to reduced T cell priming [136,137]. Secondly, they elicit the immunosuppressive cytokine IL-10, leading to a reduction in costimulatory molecule expression by APCs, and the direct inhibition of T cell proliferation [138]. The immunomodulatory potential of parasite cystatins is also evident *in vivo*, in inhibition of both allergic lung inflammation and colitis, mediated by Tregs and IL-10-producing macrophages [139].

The serpins are serine protease inhibitors [140], and one member of this family, SPN-2, is the major mRNA and secreted protein product [30,141] of *B. malayi* microfilariae. The function of SPN-2 is disputed; in collaboration with a leading serpin laboratory we reported specific inhibition of the neutrophil proteinases cathepsin G and neutrophil elastase, and no activity against a range of other enzymes such as pancreatic chymotrypsin and coagulation factors [53]. However, an independent group reported that recombinant protein was devoid of inhibitory activity [142]. Irrespective of direct anti-enzymatic activity, SPN-2 stands out as unusual because of its ability to stimulate a Th1 response in mice, corresponding to the ability of live microfilariae to drive this type of immune response [141].

4.7. Antioxidants and acetylcholinesterases

Production of reactive oxygen species (oxygen radicals, superoxide, and hydrogen peroxide) by phagocytes is a primary pathway of immune attack against parasites. Correspondingly, most parasites express high levels of antioxidants, including superoxide dismutases (SODs), catalases, glutathione and thioredoxin peroxidases, and peroxiredoxins. Secreted helminth antioxidant enzymes include *B. malayi* glutathione peroxidase [29] and SOD [143], and thioredoxin peroxidase from *F. hepatica* [46]. In the latter case, the enzyme is also responsible for inducing alternatively activated macrophages [46].

Acetylcholinesterase (AChE) breaks down the neurotransmitter acetylcholine in order to terminate neuronal signals, and is active in the neuromuscular system of helminths. AChE has been identified in the ES of many gut-dwelling nematodes, including *H. polygyrus* [144], *N. brasiliensis* [145], the lungworm *Dictyocaulus viviparus* [146], and adult *B. malayi* [147]. It has been proposed that their secretion may also hydrolyse acetylcholine from the enteric nervous system of the host [148]. Since acetylcholine-mediated signalling stimulates intestinal chloride and mucus production, AChEs may prevent fluid increases in the gut that promote parasite clearance. Finally, another *N. brasiliensis* secreted enzyme is platelet activating factor (PAF) hydrolase, which is likely to act in an anti-inflammatory capacity on the platelet population [149].

4.8. Venom allergen/ASP-like (VAL) homologues

In 1996, the Hotez laboratory described the *A. caninum* secreted protein, ASP [68], the first of an enigmatic gene family expressed across a wide variety of parasitic helminths, including human hookworm [150] filarial nematodes [30,151], trichostrongylids such as

