REVIEW ARTICLE

Parasitic helminths: a pharmacopeia of anti-inflammatory molecules

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SUMMARY

Infection with parasitic helminths takes a heavy toll on the health and well-being of humans and their domestic livestock, concomitantly resulting in major economic losses. Analyses have consistently revealed bioactive molecules in extracts of helminths or in their excretory/secretory products that modulate the immune response of the host. It is our view that parasitic helminths are an untapped source of immunomodulatory substances that, in pure form, could become new drugs (or models for drug design) to treat disease. Here, we illustrate the range of immunomodulatory molecules in selected parasitic trematodes, cestodes and nematodes, their impact on the immune cells in the host and how the host may recognize these molecules. There are many examples of the partial characterization of helminth-derived immunomodulatory molecules, but these have not yet translated into new drugs, reflecting the difficulty of isolating and fully characterizing proteins, glycoproteins and lipid-based molecules from small amounts of parasite material. However, this should not deter the investigator, since analytical techniques are now being used to accrue considerable structural information on parasite-derived molecules, even when only minute quantities of tissue are available. With the introduction of methodologies to purify and structurally-characterize molecules from small amounts of tissue and the application of high throughput immunological assays, one would predict that an assessment of parasitic helminths will yield a variety of novel drug candidates in the coming years.

Key words: helminths, immunomodulators, anti-inflammatory drugs, drug design.

INTRODUCTION

Infections with parasitic helminths continue to cause considerable morbidity and mortality in humans and their domestic livestock, translating into substantial socioeconomic losses. For instance, morbidity associated with Schistosoma mansoni or Echinococcus granulosus infections is debilitating and sometimes fatal (Carod-Artal, 2008; King and Dangerfield-Cha, 2008), and infections with hookworms retards the growth of children and can impair their cognitive functions (Oberhelman et al. 1998). The magnitude of the problems is illustrated by estimates from the World Health Organization (WHO, 1999) that, even in the midst of successful anthelmintic programs, 30% of the world’s population harbours at least 1 species of helminth parasite; a staggering 2 billion people are affected! However, numerous studies have shown that modulation of the immune response of the host by parasitic helminths can have a concomitant health benefit (reviewed by McKay, 2006). In addition to infections with live organisms, helminth-derived molecules are potent immunomodulators and can serve as templates for the design of novel anti-inflammatory drugs, and may also be candidates for vaccine development (Harnett and Harnett, 2008; Perrigoue et al. 2008). The former aspect of the host-parasite interaction is the topic of this review.

HELMINTH THERAPY: THE GOOD, THE BAD AND THE WRIGGLY

Parasitic helminths have been humans’ long-time companion and have evolved as ‘master regulators’ of host immune responses (Maizels et al. 2004). Consequently, knowledge of how the parasite modifies host immunity can be used to develop therapies for other diseases. In considering ‘helminth therapy’, one can either use viable infections or immunomodulatory molecules from the parasite; both strategies have been used to treat diseases in animal models. Infection with a variety of species of...
parasitic helminths has been shown to reduce the severity of airway, cerebral and intestinal inflammation in murine models of human disease (McKay, 2006), and a number of investigators are pursuing the mechanism(s) underlying this health benefit (Weinstock et al. 2005). Moreover, Weinstock and colleagues have shown that the ingestion of viable eggs of *Trichuris suis* by patients with inflammatory bowel disease (Crohn’s disease and ulcerative colitis) led to a reduction in disease activity, and adverse side-effects were not apparent (Summers et al. 2003, 2005). However, there can be a number of concerns regarding the use of an infection as a therapy. First, a parasite that has limited impact on the host must be used, which prohibits the use of particular species, such as *S. mansoni*, despite its potent stimulation of host immunity. Second, there are patient groups for which this approach would not be advocated, typically children, the elderly, immunocompromised individuals and pregnant women (Farah et al. 2007). Third, there are examples of helminth infections worsening disease evoked by bacteria or protozoa (Chen et al. 2005; Marshall et al. 1999) and, in at least one case, chemically-induced colitis (Hunter et al. 2007). Finally, when introducing a species to a novel environment, there is the potential for maladaptive responses and unpredicted pathological effects/changes in remote tissues. Moreover, the possibility exists for parasite establishment in, rather than expulsion from the host (Kradin et al. 2006). Despite these issues, the data on ‘helminth therapy’ are encouraging, and animal models can be used to identify novel anti-inflammatory mechanisms for translation into medical treatments, negating the need for actual infection.

The use of helminth-derived molecules as drugs overcomes the concerns with the use of live parasites. However, the issues here are often technical and revolve around a single basic question: how can pure molecules be isolated from miniscule amounts of parasite material to allow testing in biological systems and to serve as blueprints for drug development?

**IMMUNE RESPONSES TO HELMINTH PARASITES: THE BASICS**

A number of authoritative reviews of the mammalian immune system and the responses to infection with helminths are available (Hayes et al. 2004; Maizels et al. 2004; Antony et al. 2007; Khan, 2008; Perrigoue et al. 2008). As metazoans, helminths cannot be ingested by macrophages and other phagocytes, so that a variety of effector mechanisms have evolved to combat helminths. These involve the mobilization and activation of T and B lymphocytes, mast cells, eosinophils, natural killer (NK) cells, and, if the infection is at a mucosal surface, mucus-producing goblet cells. Briefly, while there is likely to be an innate immune response to helminth-derived products by macrophages and natural killer cells, the current view is that the predominant anti-worm response is the remit of an adaptive immune response. Thus, following antigen processing and presentation by professional antigen presenting cells (e.g., dendritic cells (DCΦs)), humans and mice display a stereotypic T cell response which is characterized by the development of CD4+ T helper (TH)-2 cells that produce interleukin (IL)-4, -5, -13 and others. These chemical signals mobilize other cells; for example, they direct isotype switching, so that high affinity immunoglobulin (Ig) G, E and A can be produced. These antibodies then bind to receptors on effector cells, allowing for their activation by worm antigen and the release of molecules (e.g., mast-derived histamine and trypases, eosinophil-derived cationic protein) that either disrupt the surface of the helminth and/or alter the environment, making it less hospitable to the helminth. In addition, IgG can bind directly to the surface of the helminth (opsonization), interfering with worm behaviour and targeting the worm for attack by complement and polymorphonuclear cells. Thus, the host response to infection with parasitic helminths is multi-cellular and is orchestrated by Th2 cells/cytokines. Recent studies are elucidating a role for additional cytokines such as IL-21 and IL-33 (Humphreys et al. 2008) and regulator cells, including the CD4+/Foxp3+ natural regulatory T cells and, alternatively activated macrophages (AAM) (Baumgart et al. 2006; Taylor et al. 2006; Persaud et al. 2007). While all immune cells can be considered as immunomodulatory (i.e., they affect other immune and stromal cells in their vicinity), regulatory T cells and AAMs (of which there are multiple types for each; Belkaid and Oldenhove, 2008; Reyes and Terrazas, 2007) have emerged as prototypic immunomodulators whose primary function is to switch-off or dampen immune reactions, or engage reactions that promote tissue recovery and restitution. Much research remains to be done to precisely define the biology of regulatory T cells and AAMs; however, the mobilization of both classes of cells following an infection with helminths would generate an immunoregulatory/immunosuppressive environment in which a spectrum of immune events would be inhibited. Thus, by extrapolation, the ability of products from the helminth to elicit regulatory T cell or AAM activity could contribute to worm infectivity and survival.

