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# REVIEW OF THE ROLE OF PARASITIC NEMATODE EXCRETORY/SECRETORY PROTEINS IN HOST IMMUNOMODULATION

**Ogadinma K. Okakpu and Adler R. Dillman**

University of California, Riverside 900 University Avenue, Riverside, California 92521.
Correspondence should be sent to Adler Dillman (https://orcid.org/0000-0001-7171-4332) at: *adlerd@ucr.edu*

**KEY WORDS**

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B cell
T cell
IL-10

**ABSTRACT**

Parasitic nematodes infect a variety of organisms including insects and vertebrates. To survive, they evade host immune responses to cause morbidity and mortality. Despite the vast clinical knowledge regarding nematode infections and their biological makeup, molecular understanding of the interactions between host and parasite remains poorly understood. The utilization of model systems has thus been employed to help elucidate the molecular interactions of the host immune response during parasitic nematode infection. Using model systems, it has been well established that parasitic nematodes evade host immunity by releasing excretory/secretory proteins (ESPs), which are involved in immunomodulation. Model systems have enabled researchers to characterize further the underlying mechanisms ESPs use to facilitate evasion and modulation of the host immune response. This review assessed notable ESPs from parasitic nematodes that infect vertebrates or insects and have been studied in mechanistic detail. Being able to characterize how ESPs affect the immune systems of hosts on a molecular level increases our understanding of host–parasite interactions and could lead to the identification of novel therapeutic targets and important molecular pathways.

Infections caused by parasitic nematodes are a widespread global health concern that continues to afflict humans. It has been estimated that parasitic nematodes infect more than 25% of the global population, with the concentration of infections being primarily in the global south (L'Ollivier and Piarroux, 2013; Hotez et al., 2014; Pullan et al., 2014). The difficulty of detecting parasitic infection during the early stages compounds the health effects. Part of what makes nematode infections difficult to diagnose early on is the ability of parasitic nematodes to evade the host immune system, allowing them to go undetected, which in turn leads to physiological complications that cause morbidity and mortality (Stepek et al., 2006). The global health ramifications of parasitic nematodes are further exacerbated by the possibilities of recurrent reinfection and emerging drug-resistant infections. Nematodes are thus very troublesome parasites with the ability to compromise the immune systems of insects and vertebrates (Davis et al., 2000; Hao et al., 2010; Garg and Ranganathan, 2012).

The difficulty in identifying the underlying molecular mechanisms is in part due to the lack of good model systems with established genetic, genomic, and proteomic tools that overcome logistic obstacles such as cost and time (Lok, 2007; Ward, 2015). These obstacles have been notably dealt with in other areas of investigation such as behavioral ecology and neurobiology, where insect-parasitic nematodes that are closely related to vertebrate-parasitic nematodes are used as models (Hallem et al., 2007, 2011; Dillman et al., 2012b; Castelletto et al., 2014). As a result, plausible methods for the characterization of host immunomodulation by nematodes have been made more efficient. One potential use of these model systems is to study the impact of excretory/secretory proteins (ESPs) on a host in the context of infection.

Individual components of ESPs have been observed to have mechanisms of modulating the immune system of infected hosts (Cooper and Eleftherianos, 2016). The immunomodulation promotes the survival of the parasitic nematode by strategically altering the activation of host immune responses upon infection (Cooper and Eleftherianos, 2016). ESPs have a broad spectrum of effects and have the ability to impact host responses in a multifaceted context, including the response to concurrent diseases by bystander pathogens (Jackson et al., 2006). This is highlighted by ESP-driven anti-inflammatory responses being implicated in the low occurrence of inflammatory bowel disease in populations with high rates of nematode infection (Whelan et al., 2012). Overall, specific ESPs involved in nematode immunomodulation generally vary with regards to the mode of action and type of host (Klei, 1997; Hartmann and Lucius, 2003; McSorley et al., 2013). ESPs released also vary between the various life stages of the parasitic nematode, and even between sexes (Soblik et al., 2011; Sotillo et al., 2014). The need for stage-specific ESPs most likely aligns with the specific outcomes required for the life stage

**Table I.** Table highlights entomopathogenic nematodes and excretory/secretory proteins (ESPs). Molecular immunomodulatory effects are what is observed for the corresponding type of ESP, and thus based on the type of the pathways and molecular/cellular mechanisms they affect.

