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Review

Secretory products of helminth parasites as immunomodulators

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ABSTRACT

Parasitic helminths release molecules into their environment, which are generally referred to as excretory-secretory products or ES. ES derived from a wide range of nematodes, trematodes and cestodes have been studied during the past 30–40 years, their characterization evolving from simple biochemical procedures such as SDS-PAGE in the early days to sophisticated proteomics in the 21st century. Study has incorporated investigation of ES structure, potential as vaccines, immunodiagnostic utility, functional activities and immunomodulatory properties. Immunomodulation by ES is increasingly the area of most intensive research with a number of defined helminth products extensively analyzed with respect to the nature of their selective effects on cells of the immune system as well as the molecular mechanisms, which underlie these immunomodulatory effects. As a consequence, we are now beginning to learn the identities of the receptors that ES employ and are increasingly acquiring detailed knowledge of the signalling pathways that they interact with and subvert. Such information is contributing to the growing idea that the anti-inflammatory properties of a number of ES products makes them suitable starting points for the development of novel drugs for treating human inflammatory disease.

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1. Introduction: immunomodulation in helminth infections

The global incidence of the most abundant parasitic helminth species of humans, starkly shows the success of these organisms, with a recent estimate for the gastrointestinal nematode *Ascaris lumbrocoides* staggeringly suggesting that this species alone may infect 819 million people [1], >10% of the global population. Moreover, human infection with helminths is invariably long-term, with reports of worms of some species, e.g., the filarial nematode *Wuchereria bancrofti* [2] surviving in excess of a decade. Such longevity in the face of the destructive potential of the immune system is remarkable and is most readily explained by the helminths being able to interfere with it.

It was originally considered that parasitic helminths survived in their hosts by simply blocking immune responses and indeed some of the earliest work in this area revealed that worms, e.g., *W. bancrofti*, could cause suppression of the response of cells such as lymphocytes to helminth antigens [3]. However, as the field progressed it became apparent that the effects of the helminths were more subtle than global "immunosuppression", rather it appeared that what was being documented was frequently modulatory rather than suppressive, and so the term "immunomodulation" began to appear in the literature. It is now recognized that immunomodulation by parasitic helminths is a general phenomenon that is conserved across species, classes and even phyla and has two predominant features: (i) induction of a T helper type 2 (Th2)-immune response including such components as the cytokines IL-4, IL-5 and IL-13, the antibody isotype IgE and recruitment of eosinophils and mastocytosis; (ii) generation of a regulatory response incorporating cytokines such as the anti-inflammatory IL-10 and TGF-β and cell types such as regulatory T-(Treg) and B-(Breg) cells and regulatory/alternatively activated macrophages that may act to suppress protective responses and in addition prevent potentially dangerous pathology [4]. The generation of this immunological phenotype has been the subject of great interest amongst helminth immunologists for several decades and there is universal acceptance that helminth secreted products, by virtue of their unique opportunity to interact with the host immune system, are particularly suited to playing a prominent role. This article will therefore consider the immunomodulatory properties of these molecules.

2. Studying helminth "excretory-secretory" products

During the 1970s–1980s, the practice of dividing the antigen composition of helminth parasites into three compartments – "surface", "excretory-secretory" and "somatic", arose. Excretory-secretory antigens, often abbreviated to "ES", referred to molecules, which were released into the host environment either through excretion of "waste" products or by an active secretory process for "functional" molecules. At this time, although a number of laboratories were beginning to explore their vaccine potential, the major interest in helminth ES was moving in the direction of employing them as diagnostic tools: as they could be found in the bloodstream of the parasitized host, attempts were made to employ them in immunoassays, which detect circulating antigen [5]. Nevertheless, studies on ES function were not infrequent and indeed various enzyme activities, e.g., protease, acetylcholinesterase and anti-oxidant, and in addition, immunomodulatory activities, e.g., interfering with complement activation, chemotaxis or lymphocyte responses, were reported [reviewed in Ref. [6]]. Indeed a few of the earliest studies on helminth ES were broadly in the immunomodulation area although, it was probably the 1990s before analysis of the immunomodulatory function of the ES component of parasite helminths began to dominate research efforts.

In the 1980s, ES were generally obtained by concentration and purification of spent medium obtained from parasitic helminths maintained in culture, although it was also possible to isolate them from serum or urine. Analysis invariably focused on proteins (although it was appreciated that functional small molecules of other categories were likely to be present), involved one-dimensional SDS-PAGE in combination with radiolabelling (to improve sensitivity), which could be either extrinsic (invariably iodination) or biosynthetic (usually via incorporation of [^{35}S]-methionine) [6]. This would result in a small number of polypeptides being detected, ranging in molecular weight from ~10 to several 100 kDa. A feature that was observed in most nematode species examined was "stage specificity"; here, each developmental stage of the worm exhibited a different ES polypeptide profile [6] and this was considered to reflect differences in the biology of each stage (e.g., location within the host). Also, a variety of procedures were employed to show that many ES products were glycosylated and a number of nematode-derived molecules were found to contain an additional, unusual component, phosphorylcholine (PC) [6].

Within the last decade, advances in technology have allowed more sensitive analysis of ES with the result that the number of molecules detected has increased dramatically. Two approaches have been employed, characterization of expressed sequence tags [e.g., Ref. [7]] and proteomic analysis, the latter of which is increasingly applied to parasitic nematode species as reflected by three distinct laboratories undertaking an analysis of the ES of the human filarial nematode parasite *Brugia malayi* [8–10]. Although these studies reveal some differences in ES composition, those that investigated different stages [9,10] confirmed the stage-specificity observed in the earlier studies and all three clearly illustrated the power of the approach.

3. Effects of ES products on the host immune system

Not surprisingly, immunomodulatory activity has been found in the ES of multiple species of parasitic worm, covering trematodes, cestodes and nematodes and is particularly well characterized in the model mouse nematode *Heligmosomoides polygyrus* [11] and the trematode, *Fasciola hepatica* [12]. A pleiotropic range of effects on the immune system has been described to date but in the majority of cases the role played by individual ES components has yet to be determined. Adopting the view that understanding helminth immunomodulation will necessitate focusing on individual ES products, this article will discuss current examples where information on their immunomodulatory activities and molecular aspects of mechanism of action is available.

3.1. Driving Th2 responses

As Th2 responses can offer protection against parasitic helminths [4] it could be considered that their induction is not in the interests of the worms. Indeed that they can be induced by free-living helminths [13] argues against them being an adaptation by worms to parasitism. However Th2 cytokines may help control inflammation (and promote wound healing) and this has been observed with IL-4 in a mouse schistosome model [14]. Generation of CD4$^+$ T cells with a particular phenotype is dependent on signals received from antigen presenting cells such as dendritic cells (DCs) following their interaction with pathogen-associated molecular patterns (PAMPs) [15]. Thus clearly helminth products must contain information, which upon processing allows DCs to drive a Th2 phenotype. The first defined helminth product to be described as acting in this way was ES-62, the major secreted product of