**HELMINTH-DERIVED IMMUNOMODULATORS**

An analysis of the biology of a parasite should include studying the mechanisms of the interaction of the parasite with the host immune system, so that potential parasite-derived immunomodulators (used to develop drugs to treat immuno-pathological
changes/disease) can be identified. During the last 40 years, a staggering number of helminth-derived immunomodulators have been partially characterized, both in terms of structure and bioactivity (we define an ‘immunomodulator’ as any molecule that regulates host immunity, but not antigens recognized by the host that are used to generate immune responses aimed at the destruction or elimination of the parasite from the host). Here, we focus on selected examples from the 3 main groups of parasitic helminths and draw the reader’s attention to the fact that a literature search will reveal many other examples, all worthy of discussion, and excluded only on the basis of space restrictions (the examples given below are supplemented by additional material in Tables 1–3).

**Trematodes**

*Schistosoma* spp. Schistosomes, because of their impact on human health (King and Dangerfield-Cha, 2008), have been the focus of intense research efforts, and a variety of glycoconjugates have been extracted from the eggs and adult tegument (Bennett, 1963). Soluble egg antigen (SEA) from *S. mansoni* has been implicated in the host-response to infection, and has been shown consistently to have immunomodulatory properties. For example, SEA appears to be responsible for the reduction in CD4+ lymphocytes 6 weeks following infection, an event that was attributed to enhanced Fas-FasL-induced apoptosis involving CD19+ B cells (Lundy et al. 2001). Excretory/secretory (ES) products from schistosomula contain a 23 kDa molecule that results in T cell depletion *in vivo* via Fas-FasL killing. Exposure to SEA results in reduced levels of nitric oxide (NO) and T helper cell-type 1 (TH1) chemokines (CCL3) and cytokines (tumour necrosis factor-α (TNFα), IL-12) and an increased expression of markers indicative of AAMs. These events are all compatible with the mobilization of TH2-type cytokines (*e.g.* IL-4, IL-13) which is stereotypic of the mammalian immune response to infection by helminth parasites. The induction of AAMs is of particular note, as these cells exert anti-inflammatory effects and are active in tissue restitution after injury (Gordon and Taylor, 2005). Thus, the ability to use a constitutent of SEA to induce this cellular response pharmacologically could be of direct health benefit.

Mice treated with SEA display endothelial cell proliferation and capillary formation which appear to be driven by a heat- and protease-insensitive molecule, indicative of a glycan (Kanse et al. 2005). These findings complement the work of van de Vijver et al. (2006) that detected LDN (GalNAcβ1,4GlcnAc) and LN (Galβ1,4GlcnAc) modified glycans in SEA-stimulated granulomata. SEA has been shown to induce vascular endothelial growth factor (VEGF) secretion (Loeffler et al. 2002) and fibroblast-stimulating factor (FSF-1) (Greenwel et al. 1993), which could contribute to the formation of the fibrous capsule around the granulomata, although it is unclear whether the stimulation of the production of either growth factor was due to a glycan component of the SEA. Nevertheless, one could speculate that SEA evoked growth factor release from host cells to stimulate angiogenesis could be used to enhance the recovery from tissue damage, although the potential to elicit metastatic events should not be overlooked.

In addition, while the exposure to SEA decreased the expression of ‘Toll-like receptors (TLR) on macrophages, the responses of these cells to TLR agonists (*e.g.*, lipopolysaccharide (LPS)) were not appreciably retarded (Joshi et al. 2008). These studies are intriguing, as they demonstrate a cross-talk between infection with parasitic helminths and responsiveness to bacteria and may illuminate a way in which one could exploit one type of infection to treat another. In addition, the complexity of the host response to infection, which is linked temporally or spatially and/or parasite-specific, is aptly illustrated by these studies.

The identification of a >100 kDa heat-insensitive, carbohydrate-positive fraction from *S. mansoni* (see Hayunga et al. 1979) was followed by studies characterizing the glycoproteins of schistosomes and notably led to the elucidation of lacto-N-fucopentose (LNFPIII) (carbohydrate containing the mammalian LewisX trisaccharide [Galβ1,4[Fuc×1,3 GlcNAc]] and lacto-N-neotetrose (LNT) (Ko et al. 1990). Analysis of LNT revealed that its injection into mice caused an expansion of a Gr1+ cell population which suppressed CD4+ T cell proliferation and cytokine production *via* an apoptosis-independent but cell contact-dependent mechanism (Terrazas et al. 2001). The authors postulated that this treatment ‘imprinted’ the Gr1+ cells with a TH2-type phenotype.

Since the LewisX antigen is present in the lymphocyte function-associated molecule-1 (LFA-1), the role of LNFPIII in lymphocyte proliferation was assessed and shown to stimulate IL-10 and prostaglandin E2 (PGE2) production from B-cells of infected mice without hampering antigen presentation (Velupillai and Harn, 1994). Also, the co-culture of LNFPIII-treated macrophages and NK cells enhanced NK cell activation as gauged by the expression of CD69 and interferon-γ (IFNγ) production (Atochina and Harn, 2005). Lacto-N-fucopentose III also generates anti-inflammatory responses by signalling through Toll-like receptor 4 (TLR4) to induce the phosphorylation and activation of the mitogen-activated protein kinase (MAPK), ERK (Thomas et al. 2003).

The presence of fucose in the LewisX moiety of LNFPIII is essential for bioactivity (Okano et al.
Table 1. Selected examples of immunomodulatory molecules from trematodes

