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

Everts, B.

Chapter 6

General discussion

Different modulation of DCs during different stages of infection

Schistosome infections are characterized by strong Th2 and regulatory immune responses. The *in vitro* DC-T cell polarization assay described in this thesis (**chapter 3, 4 & 5)** and used previously [1] to study the molecular mechanisms behind DC-dependent schistosome-induced immune polarization, reflects the development of these polarized immune responses *in vivo*. However, T cell polarization assays *ex vivo* with DCs isolated from subjects infected with *Schistosoma haematobium* did not confirm the ability of DCs from infected subjects to induce Th2 or Treg polarization, when compared to DCs from un-infected subjects (**chapter 2**). Instead, we observed that mDCs from infected individuals were impaired in there capacity to drive T cell activation in general, suggesting that these DCs are functionally suppressed instead of carrying a polarized phenotype due to the infection.

There are several explanations for the discrepancy between the *in vitro* and *ex vivo* findings. Although the well-controlled *in vitro* DC model is highly suitable to dissect the molecular mechanisms behind DC-dependent schistosome-induced immune polarization, it fails to incorporate the complexity of the *in vivo* situation. The function of circulating DCs in blood and as well as DCs residing in tissues will not only be influenced by interactions with pathogen-derived components, as studied in the *in vitro* model, but by other factors as well. For instance chronic schistosomiasis may negatively affect the nutritional status of the host [2-4], which is turn could result in functional suppression of DCs [5-7]. Furthermore, while during the acute stages of infection the immune response is predominantly Th2-biased, chronic infection is characterized by a strong Treg component [8], that through its anti-inflammatory mediators could potentially inhibit the T cell-activating capacity of the mDCs. Finally, although mDCs after isolation were further differentiated and activated by a combination of LPS and GM-CSF, it should not be forgotten that mDCs derived from peripheral blood may not have same intrinsic T cell-polarizing capacity as their *in vitro* cultured counterparts that are thought to represent fully differentiated DCs.

Thus, we propose that during the initial stages of infection, primarily direct interactions of helminth products with DCs will be important for its T cell-polarizing characteristics. This is probably most closely resembling the situation studied in the *in vitro* DC models, such as described in **chapter 3 to 5.** If, however, the ensuing immune response is not capable of clearing the infection and an infection becomes chronic, DCs may in addition be modulated and suppressed in their T cell-activating capacity by regulatory mediators derived from the parasites or adaptive sources of the immune system, such as Tregs, as well as by reduced nutritional status. This may more reflect the situation studied in **chapter 2.** Importantly, the suppression of DC function during these chronic stages of infection would then act as a negative feedback loop that could

contribute to the maintenance of the immune hyporesponsive state of host and as a result in persistence of the infection.

Conditioning of DCs for Th2 priming by schistosome antigens: a double-edged sword?
Overall there is a consistent picture that helminth products, including those derived from schistosomes, fail to induce conventional DC maturation [9] and inhibit DC activation induced by pro-inflammatory PAMPs, which altogether could impair Th1 development and bias the immune response towards Th2. This may suggest that the mechanisms applied by helminth antigens to instruct DCs to become conditioned for Th2-priming are similar. Our findings that Th2-inducing helminth-derived PS-lipids as well as SEA lead to a similar molecular profile in DCs, as reported in **chapter 3**, would support this notion. Based on the observations that both PS-lipids and SEA modulate LPS-induced MAPK activation, resulting in an increased ratio of *p*-ERK/*p*-p38, and that the receptors they engage (e.g. TLR2 and CLRs) have been shown to modulate MAPK activation [10-12], it is reasonable to assume that in line with the current dogma [13-16] these helminth antigens rely on PRR-signaling to modulate DCs and condition them for induction of Th2 responses. While this may be true for the lipids, the identification of the RNase omega-1 as the principal factor in SEA that instructs DCs to prime Th2 responses (**chapter 4)** in probably a PRR-signaling independent, but RNAse dependent manner (**chapter 5**), now challenges this concept for SEA. The data presented in **chapter 5** suggest that although binding of omega-1 via its glycans to DC-SIGN and/or MR triggers a signaling cascade that results in suppression of LPS-induced IL-12 expression, it is not required or sufficient for Th2 polarization. Instead, we find that the glycans present on omega-1 and their capacity to interact with CLRs are important for the recognition and internalization of the molecule by DCs to allow its subsequent biologic activity. It is this next step, the enzymatic activity of omega-1 that licenses it to suppress IL-12 production and modulate DC function for Th2 polarization by interfering with the translation machinery.

