Molecular interplay between dendritic cells and schistosomes : consequences for immune polarization

Everts, B.

Chapter

General introduction

Based on:

Helminths and dendritic cells:
Regulating the regulators

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1 Dendritic cells and their role in immune polarization

The innate immune system provides a first line of defence against invading pathogens. However, the activation of the adaptive arm of the immune system is required for generation of full protection against infections and long-lasting immunity. The cells that play a central role in activation of these adaptive immune responses are the dendritic cells (DCs). DCs are professional antigen presenting cells (APCs) that are crucially important in the initiation and polarization of adaptive immune responses. These cells are often described as sentinels of the immune system, located in peripheral tissues, where they continuously sample their environment in an immature state. Upon recognition of stimuli from the environment that trigger their activation, including components derived from pathogens, DCs undergo phenotypic changes that allow them to effectively migrate to lymph nodes and to prime T cell responses. The induction of protective adaptive immune responses by DCs against different classes of pathogens requires the activation and maintanence of specific CD4+ T helper cell (Th) subsets that can control the different types of infections. Th1 cells produce high amounts of interferon-γ (IFN-γ) and are instrumental for immunity against intracellular pathogens such as viruses and intra-cellular bacteria or parasites. Effector Th2 cells are characterized by secretion of interleukin-4 (IL-4), IL-5, and IL-13 and play an important role in providing protection against infections caused by multicellular pathogens like parasitc helminths. More recently, Th17 cells, as a third CD4+ T cell effector subset has been characterized that secretes IL-17 as a key discriminating cytokine and is thought to play a central role in clearance of extracellular bacteria and fungi. Finally, these three subsets are complemented with a fourth; the T regulatory cells (Tregs). This subset is known to be important for induction and maintainance of tolerance to self antigens, but also to keep potentially harmful inflammatrory responses mediated by the former three Th cell subsets under control. As a result, the induction of Tregs by DCs in responses to pathogens may have a dual role since it can be beneficial to the host but also to the pathogen (reviewed in [1;2]) (Fig 1).

2 Immune responses during helminth infections

Parasitic helminths, like nematodes (roundworms), trematodes (flatworms) and cestodes (tapeworms), represent a diverse group of organisms responsible for millions of human infections worldwide. The diversity within this group of parasites is well illustrated by the fact that individual species, such as schistosomes as a representative of the trematodes (its life cyle is shown in Fig 2), have developed a variety of ways to infect as well as evolved to occupy a multitude of niches in their hosts (reviewed in [3]). And yet, the host immune response mounted against these parasites is remarkably consistent. This stereotypic response involves the activation of effector