<table>
<thead>
<tr>
<th>Species</th>
<th>Worm product</th>
<th>Bioactivity</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>Schistosoma</em></td>
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<tr>
<td><em>japonicum</em></td>
<td>900 kDa ECF-SjE from homogenized eggs; pronase and heat insensitive glycoprotein; destroyed by periodate oxidation</td>
<td><em>In vitro</em> eosinophil chemotaxis</td>
<td>Ohashi and Ishii (1982)</td>
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<tr>
<td></td>
<td>440 kDa JAE-H and &lt;440 kDa JAE-L glycoproteins from adult ES products</td>
<td>Eosinophil chemotactic factors; JAE-L also induces neutrophil chemotaxis</td>
<td>Horii <em>et al.</em> (1984)</td>
</tr>
<tr>
<td><em>Schistosoma</em></td>
<td>Analogues of adrenocorticotrophin (ACTH) and α-melanotrophin (α-MSH)</td>
<td>ACTH converts to α-MSH by polymorphonuclear cells via neutral endopeptidase; α-MSH inhibits leukocyte adherence and immunosuppressive</td>
<td>Duvaux-Miret <em>et al.</em> (1992)</td>
</tr>
<tr>
<td><em>mansoni</em></td>
<td>Various surface glycans</td>
<td>Fucose and Galactose linked to bovine serum albumin reduced ERK and PKC phosphorylation and phagocytosis in <em>Lymnaea stagnalis</em> haemocytes</td>
<td>Plows <em>et al.</em> (2005)</td>
</tr>
<tr>
<td><em>Fasciola</em></td>
<td>25 kDa glutathione S-transferase in ES products</td>
<td>May degrade host mucins rich in galactose, N-acetylgalactosamine and N-acetylgalactosamin</td>
<td>Guillou <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>hepatica</em></td>
<td>ES products contain metal ion dependent glycosidases (β-galactosidase, β-N-acetylhexosaminidase and β-glucosidase) ES products</td>
<td>Prevent superoxide production by PMA-activated sheep and human neutrophils <em>in vitro</em> (a heat resistant ES component from the related species, <em>F. gigantica</em>, does the same)</td>
<td>Jefferies <em>et al.</em> (1997)</td>
</tr>
<tr>
<td><em>Diplostomum</em></td>
<td>22–24 kDa lectin with homology to β1,3 glucan binding protein localizes to the cercarial penetration glands</td>
<td>Agglutinates murine red blood cells and may facilitate tissue recognition and penetration</td>
<td>Mikes and Horak (2001)</td>
</tr>
<tr>
<td><em>pseudopathaceum</em></td>
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<tr>
<td><em>Paragonimus</em></td>
<td>27 kDa cysteine protease in ES products</td>
<td>Induces superoxide production and human eosinophil degranulation</td>
<td>Chung <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>westernmani</em></td>
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<tr>
<td><em>Opisthorchis</em></td>
<td>24 kDa thioredoxin peroxidase (TPx) isolated by genomic probing; also exists in ES products</td>
<td>Protects worm from reactive oxygen metabolites and may have other roles similar to <em>F. hepatica</em> TPx</td>
<td>Suttiprapa <em>et al.</em> (2008)</td>
</tr>
</tbody>
</table>
1999), since lacto-N-neotetrose lacking Lewis^X had no effect on DCΦ differentiation to an IL-4 producing phenotype in response to LPS. Importantly, the induction of accessory molecules is required to increase the strength of an immune response; DC-SIGN has strong affinity for intercellular adhesion molecule-3 (ICAM3) via the Lewis^X structure (Bogoevska et al. 2007), suggesting a possible mechanism used by LNFPIII to bind to DCΦ and interfere with cell-to-cell communication with other immune cells (Appelmelk et al. 2003). The multivalency of LNFPIII is important for its bioactivity, since monovalent LNFPIII was shown to be incapable of inhibiting antigen-induced peripheral blood mononuclear cell (PBMC) activation (Velupillai et al. 2000). At the signalling level, LNFPIII increases nuclear factor (NF)-κB activity in DCΦ to a level similar to LPS; however, a second phase (prolonged NF-κB activation associated with IkB degradation) was not observed. Use of the nuclear import inhibitor, SN50, and NFκB-deficient mice revealed that LNFPIII effects on DCΦ were indeed NFκB-dependent (Thomas et al. 2005). Collectively, these studies suggest that LNFPIII (or possibly fragments thereof) could be used to extrinsically manipulate DCΦ function as a way of limiting immunopathological reactions/changes or controlling immune cell responsiveness. The stimulation of IL-10 and IL-12p40 from murine peritoneal macrophages by cercarial ES products required the presence of TLR4, whereas the production of IL-6 was TLR4 independent (Jenkins et al. 2005). Furthermore, IL-6 release was reduced following protease treatment of the ES products suggesting a proteinaceous ligand, and sodium periodate ablated activity of the cercarial extract, indicating the presence of glycans. It is plausible that cercarial products contain proteins/glycoproteins that can act via TLR4, highlighting the promiscuity of TLR4 as more than an LPS receptor. Other TLRs have been implicated as receptors for schistosome ligands. Lysophosphatidylerine from S. mansoni induces DCΦ maturation and decreases IL-12p70 production via TLR2. The activity is abrogated by TLR2-blocking antibodies (van der Kleij et al. 2002), whereas host phosphatidylerine neither activates TLR2 nor induces DCΦ maturation. Additionally, phosphoserine alone is not effective in this system, indicating that the acyl and phosphoserine groups of S. mansoni lysophosphatidylerine are required for the effect. Inhibition of lysophosphatidylerine acyltransferase (LPC-AT) did not affect TLR4 expression or the binding of LPS, but it did prevent TLR4 translocation to lipid rafts and thus blocked the formation of the TLR4 complex with a resultant decrease in TNFα and IL-6 production (Jackson et al. 2008). It is possible that S. mansoni lysophosphatidylerine also signals through TLR4 in a similar manner. Studies such as these illustrate an emergent theme, in which reponses to helminth-derived molecules occur via receptors that have been defined on the basis of their ability to recognize conserved microbe-derived products; however, the data must be interpreted with caution, since the possibility of bacterial contamination of the helminth extracts is omnipresent.

_Fasciola hepatica._ ES products from adult _F. hepatica_ (FhES) contain a glycan that is cytotoxic to eosinophils and acts via tyrosine kinase and caspase-dependent mechanisms (Serradell et al. 2007). Induction of AAMs often accompanies an infection with helminths, and it has been reported that _F. hepatica_ thioredoxin peroxidase elicits AAMs production (Donnelly et al. 2005). Numerous studies have shown that AAMs are induced by IL-4/IL-13 (Stein et al. 1992); however, in the case of _F. hepatica_ thioredoxin peroxidase, this conversion appeared to be IL-4-independent (Donnelly et al. 2005). The peroxidase also increased transforming growth factor-β (TGFβ) and PGE2 while decreasing IL-10, IL-12 and IL-18 from stimulated macrophages—such events in vivo would skew the immune response in favour of immunoregulation/immunosuppression and away from TH1-dominated pro-inflammatory reactions. Complementing these findings, the in vitro treatment of bovine macrophages with FhES products inhibited IFNγ and NO production in response to bacterial products and skewed the macrophage population towards an AAM phenotype; the latter event was lost by heat treatment of the ES products (Flynn and Mulcahy, 2008). This emphasizes that we must constantly be vigilant of the possibility that parasite-derived molecules, as well as those produced by the host (e.g., cytokines), can modify the host’s immunity. Finally, cathepsin L appears to be a dominant molecule in FhES and may aid _F. hepatica_ by degrading host tissue to assist parasite entry/migration, and interfering with the host’s immune response by, for example, the degradation of IgG (Smith et al. 1993; O’Neill et al. 2001).

_Cestodes._ Pseudophyllidean and cyclophyllidean tapeworms usually elicit limited damage in the alimentary tract of their definitive host. However, they are recognized by their host (i.e., anti-worm antibodies are generated) so, the ability to suppress or negate host immunity would remain a priority for the helminth (see Table 2).

_Taenia crassiceps._ T cells from _T. crassiceps_-infected mice are hypo-proliferative and, in the presence of excretory/secretory (TcES) products, show diminished responses to mitogen stimulation in vitro (Spolski et al. 2000, Scuitto et al. 1995). Administration of TcES products to mice caused expansion of F4/80^+ Gr1^+ cells, and these cells
Table 2. Selected examples of immunomodulatory molecules from cestodes

