

MULTIVALENT ADENOVECTOR-BASED MALARIA VACCINE INDUCES HIGH LEVELS OF FUNCTIONAL ANTIBODIES



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Malaria is the most devastating parasitic disease affecting humans. Each year, there are 350-500 million clinical cases and greater than one million deaths due to malaria, primarily of children in sub-Saharan Africa. The feasibility of a blood-stage malaria vaccine is supported by the finding that individuals living in malaria-endemic communities develop clinical immunity to the parasite, and that passive transfer of immunoglobulin from such individuals results in a marked decrease in *P. falciparum* blood-stage parasitemia and resolution of symptoms.

To reduce manufacturing costs for a vaccine for the developing world and to increase vaccine breadth and potency, our development strategy is to combine multiple antigens targeted by protective immune responses into a single, multivalent adenovector. One approach has been to develop a bivalent adenovector that expresses optimized forms of two *P. falciparum* blood-stage antigens, which are targets of naturally acquired immunity. In the optimization stage, we tested whether intracellular, secreted glycosylated, or secreted non-glycosylated forms of mammalian codon-optimized PfAMA1 and PMSP1₄₂ induced superior antibody and T cell responses in mice and rabbits. Adenovector-delivered PfAMA1 induced high ELISA titers in mice and high titer functional antibodies in rabbits by Growth Inhibition Assay (GIA). Greater than 95% inhibition was achieved using 2.5 mg/ml of purified IgG. The secreted glycosylated and the secreted non-glycosylated versions of PfAMA1 induced equally high levels of functional antibodies. Adenovector-delivered PMSP1₄₂ also induced high ELISA titers in mice and functional antibody responses in rabbits, however, the relative levels of GIA activity were lower than those observed with the PfAMA1-expressing vector. The most robust responses were seen in animals immunized with the secreted glycosylated versions of PMSP1₄₂. Antigens that were expressed at the cell surface in either a glycosylated or non-glycosylated form were much better than intracellular antigens at inducing antibody responses. In contrast, T cell responses were not greatly affected by the cellular location and glycosylation status of the antigens.

Based on induction of functional antibody responses in rabbits, we chose secreted glycosylated versions of both PfAMA1 and PMSP1₄₂ for inclusion in a bivalent adenovector. We evaluated immune responses induced by this adenovector in mice and rabbits. For both T cell and antibody responses, the bivalent adenovector induced responses to each antigen that were comparable to the single antigen expression vectors. Interestingly, using this vector in a homologous prime-boost regimen, we were able to boost antibody responses by ~10 fold relative to responses observed following a single administration. These results support the advancement of this bivalent, blood-stage adenovector vaccine to clinical development.

Figure 1. Schematic Representation of Vaccine Constructs

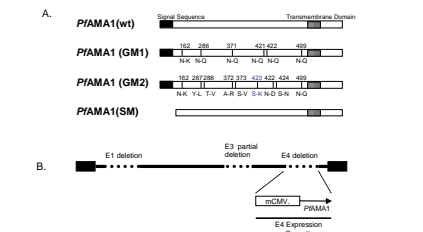


Figure 1. Schematic representation of vaccine constructs. (A) The *Plasmodium falciparum* (P) AMA1 antigens are indicated, wt indicates the wild type antigen, GM1 and GM2 represent PfAMA1 antigens with selected mutations in N-linked glycosylation sites. SM is a PfAMA1 antigen with a deletion in the signal sequence. (B) The E1-, E3-, E4-deleted adenovector containing an E4 expression cassette driven by the murine CMV promoter.