These findings not only provide important new insights into the pathways used by schistosomal components to modulate DC function, but also in the mechanisms through which schistosome-conditioned DCs may differentiate naïve Th cells into a Th2 phenotype. For induction of Th2 responses secretion of very low amounts of IL-12 by DCs is thought to be essential. However, just lowered IL-12 release appears not to be sufficient [17;18]. This has led to the hypothesis that additional polarizing signals are expressed by schistosome-conditioned DCs that are required for the Th2 polarization, equivalent to polarizing cytokines released by DCs that have been identified to lead to priming of Th1 and Th17 responses [19]. Although the costimulatory molecule OX-40L and Notch ligand Jagged-2 have been shown to meet

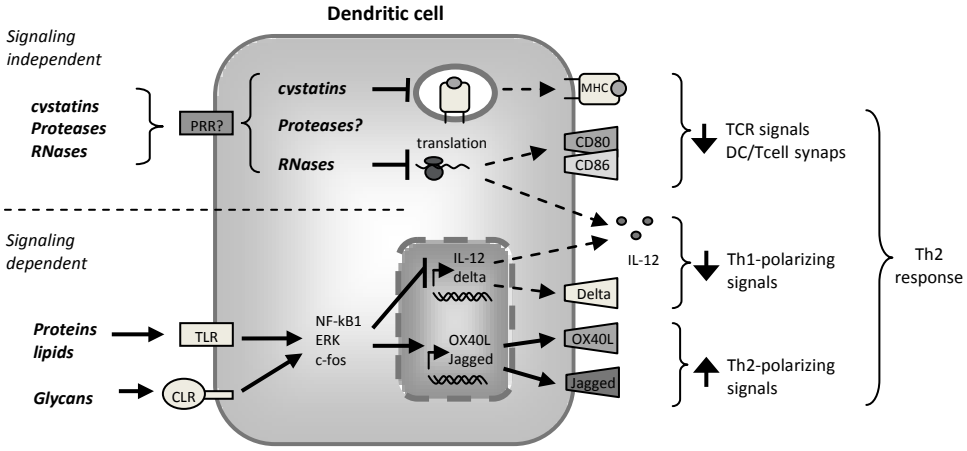

Figure 1. Proposed model of the molecular mechanisms through which DCs become conditioned by helminth products via signaling-dependent and independent pathways for priming of Th2 responses.

demands *in vitro* [20;21], their importance for SEA-induced Th2 polarization *in vivo* remains debatable [21-23]. The recent observations that omega-1-conditioned DCs have an impaired capacity to form T cell-DC conjugates [24] may provide an explanation for the so far unsuccessful search for true Th2-polarizing molecules expressed by SEA-primed DCs. Namely, reduced TCR-triggering has previously been put forward as a mechanism through which DCs could prime Th2 polarization, based on the observations that low TCR-triggering, as a result of low antigen presentation or low affinity for the peptide/MHCII complex, favors the induction of Th2 responses under non-polarizing conditions [25;26]. However, until now the relevance of these observations for helminth-induced Th2 polarization was unclear. The lowered DC-T cell interaction, together with our findings that omega-1 through its RNase activity can generally suppress protein synthesis that not only shuts down IL-12 secretion but can also impair expression of MHC class II or costimulatory molecules, leads us to propose that reduced TCR-triggering is a major mechanism through which omega-1-primed DCs and therefore also SEA-pulsed DCs drive Th2 responses.