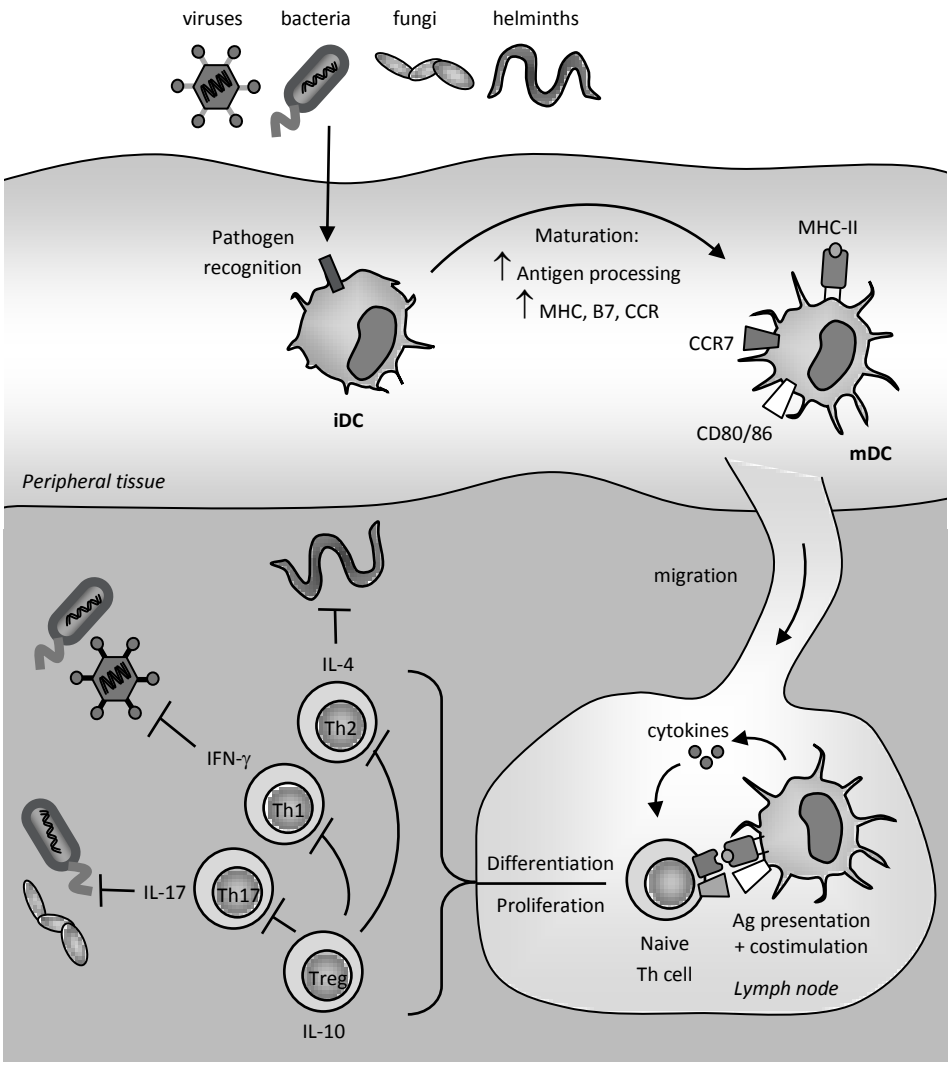

Figure 1: **Sequence of events leading to induction of T helper cell immune responses by DCs following an infection**. When immature DCs (iDC) residing in peripheral tissues sense the presence of pathogen-derived components through their pattern recognition receptors, they become activated and undergo a process of so-called maturation (mDC) that results in increased antigen processing and presentation on MHC class I & II, and expression of costimulatory molecules (B7) and chemokine receptors, such as CCR7. This triggers the maturing DC to migrate via afferent lymph vessels to draining lymph nodes, where it initiates adaptive immune responses by antigen-specific activation and polarization of naïve Th cells. Depending on the pathogen encountered by the DCs, they instruct naïve Th cells to differentiate into Th1, Th2, Th17 or Treg subsets, that are important for immunity against intracellular pathogens (viruses, bacteria & parasites), multicelullar pathogens (helminths), unicellular extracellular pathogens (fungi & bacteria), or keeping these immune responses onder control, respectively.

cells such as eosinophils, basophils, mast cells, the production of Immunoglobuling E (IgE), and Th2 cells which is collectively known as a type 2 immune response. The induction of type 2 immunity has been shown to be important for resistence to helminth infections in various model systems. However, despite induction of a Th2 response, total clearance of the parasites rarely occurs in helminth infections in man (reviewed in [4;5]). This is exemplified by the observation that these parasites have a long life span and that their infections are often chronic in nature. This implies that helminths have evolved strategies to prevent expulsion from their hosts by evasion or suppression of the host immune response that allow their long term survival. The immune modulatory effects of helminths are supported by the observation that many chronic helminth infections are associated with a hyporesponsive state of the host immune system. It is thought that this arises from the helminth's capacity to exploit the host's own system of immune regulation that is normally put in place for maintenance of immune homeostasis and self tolerance. In this respect, induction of Tregs has been shown to be one of the most common mechanisms whereby immune responses against the parasite are restrained and at the same time the host is protected against excessive inflammation and tissue damage (reviewed in [5;6]).

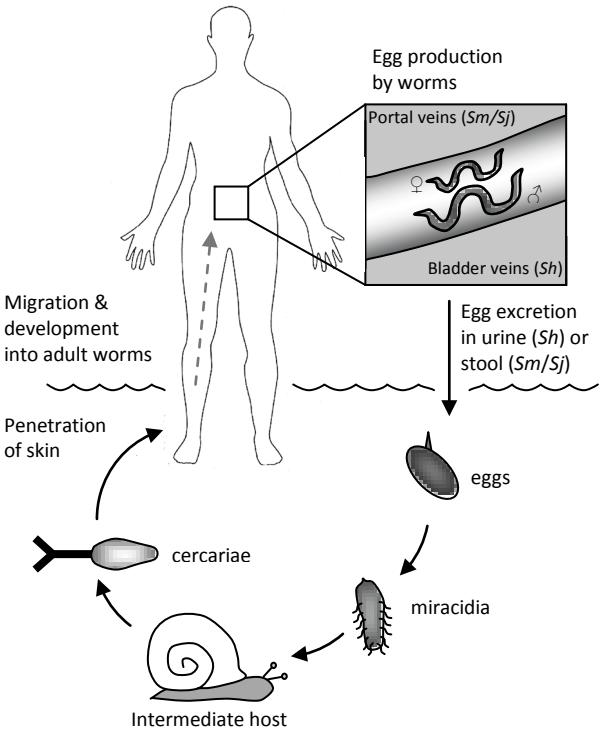

Figure 2: Life cycle of schistosomes. Upon penetration of the human skin as cercariae, the parasites transform into schistosomula, which migrate via the bloodstream through the lungs to the liver, where the mature female and male worms pair and produce eggs. The eggs penetrate through the tissues to the intestine (*S. mansoni* and *S. japonicum*) or to the bladder (*S. haematobium)*, where they are excreted with the faeces or urine. When the eggs come in contact with fresh water, they hatch releasing miracidia that can infect certain snails. Within the snails, the cerceriae develop from miracidia.

Thus, it is thought that the immunological profile induced by helminth parasites and especially the balance between Th2 and regulatory responses, will determine the outcome of the infection and as such is of critical importance for survival of both parasites and host. Given the prominent role of DCs in shaping and maintaining immune responses, the key to understanding how immune responses during helminth infections are initiated and regulated, is to understand the molecular mechanisms through which DC function is modulated by both the parasite and the host.

3 Modulation of DC function by direct interaction with helminth-derived molecules

3.1 *DC modulation via engagement of Pattern recognition receptors*

The capacity of DCs to recognize and distinguish different classes of pathogens is important to the initiation of appropriate immune responses. For this purpose DCs are equipped with several classes of innate pattern recognition receptors (PRRs), that are able to recognize and differentiate between different pathogen derived molecules, the so-called pathogen associated molecular patterns (PAMPs) (Fig 3). One of the best studied group of PRRs is the Toll-like receptors (TLRs), which trigger DC activation resulting in priming of pro-inflammatory/Th1 responses mainly in response to PAMPs from bacterial or viral origin [7]. Nonetheless, some helminth products have been shown to prime Th2 or regulatory responses through ligation of TLRs. For instance excretory/secretory-62 (ES-62), a phosphorylcholine-containing glycoprotein secreted by the nematode *Acanthocheilonema viteae*, conditions DCs to induce Th2 responses through TLR4 (Goodridge et al., 2005); a process that was found to be primarily mediated by the phosphorylcholine moiety of ES-62 [8]. Also lacto-N-fucopentaose III (LNFPIII), a carbohydrate containing the Le^x-motif found in schistosomes, has been shown to to prime Th2 responses via DCs in TLR4-dependent fashion [9]. However, the significance of this latter finding remains debatable as schistosome soluble egg antigens (SEA), harboring this same Le^x-motif, have been shown not to bind to TLR4 [10;11] and in addition are capable of modulating DCs for Th2 priming in the absence of TLR-signaling [12]. Furthermore, phosphatidylserine (PS) lipids derived from schistosomes and ascaris worms have been shown to promote Th2 responses via DCs and to bind to TLR2 [11], while specifically mono-acetylated PS-lipids from schistosomes were found to instruct DCs to preferentially induce IL-10-producing Tregs in a TLR2-dependent fashion [13]. Although TLR2 does not seem to be essential for schistosome-induced Th2 polarization *in vivo* [12;14], there is evidence that TLR2 plays an important role in induction of Tregs during natural infection. Finally, double

stranded RNA from schistosome eggs has been implicated in activation of DCs via TLR3, resulting in a Th1 polarized response [14;15].

