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How helminths use excretory secretory fractions to modulate dendritic cells

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It is well known that helminth parasites have immunomodulatory effects on their hosts. They characteristically cause a skew toward T_H2 immunity, stimulate Treg cells while simultaneously inhibiting T_H1 and T_H17 responses. Additionally, they induce eosinophilia and extensive IgE release. The exact mechanism of how the worms achieve this effect have yet to be fully elucidated; however, parasite-derived secretions and their interaction with antigen presenting cells have been centrally implicated. Herein, we will review the effects of helminth excretory-secretory fractions on dendritic cells and discuss how this interaction is crucial in shaping the host response.

Introduction

Parasitic worms have co-evolved with vertebrate hosts over millions of years. As a result, they have developed numerous survival strategies including potent factors for immunomodulation. Infections can be long-lived and comprise complex and morphologically distinct life cycle stages. Worms display multiple mechanisms not only to evade host immunity for maturation and transmission, but also to preserve the host long enough for them to do so. Their success depends not only on their specialized ways of colonizing their host but also on host-specific genetic and immunological factors. Differences in worm burden among communities with equivalent levels of exposure has been repeatedly noted for a variety of helminth infections in both animal models and humans[1-3] with familial patterns pointing to a genetic link to susceptibility. For example, linkage analysis has highlighted three genes with strong associations to Ascaris worm burden in humans,[4] TNSF13B and ABF1, a TNF family member and transcription factor, respectively, that play central roles in B cell regulation, as well as IL-7, a cytokine influencing mucosal lymphocytes whose production is associated with protection against intestinal worms.[5] These gene products have perceptible links to adaptive immunity pointing to both genetic and immunological factors as determinants of the course and severity of infection.

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Helminths affect the immune system differently to other pathogen classes. They present as large multicellular entities that cannot be readily phagocytosed yet they elicit a strong adaptive humoral response.[6] This point begs the question of how their components enter the antigen presentation pathway. The answer likely lies within worm-derived secretions, namely the excretory-secretory fraction (ES), components of which are released into the host throughout the life cycles of most parasitic helminths.

The use of "excretory-secretory" to describe the mixture of material released by helminths into their host organism fails to distinguish between components that are actively secreted and those that are released as a consequence of physiological processes such as egg laying and digestion. Individual characterization of components that are purposefully host-targeted may be more useful in understanding a given worm's adaptation to its developmental milieu, but the immunological affront inflicted on the host during infection derives from both passively and actively secreted antigens. Although ES composition of parasitic worms of different taxonomies varies significantly, secretions have been found to contain a mixture of glycoproteins, proteins, glycolipids, and polysaccharides.[7] Effects of ES on cellular physiology are vast, but here we will focus on what is known about ES influence on dendritic cells and how these elements are able to direct adaptive immunity.

Helminth ES

Parasitic worms can progress through intricate life cycles stages, occupying distinct niches in the host and taking vastly different physical forms. *Echinococcus granulosus*, a cestode tapeworm, exists in the canine intestine as a segmented adult worm but once transferred to an intermediate host like a human, its larval stage develops into fluid filled cysts.[8] Likewise, adult worms of the nematode *Trichinella spiralis* infect the intestinal tract whereas its larval stage encysts in striated muscle and can survive in this form for years.[9] Each parasitic form and corresponding niche undoubtedly involve different types of interactions with the host immune system and distinct DC subtypes, a point that is reflected in the dynamic reshaping of surface molecules and secreted elements at each stage. The interactions of adult *T. spiralis* in the intestine vs. those of its intracellular larval stage with the host immune system are inevitably quite different. Therefore, the

composition of its excretory-secretory fraction is dependent on how it needs to interface with its host to ensure progression to the next life-cycle stage.

Characterizing the ES and defining these interactions is an enormous challenge when dealing with organisms that cannot be modeled in vitro. In the example of Trichinella, adult worms can be extracted from the intestine and cultured for a few days as can larvae enzymatically digested out of muscle tissue. Neither of these stages truly reflects what is being secreted by the encysted larvae within the muscle tissue, which is arguably most biologically relevant. Many helminth parasites cannot be cultured at all, relying on mouse models and ex vivo studies to infer what is being released by the worms during natural infection. Furthermore, barring certain pioneering studies done in schistosomes,[10,11] these worms are genetically intractable, posing an added challenge.

