Exploring host-immune-microbial interactions during intestinal schistosomiasis

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English summary

Parasites are incredibly fascinating organisms, that are hailed for their undesirable attributes (e.g., bizarre appearances and unique behavioural practices) and defined by their need to live off another organism (a host) to grow, feed and thrive. To maintain their long-term survival, complete their infectious lifecycle and lessen infection associated pathology, many parasites have evolved sophisticated techniques and factors to configure, suppress and evade the host immune system. Importantly, although parasites are primarily portrayed negatively, their capacity to modulate immune cells can prove beneficial to host health. More specifically, mounting evidence suggests exposure to parasites and symbiotic microbes (e.g., bacteria and fungi) during childhood is crucial for adequate immune education and protection against inflammatory diseases. This thesis strives to better delineate the mechanisms by which the parasitic Schistosoma flatworm calibrates and modulates host immunity, with emphasis on their impact on the intestinal environment and interactions with tolerogenic B cells.

Schistosomiasis, also known as Bilharzia, is a neglected yet significant tropical disease, with over 240 million cases annually, an estimated 200,000 deaths per year, and second only to Malaria in terms of debilitation. This parasitic disease is widespread across sub-Saharan Africa, regions of South America and Asia, and heavily impacts those in impoverished communities with poor access to safe drinking water. The three most common species of *Schistosoma* blood flukes to infect humans are *S. mansoni*, S. *japonicum* and S. *haematobium*. The two former species are responsible for intestinal disease, whilst *S. haematobium* has consequences for the urinary tract. The focus of this thesis is on *S. mansoni.*

Schistosoma infection starts upon exposure to freshwater harbouring the free-swimming larval form of the parasite, cercariae. These fork-tailed parasites penetrate the skin of their prospective mammalian host, and over the course of several weeks, navigate from the skin, across the pulmonary system, to the blood vessels of the liver and intestine. Here, immature *Schistosoma* parasites (schistosomula) mature into male and female adult worms, which pair and produce egg (up to 300 per day per worm pair) for the remainder of their lifespan, which can be years without pharmaceutical intervention. In an impressive case of immune modulation and protective mechanisms by the host, schistosome eggs are enclosed by host immune cells and fibrotic material, in an immune lesion known as a granuloma. Granuloma formation is required for the translocation of eggs across the intestinal wall and elimination into faeces, where the eggs hatch and complete their lifecycle outside of the mammalian host, in a suitable freshwater snail. Alternatively, many

eggs fail to transit across the intestine and are instead swept with the blood flow to the liver where they lodge and evoke inflammatory granulomas. These egg-evoked inflammatory lesions are central to schistosome-associated pathology, and in severe advanced disease, can lead to fatality.

To enhance the fitness of their host, and thus promote their own survival, schistosomes employ a variety of techniques to calibrate and influence host immunity. This includes the endorsement of Type 2 immunity to support granuloma formation and egg egress, as well as the bolstering of tolerogenic immune cell networks, which serve to down-modulate the potentially exuberant responses directed against them and lessen egg associated pathology. Moreover, as an important bystander effect, the induction of tolerogenic cell types, including regulatory B cells (Bregs) and T cells (Tregs), provides the host relief against non-related immune diseases typified by an overactive immune system, including allergy and autoimmunity.

Experimental (murine) schistosomiasis forms an ideal system to study the pathways and molecules exploited by the parasite to drive host immune modulation. For example, the potent capacity of schistosomes to support Breg and Tregs generation can be studied over time. Similarly, as both schistosomiasis and allergy are hallmarked by Type 2 immune responses, though with completely different clinical symptoms, detailed inspection during experimental schistosomiasis may provide insight into novel cell types and pathways involved in type-2 immune driven host defence and repair, which may have repercussions for novel therapeutic allergy treatments. Finally, by bolstering our understanding of the impact of schistosome egg transit on the intestinal interface, we may identify core mechanisms involved in the regulation and repair of mucosal inflammation, including the involvement of intestinal bacteria.

The first part of this thesis provides a high-resolution narrative of the immune response evoked by schistosomiasis over the course of infection (Chapter 3**)** and offers novel insight into the consequences of schistosome egg transit on intestinal barrier integrity, microbiota structure and mucosal immunity (Chapters 2&4).

