A supramolecular chemistry approach for potentiating live attenuated whole-organism vaccines

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1

Introduction & Dissertation Outline

Introduction

Malaria has been a uniquely destructive infectious disease in humanity's history. Once widespread throughout the world (Cox, 2002), the disease is now mostly confined to tropical regions, where it continues to be a major source of mortality and morbidity: in 2020 alone an estimated 627,000 deaths were attributed to malaria (WHO World Malaria Report 2021). Some success has been had in driving down mortality/morbidity via interventions targeting the Anopheles mosquito responsible for malaria transmission (Bhatt et al., 2015), but a plateauing of annual deaths in recent years suggests that further reductions of malaria's burden will require use of an effective vaccine.

Initial efforts at developing a subunit-based vaccine against the circumsporozoite protein (CSP) found abundantly on the malaria sporozoite (SPZ) surface, known as RTS,S, did not yield high levels of protection in a large Phase 3 trial, with only 30% protection after 18 months (Rts et al., 2012). The more recent focus of malaria vaccine design has turned to whole SPZ-based vaccines that better activate the immune system. Studies in malaria-naïve individuals have shown that complete protection from malaria challenge can be achieved by immunization with such vaccines (Mordmuller et al., 2017; Roestenberg et al., 2009; Seder et al., 2013). However, field trials in endemic areas have yielded considerably lower levels of protection (Oneko et al., 2021; Sissoko et al., 2017). These findings indicate the need for further improving the immunogenicity of SPZ-based malaria vaccines.

In this dissertation, a strategy for improving SPZ-based malaria vaccine immunogenicity is presented in which the SPZ cell surface is chemically augmented with immunogenic adjuvants. The conceptual basis of this strategy is derived from advances in the field of supramolecular chemistry that have shown the power of harnessing multivalent supramolecular interactions to drive very strong interactions between manifold chemical moieties and biological entities – such as a cell's enveloping lipid bilayer (Roy et al., 2020). Building on such findings, this dissertation describes application of the principle to anchor onto both bacterial and malaria SPZ cells a supramolecular scaffold enabling chemical augmentation of their cell surfaces. These chemical scaffolds are subsequently engineered with immunogenic adjuvants whose presence on the (SPZ) cell surface induces a more vigorous immune response compared to unadulterated cells.

Dissertation Outline

This dissertation presents the development and immunological assessment of a strategy for chemically augmenting SPZ immunogenicity.

I. Chemical augmentation technology

In **Chapter 2**, the concept – namely, using supramolecular chemistry to functionalize microbial cell surfaces via an in situ pre-targeting host-guest strategy – was investigated in several bacterial model systems. Next to establishing this novel technique for pathogen surface functionalization, its effect on (normal) macrophage behavior, a key immune cell early in immune responses, was investigated. In **Chapter 3**, the chemical functionalization strategy was evaluated in vivo in a pre-targeting paradigm. Using a bacterial infection model, it was shown that polymers, when intravenously administered, could home in on infected tissue containing pre-targeted bacteria and there remain stably associated.

II. Vaccine development

Chapter 4 expanded on the concept by using the same chemical strategy to introduce an adjuvant onto the cell surface of (poorly immunogenic) bacteria. The key question here addressed was: does an adjuvant complexed in this manner induce a more pro-inflammatory response to bacteria? This was indeed so, as macrophages responded to adjuvanted bacteria with significantly increased production of the pro-inflammatory cytokine IL-6. These findings supported translation of the concept to increase SPZ-based malaria vaccines' immunogenicity. In **Chapter 5**, minor changes in functionalization strategy allowed translation of the concept to malaria SPZ, which yielded markedly improved immune responses in an in vitro macrophage model. A more extensive immunological characterization in an in vivo mouse model of malaria followed, the results of which suggested that chemically augmented SPZ did indeed possess a better immunogenicity than wild-type SPZ.

In **Chapter 6**, a summary of the findings and future outlooks, particularly in the context of advancing malaria vaccines, is outlined, including preliminary data postulating a refinement of the presented adjuvanting strategy.

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