The viability of the worms in culture varies between organisms, for example *T. spiralis* L1 stage larvae will only survive 4–5 d in culture medium whereas adult *Heligmosomoides polygrus* may be cultured for up to 20 d. In all examples discussed, the environmental cues that would be present in the native system are absent and this will undoubtedly have an effect on the secretion and metabolic pattern of the parasite. Methods for collecting ES depend on the parasite, its life cycle and the form in which ES components are released, a recently discovered route being through exosomes.[12] In some cases many stages of the parasite are accessible, whereas others prove extremely restrictive. *Nippostrongylus brasiliensis* is a rodent gastrointestinal nematode closely related to the human and sheep/goat hookworms. *N. brasiliensis* adults lay eggs in the gut that are excreted by the host. Eggs can be collected from feces and hatched. The larvae then develop from L1 to L3 (infective stage larvae) that can be cultured in liquid media and ES is collected from the supernatant. *N. brasiliensis* ES is therefore representative of the free-living stage that in nature enters the host via the skin and transits to the lungs. Adults may also be isolated from the host intestines and cultured for ES, representing parasitic components that encounter the intestinal microenvironment.

Ascaris lumbricoides, *Ascaris suum* and *Toxocara canis* are large intestinal roundworms that may be collected from the intestines or the feces of infected hosts. Adult (gut) L2 (circulating) and L3/4 (lung) stage parasites can also be extracted and cultured for ES.

Brugia malayi and *Onchocerca volvulus* are examples of filarial nematodes that are transmitted to humans via mosquito or blackfly vectors respectively. In the laboratory, adult parasites are collected from nodules in the lymphatic system of jird rodents and cultured for ES. Microfilariae and L3 larvae may also be isolated from the vector. Studies have demonstrated how filarial ES proteins vary between stages and are gender-specific.[13,14]

Various stages of the human blood fluke *Schistosoma mansoni* can be isolated for study. Eggs can be collected from the liver or feces of hosts and schistosomula can be recovered from lung tissue for ES collection. Adult schistosomes may also be collected by dissection or perfusion, but adult yields are low and the site of their residence varies between animals.

Often used in laboratory studies of cestodes are the tapeworms *Echinococcus granulosus* and *Taenia solium*, which also parasitize humans. Adult cestodes usually reside in the intestine of a definitive host, from which eggs are shed in the feces and ingested by an intermediate host. Parasites can be dissected out of the hydatid cysts of intermediate hosts and cultured, as can eggs isolated from feces that hatch, releasing oncospheres from which ES can be collected. Occasionally adults may also be extracted from the gut of infected animals.

Since it is a challenge to purify ES that is representative of the life cycle stage of interest, it is reasonable that helminth extracts, which contain a high concentration of helminth antigens, are routinely prepared for immunological analyses. These extracts can be prepared after immediate isolation of the parasite, without culturing or manipulation, and have proven useful for learning more about important parasite antigens and their effect on the immune response. For example, soluble *S. mansoni* egg antigen (SEA) and soluble schistosomule antigen (SSA) are often used, as are whole extracts prepared from nematode and cestode parasites. Although a crude extract, this will contain products from the parasite secretory organs and therefore the ES products themselves, pre-secretion. They are also rich in parasite-specific modifications that decorate ES components. Lewis^x, an abundant parasitic glycan, is found in SEA and SSA but also on omega-1 and α-1, glycoproteins that are amply secreted by schistosome eggs.[15-17] Le^x is also expressed by nematodes such as *Dictyocaulus viviparus*[18] and antisera against structures containing Le^x are raised during infection with *Taenia crassiceps* and *T. spiralis*.[19,20] In addition, many helminths have been shown to express an enzyme responsible for the production of Le^x.[21-24]

Therefore, many of the studies that have truly implicated ES in immune interactions, such as those with DCs, have either been done in vitro, or in vivo using elements shown to contain both secreted and non-secreted helminth components. For the purposes of this review, we include these studies since our current understanding of helminth-provoked effects on DCs are in large part owing to this work.