| Infected host | Parasitic ESPs released | Molecular immunomodulatory effects* | References |
|---|---|---|---|
| Insect | Sc-CHYM | Decrease of hemocyte encapsulation, PPO inhibition. | Balasubramanian et al. (2009) |
| | Sc-SRP-6 | No melanin deposition, disruption of clotting. | Toubarro et al. (2013a) |
| | Sc-KU-4 | Inhibition of hemocyte aggregation and encapsulation. | Toubarro et al. (2013b) |
| | Trypsin-like serine protease | PPO inhibition, change in hemocyte morphology, reduced hemocyte spreading and recognition. | Balasubramanian et al. (2010) |
| | Hb-ilys-1 | Suppression of PO activity. | Kenney et al. (2021) |
| | Hb-sc-1 | Suppression of PO activity, reduced AMP upregulation, reduced phagocytic activity. | Kenney et al. (2021) |
| | Hb-ugt-1 | Suppression of *Br-c*, reduced AMP upregulation. | Kenney et al. (2020) |

*Abbreviations: PPO: prophenoloxidase, PO: phenoloxidase. AMP: antimicrobial peptides.

(Soblik et al., 2011; Sotillo et al., 2014). Overall, few of these proteins have been studied in mechanistic detail. This review examined select immunomodulatory ESPs from nematode parasites of insects and vertebrates that have been mechanistically characterized and assessed the potential of insect-parasitic nematodes to serve as model systems for molecular characterization of immunomodulatory ESPs (Stock, 2005).

## MOLECULAR INTERACTIONS OF ESPS IN INSECTS

A specialized subset of insect-parasitic nematodes called entomopathogenic nematodes (EPNs) are characterized by their ability to kill hosts quickly, and their utilization of symbiotic bacteria to facilitate their parasitic lifestyles (Dillman et al., 2012a; Lewis and Clarke, 2012). Most EPNs enter an insect through natural openings, and once inside they release highly pathogenic bacteria along with ESPs into the insect's hemolymph. Although it was originally thought that the bacterial symbionts were the primary source of toxicity to insect hosts, with the nematodes serving primarily as vectors, recent studies showed that ESPs of EPNs are highly toxic to insects (Lu et al., 2017; Chang et al., 2019). Infection by EPNs does not go unnoticed by insects, however, as the insect's innate immune system uses a series of mechanisms that detect the nematode and bacterial partners to restrain their dissemination (Eleftherianos et al., 2010; Castillo et al., 2011). These immune response mechanisms fall under 2 categories: humoral and cellular (Lemaitre and Hoffmann, 2007; Jiang et al., 2010). The humoral immune response activates genes needed for synthesizing and secreting antimicrobial peptides (AMPs) from the fat body into the hemolymph (Imler and Bulet, 2005; Casanova-Torres and Goodrich-Blair, 2013; Rolff and Schmid-Hempel, 2016). Cellular immune responses are regulated by hemocyte function (Ribeiro and Brehélin, 2006). Hemocytes are the main component of the cellular response, and they are implicated in several functions like cell aggregate formation, phagocytosis, melanization, and encapsulation to help fight off infections (Marmaras and Lampropoulou, 2009; Honti et al., 2014). Melanization occurs after the production of phenol-oxidase (PO) that is produced by the cleavage of the proenzyme prophenoloxidase (proPO), which is a key component of the insect immune system (Eleftherianos and Revenis, 2011; Lu et al., 2014). Prophenoloxidase catalyzes melanization by mediating the oxidation of mono- and diphenols to quinones; they then polymerize to form melanin-generating reactive oxygen species (ROS) (Cooper et al., 2019). In *Drosophila* hemolymph coagulation, after the initial phase where cross-linking depends on transglutaminase activity, PO activity becomes the key component in the subsequent phase for further cross-linking, hardening, and melanization of the clot matrix (Dziedziech et al., 2020). Hemolymph coagulation is important in the insect immune response as it stops bleeding, seals wounds, and prevents the dissemination of pathogens and entry of microbial invaders at the wound site (Dziedziech et al., 2020). To have a successful infection, EPNs must evade, suppress, or modulate the insect immune response, at least temporarily to survive, release their mutualistic bacteria, and complete their life cycle. These bacteria are located in a receptacle near the pharyngeal bulb and are necessary for the growth and development of the nematode during infection (Sicard et al., 2003). Thus, EPNs are obliged to deploy rapid immunomodulatory strategies to protect the small cohort of symbiotic bacterial cells they release into the host, depressing host immunity, at least temporarily, so that the bacteria can resume growth and deploy their immunomodulatory arsenal to aid in protection from host immunity.