Figure 2. Glycosylation Status of PfAMA1 Variants

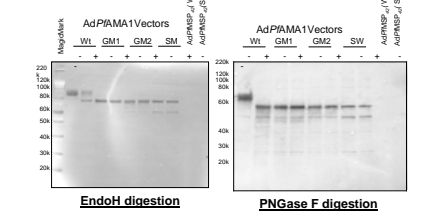


Figure 2. Analysis of PfAMA1 glycosylation status following transduction of A549 cells. Adenovectors expressing the various forms of PfAMA1 indicated in figure 1 were used to infect A549 cells and the cell lysates were subjected to digestion with Endo H and PNGase F and immunoblot analysis.
Conclusion: The PfAMA1 (wt) is glycosylated and the glycosylation mutants (GM1, GM2) and the version with the signal sequence deleted (SM) are not glycosylated

Figure 3. Cellular Localization of PfAMA1 Variants

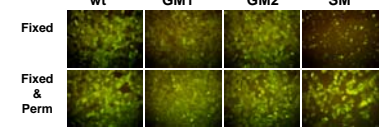


Figure 3. Cellular localization of PfAMA1. A549 cells were infected with adenovectors expressing the various forms of PfAMA1 and the cellular localization of the PfAMA1 antigens was analyzed by immunofluorescence microscopy. 24 hours post infection cells were either fixed with 4% paraformaldehyde to identify proteins that are localized at the cell surface, or fixed and permeabilized with 0.1% saponin to identify both intracellular and extracellular proteins.

Conclusion:

All PfAMA1 versions with the signal sequence are located at the cell surface

Figure 4: PfAMA1 Immunogenicity in Mice

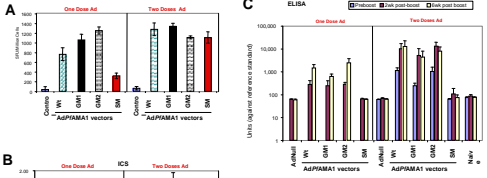


Figure 4. Immunogenicity of Adenovectors expressing various forms of PfAMA1. BALB/c mice received intramuscular immunizations of adenovector and ELISpot assays (A) and ICS (B) were performed on splenocytes harvested 2 weeks following the last administration of vector. (C) Antibody responses were measured by ELISA.

Conclusions:

- Adenovectors that express cell surface associated versions of AMA1 induce the most robust antibody and T cell responses
- AMA1 glycosylation did not affect antigen specific antibody or T cell responses following adenovector delivery

Figure 5. Functional Antibody Response in Rabbit Following Adenovector Delivery of PfAMA1

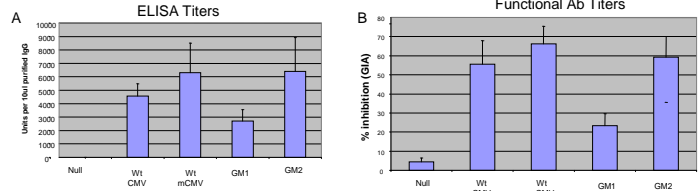


Figure 5. Immunogenicity of adenovectors expressing various forms of PfAMA1. New Zealand white rabbits received two intramuscular immunizations of 1 X 10¹⁰ pu of adenovector (A) Total PfAMA1 antibody was measured by ELISA. (B) Functional antibody responses were determined by GIA.
Conclusion: Adenovectors induce potent functional antibody response to PfAMA1