DC and Helminths: Th2 Polarizing Response

Many subtypes of DCs have been implicated in the host response to parasitic worms.[25-29] Each is characterized by a basic combination of surface markers, however new subtypes and slight variants of known subtypes are constantly being discovered. The specific phenotypes differ based on host organism (mouse or human), whether a study was done in vitro or in vivo, what location or organ they are extracted from and how they were matured. The particular adaptations of DC subtypes to their distinct microenvironments makes studying them in situ of paramount importance to understanding their behavior. This point is particularly pertinent for helminth infections given their diverse tissue specificity. In light of the experimental obstacles outlined above, localized analysis of DC response to worms is often impossible. As a result, the majority of published work on this topic heavily relies on either ex vivo observations or monocyte and bone-marrow derived DCs and their characteristic plasticity, wherein their behavior can be largely driven by microbial cues.[30]

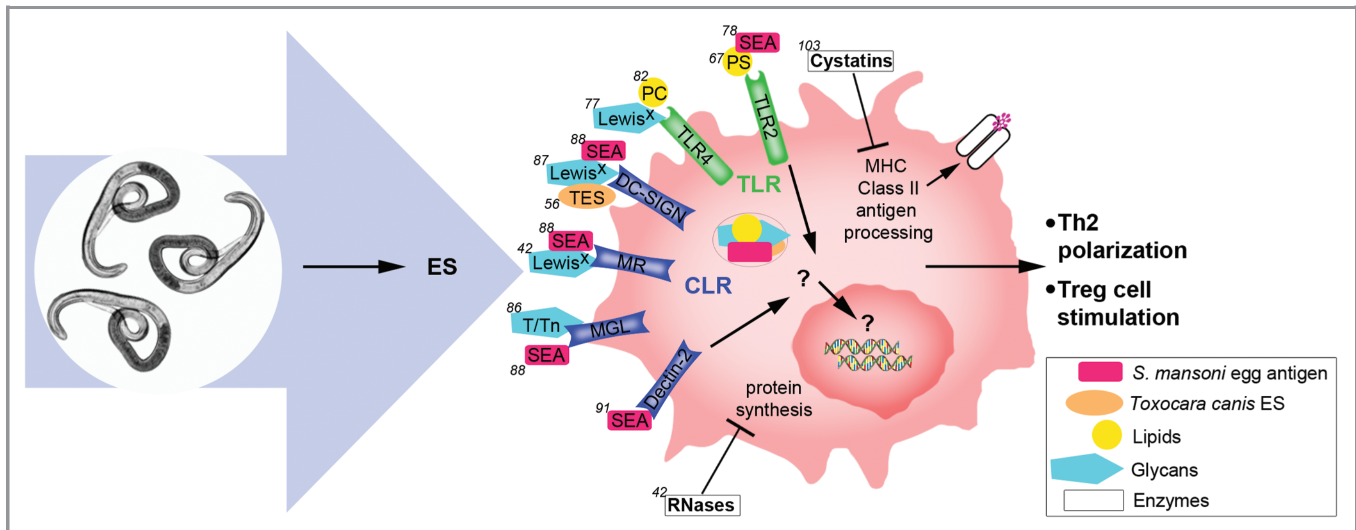

Figure 1. The molecular interactions involved in DC conditioning by components found in helminth excretory-secretory products (ES) and in *S. mansoni* soluble egg antigen (SEA) are illustrated. ES and SEA contain a mixture of immunogenic proteins and lipids, many of which are glycosylated. Many of the most potent antigens in SEA are also found in ES. Interactions are C-type lectin (CLR) or Toll-like receptor (TLR) dependent or pattern recognition receptor (PRR) independent. DCs are polarized toward a T_H2 phenotype and Treg cells are stimulated. Each interaction is numbered based on the corresponding reference in which it was reported.