It is important to note that EPNs release ESPs during the infective juvenile stage (IJ) (Lu et al., 2017; Chang et al., 2019). As IJs, EPNs are in arrested development until a host is found; upon entering the host the IJs become activated for release of ESPs (Lu et al., 2017; Chang et al., 2019). The subsequent proteins that will be described were discovered in the ESPs released by specific EPNs. Each protein displayed notable immunomodulatory properties during experimental studies that will be briefly discussed (Table I).

### Trypsin-like serine protease and Sc-CHYM

Research to identify immunomodulatory proteins led to the discovery of a trypsin-like serine protease secreted by *Steinernema carpocapsae* IJs, during infection of *Galleria mellonella* larvae (Balasubramanian et al., 2010). Endogenous serine proteases and serine protease inhibitors are important in immune response, as they activate proenzyme prophenoloxidase (proPO), converting it to phenoloxidase (PO), and this activation results in hemocyte encapsulation, and melanization (Franssens et al., 2008). Although many trypsin serine proteases that have been discovered have been assigned a group or family, the trypsin-like serine protease that was described in Balasubramanian et al. (2010) has not been fully characterized for classification. The trypsin-like serine protease displayed the ability to suppress 38.9–52.6% of

proPO experimentally, leading to interruption of the process of melanization and ultimately reduced EPN encapsulation by hemocytes (Balasubramanian et al., 2010). It altered the morphology of *G. mellonella* hemocyte F-actin filaments from a highly organized state to a disorganized state and caused a change of hemocyte spindle shape, which coincided with inhibition in the hemocyte spreading (Balasubramanian et al., 2010). This also resulted in reduced recognition of the EPN *Heterorhabditis bacteriophora* by *G. mellonella* hemocytes by 55% (Balasubramanian et al., 2010). The trypsin-like serine protease thus significantly impairs host immunity by decreasing hemocyte spreading, encapsulation, and recognition of EPNs during infection.

Another study of ESPs secreted by *S. carpocapsae* reported a discovery of a chymotrypsin serine protease virulence factor named Sc-CHYM (Balasubramanian et al., 2009; Cooper and Eleftherianos, 2016). Because this protein is a serine protease such as the 1 described prior, it is likely they both affect the activation of the proPO-PO cascade by competing with the endogenous serine proteases. In vitro, Sc-CHYM displayed the capability to inhibit proPO by suppressing its enzymatic activity (Balasubramanian et al., 2009). In vivo, Sc-CHYM reduced the melanization and encapsulation of protease-treated beads that were injected into *G. mellonella*; normally such foreign objects are encapsulated and melanized (Balasubramanian et al., 2009). Sc-CHYM was thus shown to weaken the cellular immune response of the insect host and increase the success of parasitism by *S. carpocapsae*.

### Sc-SRP-6

Another ESP from *S. carpocapsae*, Sc-SRP-6, was shown to have 2 roles in protecting EPNs from host immunity. The first role is the inhibition of hydrolysis of food particles by reducing the activity of insect digestive enzymes (Toubarro et al., 2013a). Protection from digestive enzymes keeps IJs and their ESPs safe from metabolic breakdown when they enter the alimentary canal of the host. The second role of Sc-SRP-6 is interfering with clot formation in infected insects by binding with hemolymph plasma proteins, forming complexes that prevent the incorporation of melanin into the clot matrix, which is essential for encapsulation and nodulation immune processes (Toubarro et al., 2013a; Honti et al., 2014; Satyavathi et al., 2014; Theopold et al., 2014). Inhibiting clot formation weakens host cellular immunity, adding further protection to IJs during parasitism via host immunomodulation.

