# Tracking helminths : from molecular diagnostics to mechanisms behind immune polarization

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## SUMMARY

Parasitic helminths are important organisms to study because their infections can have both adverse and beneficial effects on the human host. Helminth infections including those caused by soil transmitted helminths (STHs) and schistosomes are considered a burden, as these infections cause significant morbidity in a large proportion of the 1.5 billion people infected worldwide. However, helminth infections, by means of their ability to modify host immune responses can also provide protection against a wide spectrum of inflammatory diseases, such as celiac disease, inflammatory bowel disease, diabetes, and asthma. It is important to better understand the underlying mechanisms of these Yin (positive) and Yang (negative) consequences of helminth infections. The general objective of this thesis is to track helminths at different levels. In Chapter 2 and 3 this is achieved by focusing on molecular diagnostics to detect helminth infections, while in Chapter 4, 5, 6 and 7 the host-parasite interactions and the associated immunological footprint induced by helminths are studied.

**Chapter 2**
Microscopy-based methods have always been the first-line approach to diagnose helminth infections. However, their limitations and lack of standardization lead to the need for better methods in parasite detection, such as molecular diagnostics. In this chapter, the performance of multiplex real-time PCR and microscopy methods for the detection of STHs (hookworm, *Ascaris lumbricoides* and *Strongyloides stercoralis*) in stool samples between two endemic areas were evaluated. In Indonesia, using a microscopy-based formal ether concentration method (FEC) 35.4% of the study population was found to be positive for helminth infections, while multiplex real-time PCR detected 81.4% was helminths positive. In Mozambique, a combination of microscopy methods (Direct smear, Kato smear, FEC, Baermann method and copro-culture) *versus* a multiplex real-time PCR resulted in 77.9% vs 73.6% positivity for helminths. This work suggests that DNA detection in stool, using multiplex real-time PCR, seems a more reliable approach than stool microscopy in studying the burden and comparing helminth infections in different populations.

**Chapter 3**
*Trichuris trichiura* is one of most prevalent STHs worldwide. To date, DNA detection of this parasite from stool has been challenging due to difficulty in extraction of DNA from *T. trichiura* eggs. We optimized the sample preparation procedure for the detection of *T. trichiura* DNA. We found that the combination of ethanol preservation and a bead-beating procedure prior to parasite DNA extraction from stool resulted in a higher sensitivity for detection of *T. trichiura* infection in comparison to a control procedure (without ethanol or bead-beating) in a patient cohort from an endemic region in Indonesia: 55% versus 40% positivity for *T.trichiura*, respectively. Moreover, the optimized method showed significantly higher *T. trichiura* DNA loads. Together the findings demonstrate that this optimized method significantly improved the ability to detect *T. trichiura* infection. Importantly, this optimized procedure had a minor effect on the PCR results of other STHs compared to the control procedure.

**Chapter 4**
In Chapter 4, we investigated the lipid profile of different life cycle stages (cerariae, worms and eggs) of *Schistosoma mansoni* as well as the lipid profile of the corresponding extracts, to find

potential leads for helminth-derived lipids with immunomodulatory potential. As expected different life cycle stages exhibited distinct lipid signatures. Interestingly the lipid composition of parasite life cycle stages and corresponding extracts were highly similar. The latter mentioned finding validates the use of the widely used parasite preparations such as soluble eggs antigens (SEA) in models to explore host-parasite interactions in immunological studies. A striking finding was the presence of prostaglandins (PGs) specific to egg- and cercarial preparations. Prostaglandins are lipid mediators with known modulatory effects on immune cells. Thus, our lipid profiling study has indicated that such approach could identify immunomodulatory compounds in helminths .

**Chapter 5**

From detection to characterization of helminths, we moved on to study their detailed interaction with the human immune system. Dendritic cells are central to skewing of immune responses and have often been studied in the context of their interaction with helminths. The maturation of DCs is characterized by changes in expression of a large number of proteins. We aimed to link the expression of proteins to the Th2 priming-capacity of helminth-conditioned DCs. Using a high-throughput mass spectrometry-based method, we explored the proteome of DCs stimulated with *S. mansoni*-derived products, SEA and omega-1 (ω-1). Omega-1 is a glycoprotein derived from SEA and is known as a potent Th2 inducer. We observed an increase in the expression of two proteins involved in ribosome and mitochondrial function indicating that both SEA and ω-1 could affect protein expression and cellular metabolism. Indeed, SEA and ω-1 decreased the expression of proteins related to antigen processing and presentation. These finding, are largely consistent with the hypothesis that the promotion of Th2 responses can result from weaker immunological synapse formation between DCs and T cells.

**Chapter 6**

Based on the findings in Chapter 4, we sought to dissect the role of lipid mediators in Th2 polarization by SEA via DCs. We found that $PGE_2$ and its isomers were not only present in SEA but were also synthesized by SEA-conditioned DCs. We found that SEA binds to dectin-1 and dectin-2 receptor in DCs to trigger a signalling pathway involving Syk, ERK, $cPLA_2$ and COX-1 and -2, leading to $PGE_2$ synthesis. $PGE_2$ subsequently acts in an autocrine manner to drive expression of OX40L by DCs, which licenses DCs to prime Th2 responses. This Dectin-1/2 induced autocrine $PGE_2$ signalling axis can fully account for the ω-1 independent ability of SEA to prime Th2 responses. This pathway is also essential for Th2 polarization *in vivo* and for Th2-driven granuloma formation around eggs in the liver during *S. mansoni* infection. Together, we identified in this chapter a novel pathway through which *S. mansoni* conditions DCs to prime Th2 responses.

**Chapter 7**

Recent studies have shown that helminths are capable of inducing regulatory T cells (Tregs) by promoting production of short chain fatty acids (SCFAs) by microbiota in the gut. In particular butyrate has been shown be an important driver of Treg activation. However, how SCFAs promote this response in humans in less clearly defined. Therefore, we investigated in this chapter whether and through which mechanisms SCFAs can condition human DCs to prime Tregs. We found that butyrate conditions human DCs to prime IL-10 producing type 1 regulatory T cells (Tr1). Butyrate induces RALDH activity, a key enzyme to convert vitamin A into retinoic acid (RA). RALDH-derived

RA acts on DCs themselves to enforce RALDH expression and in T cells to promote Tr1 differentiation. Mechanistically, we found butyrate to depend on the combined action of HDAC inhibition and signalling via GPR109A to promote RALDH activity in DCs and to license these cells to prime Tr1 cells. This provides novel insights in the mechanisms through which helminths are capable of inducing tolerogenic immune responses in humans, by promoting production of SCFAs by gut microbiota.

In conclusion, the studies described in this thesis on the one hand help to improve the detection of helminth infections, essential for the studying helminths and the interaction with their human host. Moreover, the development of more sensitive diagnostics is instrumental for reliably monitoring the distribution of helminth infections, which is key to evaluate the success of programs that aim to eliminative helminth infections, currently underway in many parts of the world. On the other hand, this thesis has generated important new mechanistic insights into how the interplay between helminths and the host immune system results in priming of Th2 and regulatory T cell responses. This could pave the way for the identification of pathways that can be targeted to manipulate these immune responses, as part of developing therapeutics to treat inflammatory disorders characterized by deregulated Th2 and/or Treg responses.