Parasitic worms have long been known to promote host immune responses heavily skewed toward the T_H2 phenotype.[31] Unlike viruses and bacteria, worms do not elicit release of pro-inflammatory cytokines and responses targeted toward killing the pathogen itself. Perhaps this phenomenon is partly due to the worms being large, multicellular entities difficult to damage via direct inflammatory mechanisms.[7]

Type 2 immunity to helminths revolves around the $CD4^+$ T helper 2 cell, whereby depletion of these cells is associated with an inability to control[32] or clear[33] infection. Through cytokine secretions, this cell type is able to elicit a range of downstream responses, including B cell-derived antibody production and granulocyte-derived release of inflammatory effectors. In the context of a helminth infection, these events, in turn, result in both direct and indirect challenges against the pathogen. Immunoglobulins can directly opsonize antigens,[6] while effector-induced muscle contractions and mucus release can work toward mechanically expelling the parasite.[34,35]

Why do helminths specifically drive T_H2 responses? The answer to this question is still largely unknown. One hypothesis that has been suggested is that this happens as a default, a result of no T_H1 polarizing stimuli on the part of worms.[36] Another is that T_H2 responses promote wound repair, important during worm infections where skin and gut epithelia are often damaged.[37] The mechanisms behind T_H2 polarization likely combine elements from both these hypotheses. In an effort to distill the underlying processes involved, much work has been done toward characterizing the host receptors and parasitic ligands responsible for initiating the immune response during helminth infections. These studies have collectively implicated a range of pattern recognition receptors (PRR) and identified the dendritic cell as an essential regulator of T_H2 polarization. For instance, in vivo removal of DCs via CD11c-selected depletion results in a remarkable decrease in both T_H2 cytokine release and expansion of T helper cells upon parasitic challenge.[38] Conversely, adoptively transferred-DCs pulsed with ES antigens from the rodent nematode *N. brasiliensis* can efficiently drive T_H2 responses in naïve recipient mice.[28] A synergistic role for basophils in the cooperative generation of T_H2 immunity to worms with DCs has also recently been described. However, despite their ability to antigen present, to expand T cell populations and to elicit production of regulatory cytokines, their presence seems to be dispensable to these processes, unlike that of DCs.[39]

How Do Dendritic Cells Interface with Parasite ES?

The main function of dendritic cells is to capture, process and present antigen to T cells, serving as mediators between innate and adaptive immunity. The mechanisms by which they detect antigen are manifold; however pattern recognition receptors (PRR) are particularly important to this process. These receptors comprise several molecular families including cytoplasmic DNA sensors, Toll-like (TLR), C-type lectin (CLR), NOD-like (NLR) and RIG-1-like receptors and have evolved to recognize pathogen-associated molecular patterns (PAMPs) in order to allow DCs the ability to rapidly detect and respond to foreign antigens. In the context of helminth infections, ES-derived and parasite surface molecules are the agents responsible for the contact and interaction with host immune cells (**Fig. 1**).

Parasite-derived signals. As previously mentioned, ES contains a complex mixture of proteins, lipids and metabolic by-products. Many of these proteins and lipids are highly glycosylated. It is widely understood that it is these carbohydrate moieties, or glycans, that are most important for the interface with host DCs.[40]

Evidence shows that the functional protein portion of the ES is not necessarily required for DC recognition but does play a role in the overall response. For example, heat treated ES from the nematodes *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis* is still able to elicit a response representative of Th2 polarization in mouse BMDCs.[41] However, Everts et al. recently showed that although the carbohydrate domain alone of omega-1, a glycoprotein secreted from *Schistosoma mansoni* eggs, is sufficient for binding to DCs, the protein domain is still required for conditioning the Th2 response.[42]