### Sc-KU-4

Another protein of interest, which belongs to the Kunitz-type serine family of protease inhibitors, is Sc-KU-4. This protease inhibitor is most highly expressed by the invasive stage, the IJ, of *S. carpocapsae*. Sc-KU-4 was reported to inhibit hemocyte aggregation in *G. mellonella* hemolymph (Toubarro et al., 2013b). Beads treated with Sc-KU-4 remained individualized in *G. mellonella* plasma, whereas nontreated beads were aggregated and entrapped by clotting material, suggesting Sc-KU-4 protects foreign bodies from host clotting mechanisms (Toubarro et al., 2013b). Lastly, Sc-KU-4-treated beads were pulled down from insect plasma and observed to be strongly bound to 2 proteins linked to immune recognition: A homolog of a masquerade-like protein (MSPH) and a homolog of a serine protease-like 1b (SPH-1) (Toubarro et al., 2013b). These findings suggest that Sc-KU-4 targets insect immune recognition proteins in the plasma such as antimicrobial peptides (AMPs), inhibits hemocyte aggregation, and prevents encapsulation of EPNs. Protecting IJs from recognition proteins enables them to hide from the host, thus preventing an adequate immune response to parasitic infection.

### Hb-sc-1 and Hb-ilys-1

The EPN *Heterorhabditis bacteriophora* has also been utilized for the discovery of novel immunomodulatory ESPs by transcriptomic analysis. Transcriptome studies were able to identify multiple secreted protein factors that were upregulated during parasitism (Vadnal et al., 2017). Two notable proteins that were recently characterized are a putative lysozyme (Hb-ilys-1) and serine carboxypeptidase (Hb-sc-1) (Kenney et al., 2021). The potential immunomodulatory capabilities of these proteins were assessed utilizing *Photorhabdus luminescens* infection of *Drosophila melanogaster*. Both recombinant proteins caused increased mortality during in vivo co-injections of *D. melanogaster* with *P. luminescens*, when compared to injections of *D. melanogaster* with *P. luminescens* alone (Kenney et al., 2021). Both Hb-ilys-1 and Hb-sc-1 suppressed PO activity, which correlated with a reduced melanization response during infection. In addition to reduced PO activity, Hb-sc-1 also reduced the upregulation of certain AMPs (*Diptericin*, *Attacin*, and *Drosomycin*), indicating inadequate activation of the immune response (Kenney et al., 2021). It was also found that Hb-sc-1 reduced phagocytic activity so that hemocytes were less effective at phagocytosing pHrodo-labeled *Escherichia coli*. This indicates that Hb-sc-1 might be broadly interfering with the cellular response of the fly during infection (Kenney et al., 2021). Further molecular experimentation is needed to understand how PO activity is suppressed by both enzymes, as well as to elucidate how Hb-sc-1 causes reduced upregulation of AMPs and reduced phagocytic activity. Both Hb-sc-1 and Hb-ilys-1 cause measurable effects on host immunity during infection resulting in reduced survival.

### Hb-ugt-1

Another protein released by *H. bacteriophora* that displayed immunomodulatory effects is a putative UDP-glycosyltransferase called Hb-ugt-1. A recent study showed that injection of *D. melanogaster* with recombinant Hb-ugt-1 resulted in reduced upregulation of the AMPs Diptericin, Attacin, and Metchnikowin (Kenney et al., 2020). To assess the physiological effects of this, *D. melanogaster Relish* mutants lacking an immune deficiency (Imd) – based response, were injected with recombinant Hb-ugt-1 to assess survival. The survival of these injected flies was significantly lower over 6 days in comparison to regular survival for wild-type flies, though the reason for reduced survival in this mutant context is not fully understood (Kenney et al., 2020). In addition to AMP suppression, *D. melanogaster* larvae injected with recombinant Hb-ugt-1 showed suppression of the ecdysone-transcription factor *Broad-Complex* (*Br-c*), which upregulates components of the immune response including the Peptidoglycan Recognition Protein LC (PGRP-LC) and some AMPs (Kenney et al., 2020). Thus, the suppression of Br-c by Hb-igt-1 may be responsible for the reduction of AMP upregulation. Through the reduction of AMP upregulation, Hb-ugt-1 is likely able to compromise the host immunity during infection.

