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Review series on helminths, immune modulation and the hygiene hypothesis: Mechanisms underlying helminth modulation of dendritic cell function

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Summary

Dendritic cells (DCs) play a central role in activating CD4 T (T helper, Th) cells. As a component of their response to pathogen-associated stimuli, DCs produce cytokines and express surface molecules that provide important cues to modulate the effector functions of responding Th cells. Much is known of how DCs respond to, and influence immune response outcome to, bacterial and viral pathogens. However, relatively little is understood about how DCs respond to helminth parasites. This is an area of considerable interest since it impacts our understanding of the initiation of Th2 responses, which are stereotypically associated with helminth infections, and the regulation of allergic and autoimmune pathologies which evidence suggests are less severe or absent in individuals infected with helminths. This review attempts to summarize our understanding of the effects of helminth products on dendritic cell biology.

Keywords: dendritic cell; helminth pathogens; immunoregulation; Toll-like receptors; T helper type 1 (Th1)/Th2

Introduction

Parasitic helminths are estimated to infect three billion people.[1] The risk of mortality as a result of infection with helminths is for the most part low. However, helminth infections are often chronic and can cause insidious or frank disease that leads to considerable morbidity.[1] Thus, helminth pathogens differ from human immunodeficiency virus (HIV), *Mycobacterium tuberculosis* and *Plasmodium* spp. in being associated with low mortality but high morbidity. A consequence of this is that investment in research on and control of helminth diseases has been relatively meager, and this has been noted recently with appeals for focus on the so-called neglected tropical diseases, many of which are caused by helminth infections.[2]

'Helminth' is a working term for metazoan organisms that are worm-like. In reality, the helminth group includes highly diverse organisms belonging to distantly related phyla – Nematoda (round worms) and Platyhelminthes (flatworms).[1] Despite the lack of relatedness, infections with round worms and flatworms stereotypically lead to T helper type 2 (Th2) responses, in which responding CD4 T cells make interleukin (IL)-4, IL-5, IL-13 and a panel of additional cytokines, and concomitantly eosinophils, basophils, mast cells and goblet cells are involved and contribute to the response.[3] In such settings, responding B cells make immunoglobulin E (IgE) and IgG1. Interestingly, the ability to induce Th2 responses does not appear to be an adaptation to parasitism, as free-living helminths also possess this property.[4] Th2 responses play a crucial role in resistance to helminths,[3] but can also be immunopathological.[5] Their pathological potential is underpinned by the fact that they play causative roles in prevalent diseases of westernized societies such as allergic disease, asthma and ulcerative colitis.

CD4 T cells cannot directly recognize antigen, but rather require that it be processed and presented bound to major histocompatibility complex (MHC) class II molecules on the surface of antigen-presenting cells. Relatively few cell types – dendritic cells (DCs), macrophages and B cells, most prominently – possess the ability to present antigen/MHC class II complexes. Amongst these, DCs are considered to be the cells that possess the greatest ability to activate naïve Th cells and thereby initiate adaptive immune responses. A major role of DCs in this context is to interpret pathogen-inherent signals to provide cues for

Th cell differentiation into cells that possess effector properties that are appropriate for countering the inducing stimulus. For example, DCs exposed to Gram-negative bacteria respond by making IL-6, IL-12 and IL-23, which may promote the development of naïve Th cells into Th1 or Th17 cells which are able to initiate processes that facilitate control of bacterial infections (e.g. see refs 6 and 7). Because they occupy such a key position in the immune responses, there has been considerable interest in how DCs interact with helminth parasites, particularly in the context of understanding how adaptive immune responses in helminth-infected animals become Th2-biased.

In the interest of clarity and brevity, we have oversimplified two issues: (i) we have avoided excessive reference to different helminth species or life stages, and frequently refer only to 'helminths' or 'helminth products', and (ii) we make no reference to different DC subsets, because few specific data regarding their interactions with helminths are available.