<table>
<thead>
<tr>
<th>Cestode</th>
<th>Worm product</th>
<th>Bioactivity</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td><em>Echinococcus</em></td>
<td>ES product</td>
<td>Modifies macrophage accessory function leading to inhibition of lymphocyte proliferation <em>in vitro</em></td>
<td>Rakha <em>et al.</em> (1991)</td>
</tr>
<tr>
<td><em>multilocularis</em></td>
<td>Metacestode glycosphingolipids</td>
<td>Inhibits PBMC proliferation; decreases IL-2/IL-2 receptor expression</td>
<td>Persat <em>et al.</em> (1996)</td>
</tr>
<tr>
<td></td>
<td>14-3-3 proteins in ES products</td>
<td>Inhibits LPS-induced nitric oxide production by rat macrophages</td>
<td>Andrade <em>et al.</em> (2004)</td>
</tr>
<tr>
<td><em>Echinococcus</em></td>
<td>E4 Galectin-1,4Gal glycan from protoscolexes extract</td>
<td>Inhibits mitogenic-induced proliferation; increases IL-10</td>
<td>Dematteis <em>et al.</em> (2001)</td>
</tr>
<tr>
<td><em>granulosus</em></td>
<td>Antigen B protease inhibitor in hydatid cyst fluid</td>
<td>Inhibits neutrophil recruitment <em>in vitro</em>; increased production of IL-4 and IL-13 by human PBMCs; impairs human DCΦ differentiation but polarizes maturation of immature DCΦ to TH2</td>
<td>Shepherd <em>et al.</em> (1991); Rigano <em>et al.</em> (2001, 2007)</td>
</tr>
<tr>
<td><em>Taenia</em></td>
<td>Glucose lectin in cyst fluid</td>
<td>May have similar function as ES product of <em>E. multilocularis</em></td>
<td>Judson <em>et al.</em> (1987)</td>
</tr>
<tr>
<td><em>multiceps</em></td>
<td>19.5 kDa proteinase inhibitor from somatic extract</td>
<td>Inhibits splenocyte proliferation in response to mitogenic and antigenic stimuli</td>
<td>Leid <em>et al.</em> (1984, 1986)</td>
</tr>
<tr>
<td></td>
<td>ES products</td>
<td>Sequesters complement; decreases mitogen-induced splenocyte proliferation IL-2 production</td>
<td>Suquet <em>et al.</em> (1984)</td>
</tr>
<tr>
<td><em>Taenia</em></td>
<td>Interferon-γ analogue (p66) in ES products</td>
<td>Enhances mitogen-induced splenocyte proliferation and IFNγ and IL-10 production; increases nitric oxide production by macrophages increases</td>
<td>Spolski <em>et al.</em> (2002)</td>
</tr>
<tr>
<td><em>taeniaeformis</em></td>
<td>RNA peptide in metacestode extracts</td>
<td>Inhibits response to <em>Salmonella</em> antigens as well as mitogen-induced proliferation and inflammatory responses to metacestode antigens, correlating with decreased IL-2, IFNγ and IL-4; reduced TNFα production by macrophages following LPS stimulation</td>
<td>Arechavaleta <em>et al.</em> (1998)</td>
</tr>
<tr>
<td><em>Taenia</em></td>
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<tr>
<td><em>crassiceps</em></td>
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<tr>
<td><em>Taenia</em></td>
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<tr>
<td><em>sollium</em></td>
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were capable of blocking T cell activation in vivo. Glycans in the TsES were essential for this response, since the treatment with sodium periodate significantly reduced F4/80\(^\text{Gr1}^+\) cells in the peritoneal cavity of infected mice and the production of cytokines and AAM (Gomez-Garcia et al. 2005). Extracts from the adult worm also directed a strong TH2 polarization (IL-4 production) and enhanced antibody production (IgG\(_1\) and IgE) in mice co-treated with ovalbumin (Gomez-Garcia et al. 2006). Concomitant with these events was an increased expression of markers indicative of AAMs, a predicted decrease in IFN\(\gamma\) and iNOS expression and increased synthesis of IL-10 and TGF\(\beta\). Like the ES products, the glycan component was critical for these effects of a crude antigen preparation from the adult worm, because all were abolished or significantly inhibited by sodium periodate treatment of the extract.

Extracts from the T. crassiceps metacestodes are highly mannosylated and contain both non-fucosylated and fucosylated structures, including the Lewis\(^X\) antigen (Dissanayake et al. 2004). In contrast to the adult worm antigen, the effect of which was unaltered by the knock-out of TLR4 (Gomez-Garcia et al. 2005), the ability of the glycans in the metacestode extract to elicit IL-6 production from murine peritoneal macrophages was reduced in cells lacking TLR2 and TLR4 (Dissanayake et al. 2004). The same researchers recently found that the terminal beta-(1-4)-galactose residue in the Lewis\(^X\) analogue can evoke IFN\(\gamma\) production by murine splenocytes and activate the NF-\(\kappa\)B pathway. The IFN\(\gamma\) production was reduced by inclusion of anti-TLR2, -4 and -6 antibodies in the assay (Dissanayake and Shahin, 2007), again reiterating the notion that helmith-derived products can exert their effects via TLRs.

* Spirometra erinaceieuropaei. Excretory/secretory (SeES) products contain immunoglobulins and a heat-sensitive factor that inhibits LPS- and lipoteichoic acid (LTA)-induced TNF\(\alpha\) and IL-1\(\beta\) production from murine macrophages (Dirgahayu et al. 2004). The inhibitory effect of the SeES products was not due to the stimulation of PGE\(_2\), IL-10 or TGF\(\beta\) in the target cells (Fukushima et al. 1993). The inhibition of macrophage production of TNF\(\alpha\), IL-1\(\beta\) and NO was also shown in a study by Kina et al. (2005), in which a 90 kDa trypsin-sensitive glycoprotein was identified as the active component in the SeES products. Similarly, a 94 kDa component of SeES products could block LPS-induced MAPK (i.e., ERK1, ERK2 and p38MAPK) phosphorylation and the accompanying synthesis of TNF\(\alpha\) (Dirgahayu et al. 2002).

* Hymenolepis diminuta. Extracts from adult H. diminuta reduced ConA-induced proliferation, IL-2 and IL-4 synthesis by murine spleen cells and human PBMC cells in a concentration-dependent manner (Wang and McKay, 2005). These events were mirrored, to a large extent, by use of ES products from the adult worm. A crude phosphate-buffered saline (PBS)-soluble extract of the adult worm stimulated IL-10 release from murine splenocytes and also contained a component with some structural similarity to the p40 chain of IL-12, as detected by enzyme-linked immunosorbent assay (ELISA) and immunoblotting. The bioactivity in both the worm extract and the ES products was within a > 50 kDa fraction and was partially sensitive to boiling (Wang and McKay, 2005).

**Nematodes**

Parasitic nematodes have been the focus of extensive research efforts because of their major socioeconomic impacts globally (Hall et al. 2008). Therefore, a number of immunomodulatory molecules have been identified from this phylum (Table 3). We focus this discussion by highlighting known molecules from Nippostrongylus brasiliensis, Trichinella spiralis, Necator americanus, Trichuris suis, Ascaris suum and Acanthocheilonema viteae.

* Nippostrongylus brasiliensis. ES products from this parasite direct a range of immunomodulatory reactions. As examples, (i) they reduce LPS-induced IL-12p40 production and expression of a panel of cytokine/chemokine genes in murine bone-marrow-derived DC\(\Phi\), (ii) an undefined protein component induces IL-4, IL-10 and IgE production, with the IL-4 response requiring the presence of major histocompatibility class II (MHC II) antigens and so presumably MHC II-T cell restricted events, and (iii) suppress TH1-events via an IL-10-independent mechanism (Holland et al. 2000, 2005; Balic et al. 2004). The characterization of the ES products revealed the presence of a serine acetylhydrolase capable of reducing intestinal anaphylactic responses by inactivating platelet activating factor and blocking neutrophil and eosinophil aggregation (Blackburn and Selkirk, 1992). The value of inhibiting eosinophil activity to the helminth is intuitive, and the use of this acetylhydrolase would be beneficial in limiting the pro-inflammatory effects of granulated phagocytes in other diseases. Furthermore, NbES blocked goblet cell hyperplasia, eosinophilia, neutrophilia and airway hyper-responsiveness in murine models of asthma (Keir et al. 2004; Trujillo-Vargas et al. 2007). The active component/s was/were heat and protease insensitive, suggesting a glycan-type molecule. When NbES was administered intraperitoneally to ovalbumin-sensitized mice, they developed less pathology on ovalbumin re-challenge than untreated controls; in contrast, intranasal NbES induced significant lung inflammation (Marsland...
Table 3. Selected examples of immunomodulatory molecules from nematodes