Apart from TLRs, there is a growing body of evidence that another group of PRRs, a family of carbohydrate-binding receptors, the C-type lectins (CLRs), plays an important role in sensing of helminth glycans by DCs. For instance, SEA, which contains glycoproteins, has been demonstrated to be recognized and internalized by human DCs in a DC-specific intracellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), Mannose receptor (MR) and Macrophage Galactose-type lectin dependent manner [10;16]. Also antigen preparations of other stages of the schistosome life cycle have been shown to bind to DC-SIGN [17]. Binding of SEA to DC-SIGN was found to be dependent on sugar motifs Le^x and LDN-F [16], while chemical modification of the glycans present in SEA abolished the Th2-driving capacity of SEA [18]. This, together with the observation that Le^x-containing LNFPIII favors Th2-biased responses [19], suggests that CLRs play a dominant role in conditioning DCs for induction of Th2 responses by schistosomal antigens. Moreover, antigens from *Toxocara canis* have also been found to be recognized by DC-SIGN expressed on DCs [20], while the induction of a Th2 response *in vivo* by antigens of the parasitic nematode *Brugia malayi* as well as

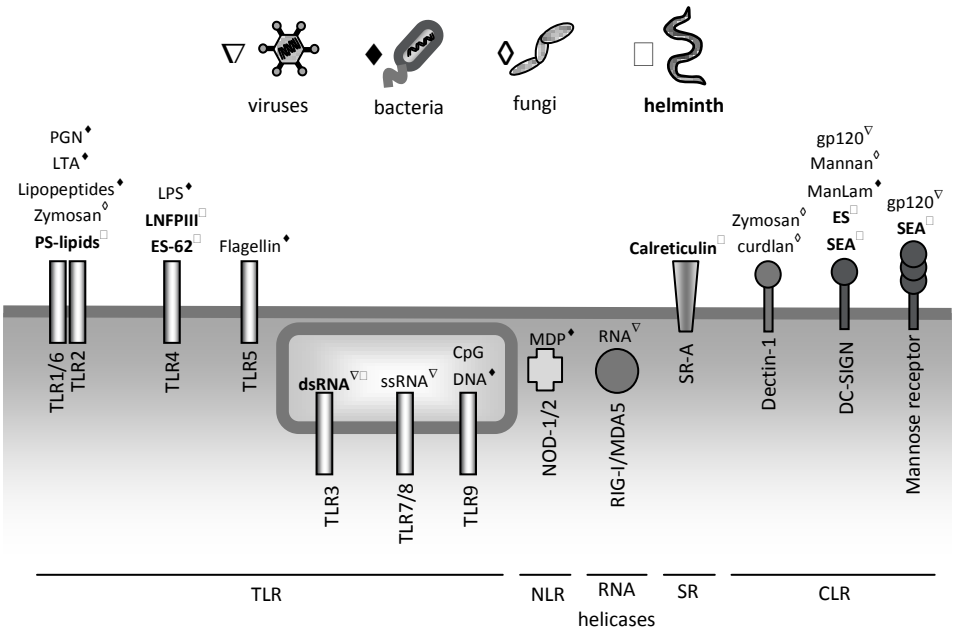

Figure 3: Examples of the most well known pattern recognition receptors and their pathogen-derived ligands. ES, excretory/sectretory products; LPS, lipopolysaccharide; LTA, liptochoic acid; MDP, muramyldipeptide; NLR, NOD-like receptors; PGN, peptidoglycan; SEA, soluble egg antigens; SR, Scavenger receptor.

the free-living nematode *Caenorhabditis elegans* appeared to be dependent on intact glycans [21]. This suggests that the Th2-bias induced by helminth glycans is a general phenomenon and that helminth glycans may serve as a conserved molecular pattern that instructs DCs via CLRs to drive Th2 polarized responses.

Finally, there is evidence that the class A scavenger receptor (SR-A), which is a member of a class of receptors that binds chemically modified low-density lipoproteins and suggested to function as PRR (reviewed in [22]), mediates recognition of helminth components that results in the induction of Th2-polarized responses. It was shown that, calreticulin, a secreted protein expressed by tissue invasive larvae of the gastrointestinal helminth *Heligmosomoides polygyrus* binds SR-A on DCs and has the capacity without adjuvant to predominantly induce specific IL-4 production *in vivo* [23].