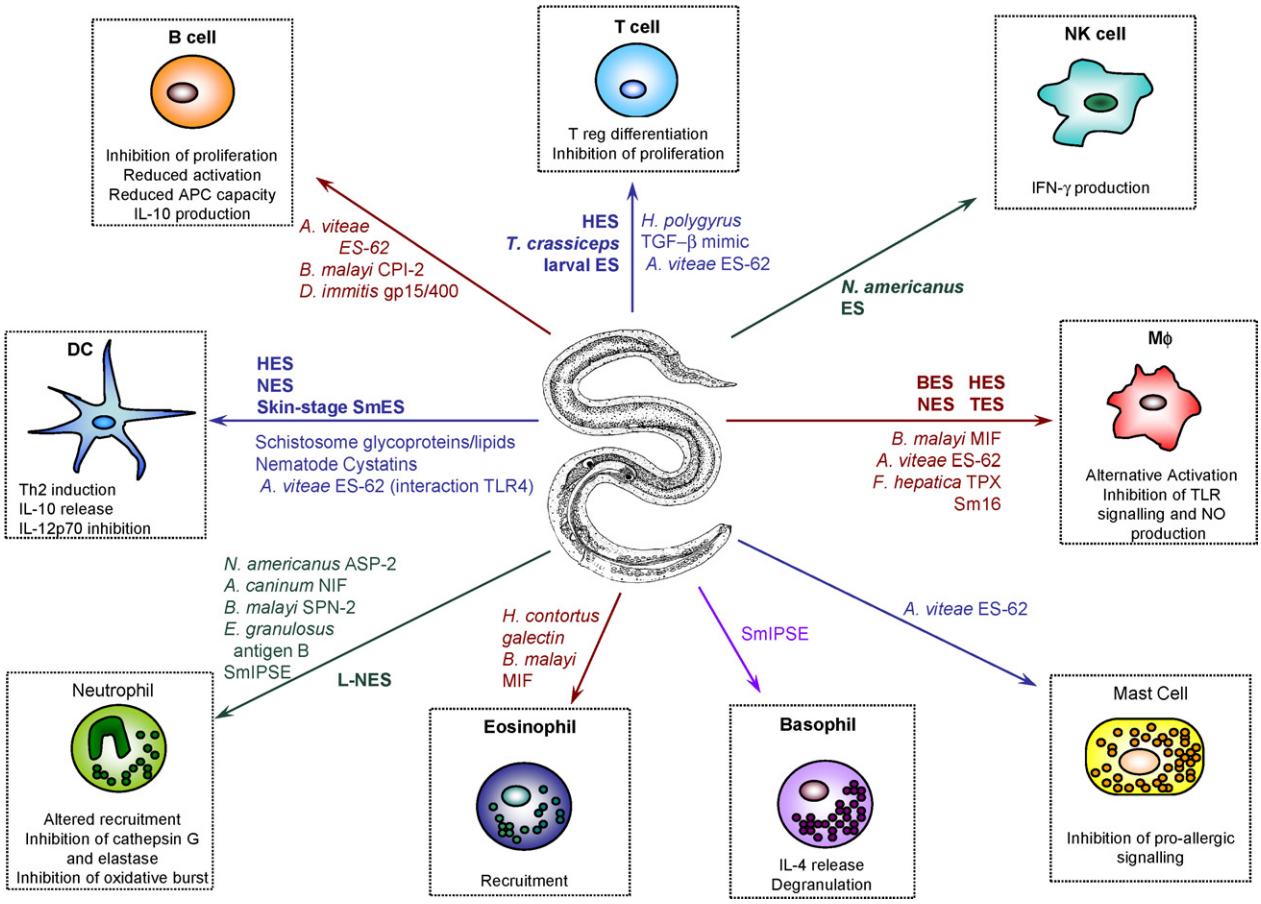

Fig. 2. Mechanisms of immune modulation by helminth ES products (in bold) and defined molecules (in plain type) discussed in the text. *Abbreviations*: **APC**, antigen presenting cell; **ASP**, *Ancylostoma* secreted protein; **BES**, *B. malayi* ES; **CPI**, cysteine proteinase inhibitor (cystatin); **HES**, *H. polygyrus* ES; **IPSE**, IL-4-inducing principle of schistosome eggs; **L-NES**, larval *N. brasiliensis* ES; **MIF**, macrophage migration inhibitory factor; **NES**, adult *N. brasiliensis* ES; **NIF**, neutrophil inhibitory factor; **Sm**, *Schistosoma mansoni*; **SPN**, serine proteinase inhibitor (serpin); **TLR**, toll-like receptor; **TGF**, transforming growth factor; **TES**, *T. canis* ES.

H. contortus [77,78], schistosomes [28,41,152], as well as free-living *C. elegans* [153]. We have termed this the Venom allergen/ASP-Like (VAL) gene family [151]. Alongside mammalian cysteine-rich sperm proteins (CRISPs), insect venom allergens and plant pathogenesis family-1 (PR-1) proteins, VAL proteins are members of the SCP (sperm coating protein)-1 superfamily. Despite sequence similarity, no coherent function for this protein family has been demonstrated. An *A. caninum* SCP-1 protein, neutrophil inhibitory factor (NIF), binds the host integrin CR3 (CD11b/CD18) and is able to inhibit neutrophil function, including oxidative burst [154,155].

The crystal structure of *N. americanus* ASP-2 reveals a charge segregation reminiscent of mammalian chemokines, suggesting that this protein may be a ligand or antagonist for G-protein coupled receptors such as the chemokine receptors [156]. Consistent with this prediction, *Na*-ASP-2 has recently been shown to induce neutrophil chemotaxis *in vitro* and *in vivo* [157], but it remains uncertain if this is a widespread property of VAL homologues. An alternative possibility is that the SCP-1 domain provides a stable structural backbone, allowing the non-conserved regions of the different VAL proteins to carry out numerous different roles [70]. Even if this were the case, the prominence of VAL products in most helminth secretions is highly suggestive of an important role in modifying host immunity.

4.9. Novel proteins

Helminths secrete numerous products lacking discernable sequence similarity to known proteins. Examples include the filarial ALT-1 and ALT-2 proteins which are highly abundant in the infective larval stage [158,159]. One route to determine the function of these proteins has been by heterologous expression in *Leishmania* parasites, studying changes in immune responsiveness resulting from filarial gene expression. *L. mexicana* parasites expressing *B. malayi* ALT-1 or ALT-2 were found to reach significantly higher levels of infection in macrophages *in vitro*, inhibiting killing mechanisms, and were more virulent *in vivo* [160]. Cells harboring transgenic parasites upregulated SOCS-1, an inhibitor of IFN-γ signalling, suggesting that the ALT proteins impair Th1 responsiveness known to be required for immunity in this system [160].

A *B. malayi* polyprotein "ladder" gp15/400 represents another unusual filarial immunomodulator. Adults synthesise this protein as a large 400-kDa precursor, subsequently processed into secreted 15-kDa subunits [161]. Released subunits can bind host retinoids [162], a property that may be shared with another family of secreted proteins, the transthyretin-like proteins [29]. Given that retinoic acid can synergise with TGF-β to induce Foxp3$^+$ Tregs [163], it is possible that such proteins could enhance vitamin A uptake by host tissues to favour conversion to RA and thus enhance Foxp3$^+$ Treg induction. The homologue of gp15/400 from *Dirofilaria immitis*, a filarial worm of dogs, stimulates mouse B cell synthesis of IgE through direct binding to CD40 [164] and can also inhibit insulin-dependent diabetes in mice [165].

5. Conclusion

The systematic analysis of ES products, which has become possible through the combination of proteomics and genomics,

is now providing us with a comprehensive catalogue of potential immunomodulators, each pointing the way towards critical interactions between parasites and the host immune system (Fig. 2). Many parasites have targeted similar host pathways, particularly within innate immunity, but the detailed mechanisms differ because each helminth species has evolved its own strategy to confound host defences. Identification of these specific mechanisms may allow the development of neutralising vaccines that promote worm clearance. Moreover, the striking protective effect of helminth infections, in many contexts, against immunopathological disorders [9,115,166], and the introduction of therapeutic helminth infections [8], sets an urgent agenda to replace live parasite therapy with non-living parasite products. The recent advances in ES are likely to have already identified the candidates, and as we have described here, provided exciting early data on the ability of these proteins to modulate host immunity.

Note added in proof

Bennuru et al. [170] have performed a comprehensive proteomic analysis of the ES proteins from L3, L3 to L4 moult, MF and adult *B. malayi*, resulting in the identification of 852 proteins. This supports the previous studies [29,30], and additionally shows the abundant secretion of ALT family members by larval parasites, as well as the release of trace amounts of *Wolbachia endosymbiont* proteins. Robinson et al. [171] have also made available an in-depth proteomic analysis of the *F. hepatica* secretome based on new transcriptomic data.