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Worm product</th>
<th>Bioactivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris suum</em></td>
<td>Pseudocoelomic fluid protein(s)</td>
<td>Induces eosinophil and neutrophil chemotaxis; reduces bronchoalveolar lavage fluid cell infiltrate, IL-5 and IL-13 as well as expression of CD40 and CD86 and is therapeutic in murine asthma models</td>
<td>Tanaka <em>et al.</em> (1979) McConchie <em>et al.</em> (2006)</td>
</tr>
<tr>
<td></td>
<td>Somatic extract from homogenized adults</td>
<td>Inhibits zymosan-induced murine arthritis; reduces hypersensitivity to ovalbumin independently of IL-10, and reduces cell proliferation, IL-2, IFNγ, eosinophil peroxidase, IL-4, IL-5 and eotaxin levels;</td>
<td>Rocha <em>et al.</em> (2008) Souza <em>et al.</em> (2002) Itami <em>et al.</em> (2005)</td>
</tr>
<tr>
<td></td>
<td>200 kDa PAS-1 from adult somatic extract</td>
<td>Inhibits airway allergy induced by the worm allergen via inhibition macrophage cytokine release</td>
<td>Lima <em>et al.</em> (2002)</td>
</tr>
<tr>
<td></td>
<td>Phosphorylcholine-conjugated glycosphingolipids from adults</td>
<td>Increases TNFα, IL-1β and IL-6 from human PBMCs; decreases B cell proliferation; is pro-apoptotic; decreases TH1 cytokine production from LPS + IFNγ treated macrophages</td>
<td>Deehan <em>et al.</em> (2002) Kean <em>et al.</em> (2006) Katz <em>et al.</em> (2001)</td>
</tr>
<tr>
<td><em>Anisakis simplex</em></td>
<td>Heat sensitive larval homogenate</td>
<td>Eosinophil chemotaxis</td>
<td>Tanaka and Torisu (1978)</td>
</tr>
<tr>
<td><em>Brugia malay</em></td>
<td>47 kDa serine protease inhibitor in ES products</td>
<td>May inhibit neutrophil cathepsin G and elastase (disputed by Stanley and Stein (2003))</td>
<td>Zang <em>et al.</em> (1999)</td>
</tr>
<tr>
<td></td>
<td>62 kDa asparaginyl-tRNA synthetase in ES products</td>
<td>Induces ERK1/2 MAPK phosphorylation via a G-protein coupled receptor; chemotactic for immature DCΦ, lymphocytes, eosinophils, neutrophils and cells positive for chemokine receptors CXCRI and CXCR2,</td>
<td>Kron <em>et al.</em> (1995) Ramirez <em>et al.</em> (2006)</td>
</tr>
<tr>
<td></td>
<td>Galectin-1 like molecule in ES products</td>
<td>Binds galactose containing glycoconjugates and may protect helminth from eosinophil and neutrophil mediated damage</td>
<td>Hewitson <em>et al.</em> (2008)</td>
</tr>
<tr>
<td></td>
<td>12 kDa macrophage migration inhibitory factor (MIF) in ES products</td>
<td>Likely to have similar function as <em>T. spiralis</em> MIF</td>
<td>Pastrana <em>et al.</em> (1998)</td>
</tr>
<tr>
<td><em>Necator americanus</em></td>
<td>ES products contain calreticulin</td>
<td>Identical to <em>H. contortus</em> calreticulin; C1q complement is bound which protects the nematode from surface damage while promoting feeding by sequestering pro-coagulants. May also bind C-reactive protein.</td>
<td>Kasper <em>et al.</em> (2001)</td>
</tr>
<tr>
<td><em>Trichinella spiralis</em></td>
<td>Encysting stage produces a 12 kDa macrophage migration inhibitory factor (MIF)-like molecule</td>
<td>Potential to regulate host macrophage responses</td>
<td>Wu <em>et al.</em> (2003)</td>
</tr>
<tr>
<td><em>Toxocara canis</em></td>
<td>O-glycans in ES products</td>
<td>May act as antibody decoys or to alter viscosity of host mucins</td>
<td>Gems and Maizels (1996) Loukas <em>et al.</em> (2000b)</td>
</tr>
<tr>
<td></td>
<td>Tumour associated antigen ((GalNAc-O-Ser/Thr)(Tn)) and GalNAc polypeptide N-acetylgalactosaminyltransferase (ppGaNTase) ES products</td>
<td>The antigen is synthesized by the enzyme via abnormal elongation of O-glycans and interacts with macrophage C-type lectins and affects T cell activation</td>
<td>Clausen and Bennett (1996) Casaravilla <em>et al.</em> (2003)</td>
</tr>
<tr>
<td><em>Teladorsagia circumcincta</em></td>
<td>Adult and L4 ES products contain thioredoxin peroxidase (TPx)</td>
<td>Impair eosinophil-mediated damage through unknown mechanism</td>
<td>Giacomin <em>et al.</em> (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Likely to have similar function to <em>F. hepatica</em> TPx in affecting TH1/TH2 cytokine balance</td>
<td>Craig <em>et al.</em> (2006)</td>
</tr>
</tbody>
</table>
et al. 2005). Finally, and intriguingly, *N. brasiliensis* may be worthy of consideration in cancer therapy. When carcinogenic cells were injected with *N. brasiliensis* extracts, eosinophils were recruited and attacked the cells (i.e., as if they were a helminth), resulting in reduced size of the carcinoma (Frenoy, 2005).

**Trichinella spiralis.** Structural and biochemical analysis of *T. spiralis* ES products (*TsES*) from larvae or adult worms revealed the presence of a nucleoside 5′-diphosphate phosphohydrolase (apyrase, 17 kDa), a 5′nucleotidase that preferentially targets AMP to generate adenosine and an adenosine deaminase (Gounaris, 2002; Gounaris et al. 2004). The ability to regulate AMP metabolism has a number of potential immunomodulatory effects, since adenosine is known to inhibit platelet aggregation, monocytes/macrophage TH1 cytokine production, and neutrophil synthesis of reactive oxygen species (Liaudet et al. 2002; Antonioli et al. 2008), and is protective in a murine model of colitis (Mabley et al. 2003). Also, adenosine stimulates epithelial electrogenic Cl⁻ secretion, which creates a driving force for water to move into the gut (Kottgen et al. 2003) and could act to flush *Trichinella* larvae as well as intestinal bacteria and protozoan pathogens from the host.

**Necator americanus.** Nematode-derived proteases serve a number of vital roles in larval exsheathment, the digestion of host tissue (facilitating invasion and migration) and in immune evasion (Gamble et al. 1989, McKerrow et al. 1990). *Necator americanus* ES (*NaES*) contains at least 7 cysteine proteases, 1 serine protease and various metalloproteases, some of which act as immunoglobulinases (Kumar and Pritchard, 1992). These enzymes exert a range of bioactivities, the majority of which are unknown. However, one can speculate that the cleavage of immunoglobulins (Igs) could result in reduced complement activation and that the activity of cells that bind Igs and are activated by antibody-cross-linkage by antigen (e.g., mast cells and eosinophils) would be reduced. Eosinophil recruitment in vivo could also be perturbed by, for example, the degradation of the chemokine, eotaxin-1 (Culley et al. 2000). Additionally, a *Necator americanus* protein was shown to evoke release of IFNγ from murine and human NK cells but not NK T cells (Hsieh et al. 2004).

**Trichuris suis and Ascaris suum.** *Trichuris suis* ES (*TrsES*) products contain heat-stable and protease-insensitive components which reduce the growth of the bacteria *Campylobacter jejuni*, *Escherichia coli* and *Staphylococcus aureus* (see Abner et al. 2001). High performance liquid chromatography (HPLC) did not reveal the presence of antibiotics (i.e., penicillin, streptomycin and amphotericin) in the
The active component was <10 kDa in size. *A. suum* pseudocelomic fluid (PCF) also contained a 6–14 kDa heat-stable, trypsin-sensitive molecule which could kill Gram-positive bacteria (e.g., *S. aureus*). The molecule was designated ASABF (*Ascaris suum* antibacterial factor) (Kato and Komatsu, 1996) and is similar to insect defensins which penetrate Gram-positive bacterial membranes. Genes encoding antibacterial cecropins had been characterized in *A. suum*, and chemically synthesized versions are active against numerous species of bacteria (Pillai *et al.* 2005). With increasing incidence of antibiotic-resistant bacteria, the putative usefulness of these nematode-derived molecules is apparent.

