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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