3.1.2 *PRR-signaling in DCs by helminth-derived factors*

Classically, following engagement of PRRs by microbial ligands, signaling cascades are triggered that involve activation of the Mitogen Activated Protein Kinases (MAPKs), p38, ERK and JNK as well as Nuclear factor-κB (NF-κB). These signaling events subsequently result in the induction of a maturation process, that is represented by stable presentation of peptides in the context of major histocompatibility complex II, up-regulation of co-stimulatory molecules, and production of polarizing cytokines, that all together enable the DCs to activate and direct T helper cell differentiation. This paradigm of DC maturation has mainly been established based on observations from responses towards unicellular pathogens, like bacteria, viruses and fungi [24]. In contrast to this classical view of DC activation, DCs primed by helminth products often fail to show signs of classical DC maturation [25]. The absence of DC maturation is in line with several studies in which potent activation of p38, a signaling molecule crucial for PRR-mediated DC activation [26], was not seen in DCs exposed to helminth products such as SEA, ES-62 and LNFPIII [9;27-29]. Instead, these helminth-derived components, were found to preferentially induce the activation of ERK, in the case of SEA and ES-62, or NF-κB1 and ERK, in case of LNFPIII. Signaling through ERK in DCs has been shown to result in suppression of IL-12 and induction of IL-10 expression, and in line with this ERK-/- mice are prone to develop autoimmunity. As a result ERK has been thought to play a role in conditioning DCs for Th2-priming [11;30;31]. Likewise, signaling through NF-κB1 appears to be important for priming of DCs for Th2 polarization, since both SEA- [32] and LNFPIII-pulsed NF-κB1 deficient DCs [29] are incapable of inducing a Th2 response.

In addition to the inability to classically activate DCs, a feature shared by many helminths, is their capacity to potently suppress TLR-mediated DC activation by microbial PAMPs. Numerous studies have reported the inhibitory effects of helminth-

derived components on TLR induced activation as determined by pro-inflammatory cytokine production and expression of MHC class II/costimulatory molecules [28;33-39]. The pathways underlying this suppression are still poorly understood. Interestingly, the suppression of TLR-mediated responses by helminth antigens, has striking similarities with the effects induced by several microbial pathogens that target DC-SIGN [40-42]. Recently, significant advances have been made in the identification of the pathways downstream of DC-SIGN that result in modulation of TLR signaling. Glyconjugates carrying the Le^x-motif were found to modulate LPS-induced signaling (e.g. elevation of IL10 / reduction in IL-12) via leukocyte specific protein-1 (LSP-1) [43]. These findings are highly reminiscent of those found for SEA and make it very likely that these signaling pathways play a role in DC modulation by schistosomes. However, it remains to be established whether similar signaling events are also involved in modulation of TLR-induced responses by other helminth antigens. Since not all helminth modulatory molecules are glycans, it is reasonable to assume that exploiting CLRs to modulate DC function is just one of the ways helminths exert their modulatory effects on microbial DC activation. Indeed, inhibition of p38 has been suggested to play a role in this phenomenon for schistosomal lipids that bind to TLR2 [11].

Overall there is a consistent picture that helminth products regardless of whether they interact with either TLRs or CLRs fail to induce conventional DC maturation [44] and inhibit DC activation induced by pro-inflammatory PAMPs, which not only impairs Th1 development but also biases the immune response towards Th2 or regulatory responses.

3.2 *DC modulation through mimicry of host factors*

One of the interesting strategies that pathogens, including helminths, have developed to modulate host immune responses is the production of homologs of host anti-inflammatory factors that can act, among others, on DCs. The most well known example of mimicry of host factors in the production of TGF-β homologs by the filarial nematode *Brugia malayi* [45]. TGF-β is a key regulatory cytokine that is central in suppression and regulation of immune responses. TGF-β is activated through mechanisms that are dependent on avb8 integrin expressed by DCs, which creates a milieu around the DCs rich in active TGF-β. Active TGF-β subsequently engages TGF-β receptors on DCs as well as naive T cells to induce Tregs (reviewed in [46]). The TGF-β homolog, *Bm*-TGH2, has been shown to be secreted by adult filarial worms and to bind to host TGF-β receptors, suggesting that TGH-2 might have an immune modulatory function in the host that is potentially mediated by DCs [47].

Heat shock proteins (HSPs) produced by helminths may provide another example of how mimicry can induce immune regulation via DCs. HSPs are highly

conserved intracellular chaperones that have extracellular roles as immune modulators. Although endogenous HSPs can function as powerful immunological adjuvants possibly by interacting with TLRs, they can also attenuate inflammatory diseases via possible effects on Treg populations (reviewed in [48]). Interestingly, some pathogen derived HSPs, such as HSP70 expressed by *Mycobacterium tuberculosis*, have been shown to induce regulatory T cell responses via DCs [49]. Since several parasitic helminths are also known to secrete HSP70 [50-52], it is tempting to speculate that helminths via this pathway can condition DCs for the induction of Tregs.

3.3 *DC modulation through PRR-signaling independent mechanisms*

Apart from modulation of DC function through receptor mediated signaling events, evidence is emerging that manipulation of DCs may also be mediated by other mechanisms, such as enzymatic activities from helminth-derived products. Helminth parasites are well known to release a wide variety of enzymatically active products, that are thought to play an important role in establishing and maintaining infection, by contributing to degradation of host-derived soluble anti-parasitic molecules, impairment of innate effector immune cells or invasion of host tissues by the parasites [53].

With regard to the effects of these molecules on DCs several studies have documented the potent suppressory effects of cystatins, a class of molecules expressed by filarial nematodes on host immune responses [54-56]. This is thought to reflect their capacity to interfere with antigen presentation by DCs through blocking the host cysteine protease activity, which is required for removal of the invariant chain that normally is necessary for peptide loading into MHC class II [57;58]. Thus, rather than promoting Th2 or Tregs by DCs, cystatins seem to suppress the capacity of DCs to prime T cell responses in general.

Furthermore, helminth pathogens express cysteine proteases, termed cathepsins, that apart from being important for processes like tissue invasion [59], have also been shown to suppress bacteria-elicited Th1 responses [60]. Whether helminth proteases can mediate these effects by targeting DCs is at the moment unclear. However, based on the observations that numerous allergens are known to be cysteine proteases [61] and that the cysteine protease Der p 1, one of the major allergens of the house dust mite *Dermatophagoides pteronyssinus*, primes monocyte-derived DCs for Th2 polarization in a protease dependent manner [62], makes it tempting to speculate that helminth-derived proteases have the potential to favor Th2 polarization through functional modulation of DCs.