The induction of Th2 responses by DCs

The ability of DCs to interpret helminth-inherent signals and induce Th2 responses has been illustrated by experiments in which mice injected with DCs that have been pulsed with extracts of helminths *in vitro* develop Th2-biased helminth-specific responses (e.g. 8–10). That these responses are directly induced by the injected DCs is indicated by the inability of MHC II-deficient DCs to prime T-cell responses in these systems.[8] The Th2-polarizing properties of helminths appear to reflect the conditioning of DCs to induce these types of immune response, because helminth products can act as Th2 adjuvants for unrelated antigens.[11–13]

The response of DCs to helminth products

The response of DCs to microbial pathogens is mediated in large part via Toll-like receptors (TLRs), with input from other pattern recognition receptors such as lectins.[14,15] The DC response initiated by ligation of different TLRs is somewhat stereotypical. It is characterized by profound changes in gene expression that lead to DC 'maturation', a term used to describe the full breadth of changes in DC biology that accompany the TLR-mediated transition from a more resting state into a more dynamic state in which the cells secrete a broad array of cytokines and chemokines, begin processing previously acquired antigen for presentation in MHC molecules, and express important costimulatory molecules such as CD80 and CD86. These changes in cell biology reflect the initiation of mitogen-activated protein kinases (MAPKs) and nuclear factor (NF)-κB signalling by TLRs, and effects of type 1 interferon (IFN) production and autocrine signalling.[16,17] TLR signalling is predominantly MyD88-dependent, although some TLRs function independently of MyD88, in a Toll/IL-1 receptor domain containing adaptor inducing IFN-beta (Trif)-dependent manner.[16] For obvious reasons, DC maturation has been considered to be essential for DCs to be able to induce T-cell responses.[18] However, it has become clear that DCs responding to helminth products do not mature in the conventional sense.

Unbiased global gene expression profiles, or proteomic analyses of mouse or human DCs following stimulation with helminths, have revealed that remarkably few genes are induced, and that those that are have little obvious connection to the ability of the cells to induce Th2 responses.[19–21] Other studies describe targeted analyses of potentially important molecules expressed in response to exposure to helminth products. For the most part these concur in finding that helminth products fail to directly activate DCs (e.g. refs 8 and 22). In contrast, proteins secreted by the nematode *Nippostrongylus brasiliensis* were found to induce partial activation of DCs, inducing the expression of CD40, CD86 and OX40L, which have been associated with Th2 promotion, and IL-6, IL12p40 and macrophage-derived chemokine.[9] Nevertheless, the overall picture is that DCs produce a very muted response to helminth products.

A striking and significant difference between Th2 responses and Th1 responses is that the former develop normally in the absence of MyD88[23–25] and, in cases where it has been examined, Trif (CK and EJP, unpublished data). The implication of this finding is that conventional TLR-initiated signalling in DCs is not necessary for them to be able to induce Th2 responses.[26] Consistent with this, analyses of signalling events within the NF-κB and MAPK pathways have revealed significant differences between DCs exposed to helminth products and those stimulated with microbial products such as bacterial lipopolysaccharide (LPS). For example, extracellular signal regulated kinase (ERK), c-Jun N-terminal kinases (JNK) and p38 are heavily phosphorylated after exposure of DCs to LPS, but in DCs exposed to schistosome egg antigen (SEA), a soluble extract of schistosome eggs that is capable of conditioning DCs to induce strong Th2 responses, ERK and to a lesser extent p38 are phosphorylated, but JNK is not.[20] In this case, the phosphorylation of ERK has been reported to be unusually sustained, and has been shown to stabilize c-FOS, which suppresses IL-12 production.[27] Lacto-*N*-fucopentaose III (LNFPIII), a milk sugar that contains the Lewis^x trisacharide that is found in SEA, and which acts as a Th2 response-promoting adjuvant when conjugated to other proteins,[12] also stimulates ERK phosphorylation.[28] A role for ERK in Th2 response development is indicated by the findings that ERK$^{-/-}$ mice exhibit increased susceptibility to experimental autoimmune encephalomyelitis and are Th1 prone,[29] although

