To IMAGE or to IMAGINE: visualization of parasite migration as a means to support (malaria) parasite vaccine development

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ENGLISH SUMMARY

Summary of this thesis entitled: 'To image or to imagine - Visualization of parasite migration as a means to support (malaria) parasite vaccine development'.

This thesis describes the development of quantitative imaging-based tools to study parasite migration under different conditions aiming to support the development of antiparasitic vaccines needed to reduce the global disease burden caused by parasitic infections.

Development of SMOOT

SMOOT (Sporozoite Motility Orienting and Organizing Tool) was developed and established as a quantitative software analysis tool for tracking the migration of malaria sporozoites *in vitro* and in human skin explant. This tool provided a readout with high kinematic detail, enabling the quantitative characterization of novel factors influencing the migration capability of malaria sporozoites:

- A complex interplay of different (macro)molecules act as regulators of sporozoite motility (chapter 1).
- Radiation attenuation impairs the capacity of sporozoites to vary their turn angle, velocity and direction, resulting in a loss of movement variability (chapter 2).
- The administration route of sporozoites, whether via mosquito inoculation or intradermal syringe injection, cause differences in the sporozoite distribution through the skin, the presence of hematomas, tissue structure and (possibly as an indirect consequence of the other differences) sporozoite migration patterns (chapter 3).
- Anti-CSP antibody concentrations far above the theoretical saturating binding concentration are needed to inhibit sporozoite motility *in vitro,* these values increase even further in human skin explant and subsets of sporozoites seem to be refractory to the effect of anti-CSP antibodies (chapter 4).

Development of a hybrid tracer for sporozoites

In chapter 5 we expanded the study of sporozoite migration beyond *in vitro* and *ex vivo* models. A hybrid tracer labeling approach for malaria sporozoites was developed which yielded viable sporozoites that were both fluorescently and radioactively labeled. With this approach the *in vivo* dissemination of malaria sporozoites could be revealed in a murine model showing that live sporozoites are able to migrate from the lungs to the liver more efficiently than non-viable sporozoites.

Relevance for vaccine development

Since migration is crucial for sporozoites to continue their life cycle within the host, factors influencing their migration capability may be important targets for (vaccine-induced) immunity.

In this thesis, imaging of parasite migration was used to gain insights that can support the development of antiparasitic vaccines. The findings from chapter 1, which demonstrate that different (macro)molecules regulate sporozoite motility, and the observations from chapter 3, which show the impact of the administration route on sporozoite migration patterns, suggest that optimizing the vaccine formulation and developing an administration method that mimics mosquito inoculation may enhance the ability of vaccine sporozoites to reach the liver after intradermal delivery and thereby improve their efficacy to induce immunity. Additionally, the results from chapter 2, that radiation attenuation impairs the sporozoite's migration capability and from chapter 5, that live sporozoites are more efficient in migrating from the lungs to the liver than non-viable sporozoites, suggest that there needs to be an optimal balance between the degree of attenuation that ensures safety of the vaccine and minimal impairment to allow sufficient antigenic exposure required for inducing protective immunity. Furthermore, quantitative assessment of the parasite inhibitory capacity of anti-CSP antibodies, as described in chapter 4, can aid in selecting the most efficacious antibodies to support optimization of subunit and passive immunization strategies.

Broader potential for imaging technology in parasitology

In chapter 6 we used the concepts developed for studying the migration of malaria sporozoites (as described in chapter 1-5) to investigate skin invasion by *Schistosoma mansoni* and *Necator americanus* larvae. We introduced a multimodal imaging approach that combined fluorescent and radioactive imaging to assess helminth skin invasion in real-time using a human skin explant model. This setup enables a comprehensive and quantitative evaluation of helminthic invasion, providing possibilities for gaining deeper insights into this process as well as serving as a useful readout for assessing the efficacy of future helminth invasion-blocking strategies. This thesis concludes with a review of the broader potential for imaging technology to advance the development of new diagnostic methods, therapeutic interventions and vaccines for combating parasitic infections (chapter 7). This review emphasizes the possibilities to improve current microscopy-based diagnostic methods and extend them with radiological imaging modalities. Additionally, *in vivo* tracking of parasites is highlighted as a potential readout for the efficacy of new antiparasitic strategies and as a source of fundamental insights for rational design.