Table 1. Helminths factors putatively modulating DC function during helminth infections

Group	Species	Component	PRR	Signaling or mode of action	Resulting DC phenotype	Th cell skewing	Refs
Trematodes	*Fasciola hepatica*	Tegumental antigen	TLR independent	↓ NF-κB p65	↓ IL-12, ↓ IL-10	↓Th1	[36]
	Schistosoma mansoni	LNFPIII	TLR4 CLR?	↑ NF-κB1 ↑ ERK	Low IL-12	Th2	[9;19;29]
		SEA	DC-SIGN, MR, MGL	↑ ERK, ↑c-fos	↓ IL-12, ↑ IL-10 ↑ OX40-L	Th2	[10;16]
		Egg ES	CLR?	↑ ERK	↓ IL-12, ↑ IL-10	Th2	[37;158] Unpu-blished
		PS lipids	TLR2	↓ p38	↓ IL-12	Th2	[11;13]
		Lyso-PS lipids	TLR2	ND	↓ IL-12	Tregs (Tr1)	[13]
		dsRNA	TLR3	STAT-1, ISG	IFNα	Th1	[14;15]
		0-3h cercarial ES	DC-SIGN	ND	Low IL-12	Th2	[17;135]
Cestodes	*Echinococcus granulosus*	AgB	TLR?	↑ IRAK1 ↑ NF-κB1	↓ IL-12 ↑ CD40	Th2	[39]
Nematodes	*Acanthocheilonema viteae*	ES-62	TLR4	↑ ERK	Low IL-12	Th2	[8;27; 159]
	Ascaris lumbricoides	PS lipids	TLR2	↓ p38	↓ IL-12 ↓ IL-10	Th2	[11]
	Brugia malayi	TGF-β homolog	TGF-βR	SMAD2/3	↑ IL-10	Tregs	[45-47]
	Heligmosomoides polygyrus	calreticulin	SR-A signaling?	ND	ND	Th2	[23]
		ES	ND	ND	↓ IL-12 ↓ IL-10	Tregs	[34;66]
	Nippostrongylus brasiliensis	NES	ND	ND	↓ IL-12 ↑ OX40-L ↑ CD86		[33;66]
all	Multiple helminth species	Cathepsins	Cleavage of specific proteins?	ND	ND	↓Th1	[59;60]
		HSP70	TLR4?	ND	↑ IL-10	Tregs	[50-52]
		Cystatins	ND	↓ peptide loading on MHC	↓ ag presentation	↓ T cell activation	[54-56] [57;58]

AgB: purified antigen B, ISG: interferon-stimulated gene, IRAK-1: interleukin-1related accociated kinase-1, ND: not determined, (N)ES: (Nippostrongylus) Excretory/Secretory products, SMAD2/3: SMA/MAD related 2/3.

Taken together, many studies provided evidence that a variety of molecules released by helminth parasites have the capacity to modulate DC function through signalling-dependent and -independent mechanisms (Table 1). Although the modes of action of the molecules may differ, the exposure of DCs to these products invariably results in suppression of antigen presentation, as well as costimulation and/or Th1 polarizing cytokine production, thereby generally suppressing T cell responses or favoring the induction of Th2 responses.

4 Endogenous factors modulating DC function during helminth infection

4.1 *Host-derived factors that modulate DC function*

In addition to modulation of DC function by direct interactions with helminth products, DCs are also likely to be influenced by host-derived factors that are produced in response to infection. During the initial stages of infection these modulatory factors may come from other innate immune cells or epithelial cells that respond to helminth products, while during the later phases of infection cytokines from adaptive immune cells, like T cells, could potentially alter the function of DCs.

4.1.1 *Host-derived factors that modulate DC function for Th2 polarization*

Although it is well established that DCs can be conditioned to prime Th2 responses by direct interaction with helminth antigens, several host-derived mediators have been identified that can exert the same polarizing effects on DCs. In this respect, thymic stromal lymphopoietin (TSLP), a IL-7 homolog has been reported to potently modulate DC function by inducing the expression of MHC class II and costimulatory molecules, but not IL-12, which results in a Th2 polarizing phenotype [63]. TSLP is mainly expressed by epithelial cells, and has been well described to play a central role in development of allergic responses [64]. On the other hand, recent evidence suggests that TSLP is crucial for generation of protective Th2 immunity against intestinal infection caused by the nematode *Trichuris muris*. Upon infection levels of TSLP mRNA were found to specifically increase in ECs and TSLPR$^{-/-}$ mice failed to clear the infection [65]. However, TSLP does not seem to be essential for every intestinal helminth, because the development of protective Th2 immune responses after infection with *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis* was still in tact in TSLPR$^{-/-}$ mice [66]. Similarly, using the same knockout mice, it was found that, although TSLPR signaling participates in the development of *Schistosoma mansoni* egg-induced Th2 responses, it plays only a minor role in the development of Th2-dependent pathology in the lung, liver, and intestine [67]. In this respect it is particularly interesting that excretory-secretory products from *H. polygyrus*, *N. brasiliensis* and *S. mansoni*, but not

from *T. muris*, have been found to suppress production of IL-12 by DCs [28;66]. This suggests that helminth-elicited TSLP plays a nonredundant role in priming of Th2 responses via DCs in infections where DCs are refractory to modulation by helminth derived products, while the need for TSLP can be bypassed during infections when DC are able to recognize and respond to helminth derived factors directly.

In addition to TSLP, ECs have also been found to be a major source of IL-25 and IL-33, two cytokines associated with the promotion of Th2 responses at mucosal sites and critical for protective immunity to intestinal helminths [68;69]. Although these cytokines seem to mainly target T cells for augmentation of Th2 responses or directly lead to activation of basophils or mast cells [70], there are now also indications that IL-33 can activate DCs, as evidenced by increased expression of MHC class II, CD80 and IL-6, but not IL-12, enabling them to drive Th2 polarization [71].