Taken together, these data could suggest that Th2 polarization by schistosomes, and possibly helminths in general, can rely on at least two distinct mechanisms that, in addition to suppression of IL-12, may not only involve induction of Th2-polarizing signals, but also weakening of TCR-triggering. It is tempting to speculate these two mechanisms are a reflection of the two main modes of action through which helminth molecules are assumed to modulate DC function: On the one hand, receptor-mediated DC modulation may, via signaling-dependent mechanisms, suppress IL-12 production and promote the expression of Th2-polarizing factors. On the other hand, signaling-independent modulation of DC function by physiological actions of enzymes (RNases

and proteases) or direct interference with antigen presentation (cystatins), may lead to reduced synaps formation and antigen presentation to T cells and thereby TCR-triggering (Fig 1). SEA has been shown to induce the expression of Th2-associated Notch ligand Jagged-1 through signaling via ERK [27], but also to suppress IL-12 in an ERK and c-fos dependent fashion [10]. Since SEA contains omega-1, it can additionally modulate DC function by suppressing antigen presentation as well as IL-12 production in an enzyme dependent fashion. This may explain why SEA is such a strong Th2 polarizing agent as it has the potential to exploit both pathways to condition DCs for Th2 polarization.

Helminth-induced immune polarization: the bigger picture

The fact that helminth infections consistently result in the generation of type 2 immune responses, has led to the concept that this type of immune response has been propagated by the host to combat this group of infections. But what is the evidence for that? The observation that mice deficient for canonical Th2 effector cytokines IL-4 and IL-13, IL-4 receptor alpha or its downstream signal transducer and activator of transcription 6 fail to clear intestinal nematode parasites *Nippostrongylus brasiliensis*, *Trichinella spiralis* and *Trichuris muris*, support the view that Th2 responses are crucial in providing immunity against these helminths. However, protective effects of Th2 responses during infections caused by helminths that have found their niche at sites other than the gastrointestinal tract are far less clear cut. For instance, the Th2 response generated during *Schistosoma mansoni* infection, mainly in response to eggs, is not sufficient to expel the worms and has even been shown to facilitate transport of the eggs across the intestinal wall, thereby contributing to the completion of the parasite life cycle [28;29]. Instead, the Th2 response during this infection causes the formation of type 2 granulomas around the eggs once they become trapped in the liver (reviewed in [30]). It is thought that this encapsulation of parasite eggs is important for constraining inflammation potentially caused by the eggs. This is illustrated by the fact that *S. mansoni*-infected mice lacking IL-4 and/or IL-13, display an uncontrolled Th1-biased response that results in hepatocyte damage and intestinal pathology [31;32]. These observations suggest that Th2 immune responses may not only be generated with the purpose to kill these parasites, but also have evolved to prevent excessive tissue damage, inflicted by helminths. However, the flip side of the coin is that during chronic stages of infection, maintenance of an inflammatory Th2 response that is too strong may cause, rather than prevent pathology due to excessive tissue fibrosis. Therefore, restraining excessive inflammatory Th2 responses during helminth infections is imperative to prevention of potentially harmful inflammation and survival of the host. Modulation of the intrinsic characteristics of the Th2 response

due to host homeostatic mechanisms during helminth infections (classified as a so-called 'modified' Th2 response) alongside the activation of regulatory responses are thought to play an important role in controlling inflammation-induced pathology [33].