_Acanthocheilonema viteae_. Nematode-derived cystatins (cysteine protease inhibitors) have been repeatedly shown to regulate immune cell activity. Cystatins from _A. viteae_ (as well as those of other filarioids, including _Onchocerca volvulus_ and _Brugia pahangi_ ) inhibit T cell proliferation, interfere with antigen processing and presentation, and enhance IL-10 production from stimulated murine spleen cells (Osborne and Devaney, 1999; reviewed by Hartmann and Lucius, 2003). Recently an _A. viteae_-derived cystatin has been demonstrated to reduce inflammation in a murine model of airway allergy (Schnoeller *et al.* 2008). Onchocystatin (Ov17) from _O. volvulus_ was specifically shown to suppress PBMC proliferation by decreasing expression of the co-stimulatory molecule B7.2 (CD86) (Schonemeyer *et al.* 2001), and infection with _O. volvulus_ resulted in IL-10/TGFβ-mediated immune hypo-responsiveness (Doetzte *et al.* 2000). In that study, the source of the IL-10 and TGFβ was most likely host cells, but the possibility of helminth-derived analogues of these cytokines should not be overlooked. For example, a TGFβ-like molecule has been isolated from the filarial nematodes _Brugia malayi_ and _Brugia pahangi_ (Gomez-Escobar *et al.* 2000).

The best described helminth immunomodulator is an _A. viteae_ ES 62 kDa glycoprotein (ES-62) product. The bioactivity of ES-62 is critically dependent on phosphorylcholine (PC) attached to an N-glycan (likely GlcNAc) via acetylglycosaminyltransferase I, such that recombinant ES-62 lacking PC is generally non-functional (Goodridge *et al.* 2004; Houston and Harnett, 2004; Houston *et al.* 2008). Homologues of ES-62 exist in related nematodes (Stepek *et al.* 2002, 2004; Hewitson *et al.* 2008). Harnett *et al.* (2004) continue to explore the bioactivity of ES-62 in cell culture systems and murine models. Thus, ES-62 has been shown to enhance the production of IL-4 by murine splenoocytes (Harnett *et al.* 1999) and decrease IFNγ, TNFα, IL-1β, IL-12 and IL-18 from DCΦ stimulated with TLR ligands (Whelan *et al.* 2000; Goodridge *et al.* 2001, 2004). Complementing the latter, macrophages from mice implanted with mini-osmotic pumps containing ES-62 were hypo-responsive to bacterial stimuli. ES-62 treatment in a murine model of arthritis resulted in significantly less inflammatory disease, which was accompanied by reduced IFNγ, TNFα and IL-6, and increased IL-10 synthesis by stimulated lymphocytes (McInnes *et al.* 2003). Significantly, IFNγ and T cell proliferation remained suppressed for up to 50 days after treatment whereas TNFα and IL-6 suppression required ongoing exposure, as did the inhibition of the production of collagen-specific antibodies. ES-62 also prevented B lymphocyte proliferation, and less IgG2a would contribute to reduced auto-immune driven arthritis (Wilson *et al.* 2003).

ES-62 inhibits phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAM) following B cell receptor ligation via the induction of the Src homology protein (SHIP-1) tyrosine phosphatase. This effectively prevents the recruitment of crucial signalling molecules, such as protein kinase C (PKC) (Goodridge *et al.* 2005a). A similar inhibition of PKC activity may relate to the ability of ES-62 to inhibit TH1 cytokine production (Deehan *et al.* 1997). In contrast, ES-62 inhibition of mast cell mediator release following the cross-linking of FceRI did not require tyrosine phosphatase activity (Melendez *et al.* 2007). Instead, the increase in intracellular Ca2+ elicited by receptor cross-linking was affected and mobilization of phospholipase D and sphingosine kinase was disrupted (Goodridge *et al.* 2005a). FcεRI-mediated NF-κB activation and the consequent synthesis of pro-inflammatory cytokines were abrogated by ES-62 through the disruption of the p50 and p65 subunits of NF-κB (Melendez *et al.* 2007). Given the pivotal role that mast cells play in immediate hypersensitivity and allergic reactions, a molecule, such as ES-62, could have many therapeutic applications.

The effect of ES-62 on mast cells appears to require TLR4 (Melendez *et al.* 2007), and this is in agreement with the examination of the effects of ES-62 on murine macrophages and DCΦ. This is not entirely unexpected, since PC can be a component on pathogen-associated molecular patterns (Goodridge *et al.* 2005b). Thus, cells deficient in TLR4 or MyD88 (a key intracellular adaptor molecule) were unaffected by ES-62. The utilization of MyD88 may be critical, since cytokine-inducing stimuli that do not require this molecule, for example, the ligation of TLR3 or the accessory molecule CD40 in macrophages, are not affected by ES-62 treatment (Goodridge *et al.* 2005b). Moreover, a natural mutation in the TIR domain in the TLR4 gene of C3H/HeJ mice renders these animals unresponsive to LPS, yet the ES-62 response remains, indicating that the helminth-derived molecule uses TLR4 in an unorthodox manner. It should be noted that the inhibitory effect of ES-62 can
be overridden, as observed in Toxoplasma gondii-infected mice, in which immunity relies on TH1 cytokines from a variety of cell types (Couper et al. 2005), or when T or B cells are challenged with two TH1-inducing antigens from O. volvulus (see Al-Riyami et al. 2008).

While the goal of this commentary is to illustrate the immunomodulatory effects of helminth-derived molecules and the potential of these molecules to serve as drugs or templates for drug design, it would be naïve to think that all parasite products would be beneficial. This point is intuitive and we will not belabour it here, but for the sake of balance in this discussion, we draw the reader’s attention to a few of many examples in which helminth-derived products elicit pathological reactions (Hill et al. 1993; Lee et al. 2006; Kim et al. 2008).

We have presented selected examples of immunomodulation by parasitic helminth-derived molecules. Since the essence of any host-parasite relationship is specificity, it follows that nuances of immunomodulation will be parasite-, and indeed life-cycle stage-specific. However, some commonality emerges from these studies and, as generalizations, helminth-derived products mobilize a TH2 cytokine response (and ablate TH-1 events), stimulate the synthesis of immunomodulatory/immunosuppressive molecules (e.g., IL-10, TGFβ, PGE2), and some evoke eosinophil (and neutrophil) apoptosis. All of these events would ameliorate inflammatory disease and, as a pharmacopoeia for new drugs, the potential of the parasitic helminths should not be underplayed. Yet, much remains to be explored, to move the field toward the development of drugs with a safety profile compatible with testing in clinical trials. At such a juncture, addressing issues relating to the in vitro stability and pharmacodynamics, and even the delivery methods of the helminth-derived molecule or drug, will be critical if new therapeutic modalities are to be achieved.

**Helminth Glycobiology**

Parasitic helminths as a group express or secrete/excrete a variety of molecules that have immunomodulatory properties. These molecules can be classical proteins or lipid-based, and many others are glycoproteins. We have opted to focus on parasite glycoproteins as molecules that could serve as models for the development of anti-inflammatory or immunoregulatory drugs, and will describe how such molecules can be isolated, characterized and might interact directly with mammalian cells.