The movement of schistosome eggs from the intestinal vasculature across the intestinal wall into host faeces is an enigmatic and intriguing process, involving the passing of multiple barriers (epithelial and endothelial), the recruitment and modulation of immune cells for granuloma formation, and the instruction of repair networks to heal egg driven damage. In Chapter 2, we discuss our current understating of schistosome egg migration, outline the host-parasite-microbial interactions that may enable this process and speculate on the consequences of successful (or unsuccessful) egg transit on the fitness of the host and unrelated diseases.

Schistosomiasis is hallmarked by a distinctive triphasic immune response. This includes a mixed, low-level Type 1 and Type 2 immune response during the initial weeks of infection, an exuberant Type 2 response from the point of egg production (week 5-6), and the emergence of potent regulatory cell networks during infection chronicity (from week 12 onwards). This narrative has slowly been pieced together from hundred of independent research articles, all focusing on different time points of infection and different organs of interest. In Chapter 3 we provide a concise and up-to-date narrative of the immune trajectory of schistosomiasis across the course of experimental infection, focusing on three tissue sites affected by infection: the liver, mesenteric lymph nodes and spleen, using histological, cellular (flow cytometry) and transcriptomic (RNA-seq) based techniques. Further, through the depletion of $CD11c^+$ antigen-presenting cells (including dendritic cells and macrophages) during the initial stages of egg production, we reveal a critical role for $CD11c^+$ cells in the coordination of granuloma responses and Th2 inflammation.

Despite the large impact of schistosomiasis on the intestine, the bulk of immunological studies on *S. mansoni* to date have focussed on the liver (principal site of egg deposition) or more distal sites, including the spleen. This, in part, stems from the notorious technical difficulty to obtain live cells from the schistosome-infected murine intestine, due to the hostile anti-parasite Type 2 immune response evoked here, including excessive mucus production, tissue remodelling, pH alterations and cellular infiltration. We have overcome these technical difficulties and were able to islate live immune cells from the guts of infected mice. In Chapter 4, we provide a detailed characterisation of the intestinal environment during experimental schistosomiasis, with specific emphasis on intestinal microbiota structure, integrity of the intestinal barrier and contrasts between mesenteric and intestinal immune responses. Through the employment of egg producing and non-egg producing infections – by making use of infections with male and female larvae (egg production) or with male larvae only (no egg production) - we show egg deposition to dramatically reduce the integrity of the intestine, modify the composition of the intestinal microbiota and reconfigure the frequency and profile of colonic and mesenteric immune cells. Further, using germ-free mice (devoid of microbiota) and faecal microbiota transfers, we demonstrate the capacity of a schistosome-associated microbiota to instruct components of Type 2 immunity.

The second part of this thesis focus on the induction of splenic regulatory cell networks (Chapter 5&6) and the potential for the microbiota of schistosome infected animals to alleviate experimental allergy (Chapter 6). Previous work from our lab shows chronic schistosome infections to support the expansion of tolerogenic Bregs, which provide relief against experimental allergy, and that both

IL-10 producing Bregs from the spleen and the lung are involved. The cellular mechanisms driving Breg induction remain relatively undefined, but previous studies and unpublished gene expression analysis from our lab points towards a role of cytokines, like Type I interferons, in Breg activation during chronic schistosomiasis. We found Type I interferons to support the expansion of Breg cells in vitro culture systems, but not within the body.

In **Chapter 6**, we continued our search to define regulatory cell inducing molecules during schistosomiasis, using egg producing and non-egg producing infections to tease signals apart. We show both type of chronic infections to expand distinct splenic regulatory cell networks, but with T cells and B cells from egg producing infections to have a superior capacity to produce IL-10. With work in Chapter 4 demonstrating that the intestinal microbiota differs between egg producing and non-egg producing infections, we next questioned whether the microbiota was involved in regulatory cell induction and alleviation of allergic disease. In comparison to allergic mice recolonised with naïve uninfected faeces, the transfer of faeces from egg producing and nonegg producing infections reduced recruitment of cells characteristic to an allergic response into the airways and imprinted a distinctive Type 1 phenotype on innate and adaptive immune cell types instead of Type 2.

Finally, in Chapter 7, we discuss our main findings, and its implications for our current understanding of host-parasite-microbial relations.