The Role of Stimulatory Immunomodulator Adjuvants in Blood Stage MSP5 Plasmodium Falciparum Vaccines

Alex Reeder*
Department of Biology
Lake Forest College,
Lake Forest, Illinois 60045

Introduction
Much research has been conducted to develop Plasmodium falciparum vaccines, which work against the parasite that causes malaria. Although there are many proteins that can be exploited for vaccines in the different stages of the P. falciparum life cycle, recent studies have pointed to merozoite surface protein 5 (MSP5) as one with great potential (Bracho), due to its high conservation across P. falciparum isolates (T) and its role in initial attachment to host red blood cells (Kadekoppala). Similar to other vaccines for diseases such as HIV and HBV (Coler, Carter and Friede), malaria vaccines have been shown to work best when paired with adjuvants, which are used to elicit an increased cellular response as well as a humoral response against the parasite, both of which are integral responses of successful malaria vaccines.

In choosing adjuvants, route of delivery and immunogenicity must be considered (Coler, Carter and Friede). Previous studies point to adjuvants with stimulatory immunomodulators, such as a saponin derivative (QS21), a meningococcal derivative (AFCo1), and a lipid A derivative (MPL), as adjuvants, all of which have been shown to stimulate cellular and humoral responses (Hui). QS21 is composed of plant-based glycosides and demonstrates low toxicity as well as maximum adjuvant activity (Coler, Carter and Friede). Other promising adjuvants include MPL, a lipopolysaccharide (LPS) from Salmonella Minnesota (Coler, Carter and Friede), and meningococcal derived AFCo1 (Brach), both of which induce T and B cell proliferation.

Identifying an adjuvant that can accommodate the MSP5 protein, increase the immune response, and have a low toxicity level will increase the possibility of formulating a vaccine that will aid in the prevention and control of malaria. Studying this interaction will allow us to further elucidate the effectiveness of different antigen adjuvant combinations.

Hypothesis
Previous studies have demonstrated MSP5 as a useful antigen (Brach), as well as the efficacy of ISA720, QS21, AFCo1, and MPL in combination with other sporozoite and merozoite proteins (Brach, 2009; Coler, Carter and Friede). The current gap in knowledge is the efficacy of an MSP5 vaccine in conjunction with QS21, AFCo1, and MPL, separately. We hypothesize that those adjuvants that contain immunomodulators (QS21, AFCo1, MPL) increase MSP5 vaccine efficacy when compared to adjuvants that lack this component, namely the water-in-oil emulsion ISA720.

Specific Aims
We will test the prediction that adjuvant formulations that contain immunomodulators provide an additional immune stimulus. We will integrate the Plasmodium falciparum merozoite surface protein 5 (MSP5) with each ISA-720, QS21, AFCo1, and MPL in four separate combinations. Our hypothesis predicts that mice immunized with stimulatory immunomodulators, specifically the meningococcal formulation, will have increased titer levels of interferon-gamma (IFN-g), interleukin-2 (IL-2), intercellular adhesion molecule 1 (ICAM-1), and immunoglobulin G (IgG). Our results will help uncover the possible mechanism behind the increased immune response associated with these adjuvants. We will compare ELISA and ELISPOT titers of mice immunized with each adjuvant. This will be a measure of each adjuvant's immunogenicity.

Experimental Proposal
For use in each experiment, the MSP5 protein will be diluted and combined separately with each ISA720, QS21, AFCo1, and MPL, and then incorporated into adjuvant formulations (Sera and method) into mice (Guidelines for Immunization of Research Animals). Each antigen/adjuvant combination will be injected into five mice at four-week intervals for a total of three immunizations. Mice will be bled to acquire sera three weeks after the last injection. Five mice will be immunized with an MSP5 vaccine without an adjuvant, to serve as a control reference point for normal comparison. Adjuvant immunogenicity will be tested using the mouse sera for ELISPOT and ELISA assay as described below. Immunogenicity will be measured in the form of IFN-g, IL-2, ICAM-1, and IgG and compared to the levels of these molecules in non-adjuvant immunized mice.

IFN-g, IL-2, and ICAM-1 were chosen due to their roles in stimulating and activating T cells (cellular) as well as antigen presenting cells. If immunostimulatory adjuvants are shown to increase these immune molecules, our hypothesis will be supported. In contrast to the T-cell related immune molecules, IgG is representative of the B cell (humoral) response (Immunoglobulin G). This is the most abundant antibody type within the body and is secreted by B cells. They function in malaria immunity by binding to and neutralizing free pathogens that are released by merozoite-infected cells burst in the blood stream. If an immunostimulatory adjuvant is associated with an increase in IgG, we will conclude that this adjuvant stimulates B-cell secretion, resulting in more IgG and more antigen neutralization, which would support our hypothesis.

ELISPOT Assay Measuring Splenocytes
This technique, as taken from a previous study (Hui), will be used to measure the number of IFN-gamma (IFN-g) and IL-4 producing cells. IFN-g or IL-4 specific antibodies are immobilized on the surface of a 96-well microtiter plate. IFN-g or IL-4 producing cells (splenocytes) from the mouse serum will be added to the wells in the presence of the MSP5 antigen in order to induce production of cytokines, which will bind to the immobilized antibody. An enzyme-linked anti-cytokine antibody is then added, which creates an “antibody sandwich” around the cytokine. A substrate catalyzes a color change, which manifests itself as a “spot” within the well. More spots indicate more cytokine producing cells. The negative control wells will have the same composition, except no MSP5 antigen will be added; thus, no cytokine production will take place, resulting in no spots. The positive control will include phytohemagglutinin instead of the antigen, which will elicit an immune response in the same way that MSP5 would.
Serum ELISA Assay

This technique, also taken from a previous study (Hui), will be used to measure the level of antibody response to the antigen, namely level of IgG as well as that of ICAM-1. An ELISA assay is similar to the ELISpot assay in that it can use “sandwich antibodies” for detection. Specific monoclonal antibodies for IgG or ICAM, called capture antibodies, are bound to the membrane of the 96-well microtiter plates. IgG or ICAM-1 producing cells are added in the presence of the MSP5 antigen to induce IgG or ICAM-1 production within the well, the results of which bind to capture antibodies. Enzyme-linked detection antibodies are added and bind to the captured molecule; this creates the “sandwich antibody”. A colorless substrate is added that induces a color change that is quantified by absorption. Darker wells indicate more IgG or ICAM-1 was produced. The negative control will consist of a well without the MSP5 antigen present, while the positive control will consist of the addition of phytohemagglutinin instead of the antigen, for the same purpose as the positive control in the ELISpot assay.

Conclusion

We believe that this research will add to the understanding of how to formulate vaccines that are targeted specifically against blood stage Plasmodium falciparum proteins. These adjuvants and immune molecules were selected due to their promising results in previous studies as well as their integral role in cellular and humoral immunity. These findings about adjuvants can be used to formulate other vaccines for the other stages of malaria that use different antigens. Although our research is promising, we acknowledge that some adjuvants have been shown to have some negative side effects (Coler, Carter and Friede). Future studies may focus on reducing the reactivity of promising adjuvants such as AFCo1.

References


Hui, G. (2007). The requirement of CD80, CD86, and ICAM-1 on the ability of adjuvant formulations to potentiate antibody responses to a Plasmodium faciparum blood-stage vaccine. Vaccin, 25 (51), 8549-8556.