One of the key effector cells in Th2 responses during helminth infections are eosinophils. They are rapidly recruited to the site of infection where they release a variety of inflammatory and toxic molecules that help to recruit other immune cells as well as to kill the parasite. Interestingly, eosinophils also secrete factors that can polarize DCs. A recent study has identified Eosinophil-derived neurotoxin (EDN) as an alarmin that activates the TLR2-MyD88 signal pathway in dendritic cells resulting in priming of Th2 responses [72]. Furthermore, in response to helminths, eosinophils can express lipid compounds that are derived from fatty acids, including prostaglandin E2 (PGE2). PGE2 has been shown to downregulate IL-12 and increase IL-10 release in both murine [73] and human DCs *in vitro* [74] and to result in conditioning of DCs for Th2 reponses [75]. Although, increase in levels of EDN and PGE2 has been documented in tissues of humans with helminth infections [76-78], the contribution of these factors during helminth infections in priming of DCs for Th2 polarization has so far not been addressed specifically.

Mast cells are another cell type activated during Th2 responses in helminth infections and documented to produce DC-modulating factors. In this respect, of particular interest is PGD2 [79]. PGD2 has been shown to inhibit epidermal Langerhans cell migration during schistosoma infection [80] as well as to suppress IL-12 secretion by DCs [81] resulting in polarization of T helper cells towards Th2 [82;83].

4.1.2 *Host-derived factors that modulate DC function for Treg induction*

While helminth infections are the most potent natural inducers of Th2 responses in which host-derived inflammatory mediators, as described above, can play a role, they are also well known for their capacity to modulate and suppress immune responses through induction of regulatory T cell responses and cytokines. Key effector cytokines in these regulatory responses are IL-10 and transforming growth factor (TGF)-β [46].

There is a wealth of data showing that during chronic helminth infections in humans [84-90], levels of systemic IL-10 and/or TGF-β are elevated and participate in suppression of immune responses against helminth as well as bystander antigens. A variety of cells have been found to produce these regulatory cytokines during helminth infections. It is well established that induced Tregs are a main source of these anti-inflammatory mediators and that they are dependent on these cytokines to exert their suppressive function [91]. In addition, IL10 or TGF-β derived from macrophages [54], B cell [92;93] and DCs themselves [94], have also been shown to contribute to the anti-inflammatory milieu [95]. Several mechanisms have been proposed through which IL-10 and TGF-β exert their suppressive effects during helminth infections, of which modulation of DC function is one [96;97]. Both *in vitro* and *in vivo* studies have demonstrated that exposure of DCs to IL-10 or TGF-β results in suppression of DC maturation and promotes the induction of T cell anergy [98] or Treg responses [99-101]. Although based on these findings, a role for host-derived regulatory cytokines in modulation of DC function during helminth infections seems plausible; there is only one study that has specifically addressed this. In this report they show that down-regulation of expression of costimulatory molecules and T cell activation by antigen-presenting cells by components of *Ascaris suum* is absent in IL-10 deficient mice [102], implying a direct effect of IL-10 on DC function in this model. Additional studies will be needed to address whether modulation of DC function for induction of Treg responses and immune hyporesponsivess during helminth infections via host-derived IL-10 and TGF-β are a common mechanism at play.

Furthermore, increased expression levels of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) have been found on Tregs during the chronic helminth infection in several human [87;103-105] and murine studies [106-110]. CTLA-4, which competes with B7 molecules for CD28 engagement, is a well known inhibitor of T cell responses, that is predominantly expressed by Tregs. Indeed a role for CTLA-4 in immune hyporesponsiveness came from studies that showed that neutralization of CTLA-4 could restore effector T cell proliferation and cytokines responses both *in vitro* and *in vivo* [111]. With regard to the mode of action, interactions with DCs represent an important pathway through which CTLA-4 expressing cells exert their suppressive function [111]. It has been reported that Tregs, by virtue of CTLA-4, suppress expression of costimulatory molecules [112;113] and prevent priming of T cell responses by competing with naïve T cells for formation of conjugates with DCs [112;114]. So far, no studies have been conducted to discern a direct role for CTLA-4 in modulation of DC function during helminth infections. Nonetheless, it is likely that these direct interactions act in concert with anti-inflammatory mediators to modulate DC function during the chronic stages of infection.