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References

[1] Behnke JM, Barnard CJ, Wakelin D. Understanding chronic nematode infections: evolutionary considerations, current hypotheses and the way forward. Int J Parasitol 1992;22:861–907.
[2] Maizels RM, Yazdanbakhsh M. Regulation of the immune response by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 2003;3:733–43.
[3] Imai S, Fujita K. Molecules of parasites as immunomodulatory drugs. Curr Top Med Chem 2004;4:539–52.
[4] Johnston MJG, Macdonald JA, McKay DM. Parasitic helminths: a pharmacopoeia of anti-inflammatory molecules. Parasitology 2008:1–23.
[5] Maizels RM, Blaxter ML, Scott AL. Immunological genomics of *Brugia malayi*: filarial genes implicated in immune evasion and protective immunity. Parasite Immunol 2001;23:327–44.
[6] Jenkins SJ, Hewitson JP, Jenkins GR, Mountford AP. Modulation of the host's immune response by schistosome larvae. Parasite Immunol 2005;27:385–93.
[7] Hoerauf A, Satoguina J, Saeftel M, Specht S. Immunomodulation by filarial nematodes. Parasite Immunol 2005;27:417–29.
[8] Elliott DE, Summers RW, Weinstock JV. Helminths as governors of immune-mediated inflammation. Int J Parasitol 2007;37:457–64.
[9] Harnett W, Harnett MM. Therapeutic immunomodulators from nematode parasites. Expert Rev Mol Med 2008;10:e18.
[10] Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor M, Allen JE. Helminth parasites: masters of regulation. Immunol Rev 2004;201:89–116.
[11] King CL, Medhat A, Malhotra I, et al. Cytokine control of parasite-specific anergy in human urinary schistosomiasis. IL-10 modulates lymphocyte reactivity. J Immunol 1996;156:4715–21.
[12] Sartono E, Kruize YCM, Kurniawan-Atmadja A, Maizels RM, Yazdanbakhsh M. Depression of antigen-specific interleukin-5 and interferon-g responses in human lymphatic filariasis as a function of clinical status and age. J Infect Dis 1997;175:1276–80.
[13] Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. J Immunol 2006;176:3248–56.
[14] Wilson MS, Taylor M, Balic A, Finney CAM, Lamb JR, Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med 2005;202:1199–212.
[15] van den Biggelaar A, van Ree R, Roderigues LC, et al. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. Lancet 2000;356:1723–7.
[16] Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol 2007;61:97–108.
[17] Sartono E, Kruize YCM, Kurniawan A, et al. Elevated cellular responses and interferon-g release after long-term diethylcarbamazine treatment of patients with human lymphatic filariasis. J Infect Dis 1995;171:1683–7.
[18] Semnani RT, Law M, Kubofcik J, Nutman TB. Filaria-induced immune evasion: suppression by the infective stage of *Brugia malayi* at the earliest host–parasite interface. J Immunol 2004;172:6229–38.
[19] Lightowlers MW, Rickard MD. Excretory–secretory products of helminth parasites: effects on host immune responses. Parasitology 1988;96:S123–66.
[20] Williamson AL, Lustigman S, Oksov Y, et al. *Ancylostoma caninum* MTP-1, an astacin-like metalloprotease secreted by infective hookworm larvae, is involved in tissue migration. Infect Immun 2006;74:961–7.
[21] Culley FJ, Brown A, Conroy DM, Sabroe I, Pritchard DI, Williams TJ. Eotaxin is specifically cleaved by hookworm metalloproteases preventing its action *in vitro* and *in vivo*. J Immunol 2000;165:6447–53.
[22] Gregory WF, Blaxter ML, Maizels RM. Differentially expressed, abundant *trans*-spliced cDNAs from larval *Brugia malayi*. Mol Biochem Parasitol 1997;87:85–95.
[23] Ghedin E, Wang S, Spiro D, et al. Draft genome of the filarial nematode parasite *Brugia malayi*. Science 2007;317:1756–60.
[24] Verjovski-Almeida S, DeMarco R, Martins EAL, et al. Transcriptome analysis of the acoelomate human parasite *Schistosoma mansoni*. Nat Genet 2003;35:148–57.
[25] Parkinson J, Mitreva M, Whitton C, et al. A transcriptomic analysis of the phylum Nematoda. Nat Genet 2004;36:1259–67.
[26] Harcus YM, Parkinson J, Fernández C, et al. Signal sequence analysis of expressed sequence tags from the nematode *Nippostrongylus brasiliensis* and the evolution of secreted proteins in parasites. Genome Biol 2004;5:R39.
[27] Nagaraj SH, Gasser RB, Ranganathan S. Needles in the EST haystack: large-scale identification and analysis of excretory–secretory (ES) proteins in parasitic nematodes using expressed sequence tags (ESTs). PLoS Negl Trop Dis 2008;2:e301.
[28] Cass CL, Johnson JR, Califf LL, et al. Proteomic analysis of *Schistosoma mansoni* egg secretions. Mol Biochem Parasitol 2007;155:84–93.
[29] Hewitson JP, Harcus YM, Curwen RS, et al. The secretome of the filarial parasite, *Brugia malayi*: proteomic profile of adult excretory–secretory products. Mol Biochem Parasitol 2008;160:8–21.
[30] Moreno Y, Geary TG. Stage- and gender-specific proteomic analysis of *Brugia malayi* excretory–secretory products. PLoS Negl Trop Dis 2008;2:e326.
[31] Hokke CH, Fitzpatrick JM, Hoffmann KF. Integrating transcriptome, proteome and glycome analyses of *Schistosoma* biology. Trends Parasitol 2007;23:165–74.
[32] Jang-Lee J, Curwen RS, Ashton PD, et al. Glycomics analysis of *Schistosoma mansoni* egg and cercarial secretions. Mol Cell Proteom 2007;6:1485–99.
[33] Liu LX, Serhan CN, Weller PF. Intravascular filarial parasites elaborate cyclooxygenase-derived eicosanoids. J Exp Med 1990;172:993–6.
[34] Robijn MLM, Koeleman CAM, Hokke CH, Deelder AM. *Schistosoma mansoni* eggs excrete specific free oligosaccharides that are detectable in the urine of the human host. Mol Biochem Parasitol 2007;151:162–72.
[35] Gomez-Escobar N, Gregory WF, Maizels RM. Identification of *Bm-tgh-2*, a filarial nematode homolog of *C. elegans daf-7* and human TGF-b, expressed in microfilarial and adult stages of *Brugia malayi*. Infect Immun 2000;68:6402–10.
[36] Harrop R, Jennings N, Mountford AP, Coulson PS, Wilson RA. Characterization, cloning and immunogenicity of antigens released by transforming cercariae of *Schistosoma mansoni*. Parasitology 2000;121:385–94.
[37] Jenkins SJ, Mountford AP. Dendritic cells activated with products released by schistosome larvae drive Th2-type immune responses, which can be inhibited by manipulation of CD40 costimulation. Infect Immun 2005;73:395–402.
[38] McKerrow JH, Caffrey C, Kelly B, Loke P, Sajid M. Proteases in parasitic diseases. Annu Rev Pathol 2006;1:497–536.
[39] Pleass RJ, Kusel JR, Woof JM. Cleavage of human IgE mediated by *Schistosoma mansoni*. Int Arch Allergy Immunol 2000;121:194–204.
[40] Knudsen GM, Medzihradszky KF, Lim KC, Hansell E, McKerrow JH. Proteomic analysis of *Schistosoma mansoni* cercarial secretions. Mol Cell Proteom 2005;4:1862–75.
[41] Curwen RS, Ashton PD, Sundaralingam S, Wilson RA. Identification of novel proteases and immunomodulators in the secretions of schistosome cercariae that facilitate host entry. Mol Cell Proteom 2006;5:835–44.
[42] Hansell E, Braschi S, Medzihradszky KF, et al. Proteomic analysis of skin invasion by blood fluke larvae. PLoS Negl Trop Dis 2008;2:e262.
[43] Brännström K, Sellin ME, Holmfeldt P, Brattsand M, Gullberg M. The *Schistosoma mansoni* protein Sm16/SmSLP/SmSPO-1 assembles into a 9-subunit oligomer with potential to inhibit Toll-like receptor signaling. Infect Immun 2009.
[44] Dunne DW, Jones FM, Doenhoff MJ. The purification, serological activity and hepatotoxic properties of two cationic glycoproteins (a1 and w1) from *Schistosoma mansoni* eggs. Parasitology 1991;103:225–36.