Immunodominant helminth glycans were first identified, and have largely been characterized via the analysis of protective antibodies from the serum of immune animals.[21] Lewis^x or Le^x was one of the first helminth glycan antigens to be described in the immune sera of mice infected with *S. mansoni*.[43-45] Later, it was found that LacdiNAc (GalNAcβ1–4GlcNAc-R, LDN) and fucosylated LacdiNAc (LDNF) are also immunodominant glycoproteins and glycolipids from soluble extracts of *S. mansoni* and in immune sera to *Trichinella spiralis* and *Hemonchus contortus*.[46-50] Lacto-N-fucopentaose III (LNFPIII) is found in the urine of *S. mansoni* infected animals and in those vaccinated with irradiated larvae[51] but also in human milk and the urine of pregnant women. LNFPIII contains the terminal trisaccharride Le^x. LDN and LDN-F share similar motifs to mammalian glycoconjugates and, along with LNFPIII, may be described as host-like helminth glycans. Other host-like helminth antigens include the T and Tn antigens which are GalNAc-O-Ser/Thr motifs often found on surface glycoproteins of mammalian cancer cells.[52] T and Tn antigens are common to cestodes, nematodes and trematodes.[53] There is some speculation that the expression of host-like antigens may aid protection of the parasite from immune attack and clearance.[54,55] There are however helminth glycans that are parasite or even genus specific such as the O-methylated glycans from the nematode *Toxocara canis*[56] and the glycan terminal tyvelose (3,6-dideoxy-D-arabino-hexose) from *Trichinella spiralis*.[57-59] Alternatively, parasite glycans may be helminth-specific but common among different groups, such as the α(1–3)-linked core fucosylation of LacdiNAc[60] and chitin, a polysaccharide that is found in the cuticle of parasitic and non-parasitic worms.[61,62] Phosphorylcholine {PC, $[(CH_3)_3N^+CH_2CH_2PO_4^-]$} is a small zwitterionic molecule.[63] It is found in the LPS of various pathogenic bacteria as well as helminth ES products such as ES-62, an abundant ES glycoprotein antigen from *Acanthocheilonema viteae*, a rodent filarial nematode. It is also found on the ES-62 homolog from *Brugia pahangi* and the N-acetylglucosaminyl-transferase from *Brugia malayi* and seems to be nematode specific.[64-66] Other lipid antigens include phosphatidylserine (PS), which is found in *S. mansoni* and the dog heartworm *Dirofilaria imitis*.[67,68]

Host DC receptors: TLR. Although many helminth antigens have been identified, our knowledge of the host DC receptors responsible for their recognition, binding and internalization is more limited. There are 11 known mammalian TLRs that recognize bacterial, viral and fungal pathogen-derived antigens. TLR2, TLR4 and TLR9 are all expressed on DCs and have been well characterized for their interaction with potent T_H1-inducing agents such as bacterial LPS. DC TLR2 has also been linked to T_H2 immunity in response to certain pathogenic stimuli, and more recent evidence suggests that TLR4 may also be important for cross-talk between T_H1 and T_H2 immunity.[69,70] Pre-treatment with helminth ES has been shown to inhibit classical LPS signaling through DC TLR receptors.[71-74]

In vitro activation of DCs by SEA and SSA has been shown to be TLR4 independent.[75,76] In contrast, pulsing DCs with synthetic forms of the Le^x containing carbohydrates LNFPIII and LNFPIII-Dex (both found in SEA and SSA) was shown to drive DC activation in a TLR4-dependent manner.[77] Additionally, Gao et al. showed that TLR2 deficient mouse BMDCs were unable to produce IL-12p70 and IL-10 in response to SSA and SEA.[78] This result was not observed in TLR4-deficient BMDCs, suggesting that TRL2 but not TLR4 is essential for the Th2-specific DC response to SSA and SEA but that LNFPIII alone may signal via TLR4.

ES-62 carries the phospholipid PC and is expressed by *A. viteae* in a stage-specific manner. A number of studies have evaluated the molecular interaction of ES-62 with DCs.[64,79] ES-62 alone is able to stimulate DCs and induce a Th2 phenoype.[80,81] This glycoprotein was found to signal via DC TLR4 and its adaptor MyD88 but not via TLR2.[82] The mechanism of this interaction however was shown to be unconventional, i.e., mutated TLR4 that can no longer respond to LPS, still responds to ES-62. Further studies by the same group showed that the PC portion alone (as PC conjugated to ovalbumin) was still able to redirect DC maturation, and this effect was also found to be TLR4 and MyD88 dependent. The phospholipid PS from *S. mansoni* was also shown to activate DCs toward a Th2 phenotype, but unlike PC, it is most likely this occurs via TLR2 signaling.[67] There is therefore a role for both TLR2 and TLR4 in the recognition of helminth antigens by DCs and there may be some interplay between the two receptors.