**Glycan types**

An important type of protein post-translational modification is glycosylation, in which sugar residues are added on to a core protein/sugar sequence, resulting in antennary structures of differing complexity in terms of carbohydrate variety, linkage, presence of sulphated groups and antennae numbers. N-linked glycans are attached to asparagine (Asn) via the tripeptide sequence Asn-X-Ser/Thr, where X represents any amino acid except proline. N-glycans containing LDN (GalNAcβ1,3GlcNAc) in antennary regions are considered “complex” and are often capped by sialic acid or fucose via sialyl- and fucotransferases. O-linked glycans are attached to serine (Ser) or threonine (Thr) and, unlike N-glycans, do not share a common core sugar. O-glycan diversity is due to the presence of 8 possible core sugars. ‘Hybrids’ contain mannose and N-acetyllactosamine attached to a Manα2GlcNAc4 chitobiose core.

**Glycan isolation**

Technological advances in the last 30 years have revolutionized glycoconjugate analysis, and considerable data can now be gleaned from minute amounts of material. Thus, metabolic radio-isotope labelling of carbohydrates using [3H], [13C] and [14C], HPLC and fast performance liquid chromatography (FPLC), lectin affinity chromatography, followed by borate elution to increase the yield of glycans, coupled with more traditional approaches (e.g., high temperature [95 °C] hydrazinolysis, enzymatic cleavage and the use of periodate/borohydride to linearize sugar rings and remove reactive aldehydes) can yield considerable data on the nature of complex glycopolymers. Thus, mannose and fucose residues can be labelled using [2-3H]-mannose, sialic acid, GalNAc and GlcNAc with [6-3H]-glucosamine, whereas [6-14C]-galactose labels Gal and Glc. Further fractionation of labelled glycans can be accomplished via chromatography using agarose saturated with a single lectin type that reacts with carbohydrates in specific linkages (e.g., Phaseolus vulgaris agglutinin-agarose for tri- and tetra-antennary N-glycans possessing α-mannose substituted at C-2 and C-6 with Galβ1,4GlcNAc) (Morelle and Michaeliski, 2004). O-linked glycans have been analysed following β-elimination/release by mild alkaline and sodium borohydride treatment. However, this procedure releases some N-linked glycans and destroys O-acetyl groups, both of which can be avoided without lowering the yield of released glycans by using ammonia-borate β-elimination, with the added benefit that this treatment improves analysis via MALDI-MS (matrix assisted laser desorption/ionization time of flight) or CE-ESI-MS (capillary electrophoresis coupled to electrospray ionization mass spectrometry) (Huang et al. 2002).

All of these methodologies have been used to characterize helminth-derived glycans (Kang et al. 1993; Schallig and van Leeuwen, 1996; Casaravilla...
et al. 2003). For example, PNGase F and A treatment or methanolysis-evoked release of radio-isotope labelled N-glycans revealed fucosylated structures in the tegument of adult S. mansoni (see Nyame et al. 1989) and in H11 from Haemonchus contortus (see Haslam et al. 1996), while Tetragonolobus purpureas agglutinin-agarose electrophoresis and immunoblotting of metabolically labelled fractions with immune antisera demonstrated the Lewis\(^X\) antigen in schistosome glycoproteins (Srivatsan et al. 1992).

Other exquisitely sensitive techniques, such as fast atom bombardment (FAB)-MS, have provided structural information on glycans from Dicyoecaulus viviparus, H. contortus, T. spiralis, S. mansoni and O. volvulus (see Haslam et al. 1996, 1999, 2000) as well as demonstrating O-glycan abundance in Toxocara canis ES products and in the glycoalx of schistosome cercariae (Khoo et al. 1995). FAB-MS also demonstrated tyvelose (3,6-dideoxy-D-arabinohexose) on tri- and tetra-antennary glycans of T. spiralis, a modification previously regarded as unique to Gram-negative bacteria (Wisnewski et al. 1993). Although FAB-MS provides high sensitivity, MALDI-TOF mass spectrometry is ~10–100 times more sensitive (Huberty et al. 1993) and allowed the structure of the IL-4-inducing ES glycoprotein of S. mansoni, IPSE\(\alpha\)1 to be characterized (Wuhrer et al. 2006).

Peptide mass fingerprinting (PMF) determines protein identity following resolution by polyacrylamide electrophoresis. Resolved proteins are excised, cleaved by proteolysis, their masses determined by MALDI-TOF-MS and compared against a protein database. When combined with lectin chromatography, mass spectrometry revealed the Lewis\(^Y\) antigen (Fuc\(\alpha\)1,2Gal\(\beta\)1,4(Fuc\(\alpha\)1,3)GlcNAc\(\beta\)1) as a modification of tegumental, but not ES glycoproteins from S. bovis (see Ramajo-Hernandez et al. 2007). Analysis by nanoESI (nano ElectroSpray Ionization) mass spectrometry enabled the characterization of the structures of immunogenic glycoosphingolipids from S. mansoni (see Wuhrer et al. 2002, 2004). When nanoESI is combined with fourier transform ion cyclotron resonance (FTICR), analysis of intact, heavily sialylated glycoproteins can proceed without the need for chromatography, enzymatic treatment or derivitization (Nagy et al. 2004). FTICR can include fragmentation by electron capture dissociation (ECD), which cleaves protein backbones of glycoproteins without removing glycosylated regions, allowing glycosylation site determination. Combining ECD with external accumulation (XA) reduces ‘background’ while increasing resolution of unstable products, and was used to analyse <10 kDa glycoproteins containing sialic acids O-linked to GalNAc (Haselmann et al. 2001). Recently, quadrupole ion trap (QIT) technology has been coupled to ESI and MALDI-TOF MS for glycoprotein analysis, in order to facilitate further molecular resolution of the isolated molecules (Demelbauer et al. 2004). When coupled to MALDI-TOF, nuclear magnetic resonance (NMR) radio-isotope labelling and glycosidase treatment, the structures of glycosphingolipids from A. suum were obtained (Friedl et al. 2003). The technology has culminated in quadrupole orthogonal acceleration time of flight mass spectrometry (Q-TOF) (Morris et al. 1997), which may be the most sensitive means for glycoprotein analysis.

These techniques can be applied to pure compounds, and many are suitable for analysis of mixtures of molecules. For example, CE-ESI-MS is superior to both CE and HPLC, and provides enhanced resolving power and separation of molecules based on electrophoretic mobility. Sheathless CE-ESI avoids sample dilution while increasing ionization and resolution efficacy. These qualities make CE-ESI-MS analysis attractive for glycoprotein investigations (Bateman et al. 1998). However, mass spectrometry has proven to be indispensable for biological analysis and has enabled comparisons of glycans among helminths (see Morelle and Michaelski, 2004). From this brief account, it is clear that the combination of these techniques with genomics, proteomics and glycomics have brought us to a point where accelerated progress in the elucidation of helminth-derived molecules (e.g., glycans) would be anticipated.

Are Siglecs receptors for helminth-derived molecules?