[45] Dalton JP, Neill SO, Stack C, et al. *Fasciola hepatica* cathepsin L-like proteases: biology, function, and potential in the development of first generation liver fluke vaccines. Int J Parasitol 2003;33:1173–81.
[46] Donnelly S, O'Neill SM, Sekiya M, Mulcahy G, Dalton JP. Thioredoxin peroxidase secreted by *Fasciola hepatica* induces the alternative activation of macrophages. Infect Immun 2005;73:166–73.
[47] Gourbal BE, Guillou F, Mitta G, et al. Excretory–secretory products of larval *Fasciola hepatica* investigated using a two-dimensional proteomic approach. Mol Biochem Parasitol 2008;161:63–6.
[48] Miller S, Schreuer D, Hammer berg B. Inhibition of antigen-driven proliferative responses and enhancement of antibody production during infection with *Brugia pahangi*. J Immunol 1991;147:1007–13.
[49] Allen JE, MacDonald AS. Profound suppression of cellular proliferation mediated by the secretions of nematodes. Parasite Immunol 1998;20:241–7.
[50] Pastrana DV, Raghavan N, FitzGerald P, et al. Filarial nematode parasites secrete a homologue of the human cytokine macrophage migration inhibitory factor. Infect Immun 1998;66:5955–63.
[51] Bellafiore S, Shen Z, Rosso MN, Abad P, Shih P, Briggs SP. Direct identification of the *Meloidogyne incognita* secretome reveals proteins with host cell reprogramming potential. PLoS Pathogens 2008;4:e1000192.
[52] Rahmah N, Lim BH, Khairul Anuar A, et al. A recombinant antigen-based IgG4 ELISA for the specific and sensitive detection of *Brugia malayi* infection. Trans R Soc Trop Med Hyg 2001;95:280–4.
[53] Zang XX, Yazdanbakhsh M, Kiang H, Kanost MR, Maizels RM. A novel serpin expressed by the blood-borne microfilariae of the parasitic nematode *Brugia malayi* inhibits human neutrophil serine proteinases. Blood 1999;94: 1418–28.
[54] Gems DH, Ferguson CJ, Robertson BD, Page AP, Blaxter ML, Maizels RM. An abundant, *trans*-spliced mRNA from *Toxocara canis* infective larvae encodes a 26 kDa protein with homology to phosphatidylethanolamine binding proteins. J Biol Chem 1995;270:18517–22.
[55] Stepek G, Houston KM, Goodridge HS, Devaney E, Harnett W. Stage-specific and species-specific differences in the production of the mRNA and protein for the filarial nematode secreted product, ES-62. Parasitology 2004;128:91–8.
[56] Finkelman FD, Shea-Donohue T, Goldhill J, et al. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Annu Rev Immunol 1997;15:505–33.
[57] Holland MJ, Harcus YM, Riches PL, Maizels RM. Proteins secreted by the parasitic nematode *Nippostrongylus brasiliensis* act as adjuvants for Th2 responses. Eur J Immunol 2000;30:1977–87.
[58] Balic A, Harcus Y, Holland MJ, Maizels RM. Selective maturation of dendritic cells by *Nippostrongylus brasiliensis* secreted proteins drives T helper type 2 immune responses. Eur J Immunol 2004;34:3047–59.
[59] Uchikawa R, Matsuda S, Arizono N. Suppression of gamma interferon transcription and production by nematode excretory–secretory antigen during polyclonal stimulation of rat lymph node T cells. Infect Immun 2000;68:6233–9.
[60] Wohlleben G, Trujillo C, Muller J, et al. Helminth infection modulates the development of allergen-induced airway inflammation. Int Immunol 2004;16:585–96.
[61] Trujillo-Vargas CM, Werner-Klein M, Wohlleben G, et al. Helminth derived products inhibit the development of allergic responses in mice. Am J Respir Cell Mol Biol 2007;175:336–44.
[62] Keir PA, Brown DM, Clouter-Baker A, Harcus YM, Proudfoot L. Inhibition of neutrophil recruitment by ES of *Nippostrongylus brasiliensis*. Parasite Immunol 2004;26:137–9.
[63] Marsland BJ, Camberis M, Le Gros G. Secretory products from infective forms of *Nippostrongylus brasiliensis* induce a rapid allergic airway inflammatory response. Immunol Cell Biol 2005;83:40–7.
[64] Telford G, Wheeler DJ, Appleby P, Bowen JG, Pritchard DI. *Heligmosomoides polygyrus* immunomodulatory factor (IMF), targets T-lymphocytes. Parasite Immunol 1998;20:601–11.
[65] Rzepecka J, Lucius R, Doligalska M, Beck S, Rausch S, Hartmann S. Screening for immunomodulatory proteins of the intestinal parasitic nematode *Heligmosomoides polygyrus*. Parasite Immunol 2006;28:463–72.
[66] Segura M, Su Z, Piccirillo C, Stevenson MM. Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression. Eur J Immunol 2007;37:1887–904.
[67] Rzepecka J, Rausch S, Klotz C, et al. Calreticulin from the intestinal nematode *Heligmosomoides polygyrus* is a Th2-skewing protein and interacts with murine scavenger receptor-A. Mol Immunol 2008.
[68] Hawdon JM, Jones BF, Hoffman DR, Hotez PJ. Cloning and characterization of *Ancylostoma*-secreted protein. A novel protein associated with the transition to parasitism by infective hookworm larvae. J Biol Chem 1996;271:6672–8.
[69] Diemert DJ, Bethony JM, Hotez PJ. Hookworm vaccines. Clin Infect Dis 2008;46:282–8.
[70] Mulvenna J, Hamilton B, Nagaraj SH, Smyth D, Loukas A, Gorman JJ. Proteomics analysis of the excretory/secretory component of the blood-feeding stage of the hookworm, *Ancylostoma caninum*. Mol Cell Proteom 2008;8:109–21.
[71] Williamson AL, Brindley PJ, Knox DP, Hotez PJ, Loukas A. Digestive proteases of blood-feeding nematodes. Trends Parasitol 2003;19:417–23.
[72] Basavaraju S, Zhan B, Kennedy MW, Liu Y, Hawdon J, Hotez PJ. Ac-FAR-1, a 20 kDa fatty acid- and retinol-binding protein secreted by adult *Ancylostoma caninum* hookworms: gene transcription pattern, ligand binding properties and structural characterisation. Mol Biochem Parasitol 2003;126: 63–71.
