Wiles and wanderings: immune-evasive maneuvers of skin-penetrating parasites

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Appendix

ENGLISH SUMMARY

Skin-penetrating parasites have something in common; they all need to evade the initial immune response in the skin in order to avoid being evicted by their hostile host and establish an infection. To do so, they are equipped with the necessary cunning stratagems. For example, they can act directly on immune cells to alter their function and they can optimize their migration patterns to their hostile environment. This thesis is aimed at unravelling those mechanisms.

Malaria and Schistosomiasis are two deadly and debilitating parasitic diseases. With over 200 million (malaria) and over 240 million (schistosomiasis) cases annually, the global disease burden remains very high and the need for potent vaccines is evident. Whole weakened parasites can be used to vaccinate individuals against parasitic diseases like malaria. However, delivery of these parasites in the skin, as is commonly done in vaccinations, reduces their protectivity. We hypothesize that this reduction is caused by parasite-mediated immune-regulatory mechanisms that are initiated upon their first encounter with immune cells in the skin.

The skin is an important and active immune organ. Its main function is to maintain the immunological balance between swift and powerful elimination of disease-inducing microbes called pathogens and gentle tolerance towards commensals, the "good microbes" living on our body. In order to do its job properly, the human skin contains a wide variety of immune cells. Among these cells are antigen presenting cells (APCs). They have the ability to take up foreign particles (antigens), process them into small fragments, and present these fragments to T cells. T cells can then launch an immune attack when they recognize these fragments as pathogenic. The type of T cell response, either inflammation (an active immune attack) or tolerance, depends on APC signaling during presentation. Certain molecules on the APC's surface can signal T cells to induce a tolerizing immune response. These molecules, termed immune checkpoint-molecules, like PD-L1, can deactivate T cells. We investigated whether skin penetrating parasites exploit these existing mechanisms in human skin in order to enhance their survival.

In the first part of this thesis, *wiles*, we demonstrate that *Plasmodium falciparum* and *Schistosoma mansoni*, the causative agents of Malaria and Schistosomiasis or Bilharzia respectively, exploit existing APC mechanisms in the skin to induce T cell tolerance. We show that both *Plasmodium* sporozoites and *Schistosoma* larvae increase PD-L1 on the surface of skin APCs and the production of regulatory cytokines by these cells. In addition, we confirm the immune regulatory propensity of these parasite stimulated APCs by looking at their effect on T cell activation (**chapter 2** and **4**). **Chapter 3**

investigates whether the method of intradermal delivery of parasites into the skin affects APC responses by investigating cellular responses to mosquito bite or needle injected parasites in human skin explants. In **chapter 4** we show that the attenuation (weakening) of *Schistosoma* parasites decreases their regulatory effect in the skin. This may help to explain why attenuated parasites are capable of initiating protection, where repeated natural infection is not.

In the second part of this thesis, *wanderings*, we employed imaging techniques to investigate the motility of malaria parasites in human skin. In **chapter 5** we presented a semi-automatic software program that detects and tracks *plasmodium* sporozoites in microscopy videos of sporozoites moving through human tissue. This method allowed us to comprehensibly quantify movement patterns and compare radiation attenuated with non-attenuated genetically-modified fluorescent parasites, ultimately showing that irradiated sporozoites revert to less variable, "default" movement patterns. In **chapter 6** we showed a novel molecular imaging method in order to label wild type *plasmodium* parasites using a mitochondrial dye, enabling us to visualize wild type parasite movement in human skin.

Lastly, in **chapter 7** our findings are discussed in the broader context of our current understanding of parasite immunity as well as the implications for live-attenuated parasite vaccine development.