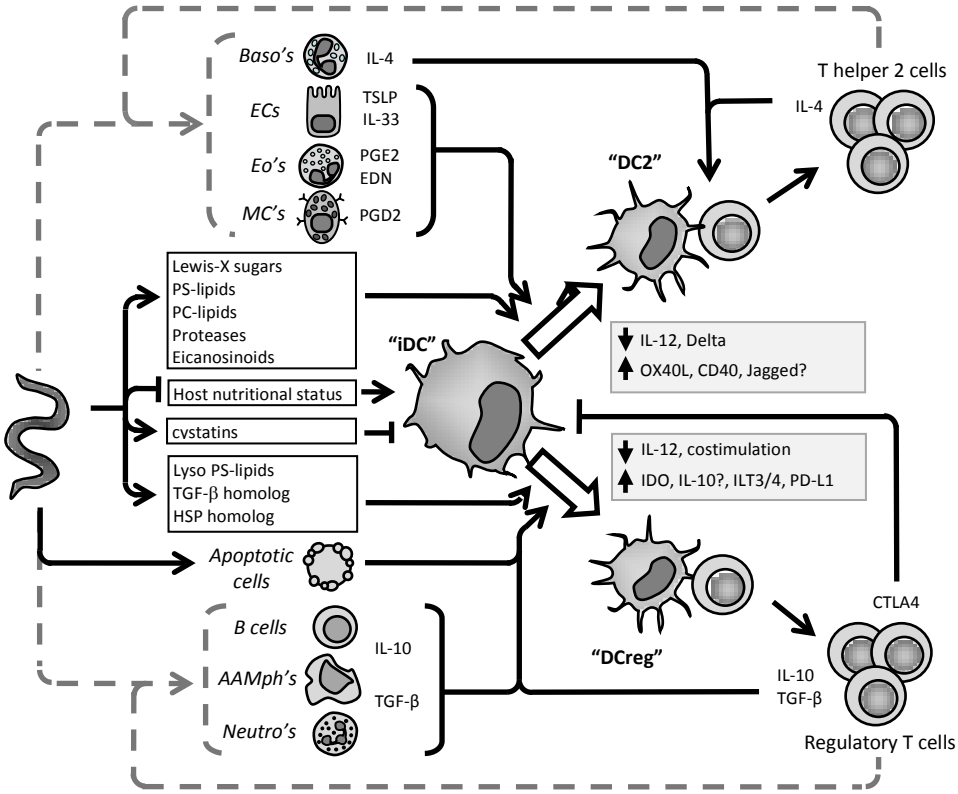

Figure 4: The T cell-polarizing properties of DCs is influenced by both parasite- and host-derived factors during helminth infections. The capacity of helminths to modulate DC function has mainly been based on studies that focus on products from worms that directly interact with DCs. However, in a physiological setting host-derived cues from the tissues the DCs reside in elicited during helminth infections, are also likely to strongly influence the T cell-polarizing potential of these cells. The molecular mechanisms through which DCs are thought to instruct naïve Th cells to differentiate into Th2 or Treg cells in the context of helminth infections are shown as well. AAM's, alternatively activated macrophages; Baso's, Basophils; Eo's, eosinophils; EC's, Epithelial cells; MC's, Mast cells; Neutro's, Neutrophils.

4.2 *Modulation of DC function by apoptotic cells*

In contrast to micro-organsisms, multicellular pathogens like helminths are more likely to induce serious tissue damage, due to their capacity to migrate through tissues ot to cause lesions in the gut wall by feeding. As a consequence cells in these tissues will be exposed to several forms of cellular stress, which can ultimately result in apoptosis-mediated cell death. It has been documented that internalization of apoptotic bodies or recognition of phosphatidylserine from apoptotic cells by DCs [115] suppresses their maturation and IL-12 production [116] and conditions them to drive Treg responses [117]. As such, this pathway may represent an important component of the regulatory responses observed during helminth infections. In fact, since it is known filarial

parasites have the capacity to directly induce apoptosis in DCs themselves [118;119], it is tempting to speculate that some helminths may even exploit the mechanism of apoptotis-mediated immune regulation to modulate DC function to subvert immune responses.

4.3 *Nutritional status of host due to helminth infection*

Chronic helminth infections, especially those caused by the species residing in the gastro-intestinal tract, can result in malnutrition, primarily as a result of impaired uptake of nutrients [120-122]. It is known that undernutrition can result in an impaired function of both the innate and adaptive immune system [123]. With respect to DC function, it has recently been shown that *in vitro* cultured DCs derived from children with severe malnutrition produce less pro-inflammatory cytokines, are less mature and have an impaired T cell-activating capacity compared to DCs from to well-nourished controls [124]. Likewise, in animal models of generalized [125], protein [126] or zinc starvation [127], a consistent defect in function of antigen presenting cells was observed, as evidenced by a diminished capacity to prime T cell cytokine production and proliferation. The molecular mechanisms underlying these observations and to what extent these converge with specific helminth-induced effects on DCs has yet to be elucidated but presents an interesting area of research when considering the impact that helminth infections have on DC function.

In summary, not only parasite but also endogenous factors will influence DC function during helminth infection and it is likely that the immune polarization induced by DCs is determined by the balance between the different components to which they are exposed (Fig 4).

5 T helper cell polarization by helminth-conditioned DCs

5.1 *Mechanisms behind DC-driven Th2 induction during helminth infection*

The requirements for DCs to drive Th1 or Th17 polarized responses in response to viruses, bacteria or fungi have been well established over the last few years [128]. In contrast, the pathways through which DCs in response to helminth antigens prime Th2 responses are still not fully understood [129]. A general characteristic of DCs exposed to helminth products, as opposed to Th1-polarizing TLR-activating microbial stimuli, is their muted activation profile, with low maturation marker expression and cytokine production. The latter characteristic, especially low production of IL-12 is thought to be a key feature of Th2-polarizing DCs, since addition of Th1-polarizing cytokine, IL-12, blocks the development of a Th2 response following injection of *S. mansoni* eggs [130].

These observations initially led to the so-called 'default' hypothesis that states that Th2 responses automatically occur in the absence of Th1 polarizing signals. However, the fact that helminth products are capable of modulating TLR-induced DC activation that deviates Th1 towards a Th2-skewed immune response [11;28] and that IL-12 deficient mice do not develop a immune Th2 profile in response to microbial pathogens [131], argues against this hypothesis and suggests that there are specific signals involved in Th2 polarization. In recent years several factors have been proposed to play a role in Th2 polarization by helminth-primed DCs. For instance, SEA-pulsed DCs that cannot express CD40, fail to induce Th2 responses both *in vitro* [132] and *in vivo* [133], while DCs deficient for CD40 primed with bacterial components from *Propionibacterium acnes* are still capable of driving Th1 responses. Also during a natural schistosoma infection CD40/CD154 interactions appear to be essential for normal Th2 development [134]. However, in studies with DCs primed with cercarial products or egg injections, treatment with agonistic CD40 antibodies was found to result in a Th1 skewed immune profile [135;136]. The discrepancy could be explained by the possibility that triggering of CD40 by these antibodies is more potent than the one mediated by CD40:CD40L interactions during helminth infections, thereby exceeding the threshold of DC activation required for effective Th2 polarization. Since CD40 signals provided by CD154 expressed by T cells is thought to be a general process to license DCs to express T cell-polarizing signals, the importance of CD40 in Th2 polarization may just be a reflection of a this general requirement. In this respect, OX40L has been shown to be upregulated in response to CD40 ligation in SEA-primed DCs and to play an important role in Th2 polarization *in vitro* [137]. Interestingly, also TSLP-conditioned DCs were shown to be dependent on OX40:OX40L interactions to drive Th2 polarization *in vitro* [138]. However, during schistosome [139], as well as gastrointestinal nematode infection [140], OX40L appears to be particularly important for full Th2 development, rather than to be serving as a Th2-polarizing signal per se. Furthermore, Notch ligands delta-4 and jagged-2 have been reported to play a role in Th1 and Th2 polarization by DCs, respectively [141;142]. Yet, although jagged-2 is upregulated in human as well as murine DCs exposed to SEA [11;143], the Th2 polarizing capacity of SEA-primed DC deficient for Jagged-2 was unaffected [11;143;144]. On the other hand, delta-4 expression was found to be suppressed in human DCs exposed to lipids derived from schistosome and ascaris worms [11]. This, together with the observation that delta not only promotes Th1 but also antagonize Th2 polarization [145], suggests that selective inhibition of delta-4 may be a prerequisite for the priming of Th2 development (Fig 4).