Some glycans from parasitic helminths appear to signal through TLRs. However, there are additional mammalian receptors which primarily recognize carbohydrates that are involved in immunomodulation, and these may have been ‘co-opted’ into the parasites strategy to overcome or skew host immunity. Siglec (sialic acid immunoglobulin-like lectin) receptors are expressed by leukocytes and recognize sialic acids; for example, Siglecs 1,3 and 4 recognize terminal α 2,3 sialic acid (Powell and Varri, 1994; Freeman et al. 1995; Collins et al. 1997); Siglec-2 binds α 2,6 sialic acid (Kelm et al. 1994); and, Siglecs 5, 7 and 9 recognize both sugar moieties (Cornish et al. 1998; Zhang et al. 2000). The cytoplasmic tails of Siglecs possess immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and, so, Siglec activation is often associated with immunosuppression. For instance, the ligation of Siglec 9, and to a lesser extent Siglec 5, reduced TNFα production by macrophages while increasing IL-10 synthesis (Ando et al. 2008). Also, the repression of Siglec 3 (or CD33) expression with siRNA increased the spontaneous release of IL-1β from monocytes (Lajaunias et al. 2005). These findings, and others (see Ikehara et al. 2004), lend credence to the hypothesis that Siglec-mediated
recognition of ‘self’ sialic acid by leukocytes may serve to generally dampen immune responses and perhaps promote tolerogenic events.

The ligation of Siglec 8 on eosinophils (and Siglec 9 on neutrophils; von Gunten et al. 2005) induces apoptosis (Nutku et al. 2003), and the ability of helminths to trigger such a response would be to their benefit. The published literature on parasitic helminths possessing sialic acid-based glycoproteins is conflicting, with many studies suggesting that helminths do not synthesize them, and there is an equally convincing body of evidence demonstrating that sialic acid reactivity can be detected in worm extracts or ES products from many helminth species (McDiarmid and Podesta, 1984; Apinhasmit et al. 2000; Elayoubi and Craig, 2004; Johnston, unpublished observations). We feel that this issue is deserving of clarity that will only come with additional well-controlled experimentation. Currently, there are no data on helminth-derived molecules that can bind Siglecs, and thus we can only speculate on the possibility of helminth-derived glycans-Siglec receptor interactions as a mechanism for helminth parasites to elicit a general immunosuppression in their hosts.

Are galectins receptors for helminth-derived molecules?

Beta-galactose binding lectins (galectins) are secreted carbohydrates that bind cell surface and matrix-associated glycans. Fifteen galectins have been identified in mammals and each possesses a conserved carbohydrate recognition domain (CRD) which binds N-acetyllactosamine (LN), Galβ1,4GlcNAc or Galβ1,3GlcNAc on N- and O-linked glycans (Milos and Zaliik, 1983). Galectins can be monovalent (one CRD), bivalent or chimeric (one CRD plus a unique amino terminal sequence). There are many examples of galectin modulation of immune activity, such as the induction of apoptosis, enhancement of macrophage phagocytosis, TH2-TH1 skewing and the induction of regulatory T cells (Rabinovich et al. 2007). Current data suggest that the helminth-derived glycans can interfere with galectin-driven events. Galectin-1 ligation by helminth galactose can potentially decrease TNFα and IFNγ and prevent mast cell degranulation and neutrophil activity (Rabinovich et al. 2002). The galectin-3 receptor recognizes lactose-containing glycans (LN and LDN) from S. mansoni. This interaction has been implicated in granuloma formation (van den Berg et al. 2004) (recently disputed by Bickle and Helmby, 2007) and in eosinophil-mediated cytotoxicity (Truong et al. 1993), although the effect on eosinophils could be reduced by the galectin-3 inhibition of IL-5 production (Cortegano et al. 1998). In addition, mice in which galectin-3 expression is reduced (via genetic manipulation) display reduced numbers of splenic T and B cells, less liver pathology and increased levels of TH1-type cytokines following S. mansoni infection (Breuilh et al. 2007). Similarly, galectin-3 appears to provide a degree of protection against Clonorchis sinensis-induced cholangiocarcinoma (see Junking et al. 2008). With the exception of galectin-3 (Yang et al. 1996), galectin activation of a cell often induces apoptosis (reviewed by Hernandez and Baum, 2002), an event that can be inhibited by α 2,6 sialic acid (Leffler and Barondes, 1986). Since many helminths produce sialic acids, the release of these glycans from the worm could increase the life-span of immune cells.

Many helminths also secrete galectins (Turner et al. 2008). For example, a 34 kDa galectin in O. volvulus is temporally expressed by third-stage larvae (L3) and adults (Joseph et al. 2000), shares 40% homology to a Teladorsagia circumcincta- and H. contortus-derived galectin (Greenhalgh et al. 1999), and is recognized by sera from infected individuals. The related galectin from O. volvulus, OvGalBP, binds IgE and may protect against eosinophil and neutrophil-mediated damage of the helminth (Klion et al. 1994). Galectin-1 administration is therapeutic in a plethora of murine models of autoimmune disease, where it is associated with reduced TH1 and TH17 events and increased CD4+ T cell apoptosis (Salatino et al. 2008). A galectin-1 like molecule has been identified in nematodes (Table 3). Moreover, chitin (a GlcNAc polymer) is abundant in helminths and profoundly affects immunity by mobilizing AAMs, eosinophils and basophils in vivo (Reese et al. 2007).

Are C-type lectins receptors for helminth-derived molecules?

C-type lectins (CTLs) are Ca2+-dependent carbohydrate receptors involved in signalling, antigen presentation and cell-cell adhesions (Pyz et al. 2006). CTLs include the collectins, which bind mannan proteins, the selectins, which bind sialylated/sulphated glycans, and the macrophage mannose receptor (MMR). A few studies have shown that parasitic helminths produce lectin-type molecules and molecules that bind host lectins, thus imparting the potential to affect processes as diverse as macrophage activation, immune cell trafficking and dendritic cell activity. For instance, mannann-binding lectin (MBL) interacts with glycans on S. mansoni and T. spiralis, resulting in complement fixation (Gruden-Movsesijan et al. 2003). Similarly, the macrophage galactose-type lectin (MGL), which is also expressed on DCΦ, binds the LewisX antigen and α/β GalNAc, both of which occur in S. mansoni LDN and LDNF (van Vliet et al. 2005). Lectin-targeting of the worm surface would be aimed at worm destruction, although the shedding of the
glycan from the parasite could be a component of an immune evasion strategy. Parasite-secreted CTL-like products have been identified and include ES products of ∼32 and 70 kDa from *Toxocara canis* which resemble the MMR (CD206) and the low affinity IgE receptor (CD23), respectively (Loukas et al. 1999, 2000a). Lectins encoded by ESTs in *Ancylostoma ceylanicum* and *Ascaris suum* exist in the GenBank database and a *Necator americanus* lectin is predicted from transcriptome analysis (Daub et al. 2000). Exactly how helmint-derived glycans or lectin-like molecules modulate mammalian immune responses is not known; however, there is potential for pure forms of these molecules to be used as potent drugs. For example, glycans and SEA from *S. mansoni* can bind to DC-SIGN (Meyer et al. 2005; van Liempt et al. 2007). DC-SIGN is important in recognizing mannose-containing carbohydrates and is also an activation signal that helps direct DCF migration from tissues to lymph nodes to initiate primary adaptive immune responses.

**Conclusions**

The concept of helmint-derived molecules as a source of immunomodulatory agents is fully established (Hartnett and Harnett, 2008), and the literature is flush with examples of how crude extracts and partially characterized molecules from many species of parasitic helmint can affect virtually all aspects of innate and adaptive immune responses. To date, this wealth of data has not been converted into a slew of new drugs. While the promise of parasitic helminters as a pharmacopeia has yet to be fulfilled, this should not deter future research in this area, as the potential pay-back in terms of new drugs is immense. Indeed, the advent of technology for the purification and characterization of molecules from small amounts of tissues and the synthesis of agents for testing in high throughput immunological assays. We subscribe to, and will leave the reader with the view presented by Falcone and Pritchard (2005): “The fact that the compounds from parasites might have evolved to unlock the human immune system selectively will also result in less attrition when compounds are tested for safety and adverse activity profiles”.

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