definitive experiments exploring Th2 response development in these animals following helminth infection remain to be carried out. Moreover, not all Th2-conditioning helminth products stimulate ERK phosphorylation in DCs.[30] Exposure of DCs to helminth products has also been reported to stimulate NF-κB activation. For example, LNFPIII stimulates rapid, transient NF-κB nuclear translocation and activation in the absence of inhibitor of nuclear factor kappa B (IκB) degradation.[31] Consistent with these findings, neither SEA nor LNFPIII-dextran (dex) pulsed NF-κB1$^{-/-}$ DCs are able to induce Th2 responses.[31,32] Why NF-κB1 is essential for the ability of DCs to prime Th2 responses remains to be determined.

How are helminth products recognized by DCs?

Despite the lack of evidence to support a role for conventional TLR-initiated signalling in DCs in Th2 response induction, TLRs have been implicated in the recognition of helminth products by DCs. LNFPIII and excretory/secretory (ES)-62, a phosphorylcholine-containing protein secreted by the nematode *Acanthocheilonema viteae*, condition DCs to induce Th2 responses through TLR4.[28,33] Consistent with this, TLR4 has been implicated in the Th2 response induction in airways hypersensitivity.[34] However, helminth-associated Th2 responses do not appear to be abrogated in the absence of TLR4, indicating that TLR4 does not play a uniformly important role in the ability of the host to recognize helminth pathogens and respond appropriately (refs 35 and 36, and CK and EJP, unpublished observations). Lysophosphatidylserine from schistosomes has been reported to trigger DC activation by binding to TLR2 in DCs,[37] and the defined TLR2 ligand pam3cys has been shown to share with SEA the ability to stimulate prolonged ERK phosphorylation in DCs, suggesting that key events in the helminth-mediated conditioning of DCs may be mediated by TLR2.[27] Nevertheless, Th2 responses do develop, and actually are exaggerated in TLR2$^{-/-}$ mice infected with *Schistosoma mansoni* [although these animals were found to exhibit a defect in regulatory T (Treg) cell development, which is consistent with the findings of van der Kleij *et al.*].[38] TLR3 has also been implicated in the response to schistosome eggs, which have been reported to contain double-stranded RNA which activates NF-κB signalling in DCs, leading to IFN-β production.[39] However, TLR3 deficiency, like TLR2 deficiency, results in exaggerated Th2 responses during schistosome infection.[38]

There has been great interest recently in the possibility that C-type lectins represent the major class of pattern recognition receptors for helminth products. In many ways, this story has its origins in Harn's important early studies showing that the induction of Th2 responses is largely attributable to carbohydrates in SEA functioning as adjuvants. He found that SEA treated with metaperiodiate lost the ability to induce Th2 responses when introduced to mice by intranasal administration.[40] Further work identified N-glycans containing fucose, expressed in multiple schistosome life stages, as possessing many of the Th2-inducing properties of SEA, as described above,[28,31,41,42] and generally indicated a role for glycans in the priming of Th2 responses by helminths.[4] In 2003 a key paper by van Kooyk and colleagues showed that DC-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN) on DCs specifically recognized mannose- and fucose-containing glycoconjugates, and could bind to a schistosome extract.[43] Later it was shown that monoclonal antibodies against the carbohydrate antigens Lewis^X and LDNF [GalNAcbeta1-4(Fucalpha1-3)GlcNAc] could block the binding of SEA to DC-SIGN.[44] DC-SIGN also serves as a receptor for other schistosome glycans, including pseudo-Lewis^Y glycolipids on the infectious larval stage of these parasites.[45] Thus DC-SIGN is a receptor for Lewis/LDNF-like structures in schistosomes. Interestingly, DC-SIGN has also been described as the receptor for Ara h1, the major glycoprotein allergen from peanuts.[46] More recently it has become clear that DCs internalize SEA through the combinatorial effects of three C-type lectins, namely DC-SIGN, macrophage galactose-type lectin (MGL) and the mannose receptor,[47] and it seems likely that the overt antigenicity of SEA, which (like certain other helminth products) can powerfully induce immune responses in the absence of added adjuvant, relates to the fact that multiple receptors are capable of mediating its uptake into DCs.