Figure 6. Cellular Localization and Glycosylation Analysis of PMSP1₄₂ Variants

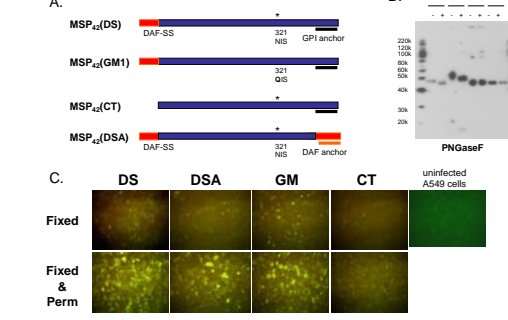


Figure 6. PMSP1₄₂ Antigen Optimization (In Vitro Data). (A) Schematic representation PMSP1₄₂ antigens evaluated in the adenovector delivery system. CT is the unmodified C-terminal 42kd fragment of PMSP1. DS contains a signal sequence from the delay activating factor (DAF). DSA contains both the DAF signal sequence and GPI anchor. GM contains the DAF signal sequence and a mutation in the PMSP1₄₂ glycosylation site (B) Adenovectors expressing the various forms of PMSP1₄₂ were used to infect A549 cells and the cell lysates were subjected to digestion with PNGase F and immunoblot analysis. (C) The cellular localization of the PMSP1₄₂ antigens was analyzed by immunofluorescence microscopy. 24 hours post infection cells were either fixed with 4% paraformaldehyde to identify proteins that are localized at the cell surface, or fixed and permeabilized with 0.1% saponin to identify both intracellular and extracellular proteins.

Conclusions:

- PMSP1₄₂ antigens are not efficiently expressed at the cell surface
- DS and DSA versions of PMSP1₄₂ are glycosylated

Figure 8. Functional Antibody Response in Rabbit Following Adenovector Delivery of PMSP1₄₂

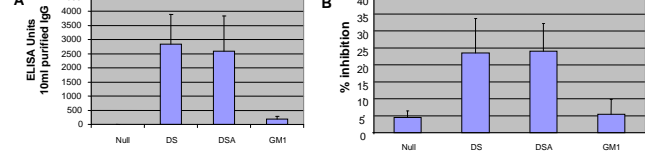


Figure 8. Immunogenicity of Adenovectors expressing various forms of PMSP1₄₂. New Zealand white rabbits received two intramuscular immunizations of 1 X 10¹⁰ pu of adenovector (A) Total antibody titers were measured by ELISA. (B) Functional antibody levels were determined by GIA.

Figure 7: PMSP1₄₂ Immunogenicity in Mice

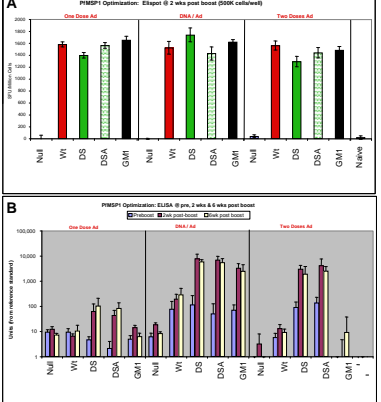


Figure 7. Immunogenicity of Adenovectors expressing various forms of PMSP1₄₂. BALB/c mice received intramuscular immunizations of 1 X 10¹⁰ pu of adenovector. (A) T cell responses were measured by ELISpot assays 2 weeks following the last administration of vector. (B) Antibody responses were measured by ELISA at various time points.

Figure 9. Bivalent Adenovectors Expressing both PMSP1₄₂ and PfAMA1 Induces Function Antibody Responses in Rabbits

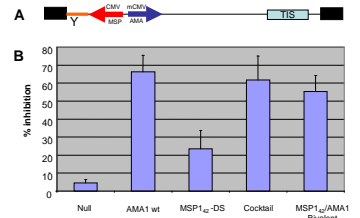


Figure 9. Immunogenicity of bivalent adenovectors expressing optimized forms of PMSP1₄₂ and PfAMA1. New Zealand white rabbits received two intramuscular immunizations of 1 X 10¹⁰ pu of bivalent adenovector. (A) Schematic of bivalent adenovector (B) Functional antibody levels were determined by GIA.

Conclusions

- Adenovectors induce robust T cell and functional antibody responses
- Adenovectors induced functional antibody responses against both PfAMA1 and PMSP1₄₂ antigens, although most of GIA activity was directed against PfAMA1
- Cell surface expression of *plasmodium* antigens was important for generation of potent antibody responses
- PfAMA1 glycosylation did not reduce T cell or functional antibody responses
- One adenovector, termed a bivalent adenovector, can be engineered to deliver two *plasmodium* antigens from two expression cassettes located in the adenovector genome
- Bivalent adenovectors can generate potent T cell and antibody responses against both antigens