CLR. CLRs contain an extracellular carbohydrate recognition domain (CRD) that binds antigenic glycans, a process that requires calcium. DC CLRs that are involved in pathogen recognition include DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN), which binds high mannose glycans.[83] DC-SIGN plays an important role in viral recognition and binding to the HIV antigen gp 120 has been well characterized.[84] DC-SIGN activation can in turn activate TLR signaling.[85] Macrophage galactose binding lectin receptor (MGL) recognizes, among others, the helminth and tumor Tn antigen motif.[86] Mannose receptor (MR) also recognizes mannose-containing glycans.[54]

Van Die et al. and others have shown that DC-SIGN is also a receptor for Le^x and that antibodies against Le^x inhibit the binding of SEA to DC-SIGN.[83,87] The mechanism of binding, however, is not the same as that observed in the interaction between DC-SIGN and HIV gp120. The carbohydrate recognition domain (CRD) of DC-SIGN is still responsible for binding Le^x, but site-directed mutagenesis of the receptor showed that the regions within the CRD that mediate interaction are not conserved. Furthermore, the internalization of SEA is not only mediated by DC-SIGN, but also by MGL and MR.[88] DC-SIGN is also able to bind total ES products from *Toxocara canis* but not synthetic

versions of the most abundant *T. canis* glycans.[56] DC-SIGN, MGL and MR receptors appear to provide some form of redundancy whereby the inhibition of any one of these receptors at any one time still allows the helminth-associated activation of the DC.[88] Everts et al. recently demonstrated that the MR, but not DC-SIGN, is responsible for the binding and internalization of the *S. mansoni* secreted glycoprotein omega-1 by DCs.[42] Omega-1 is a T2 RNase enzyme decorated with a Le^x-containing glycan.[89] Although the carbohydrate domain alone is sufficient for binding DCs, the RNase activity of the glycoprotein is required to induce Th2 polarization in vitro. Another DC CLR receptor that has been shown to bind SEA is Dectin-2. Dectin-2 is a PRR whose role in fungal antigen recognition and innate immunity has been well characterized.[90] Ritter et al. showed that the Dectin-2/FcRγ complex on BMDCs is required for SEA-induced production of the inflammatory cytokine IL-1β.[91] This was not the case when Dectin-1, CD36 or indeed MR were tested. Interestingly, IL-1β production was not observed after heat or proteinase K inactivation of the SEA, again implicating an important role for the functional proteins in SEA.

What Happens to Dendritic Cells upon ES Exposure?

Of all antigen-presenting cells, DCs express the highest levels of MHC class II and are the most efficient processors of exogenous antigens. Once they have received the necessary stimuli via PRRs, DCs undergo a process of maturation to become efficient antigen presenters via a series of subcellular and morphological changes. They translocate class II MHC molecules loaded with antigenic peptides to the cell surface, they upregulate CD40, CD80 and CD86 costimulatory molecules on the cell surface, and they release pro-inflammatory cytokines and chemokines including IL-12, IL-6, IL-23 and TNF.[92] These events enable the DC to communicate with other cells types and to determine the course of the immune response. Classically TLR-activated DCs will release IL-12 which signals effector T cells to expand and also recruits macrophages to the site of infection. In addition, IL-12 acts as a signal to neighboring cells to release other pro-inflammatory cytokines (IFN-γ and TNF-α) that drive a T_H1 response.