**Table II.** Table highlights notable vertebrate parasitic nematode excretory/secretory proteins (ESPs). Molecular immunomodulatory effects are what is observed for the corresponding type of ESP, and thus based on the type of the pathways and molecular/cellular mechanisms they affect.

| Infected host | Parasitic ESPs released | Molecular immunomodulatory effects | References |
|---|---|---|---|
| Vertebrates | ES-62 | Inhibition of B and T cell activation and proliferation. Inhibition of mast cell degranulation and the release of pro-inflammatory mediators. Inhibition of IL-12p70 and pro-inflammatory Th1 responses. ES-62 regulates gene induction by modulating the binding of NF-B to the IL-12 promoter. | Goodridge et al. (2005a) Goodridge et al. (2005b) Harnett et al. (2004) Marshall et al. (2005) Melendez et al. (2007) Whelan et al. (2000) Wilson et al. (2003a) Wilson et al. (2003b) |
| | Cystatins | Reduced T cell priming. Inhibition of T cell proliferation. Enhanced production of anti-inflammatory Il-10. Reduced induction of active immune response. | Dainichi et al. (2001) Manoury et al. (2001) Schnoeller et al. (2008) Schönemeyer et al. (2001) |
| | Ac-AIP-1 | Reduction of local infiltration of inflammatory cells. Suppression of pro-inflammatory cytokines. Production of anti-inflammatory IL-10. | Ferreira et al. (2017) |
| | Ac-AIP-2 | Suppression of T cell proliferation. Reduced DC co-stimulatory marker expression. | Navarro et al. (2016) |

## MOLECULAR INTERACTIONS OF EXCRETORY/SECRETORY PROTEINS IN VERTEBRATES

With over 1 billion people infected worldwide, vertebrate-parasitic nematodes continue to be a major public health concern globally, specifically in nations in the global south (L'Ollivier and Piarroux, 2013; Hotez et al., 2014; Pullan et al., 2014). Understanding how these nematodes evade vertebrate immunity is thus a major priority. The invasion of host tissues by parasitic nematodes activates the complement system, which identifies pathogens and directs the innate immune response. Leukocytes (encoded by the MHC class I and II genes in humans) are then recruited to the site of infection to release cytokines to enhance an inflammatory response along with a variety of other processes (Martinez et al., 2009). Participation of mast cells and eosinophils also occurs because of their roles as potent effectors of a range of cytokines and chemokines. Direct activation of leukocytes is triggered by host tissue damage by the invading nematode, which then leads to the recruitment of other kinds of leukocytes, such as neutrophils, macrophages, basophils, innate lymphoid cells, and dendritic cells, which leads to the production of toxic free radicals, phagocytosis, and the eventual development of adaptive immune response by the production of antibodies (de Veer et al., 2007; Perrigoue et al., 2008; Grencis, 2015). Vertebrate-parasitic nematodes, however, have evolved immunomodulatory mechanisms, effected through their ESPs (Table II), that can interrupt 1 or more effectors of the innate immune response (Maizels et al., 2004).

### ES-62

The glycoprotein ES-62 is an ESP released by the postinfective life-cycle stages of the rodent filarial nematode *Acanthocheilonema viteae* with immunomodulatory properties highlighted by the ability to interact with a variety of immune cells, thus being able to regulate the host immune system via the cellular response (Goodridge et al., 2005b; Pineda et al., 2014). ES-62 specifically alters molecular events that control B cell and T cell receptor signaling, which leads to significant inhibition of B cell and T cell activation and proliferation (Al-Riyami and Harnett, 2012). ES-62-mediated modulation requires the presence of Toll-like Receptor TLR4, but not TLR2 and TLR6, and can affect antigen-presenting cells, as well as the inhibition of mast cell degranulation by the formation of a complex with TLR4 at the plasma membrane (Goodridge et al., 2005a; Melendez et al., 2007).