Inhibition of DC activation by helminth products

The available evidence indicates that helminths not only fail to induce conventional signs of activation in DCs, but additionally are capable of markedly inhibiting DC maturation by TLR ligands. Specifically, TLR-mediated activation, as determined by IL-12 production and/or MHC class II or costimulatory molecule expression, or by microarray analyses, has been reported to be significantly suppressed when DCs are costimulated with a broad variety of helminths and/or their products.[20,22,35,47–50] In many cases, decreased responsiveness to TLR ligands is associated with increased production of the anti-inflammatory cytokine IL-10, and this cytokine can contribute to the overall suppressive effects observed (e.g. refs 20 and 50). Mechanistically, the pathways that allow helminths to suppress TLR signalling remain unclear. However, detailed work on C-type lectins has revealed that signalling initiated by these receptors can positively or negatively influence TLR signalling, depending on the context.[15] Strikingly, many of the effects of helminths on DCs are mirrored by the effects of mannose capped lipoarabinomannan (ManLAM), a *Mycobacterium tubercu-*

losis cell wall component and DC-SIGN ligand.[51] ManLAM antagonizes TLR4 signalling, promoting IL-10 production as it does so, and is consequently believed to play an important role in promoting the survival of the bacterium. Recent evidence indicates that ManLAM activates the serine and threonine kinase Raf-1, which leads to acetylation of NF-κB p65, but that this occurs only if NF-κB has first been activated by TLR signalling.[52] Acetylation of p65 prolongs the transcriptional activity of NF-κB and leads to increased IL-10 production. These findings are highly reminiscent of those reported for SEA, also a DC-SIGN ligand, in that SEA does not induce IL-10 production itself, but rather enhances IL-10 production promoted by TLR ligands.[20] At this time, then, it is appropriate to hypothesize that C-type lectins play a role in mediating the immunomodulatory effects of helminth products on DCs. While this does not entirely mesh with the fact that some helminth modulatory molecules are clearly not glycans (e.g. the active component of the immunomodulatory molecule ES-62 from *A. vitei* is phophorylcholine),[30] it should be pointed out that, despite their name, not all C-type lectins have been documented to recognize sugars,[15] leaving open the possibility that they may interact with other classes of molecules.

Requirements for Th2 response induction by DCs pulsed with helminth antigen

Signalling through the IL-4 receptor (IL-4R) on $CD4^+$ T cells was found to be important for the full establishment of Th2 responses.[53] This raised the possibility that helminth products stimulate DCs to make IL-4, which then plays a critical role in priming the Th2 response. This would be comparable to the way in which DCs pulsed with bacterial products make IL-12, IL-23 and IL-6 and focus Th responses in Th1 or Th17 directions. However, this turned out not to be the case, as (i) there is little evidence for DCs making IL-4, and (ii) bone marrow-derived DCs from $IL\text{-}4^{-/-}$ mice are as capable as wild-type DCs of inducing helminth-specific Th2 responses.[54] Indeed, at this time there is little evidence for essential roles for any DC-produced cytokines in Th2 response induction. However, it is clear that the expression of certain costimulatory molecules can be critical. For example, DCs that cannot express CD40 are incapable of inducing Th2 responses following pulsing with SEA,[55] and, consistent with this, mice lacking CD154 or CD40 fail to develop Th2 responses when infected with schistosomes.[55] OX40L expression by DCs also appears to play an important role in this process.[56] Functionally, CD40 is believed to be serving as a receptor to allow DC maturation in response to interactions with CD154 and OX40L is considered to serve an essential costimulatory function for Th cell activation in the context of a DC that has been exposed to a helminth product rather than an activating microbial stimulus.