In the context of helminth infections, DCs exhibit a very different phenotype. As a general rule, they fail to classically mature in that they do not upregulate coreceptors nor do they release pro-inflammatory cytokines.[93] This phenomenon seems to be common to most helminth infections, irrespective of their taxonomic classification (nematode, cestode, trematode). DCs exposed to ES derived from *Taenia crassiceps*, a cestode tapeworm, fail to upregulate CD83, HLA-DR, CD80 or CD86.[73] This immature phenotype persists even when DC are subsequently stimulated with LPS. DC stimulated with schistosome SEA exhibit the same behavior, with low expression of CD80, CD86, class II MHC and CD40[94] and no IL-12 production. Like their cestode-exposed counterparts, they too fail to classically respond to LPS stimulation. Nematode ES also inhibits DC maturation, with ES from the filarial worm *Brugia malayi* blocking IL-12 production,[95] and ES from *Trichinella spiralis* inhibiting both the upregulation of costimulatory molecules as well as LPS-responsiveness.[96] By stimulating ES-exposed DC to various TLR agonists, the authors of this study show that the inhibitory effects of the *T. spiralis* secretions are specific to TLR4. However, TL4-mediated non-responsiveness in unlikely to be caused by differences in absolute levels of TLR4 surface expression since SEA-exposed DC, that also present with the same phenotype, have equivalent levels of this receptor on their surface as compared with unexposed controls.[70] More likely, the downstream signaling pathways that are stimulated following helminth ES exposure are liable for the observed differences. TLR signaling is known to proceed via mitogen activated protein kinases (MAPK) ERK and p38. Induction of T_H1 responses, namely the release of IL-12 by DCs, results from p38 phosphorylation, whereas the ERK pathway is associated with T_H2 polarization via stabilization of the c-fos transcription factor that suppresses IL-12 release.[97,98] Consistent with these signaling associations, SEA favors ERK phosphorylation.[70,99] Therefore, the anti-T_H1 bias may partially arise from the subcellular signaling cascades that are triggered upon ES exposure.

In addition to their rapid, PPR-induced activities, DCs ingest, process and present antigen to $CD4^+$ helper T cells. Protein peptides are presented in association with class II MHC molecules, but glycolipids—which helminth ES is rich in—can also be presented via cell-surface CD1d.[100] This protein seems to play a critical role in DC-mediated T_H2 priming, since DCs from $CD1d^{-/-}$ mice were unable to drive expansion of SEA-specific T_H2 lymphocytes.[101] The fact that proper antigen processing and presentation are necessary components to an effective host response is another aspect of DC functionality with which parasitic worms can interfere. As mentioned above, helminth infections are associated with decreased MHC class II expression. Low levels of expression may either result from interference with protein synthesis and molecular assembly[102] or from worm-induced inhibition of class II MHC-peptide complex translocation to the cell surface. In line with the latter idea, the rodent intestinal nematode *Nippostrongylus brasiliensis* secretes nippocystatin, a cysteine protease inhibitor that effectively decreases antigen processing. Furthermore, mice with anti-cystatin circulating antibodies are able to better control their infection[103] suggesting that foreign peptides are not efficiently presented and downstream effector mechanisms that control worm burden are disrupted. *Onchocerca volvulus* and *Brugia malayi* are also known to secrete similar protease inhibitors which interfere with host endolysosomal proteases and potentially inhibit proper processing and loading of worm proteins onto class II MHC.[104,105]

How Do ES-Exposed Dendritic Cells Affect the Downstream Immune Response?

Despite the demonstrated necessity for DCs in the development of T_H2-polarization to helminth infections, the specific signals they send and the mechanisms through which they interact with other cells are still quite poorly characterized. The host response to parasitic worms characteristically begins with an active T_H2 effector phase that is then downregulated.[106] The presence of certain ligands on the surface of DCs has been found to be

essential for proper anti-helminth T_H2 responses to develop. One such molecule is OX40L, where an $OX40L^{-/-}$ model demonstrated that expression of this ligand on DCs is central to the development of a T_H2 effector response.[107] Similarly, DCs derived from a $CD40^{-/-}$ mouse were also found to be deficient in their ability to induce T_H2 responses.[108] Both these studies were done in the context of *S. mansoni* infections, but given the consistency of the host response to parasitic worms irrespective of taxonomy, it is likely that the importance of CD40 and OX40L will carry over to other helminth infections as well. Another mechanism by which DC may polarize a T_H2 response is through mechanical means.[109] DC exposed to SEA fail to display the ruffled appearance and adherence of classically activated DC. This altered cytoskeletal morphology correlates with decreased ability to form stable conjugates with CD4 T cells. The authors of this study hypothesize that fewer interactions between DC and T cells sends a low-dose antigenic signal to DCs which is known to favor T_H2 responses.[110,111]

As previously discussed, DCs are central to the initial T_H2 polarization, but they are apparently also essential for subsequently moderating its strength.