ES-62 is heavily conjugated with and modifies phosphorylcholine (PC), which leads to inhibition of the proliferation of $CD4^+$ T cells and conventional B2 cells in vivo. It also reduces IL-4 of $CD4^+$ cells and interferon-gamma (IFN-$\gamma$) production (Wilson et al., 2003a, 2003b; Harnett et al., 2004; Marshall et al., 2005). ES-62 also promotes the proliferation of peritoneal B1 cells and their subsequent production of IL-10 (Wilson et al., 2003b). ES-62 also targets antigen-presenting cells (APCs) to inhibit their ability to produce IL-12p70 in response to lipopolysaccharides (LPS). This is done with pretreatment of DCs and macrophages with ES-62, where ES-62-pulsed bone marrow–derived DCs can drive Th2 differentiation in vitro (Whelan et al., 2000; Goodridge et al., 2003). Utilizing its PC residues, ES-62 interacts with toll-like receptor (TLR) 4 to inhibit pro-inflammatory Th1 responses. In mast cells, binding of TLR4 by ES-62 results in degradation and sequestration of intracellular protein kinase C-$\alpha$ (PKC$\alpha$), which as a result inhibits degranulation and release of inflammatory mediators (Goodridge et al., 2005a; Melendez et al., 2007).

Although ES-62 can impair the host immune response, it has also shown the ability to reduce the outbreak of various autoimmune or allergy-related diseases (Harnett and Harnett, 2006). ESPs such as ES-62 thus not only have a role in host immunomodulation, but potentially can be utilized to design novel anti-inflammatory drugs (Al-Riyami and Harnett, 2012; Al-Riyami et al., 2013). Ultimately, ES-62 displays the ability to regulate B cell and T cell receptor signaling, as well B cell and T cell activation and proliferation, significantly.

### Cystatins

Cystatins are cysteine protease inhibitors. Cystatins have been found among the ESPs of third-stage larvae (L3) vertebrate-parasitic nematodes and have been identified to have immuno-

modulatory properties on the cellular response (Wang et al., 2017; Maizels et al., 2018). They inhibit 2 classes of cysteine proteases: Legumains, which are utilized for antigen processing and presentation, and cathepsins L and S, which are utilized for processing polypeptides. Inhibition of legumains reduces the formation of the MHC class II molecules, which reduces the induction of an active immune response (Dall and Brandstetter, 2016). Cystatins can also enhance the production of anti-inflammatory cytokine IL-10, which in turn restricts T cell–mediated responses (Schierack et al., 2003). Cystatins secreted by *Heligmosomoides polygyrus* have been shown to modulate the activity of dendritic cells. Recombinant cystatin exposed to dendritic cells resulted in the expression of fewer MHC class II molecules as well as CD 40 and CD 86, 2 proteins necessary for T cell differentiation (Sun et al., 2013; Flávia Nardy et al., 2015). Another cystatin secreted by *A. vitae* alters the expression of key cytokines resulting in modulation of pro-inflammatory effects (Behrendt et al., 2016). Recombinant cystatin resulted in downregulation of the pro-inflammatory cytokines iNOS and cyclooxygenase synthase (COX)-2 and induced an upregulation of IL-10, which further promoted an anti-inflammatory effect in microglia (Cooper and Eleftherianos, 2016).

Cystatins produce immunomodulatory effects through 2 mechanisms (Hartmann and Lucius, 2003; Gregory and Maizels, 2008). First is the inhibition of cysteine proteases (cathepsins and aspartyl endopeptidase) necessary for host APC antigen processing and presentation, which results in reduced T cell priming (Dainichi et al., 2001; Manoury et al., 2001). The second mechanism is the induction of immunosuppressive IL-10, reducing co-stimulatory molecule expression by APCS, and inhibiting T cell proliferation (Schönemeyer et al., 2001). Immunomodulation in vivo has also been characterized by inhibition of both allergic lung inflammation and colitis, which is both mediated by Tregs and IL-10–producing macrophages (Schnoeller et al., 2008). Through their inhibition of cysteine proteases, cystatins can modulate T cell differentiation and proliferation.