[73] Zhan B, Badamchian M, Meihua B, et al. Molecular cloning and purification of Ac-TMP, a developmentally regulated putative tissue inhibitor of metalloprotease released in relative abundance by adult *Ancylostoma* hookworms. Am J Trop Med Hyg 2002;66:238–44.
[74] Hsieh GC-F, Loukas A, Wahl AM, et al. A secreted protein from the human hookworm *Necator americanus* binds selectively to NK cells and induces IFN-g production. J Immunol 2004;173:2699–704.
[75] Ruyssers NE, De Winter BY, De Man JG, et al. Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice. Inflamm Bowel Dis 2009;15:491–500.
[76] Schallig HDFH, Van Leeuwen MAW, Cornelissen AWCA. Protective immunity induced by vaccination with two *Haemonchus contortus* excretory–secretory proteins in sheep. Parasite Immunol 1997;19:447–54.
[77] Schallig HDFH, van Leeuwen MAW, Verstrepen BE, Cornelissen AWCA. Molecular characterization and expression of two putative protective excretory secretory proteins of *Haemonchus contortus*. Mol Biochem Parasitol 1997;88:203–13.
[78] Yatsuda AP, Krijgsveld J, Cornelissen AWCA, Heck AJ, De Vries E. Comprehensive analysis of the secreted proteins of the parasite *Haemonchus contortus* reveals extensive sequence variation and differential immune recognition. J Biol Chem 2003;278:16941–51.
[79] Yatsuda AP, Eysker M, Vieria-Bressan MCR, De Vries E. A family of activation associated secreted protein (ASP) homologues of *Cooperia punctata*. Res Vet Sci 2002;73:297–306.
[80] Saverwyns H, Visser A, Nisbet AJ, et al. Identification and characterization of a novel specific secreted protein family for selected members of the subfamily Ostertagiinae (Nematoda). Parasitology 2008;135:63–70.
[81] Craig H, Wastling JM, Knox DP. A preliminary proteomic survey of the in vitro excretory/secretory products of fourth-stage larval and adult *Teladorsagia circumcincta*. Parasitology 2006;132:535–43.
[82] Del Prete G, De CM, Mastromauro C, et al. Purified protein derivative of *Mycobacterium tuberculosis* and excretory–secretory antigen(s) of *Toxocara canis* expand human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. J Clin Invest 1991;88:346–50.
[83] Maizels RM, Tetteh KKA, Loukas AC. *Toxocara canis*: genes expressed by the arrested infective larval stage of a parasitic nematode. Int J Parasitol 2000;30:495–508.
[84] Tetteh KKA, Loukas A, Tripp C, Maizels RM. Identification of abundantly expressed novel and conserved genes from infective stage larvae of *Toxocara canis* by an expressed sequence tag strategy. Infect Immun 1999;67:4771–9.
[85] Loukas AC, Doedens A, Hintz M, Maizels RM. Identification of a new C-type lectin, TES-70, secreted by infective larvae of *Toxocara canis*, which binds to host ligands. Parasitology 2000;121:545–54.
[86] Loukas AC, Mullin NP, Tetteh KKA, Moens L, Maizels RM. A novel C-type lectin secreted by a tissue-dwelling parasitic nematode. Curr Biol 1999;9:825–8.
[87] Gems DH, Maizels RM. An abundantly expressed mucin-like protein from *Toxocara canis* infective larvae: the precursor of the larval surface coat glycoproteins. Proc Natl Acad Sci USA 1996;93:1665–70.
[88] Loukas AC, Hintz M, Tetteh KKA, Mullin NP, Maizels RM. A family of secreted mucins from the parasitic nematode *Toxocara canis* bear diverse mucin domains but share similar flanking six-cysteine (SXC) repeat motifs. J Biol Chem 2000;275:39600–7.
[89] Khoo K-H, Maizels RM, Page AP, Taylor GW, Rendell N, Dell A. Characterisation of nematode glycoproteins: the major *O*-glycans of *Toxocara* excretory secretory antigens are methylated trisaccharides. Glycobiology 1991;1: 163–71.
[90] Schabussova I, Amer H, van Die I, Kosma P, Maizels RM. *O*-Methylated glycans from *Toxocara* are specific targets for antibody binding in human and animal infections. Int J Parasitol 2007;37:97–109.
[91] Parkhouse RME, Clark NWT. Stage specific secreted and somatic antigens of *Trichinella spiralis*. Mol Biochem Parasitol 1983;9:319–27.
[92] Arden SR, Smith AM, Booth MJ, Tweedie S, Gounaris K, Selkirk ME. Identification of serine/threonine protein kinases secreted by *Trichinella spiralis* infective larvae. Mol Biochem Parasitol 1997;90:111–9.
[93] Gounaris K, Selkirk ME, Sadeghi SJ. A nucleotidase with unique catalytic properties is secreted by *Trichinella spiralis*. Mol Biochem Parasitol 2004;136:257–64.
[94] Tan TH, Edgerton SA, Kumari R, et al. Macrophage migration inhibitory factor of the parasitic nematode *Trichinella spiralis*. Biochem J 2001;357:373–83.
[95] Selkirk ME, Hussein AS, Chambers AE, et al. *Trichinella spiralis* secretes a homologue of prosaposin. Mol Biochem Parasitol 2004;135:49–56.
[96] Gounaris K, Thomas S, Najarro P, Selkirk ME. Secreted variant of nucleoside diphosphate kinase from the intracellular parasitic nematode *Trichinella spiralis*. Infect Immun 2001;69:3658–62.
[97] Robinson MW, Connolly B. Proteomic analysis of the excretory–secretory proteins of the *Trichinella spiralis* L1 larva, a nematode parasite of skeletal muscle. Proteomics 2005;5:4525–32.
[98] Robinson MW, Gare DC, Connolly B. Profiling excretory/secretory proteins of *Trichinella spiralis* muscle larvae by two-dimensional gel electrophoresis and mass spectrometry. Vet Parasitol 2005;132:37–41.
[99] Terrazas LI, Bojalil R, Govezensky T, Larralde C. Shift from an early protective Th1-type immune response to a late permissive Th2-type response in murine cysticercosis (*Taenia crassiceps*). J Parasitol 1998;84:74–81.
[100] Spolski RJ, Corson J, Thomas PG, Kuhn RE. Parasite-secreted products regulate the host response to larval *Taenia crassiceps*. Parasite Immunol 2000;22:297–305.