Since type 2 immune responses may partly be generated with the purpose to prevent excessive tissue damage, it would make sense that mechanisms to repair damaged host tissues are an integral part of an appropriate immune response against helminths. This concept of tissue-repair is in line with the observation that alternatively activated macrophages, that play an important role in type 2 associated processes such as fibrosis [34], are highly similar to the macrophages required for wound healing found in lesions caused by non-infectious injuries [35]. On this basis one could hypothesize that not only tissue repair mechanisms are part of a Th2 response seen during helminth infections, but that tissue damage and cellular stress inflicted by the parasites may actually also be triggers for induction of type 2 responses. The fact that omega-1 as potentially toxic agent for hepatocytes [36] as well as for DCs (chapter 5) has now been identified as the single most important Th2-inducing factor secreted by those eggs (chapter 4) would be in agreement with this hypothesis. Likewise, promotion of oxidative stress in DCs has been associated with induction of type 2 inflammation during allergic responses [37]. Lastly, the recent findings that TLSP is not only elicited from ECs during helminth infections, but also following trauma and injury in a non-infectious setting would also be consistent with a direct link between tissue damage/cell stress and the induction of Th2 responses during helminth infections [38]. This view, that the immune system has evolved not only to induce type 2 immunity in response to parasites themselves, but also to tissue damage caused by the parasites, may also shed new light on the so far unsuccessful search for true Th2-priming PRRs that specifically recognize helminth components, equivalent to the Th1-polarizing TLRs that recognize bacterial and viral PAMPs, since sensing of stress signals by tissue damage, such as damage associated molecular patterns (DAMPs) [39], may provide an alternative mechanism through which APCs could become conditioned for induction of Th2 responses. However, it should be noted that the processes involved in wound healing and helminth infections overlap only partially, as exemplified by the fact that anti-helminth effector mechanisms such as that driven by IgE and eosinophils are normally not involved in tissue repair. This suggests that apart from sensing of tissue damage, additional signals provided by helminth-derived products through interactions with PRRs (such as described in chapter 3), are probably still of importance for development of full type 2 immunity against helminths.

Despite vigorous induction of Th2 immune responses by the host, helminth infections, including the ones caused by intestinal parasites, generally result in

persistent infections. Although this may in part rise from the intrinsic limitations of Th2 responses to confer immunity against certain helminth species, it is also thought to reflect the capacity of helminths to actively promote the induction of regulatory immune responses. Normally during non-infectious conditions these regulatory responses are an integral part of the host's immune system that keeps detrimental immune responses against self- or innoxious antigens in check. To increase the chances of survival, parasitic worms have evolved to exploit this Achilles' heel of the immune system, by promoting the induction and expansion of Treg responses that results in suppression of Th2 effector responses against the parasites as well as the function of innate cells, such as DCs (described in **chapter 2**) (reviewed in [33;40]). The importance of this mechanism in survival of the parasite is exemplified by the fact that after depletion of Tregs or neutralization of their suppressory molecules, Th2 immunity and thereby protection against the infection can be restored [41-43]. Although Treg responses are beneficial to the parasite, the promotion of this regulatory arm is thought to be important for the host as well since in several helminth infection models IL-10-deficient mice display severe pathology and increased mortality as a result of an overzealous inflammatory response to the parasite [44-46]. Thus, it appears that regulatory responses can benefit both the host and the parasite. Therefore, for the host an immune profile with a well controlled or 'modified' Th2 and a Treg component may be the best compromise between containment of the infection and prevention of excessive inflammation and pathology, that in the end ensures survival of the host and, as a consequence, of the parasite as well as, long enough to allow its transmission.

Concluding remarks

A well balanced Th2 and Treg response will in the end be critical for survival of both the parasite and the host. It is therefore not surprising that from a host point of view DCs, as the orchestrators of immune responses, are well equipped to efficiently sense helminth infections such as the ones caused by schistosomes in a variety of ways. This may not only involve direct PRR-mediated interactions with helminth-derived products (**chapter 3, 4 & 5**) but also sensing of stress signals induced by the worms or its components (**chapter 4 & 5**) for DCs to acquire a Th2-polarizing phenotype. During the more chronic stages of infection, in addition to helminth-derived regulatory components, host factors like an activated immune regulatory network and/or a reduced nutritional status may act on and suppress the function of DCs (**chapter 2**), which altogether may contribute to the persistence of the parasite. While we are only beginning to understand the mechanisms underlying these phenomena, it is evident that a better insight into the interplay between host and parasite at the level of DCs

will be imperative for unraveling the mechanisms underlying other Th2-mediated disorders, such as allergic diseases.

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