### Anti-inflammatory proteins (Ac-AIP-1 and Ac-AIP-2)

Gastrointestinal hookworms have evolved to cause minimal harm to their host in low-burden infections, through the secretion of immunomodulatory ESPs (Ferreira et al., 2017). This allows for the long-term survival of the parasites in a host while potentially protecting the host from inflammatory diseases (Navarro et al., 2016; Ferreira et al., 2017). Two anti-inflammatories, ESPs Ac-AIP-1 and Ac-AIP-2, were found in the blood-feeding stage (L4) of hookworm *Acylostoma caninum*. They were recombinantly expressed and experimentally shown to display immunomodulatory effects on the cellular response (Smallwood et al., 2017; Maizels et al., 2018). Recombinant Ac-AIP-1 was assessed in mouse models of colitis (Mulvenna et al., 2009; Ferreira et al., 2017). Colitis inflammation was suppressed in these models by Ac-AIP-1 at 1 mg $kg^{-1}$, and local infiltration of inflammatory cells was significantly reduced. Colitic inflammation was assessed as weight loss, colon atrophy, edema, ulceration, and necrosis, as well as abdominal adhesion. Recombinant Ac-AIP-1 promoted the production of anti-inflammatory colon IL-10, transforming growth factor (TGF)-β, and thymic stromal lymphopoietin (TSLP). It also suppressed several cytokines, including the tumor necrosis factor (TNF)-α, IL-13, and IL-17A, granulocyte macrophage colony-stimulating factor (GM-CSF), CX motif chemokine (CXCL)-11, COX-2 mRNA transcripts, and IFN-γ. Ac-AIP-1 thus displayed immunosuppressing characteristics by promoting the production of anti-inflammatory mediators IL-10 and TGF-β, and suppression of pro-inflammatory cytokines.

Ac-AIP-2 is 1 of the most abundant proteins in the *A. caninum* secretome (secreted proteome) and demonstrated immunomodulatory capabilities in a mouse model of asthma (Mulvenna et al., 2009; Navarro et al., 2016). Ac-AIP-2 suppressed airway inflammation, reduced DC co-stimulatory marker expression, and demonstrated ex vivo suppression of human T cell proliferation with dust mite allergy (Navarro et al., 2016). Mouse models showed that Ac-AIP-2 was primarily captured by mesenteric CD103+ DCs, that airway inflammation suppression was primarily dependent on DCs, and mesenteric lymph node originated (MLNs) Foxp3+ regulatory T cells (Navarro et al., 2016). Thus, potential anti-inflammatory therapeutic effects of Ac-AIP-2 were mechanistically characterized to be dependent on capture by mesenteric DC and Treg cells, which is also a mechanism by which Ac-AIP-2 modulates the immune response of the host upon secretion.

## CONCLUSION AND FUTURE DIRECTIONS

The characterization of tens of immunomodulatory ESPs (Fig. 1) from among the hundreds that have been identified highlights the challenge of ESP mechanism elucidation. Major obstacles to research success include the cost and time required to characterize ESPs of vertebrate parasitic nematodes at the molecular level fully. Recent studies show the promise of insect model systems to rationally identify immunomodulatory ESPs for recombinant expression and characterization (Lu et al., 2017; Chang et al., 2019; Parks et al., 2021). A process of in vitro activation of EPNs has been optimized, allowing for the time-friendly acquisition of high quantities of ESPs for downstream applications such as mass spectrometry identification of protein composition or fractionation of ESPs for targeted identification (Lu et al., 2017; Chang et al., 2019). Although other methods of identification and collection are utilized with vertebrate systems, the insect system, along with proteomics and transcriptomics, presents a time- and cost-effective model for researchers to screen, identify, or isolate novel proteins (Moreno et al., 2011; Falcón et al., 2014; Sotillo et al., 2014; Lu et al., 2017; Kenney et al., 2019). Also, in vitro ESP collection methods for vertebrate-parasitic nematodes have yet to be experimentally validated regarding their relevance to in vivo conditions, where EPN insect model systems have been so validated (Soblik et al., 2011; Borloo et al., 2013; Sotillo et al., 2014; Lu et al., 2017; Chang et al., 2019). EPN model systems have many advantages; there are still, however, areas of research where they can be developed. Immune priming, which is characterized by the increase in survival and host immune response after a second specific encounter, is a phenomenon in invertebrates that EPNs have recently been used to examine (Kurtz and Armitage, 2006; Cooper and Eleftherianos, 2017; Texca Tatevari et al., 2021). A recent study showed that an EPN did not generate immune priming, future experiments can elucidate factors for this or if they can elicit immune priming under certain conditions (Texca Tatevari et al., 2021). More