[101] Spolski RJ, Thomas PG, See EJ, Mooney KA, Kuhn RE. Larval *Taenia crassiceps* secretes a protein with characteristics of murine interferon-gamma. Parasitol Res 2002;88:431–8.
[102] Siracusano A, Margutti P, Delunardo F, et al. Molecular cross-talk in host–parasite relationships: the intriguing immunomodulatory role of Echinococcus antigen B in cystic echinococcosis. Int J Parasitol 2008;38:1371–6.
[103] Schramm G, Falcone FH, Gronow A, et al. Molecular characterization of an interleukin-4-inducing factor from *Schistosoma mansoni* eggs. J Biol Chem 2003;278:18384–92.
[104] Schramm G, Mohrs K, Wodrich M, et al. IPSE/alpha-1, a glycoprotein from *Schistosoma mansoni* eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo. J Immunol 2007;178:6023–7.
[105] Smith P, Fallon RE, Mangan NE, et al. *Schistosoma mansoni* secretes a chemokine binding protein with antiinflammatory activity. J Exp Med 2005;202:1319–25.
[106] Fitzsimmons CM, Schramm G, Jones FM, et al. Molecular characterization of omega-1: a hepatotoxic ribonuclease from *Schistosoma mansoni* eggs. Mol Biochem Parasitol 2005;144:123–7.
[107] Harnett W, McInnes IB, Harnett MM. ES-62, a filarial nematode-derived immunomodulator with anti-inflammatory potential. Immunol Lett 2004;94:27–33.
[108] Wilson EH, Deehan MR, Katz E, et al. Hyporesponsiveness of murine B lymphocytes exposed to the filarial nematode phosphorylcholine-containing secreted product ES-62 in vivo. Immunology 2003;109:238–45.
[109] Marshall FA, Grierson AM, Garside P, Harnett W, Harnett MM. ES-62, an immunomodulator secreted by filarial nematodes suppresses clonal expansion and modifies effector function of heterologous antigen-specific T cells in vivo. J Immunol 2005;175:5817–26.
[110] Wilson EH, Katz E, Goodridge HS, Harnett MM, Harnett W. In vivo activation of murine peritoneal B1 cells by the filarial nematode phosphorylcholine-containing glycoprotein ES-62. Parasite Immunol 2003;25:463–6.
[111] Whelan M, Harnett MM, Houston KM, Patel V, Harnett W, Rigley KP. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. J Immunol 2000;164:6453–60.
[112] Goodridge HS, Harnett W, Liew FY, Harnett MM. Differential regulation of interleukin-12 p40 and p35 induction via Erk mitogen-activated protein kinase-dependent and -independent mechanisms and the implications for bioactive IL-12 and IL-23 responses. Immunology 2003;109:415–25.
[113] Goodridge HS, Marshall FA, Else KJ, et al. Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. J Immunol 2005;174:284–93.
[114] Melendez AJ, Harnett MM, Pushparaj PN, et al. Inhibition of FceRI-mediated mast cell responses by ES-62, a product of parasitic filarial nematodes. Nat Med 2007;13:1375–81.
[115] McInnes IB, Leung BP, Harnett M, Gracie JA, Liew FY, Harnett W. A novel therapeutic approach targeting articular inflammation using the filarial nematode-derived phosphorylcholine-containing glycoprotein ES-62. J Immunol 2003;171:2127–33.
[116] Hintz M, Schares G, Taubert A, et al. Juvenile female *Litomosoides sigmodontis* produce an excretory/secretory antigen (Juv-p120) highly modified with dimethylaminoethanol. Parasitology 1998;117:265–71.
[117] Houston KM, Babayan SA, Allen JE, Harnett W. Does *Litomosoides sigmodontis* synthesize dimethylethanolamine from choline? Parasitology 2008;135:55–61.
[118] Thomas PG, Carter MR, Atochina O, et al. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a toll-like receptor 4-dependent mechanism. J Immunol 2003;171:5837–41.
[119] Van de Vijver KK, Deelder AM, Jacobs W, Van Marck EA, Hokke CH. LacdiNAc- and LacNAc-containing glycans induce granulomas in an in vivo model for schistosome egg-induced hepatic granuloma formation. Glycobiology 2006;16:237–43.
[120] Jenkins SJ, Hewitson JP, Ferret-Bernard S, Mountford AP. Schistosome larvae stimulate macrophage cytokine production through TLR4-dependent and -independent pathways. Int Immunol 2005;17:1409–18.
[121] van der Kleij D, Latz E, Brouwers JFHM, et al. A novel host–parasite lipid cross talk: schistosomal lysophosphatidylserine activates Toll-like receptor 2 and affects immune polarization. J Biol Chem 2002;277:48122–9.
[122] Vermeire JJ, Cho Y, Lolis E, Bucala R, Cappello M. Orthologs of macrophage migration inhibitory factor from parasitic nematodes. Trend Parasitol 2008;24:355–63.
[123] Zang XX, Taylor P, Meyer D, et al. Homologues of human macrophage migration inhibitory factor from a parasitic nematode: gene cloning, protein activity and crystal structure. J Biol Chem 2002;277:44261–7.
[124] Cho Y, Jones BF, Vermeire JJ, et al. Structural and functional characterization of a secreted hookworm macrophage migration inhibitory factor (MIF) that interacts with the human MIF receptor CD74. J Biol Chem 2007;282: 23447–56.
[125] Prieto-Lafuente L, Gregory WF, Allen JE, Maizels RM. MIF homologues from a filarial nematode parasite synergize with IL-4 to Induce alternative activation of host macrophages. J Leukoc Biol 2009;85:844–54.
[126] Nair MG, Cochrane DW, Allen JE. Macrophages in chronic type 2 inflammation have a novel phenotype characterized by the abundant expression of Ym1 and Fizz1 that can be partly replicated in vitro. Immunol Lett 2003;85:173–80.
[127] McSorley HJ, Harcus YM, Murray J, Taylor MD, Maizels RM. Expansion of Foxp3$^+$ regulatory T cells in mice infected with the filarial parasite, *Brugia malayi*. J Immunol 2008;181:6456–66.