DCs exposed to a wide array of helminth ES products have been shown to promote the expansion of $CD4^+CD25^+Foxp3^+$ regulatory T cells.[74,95,96,112] In turn, regulatory T cells are necessary for averting the pathology and tissue damage arising from an unchecked T_H2 response in addition to contributing to the suppression of a T_H1 response.[113] DC matured in the presence of *S. mansoni* phosphatidylserine seem to acquire the capacity to potently drive naïve T-cells to become regulatory in nature[67] and *T. spiralis* and *H. polygrus* stimulate expansion of existing Treg cell populations in vitro and ex vivo, respectively.[96,114] DC conditioning by ES also results in release of regulatory cytokines such as IL-4 and IL-10. These cytokines are most commonly produced either by DC-stimulated T cells or by DCs themselves.[99]

Although helminths elicit Type 2 immunity, prolonged infection necessitates a subsequent dampening of these responses in order to preserve host integrity.[115] Whether this downregulation is a direct effect of the parasite or, more likely, a defense mechanism against tissue damage on the part of the host, is not entirely clear.

Parasite ES contains host glycan "mimics" and their interaction with DCs are thought to play a central role in the downregulation of T_H2 responses. These "self glycans" engage C-type lectins on the surface of DCs and stimulate expansion of T regulatory cell populations which, in turn, promote tolerance and downregulate inflammatory responses.[54] Therefore, worm infections can help balance Th1-mediated pathologies given their Th2-promoting bias, and they can also control Th2-mediated conditions due to their stimulation of T regulatory populations.

Multiple sclerosis (MS) and murine experimental autoimmune encephalomyelitis (EAE), a mouse model system for MS, are both characterized by high Th1 and Th17 cell responses.[116] EAE can be significantly suppressed by treatment with *T. spiralis*, *S. mansoni* and *T. suis* ES.[117] Inhibition of DC-derived TNF-α and IL-12, coupled with upregulation of OX40L suggests that DCs may play a central role in the observed immune modulation. Likewise, DC-driven T_H2 responses also seem to protect against development of Type 1 diabetes in non-obese diabetic (NOD) mice. If exposure to *S. mansoni* antigens is established at a young age, DCs stimulate IL-10 release and Treg cell expansion which protects against the otherwise spontaneous development of the disease.[118,119]

Several studies have shown an association between helminth infection and the suppression of Th2-driven allergic responses.[120,121] Perhaps the most convincing evidence of a link comes from the central principles underlying the "hygiene hypothesis" which stipulate that industrialization—which brings a reduction in infections—is associated with corresponding increases in allergies and autoimmune diseases.[122] For instance, it is known that infection with *Ascaris suum* suppresses allergic immune responses in mice[123] in an IL-10 independent fashion. Reduced activation of DCs was deemed responsible for this difference, since cytokine production and receptor expression was suppressed in DCs exposed to parasite-derived products.

Conclusion

Although much remains to be discovered about the signals DCs receive and send in order to modulate the immune response during helminth infection, their behavior is incrementally being defined. Herein, we have attempted to comprehensively review these efforts, focusing on (1) the recognition elements between the parasites and the DC, (2) the subcellular changes these interactions induce within the DC and finally (3) how these changes translate into signals that drive T_H2 polarization and subsequent control. Like all pathogens, worms have evolved skillful and innovative ways by which to modulate host immunity to their own benefit. They secrete ES whose glycosylated components regulate fundamental processes of antigen recognition, processing and presentation. By expressing parasite specific as well as "host" glycans, they induce DCs to stimulate both inflammation and tolerance. The ability to induce and then downregulate T_H2 responses, which allows worms to establish persistent infections, can also be exploited therapeutically for certain allergic and autoimmune diseases. Further research is necessary to isolate specific components that can be used as "parasite therapeutics," and unpair them from the pathology that also accompanies helminth infection. It is likely that DCs—and the molecular mechanisms by which they interact with parasite components—hold many more clues of how to attain this goal.

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