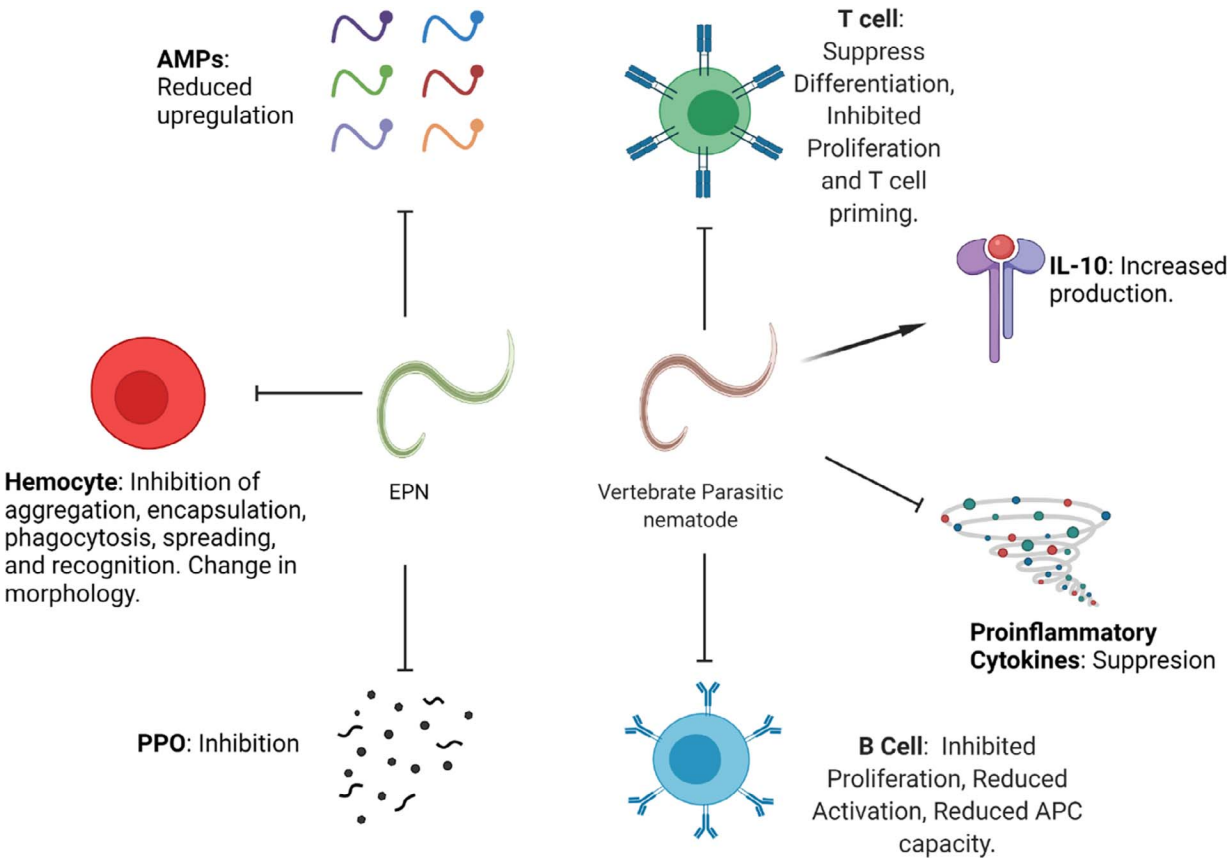


**Figure 1.** Visual representation of the molecular effects on host immunity by excretory/secretory proteins (ESPs). Left side displays the molecular interactions of ESPs released by entomopathogenic nematodes (EPNs) on insect immunity. Right side displays the molecular interactions of ESPs released by vertebrate parasitic nematodes on mammalian immunity. Color version available online.

research is needed regarding immune priming, but EPNs are still a promising and relevant model system that is closely related to nematode parasites of humans, and even releasing many of the same ESPs into their hosts (Blaxter and Koutsovoulos, 2015; Lu et al., 2017).

If successfully utilized, EPN–insect model systems can allow for the identification of novel mechanisms of immunomodulation and facilitate their characterization. With a better foundation for selecting individual proteins or molecules for experimentation, vertebrate studies can be more precise and efficient in elucidating molecular pathways involved with vertebrate-parasitic nematodes. This may allow for the development of vaccines that can promote parasitic nematode clearance, better treatments of infection, or better treatments of autoimmune disease using drugs derived from immunosuppressing ESPs.

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