[128] Freitas T, Jung E, Pearce EJ. TGF-b signaling controls embryo development in the parasitic flatworm *Schistosoma mansoni*. PLOS Pathogens 2007;3:e52.
[129] Loukas A, Maizels RM. Helminth C-type lectins and host–parasite interactions. Parasitol Today 2000;16:333–9.
[130] Bulgheresi S, Schabussova I, Chen T, Mullin NP, Maizels RM, Ott JA. A new C-type lectin similar to the human immunoreceptor DC-SIGN mediates symbiont acquisition by a marine nematode. Appl Environ Microbiol 2006;72:2950–6.
[131] Brown AC, Harrison LM, Kapulkin W, et al. Molecular cloning and characterization of a C-type lectin from *Ancylostoma ceylanicum:* evidence for a role in hookworm reproductive physiology. Mol Biochem Parasitol 2007;151:141–7.
[132] Greenhalgh CJ, Loukas A, Donald D, Nikolaou S, Newton SE. A family of galectins from *Haemonchus contortus*. Mol Biochem Parasitol 2000;107:117–21.
[133] Turner DG, Wildblood LA, Inglis NF, Jones DG. Characterization of a galectin-like activity from the parasitic nematode, *Haemonchus contortus*, which modulates ovine eosinophil migration in vitro. Vet Immunol Immunopathol 2008;122:138–45.
[134] Hartmann S, Lucius R. Modulation of host immune responses by nematode cystatins. Int J Parasitol 2003;33:1291–302.
[135] Gregory WF, Maizels RM. Cystatins from filarial parasites: evolution, adaptation and function in the host–parasite relationship. Int J Biochem Cell Biol 2008;40:1389–98.
[136] Dainichi T, Maekawa Y, Ishii K, et al. Nippocystatin, a cysteine protease inhibitor from *Nippostrongylus brasiliensis*, inhibits antigen processing and modulates antigen-specific immune response. Infect Immun 2001;69:7380–6.
[137] Manoury B, Gregory WF, Maizels RM, Watts C. *Bm*-CPI-2, a cystatin homolog secreted by the filarial parasite *Brugia malayi*, inhibits class II MHC-restricted antigen processing. Curr Biol 2001;11:447–51.
[138] Schönemeyer A, Lucius R, Sonnenburg B, et al. Modulation of human T cell responses and macrophage functions by onchocystatin, a secreted protein of the filarial nematode *Onchocerca volvulus*. J Immunol 2001;167:3207–15.
[139] Schnoeller C, Rausch S, Pillai S, et al. A helminth immunomodulator reduces allergic and inflammatory responses by induction of IL-10-producing macrophages. J Immunol 2008;180:4265–72.
[140] Zang X, Maizels RM. Serine proteinase inhibitors from nematodes and the arms race between host and pathogen. Trends Biochem Sci 2001;26: 191–7.
[141] Zang XX, Atmadja AK, Gray P, et al. The serpin secreted by *Brugia malayi* microfilariae, Bm-SPN-2, elicits strong, but short-lived, immune responses in mice and humans. J Immunol 2000;165:5161–9.
[142] Stanley P, Stein PE. BmSPN2, a serpin secreted by the filarial nematode *Brugia malayi*, does not inhibit human neutrophil proteinases but plays a noninhibitory role. Biochemistry 2003;42:6241–8.
[143] Ou X, Tang L, McCrossan M, Henkle-Dührsen K, Selkirk ME. *Brugia malayi*: localisation and differential expression of extracellular and cytoplasmic CuZn superoxide dismutases in adults and microfilariae. Exp Parasitol 1995;80:515–29.
[144] Lawrence CE, Pritchard DI. Differential secretion of acetylcholinesterase and proteases during the development of *Heligmosomoides polygyrus*. Int J Parasitol 1993;23:309–14.
[145] Hussein A, Harel M, Selkirk M. A distinct family of acetylcholinesterases is secreted by *Nippostrongylus brasiliensis*. Mol Biochem Parasitol 2002;123:125–34.
[146] McKeand JB. Vaccine development and diagnostics of *Dictyocaulus viviparus*. Parasitology 2000;120:S17–23.
[147] Rathaur S, Robertson BD, Selkirk ME, Maizels RM. Secreted acetylcholinesterases from *Brugia malayi* adult and microfilarial parasites. Mol Biochem Parasitol 1987;26:257–65.
[148] Selkirk ME, Lazari O, Hussein AS, Matthews JB. Nematode acetylcholinesterases are encoded by multiple genes and perform non-overlapping functions. Chem-Biol Interact 2005;157–158:263–8.
[149] Grigg ME, Gounaris K, Selkirk ME. Characterization of a platelet-activating factor acetylhydrolase secreted by the nematode parasite *Nippostrongylus brasiliensis*. Biochem J 1996;317:541–7.
[150] Bin Z, Hawdon J, Qiang S, et al. *Ancylostoma* secreted protein 1 (ASP-1) homologues in human hookworms. Mol Biochem Parasitol 1999;98:143–9.
[151] Murray J, Gregory WF, Gomez-Escobar N, Atmadja AK, Maizels RM. Expression and immune recognition of *Brugia malayi* VAL-1, a homologue of vespid venom allergens and *Ancylostoma* secreted proteins. Mol Biochem Parasitol 2001;118:89–96.
[152] Chalmers IW, McArdle AJ, Coulson RM, et al. Developmentally regulated expression, alternative splicing and distinct sub-groupings in members of the *Schistosoma mansoni* venom allergen-like (SmVAL) gene family. BMC Genom 2008;9:89.
[153] Maizels RM, Gomez-Escobar N, Gregory WF, Murray J, Zang X. Immune evasion genes from filarial nematodes. Int J Parasitol 2001;31:889–98.
[154] Moyle M, Foster DL, McGrath DE, et al. A hookworm glycoprotein that inhibits neutrophil function is a ligand of the integrin CD11b/CD18. J Biol Chem 1994;269:10008–15.
[155] Rieu P, Sugimori T, Griffith DL, Arnaout MA. Solvent-accessible residues on the metal ion-dependent adhesion site face of integrin CR3 mediate its binding to the neutrophil inhibitory factor. J Biol Chem 1996;271:15858–61.
[156] Asojo OA, Goud GN, Dhar K, et al. X-ray structure of Na-ASP-2, a pathogenesis related-1 protein from the nematode parasite, *Necator americanus*, and a vaccine antigen for human hookworm infection. J Mol Biol 2005;346: 801–14.

