

Summary

The classic reaction of the host to helminth infections is the induction of T helper 2 immune (Th2) responses. In addition, helminth infections elicit strong regulatory T cell (Treg) responses, which are believed to contribute to immune hyporesponsiveness observed during these infections and to reflect the capacity of these parasites to manipulate the host immune response in order to prevent expulsion. Since dendritic cells (DCs) have the unique capacity to sense invading pathogens and subsequently prime and regulate adaptive immune responses, DCs are thought to play a prominent role in these processes. However the mechanisms behind the interplay between DCs and helminths and how that translates into Th2, Treg responses or T cell hyporesponsiveness are not fully understood. The research described in the thesis focuses on the molecular interactions between human DCs and the parasitic helminths of the genus *Schistosoma* and on how this affects the T helper cell-polarizing properties of these DCs.

Chapter 1 provides a general introduction into the essential role of DCs in sensing of invading pathogens and in induction and regulation of adaptive immune responses and describes the type of immune responses that are associated with schistosomiasis and helminth infections in general. Furthermore, an overview is given of the current knowledge of how DCs recognize helminth infections and how helminths modulate DC function, via direct interactions or indirectly via host-derived cues. Also, the molecular mechanisms through which helminth-conditioned DCs are thought to prime Th2 or Treg responses are discussed.

Chapter 2 reports on the effects of chronic *Schistosoma* infection on the phenotype and function of human blood DCs. It was found that plasmacytoid (pDCs) and myeloid DCs (mDCs) from *Schistosoma haematobium* infected subjects were present at lower frequencies in peripheral blood and that mDCs displayed lower expression of HLA-DR compared to those isolated from uninfected individuals. In addition, mDCs, but not pDCs, isolated from infected subjects, had a reduced capacity to respond to TLR ligands. Furthermore, the T cell-activating capacity of TLR-matured mDCs from infected subjects was lower. This suggests that *S. haematobium* infection is associated with functional impairment of human DCs *in vivo* and sheds new light onto the underlying mechanisms of T cell hyporesponsiveness during chronic schistosomiasis.

To study the consequences of exposure of schistosome-derived components on DC function in more detail at the molecular level, an *in vitro* human monocyte-derived DC model was used in the subsequent chapters. In **Chapter 3** the signaling pathways are

characterized in DCs that are activated by Th2-promoting helminth-derived components and compared with the pathways induced in DCs by Th1-polarizing bacterial extracts. Phosphatidyl serine-lipid fractions derived from the helminths *Schistosoma mansoni* and *Ascaris lumbricoides* were found to activate TLR2 and to reduce the ratio of activated Mitogen Activated Protein Kinases (MAPK) *p*-ERK / *p*-p38 induced by LPS, just like soluble egg antigens (SEA) derived from schistosomes. In addition, analysis of the mRNA expression levels of a set of 25 genes potentially involved in modulation of T cell polarization revealed that the Schistosome- and *Ascaris*-derived lipids suppressed mRNA expression of Th1-associated notch ligand delta-4 and induced expression of the Th2-associated transcription factor c-fos. Conversely, the bacterial extracts, while also activating TLR2, had an opposite effect on the LPS-induced *p*-ERK / *p*-p38 MAPK ratio and expression of delta-4 and c-fos mRNA. Importantly, each of these molecular characteristics in DCs strongly correlated with their Th1/Th2 polarizing capacity. Thus, the activation of the same TLRs on DCs by different antigenic mixtures can lead to very different polarization of the T cell response. Moreover, this study suggests that specific elements of the molecular signature induced very early in DCs after exposure to pathogen-derived extracts can be used to predict the T cell-polarizing capacity of those compounds.

Although the schistosome eggs and their derived antigens (SEA) are well-known potent inducers of Th2 responses *in vitro* and *in vivo*, the identity of the factors from eggs that drive the Th2 responses have remained elusive. **Chapter 4** describes the identification of the major component secreted by *S. mansoni* eggs that drives Th2 responses. Analysis of IPSE/alpha-1 and omega-1 as the two most abundant proteins present in egg secretions in the *in vitro* human DC model, showed that omega-1, but not IPSE-1/alpha-1, suppressed LPS-induced DC maturation and IL-12 production, similar to SEA. Importantly, omega-1 as well as recombinant omega-1 was found to condition DCs to drive Th2 responses. Furthermore, in IL-4 dual reporter mice natural and recombinant omega-1 were shown to generate Th2 responses *in vivo* as well even in the absence of IL-4R signaling. Interestingly, when omega-1 was depleted from SEA an impaired capacity for Th2 priming *in vitro*, but not *in vivo*, was observed, suggesting that omega-1 is the major factor in SEA that conditions DCs for induction of Th2 responses, but also that additional factors within SEA exist that can compensate for the omega-1-mediated effects.

In **Chapter 5** the molecular mechanisms through which omega-1 conditions DCs to prime Th2 responses are described. Omega-1 is glycosylated and harbours RNase activity, characteristics that may play a role in its Th2-polarizing capacity. The use of an

RNase and glycosylation mutant, revealed that both the glycosylation and the RNase activity of omega-1 are required for Th2-priming of DCs, while alone neither of these characteristics could induce a Th2 response. It was found that omega-1 can bind to both Mannose Receptor (MR) and DC-SIGN via its glycans, but that uptake of omega-1 by DCs is only mediated via MR. Furthermore, omega-1 was found to impair protein synthesis following MR-dependent uptake which reflects its ability to cleave rRNA. Signaling via CLRs has been thought to playing a role in conditioning DCs for priming of Th2 responses. However, although omega-1 was found to signal through DC-SIGN via its Le^x-carbohydrate motifs, this is dispensable for instructing DCs to drive Th2 polarization. Together, these data suggest that omega-1 conditions DCs for Th2 polarization by interfering with ribosomal function following internalization by MR, which is dependent on its RNase activity and glycosylation, respectively. Moreover, it gives new insights in the molecular processes involved in schistosome-induced Th2 responses in general as it provides evidence that infliction of a stress response within DCs, such as induced by omega-1 via interference with ribosomal function, can result in cells characterized by low IL-12 expression and antigen presentation/costimulation, a DC phenotype known to favor the induction of Th2 responses.