A great deal of what we know about Th2 polarization by DCs in response to helminths has been based on *in vitro* culture systems that ignore the complexity of the

in vivo immune response. In this light, it is possible that tissue factors provide the polarizing signals that licence DCs to prime Th2 responses. For instance, IL-4 derived from innate cells such as basophils can serve as a Th2-polarizing signal [146]. In fact, basophils may not only produce Th2-polarizing factors to support DC-mediated Th2 polarization, but recent findings in a *T. muris* model point in the direction that in some settings they may even overtake the function of DCs, by acting as professional APCs [147]. This illustrates that the model of DC-T cell may be too simple and that we need to look further than DCs to fully understand the mechanisms behind Th2 polarization by helminths.

5.2 *Mechanisms behind DC-driven Treg induction during helminth infection*

Numerous studies have shown that helminth infections lead to the induction and expansion of Tregs (reviewed in [5;6]). Yet, there is still little known about the role of DCs during helminth infections in this phenomenon and the underlying molecular mechanisms. The few reports that have identified helminth-derived components that directly act on DCs to drive IL-10-producing adaptive Tregs (Tr1 cells) [13;34], did not address the molecular mechanisms by which these DCs prime regulatory responses. Apart from helminth-derived products, host derived-cytokines such as IL-10 and TGF-β, or apoptotic cells generated during helminth infections, are likely to play an important role in DC-driven Treg induction. It is known that exposure of DCs to anti-inflammatory cytokines results in a immature or semimature phenotype that in fact resembles the phenotype of DCs conditioned by Th2-polarizing helminth products [148]. Yet, in contrast to helminth-primed DCs, the anti-inflammatory cytokine-treated DCs are highly tolerogenic. It is not fully understood on what molecular basis these apparent phenotypically similar DCs are able to induce different T cell responses. However, it has been found for IL-10-conditioned DCs that depending on the DC type studied, priming of Tregs relies on the surface expression of immunologlobulin like transcript 3 (ILT3) and ILT4 [149], programmed death ligand 1 (PD-L1) [150] or the tryptophan depleting enzyme indoleamine 2,3-dioxygenase [151]. The finding that *Schistosoma mansoni* worms can induce tolerogenic T cells via selective up-regulation of PD-L1 on APCs [152] suggests that at least one of these pathways may play a role in schistosome-induced tolerogenic responses (Fig 4). In addition, IL-10 and TGF-β may be acting on the T cell polarization process directly by providing the polarizing signals that license DCs to induce Treg differentiation [153-155]. Apart from *de novo* induction of Tregs by DCs, helminth infections have also been demonstrated to lead to expansion of pre-existing naturally occurring Tregs [95;156]. DCs are well known to have the capacity to mediate this expansion *in vitro*, and are likely to contribute to this also *in*

vivo (reviewed in [157]). Yet, the exact requirements for this process are at the moment unclear.

6 Scope of this thesis

The studies in this thesis focus on the interactions between human DCs and the parasitic fluke of the genus Schistosoma and how this affects the T helper cell-polarizing properties of these DCs.

Infections caused by schistosomes are generally immunologically characterized by strong Th2-polarized responses. In addition, during chronic stages of infection a form of T cell hyporesponsiveness is observed. However, the consequences of chronic schistosomiasis on human DC phenotype and function are unknown. In **chapter 2** the effects of chronic *S. haematobium* infection on the phenotype and function of human plasmacytoid and myeloid DCs are examined, the two major DC subsets in human peripheral blood DCs. In this study we particularly focus on the T cell-priming properties of these DCs, as this may provide new clues about the mechanisms underlying T cell hyporesponsiveness associated with chronic schistosomiasis.

Apart from immune hyporesponsiveness, schistosomes are well known for their capacity to drive strong Th2-polarized immune responses via functional modulation of DCs. Yet the molecular mechanisms underlying DC-driven Th2 polarization induced by schistosomes are still poorly defined. Therefore, the study decribed in **chapter 3** compares the molecular charactistics of human monocyte-derived DCs exposed to Th1-polarizing bacterial components with DCs stimulated with Th2-polarizing helminths extracts including those from *S. mansoni*, with the aim to identify molecular correlates in DCs for the Th1 or Th2 polarization they induce.

The Th2-polaring capacity of schistosomes can be backtracked to the eggs, which are known to harbour components that strongly condition DCs to initiate this type of immune polarization. A full understanding of how egg extracts of schistosomes promote Th2 responses via DCs, has been hindered by the lack of information on the identity of the components within that mixture that act on DCs to trigger Th2 differentation. In the study in **chapter 4** the identification of the most important component secreted by the schistosoma eggs is described that drives Th2-polarizaton via conditioning of DCs. **Chapter 5** characterizes the underlying molecular mechanisms through which this component instructs DCs to drive Th2 polarization.

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