[157] Bower MA, Constant SL, Mendez S. *Necator americanus*: the Na-ASP-2 protein secreted by the infective larvae induces neutrophil recruitment in vivo and in vitro. Exp Parasitol 2008;118:569–75.
[158] Gregory WF, Atmadja AK, Allen JE, Maizels RM. The *abundant larval transcript* 1/2 genes of *Brugia malayi* encode stage-specific candidate vaccine antigens for filariasis. Infect Immun 2000;68:4174–9.
[159] Wu Y, Egerton G, Pappins DJC, et al. The secreted larval acidic proteins (SLAPs) of *Onchocerca* spp. are encoded by orthologues of the *alt* gene family of *Brugia malayi* and have host protective potential. Mol Biochem Parasitol 2004;134:213–24.
[160] Gomez-Escobar N, Bennett C, Prieto-Lafuente L, Aebischer T, Blackburn CC, Maizels RM. Heterologous expression of the filarial nematode *alt* gene products reveals their potential to inhibit immune function. BMC Biol 2005;3(8):1–16.
[161] Selkirk ME, Gregory WF, Jenkins RE, Maizels RM. Localization, turnover and conservation of gp15/400 in different stages of *Brugia malayi*. Parasitology 1993;107:449–57.
[162] Kennedy MW, Allen JE, Wright AS, McCruden AB, Cooper A. The gp15/400 polyprotein antigen of *Brugia malayi* binds fatty acids and retinoids. Mol Biochem Parasitol 1995;71:41–50.
[163] Mucida D, Park Y, Kim G, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 2007;317:256–60.
[164] Imai S, Tezuka H, Furuhashi Y, Muto R, Fujita K. A factor of inducing IgE from a filarial parasite is an agonist of human CD40. J Biol Chem 2001;276:46118–24.
[165] Imai S, Tezuka H, Fujita K. A factor of inducing IgE from a filarial parasite prevents insulin-dependent diabetes mellitus in nonobese diabetic mice. Biochem Biophys Res Commun 2001;286:1051–8.
[166] Maizels RM. Infections and allergy—helminths, hygiene and host immune regulation. Curr Opin Immunol 2005;17:656–61.
[167] Delcroix M, Medzihradsky K, Caffrey CR, Fetter RD, McKerrow JH. Proteomic analysis of adult *S. mansoni* gut contents. Mol Biochem Parasitol 2007;154:95–7.
[168] Smith SK, Nisbet AJ, Meikle LI, et al. Proteomic analysis of excretory/secretory products released by *Teladorsagia circumcincta* larvae early post-infection. Parasite Immunol 2009;31:10–9.
[169] Guillou F, Roger E, Mone Y, et al. Excretory–secretory proteome of larval *Schistosoma mansoni* and *Echinostoma caproni*, two parasites of *Biomphalaria glabrata*. Mol Biochem Parasitol 2007;155:45–56.
[170] Bennuru S, Semnani R, Meng Z, Ribeiro JM, Veenstra TD, Nutman TB. *Brugia malayi* excreted/secreted proteins at the host/parasite Interface: stage- and gender-specific proteomic profiling. PLoS Negl Trop Dis 2009;3:e410.
[171] Robinson MW, Menon R, Donnelly SM, Dalton JP, Ranganathan S. An integrated transcriptomic and proteomic analysis of the secretome of the helminth pathogen, *Fasciola hepatica*: proteins associated with invasion and infection of the mammalian host. Mol Cell Proteomics 2009 in press.