Recent reports have emphasized roles for Notch in Th1[57,58] and in Th2 cell[59–62] differentiation and highlighted the potential for differential Notch ligand engagement to control disparate outcomes downstream of common Notch receptors. These studies have linked the preferential expression of the Notch ligand Delta4 by DCs as being important for Th1 response induction, and the expression of Jagged2 as playing a role in Th2 responses.[59] However, recent reports have been unable to identify a role for Jagged 2 in Th2 response induction by SEA-pulsed DCs.[63,64] In contrast, there appears to be a major role for TLR-induced Delta1 and Delta4 expression on DCs in the ability of TLR-stimulated DCs to suppress the differentiation of naïve Th cells into Th2 cells.[65,66] Consistent with the ability of SEA to suppress TLR activation of DCs, SEA is able to suppress the expression of Delta1 and Delta4 by TLR-stimulated DCs (JS and EJP, data not shown), and in this way promote Th2 response development.

The role of other cell types in DC activation in response to helminths

The most straightforward way to envisage DCs responding to helminths is through direct interactions. Clearly these types of interaction do occur and DCs are capable of directly responding to helminth products in the absence of other cell types. However, there is increasing interest in the possibility that, following infection, in a co-ordinated fashion, other cell types respond to the invading pathogen and interface with DCs to profoundly effect their behaviour and the outcome of the immune response. Such a series of events was illustrated recently when, during the development of immunity to the gut helminth *Trichuris muris*, NF-κB1 signalling in intestinal epithelial cells was shown to play a crucial role in the production of thymic stromal lymphopoietin, a cytokine that promotes Th2 responses, in this case by preventing the production of Th1- and Th17-polarizing cytokines by DCs.[67] In another example, the requirement for CD4 T cells to express CD40 in order to induce SEA-specific effects reflects a requirement for DCs to interact with CD154-expressing cells.[55] Recently it was shown that both T cells and non-lymphoid cells can serve this latter function.[68]

Helminths and host immune regulation – the big picture

There continues to be great interest in the possibility that the exacerbation of TLR signalling underlies the ability of helminths to modulate the exaggerated immune responses observed in inflammatory autoimmune conditions.[69] Experimentally this has been illustrated in, for example, the non-obese diabetic (NOD) mouse, where infection

with *S. mansoni* prevents the development of diabetes.[48] It is particularly interesting that helminth infections can also mitigate some allergic conditions.[70] For example, there is a low prevalence of positive skin test to aeroallergens in individuals living in areas endemic for *Ascaris lumbricoides*[71] or *S. mansoni* infection.[72] Based on experimental studies in mice, It seems likely that this reflects the development of broadly effective Treg cells in response to helminth infection[73,74] Whether this reflects a particular ability of helminth product-exposed DCs to induce Treg cells remains to be established.

The interactions of helminths with DCs – a summary

Helminth products appear to be inherently adjuvantized, as they can promote strong Th2 responses to themselves and to bystander antigens, in the absence of any additional adjuvant. Consistent with the view that DCs are a primary interface between infection and the induction the adaptive immune response, DCs exposed to helminth products develop the ability to induce Th2 responses. Although there are data supporting the view that TLRs act as receptors for helminth products, and play a role in conditioning DCs to induce Th2 responses, accumulating evidence suggests that C-type lectins are likely to play the dominant role in this regard. These pattern recognition receptors not only facilitate antigen uptake by DCs, but also can suppress the ability of DCs to respond to TLR ligands. Consequently, a role for these receptors accommodates two of the noted features of helminth antigens: (i) their antigenicity, which would be expected to be accentuated if they were to be delivered by receptor-mediated uptake directly into DCs, and (ii) their ability to counteract aspects of classical TLR-mediated DC activation and generally suppress DC maturation. The latter property of helminth products is consistent with, and may underlie, their ability to promote Th2 responses, which in general are strongly exacerbated by TLR signalling.

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