Investigating the Effects of Increasing Anti-AMA1, Anti-MSP1, and Anti-MSP2 In Preventing Malaria Incidence

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Investigating the Effects of Increasing Anti-AMA1, Anti-MSP1, and Anti-MSP2 In Preventing Malaria Incidence

April Skipper, College of Natural and Health Sciences, Southeastern University

Abstract:

Malaria is a life-threatening illness that 3.2 billion people, half of the world's population, are at risk of contracting. In 2015, there were 214 million malaria cases and 438,000 deaths caused by the disease. It is caused by Plasmodium parasites which infect humans through the bite of the Anopheles mosquito. The four species of Plasmodium that are known to cause malaria are P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi. The symptoms of malaria greatly resemble symptoms of a common cold, so accurate diagnosis can be a challenge. Symptoms commonly include fever, headache, vomiting, but can progress to include anemia, respiratory distress, cerebral problems, multi-organ failure, and death in cases of severe malaria.

The most available and effective treatment for malaria currently is artemisinin-based combination therapy (ACT). Due to widespread misdiagnosis and overuse of effective drugs, many strains of Plasmodium have become resistant to common antimalarial drugs. Multi-drug resistant strains may become a larger problem, so development of an effective malaria vaccine is an important step in malaria control and hopeful eradication.

In some individuals, immunity against severe malaria can be acquired by the age of 5 years. The rapid development of immunity against severe malaria shows that a vaccine targeting young children is feasible. The exact immunological basis of this protective immunity is still unknown. It was seen through epidemiological studies that the antibody anti-AMA1 was present in a larger
proportion in immune individuals than in non-immune individuals. It was also found that merozoite surface proteins 1 and 2 (MSP1 and MSP2) seem to be major targets of antibody-mediated complement-dependent inhibitory activity. This study aims to test whether these antibodies do successfully prevent malaria infection in mouse models, both individually and in combination.

**Specific Aims:**

1. To determine whether an increase in anti-AMA1, anti-MSP1, and anti-MSP2 antibodies independently decrease the incidence of malaria in mouse models.

   - Mouse models will be injected with one of these three antibodies
   - Mice will be put in a container where *Plasmodium*-carrying mosquitoes are added
   - Blood smears will be taken from mice and analyzed for the presence of *Plasmodium*

2. To determine whether an increase in a combination of anti-AMA 1, anti-MSP1, and anti-MSP2 decrease the incidence of malaria in mouse models.

   - Mouse models will be injected with a combination of the three antibodies
   - Mice will be put in a container where *Plasmodium*-carrying mosquitoes are added
   - Blood smears will be taken from mice and analyzed for the presence of *Plasmodium*

3. To determine whether injection of an antibody formulation in pregnant mice will induce malaria immunity in their pups.

   - Pregnant mouse models will be injected with the most effective antibody combination
- Mouse pups will be born and put in a container where *Plasmodium*-carrying mosquitoes are added.

- Blood smears will be taken from mice and analyzed for the presence of *Plasmodium*. 
Introduction

Malaria Statistics:

As of January 2016, 3.2 billion people are at risk for contracting malaria.\(^1\) These people live in areas of high malaria transmission, which includes 106 countries.\(^2\) In 2015 alone there were 214 million malaria cases with 438,000 deaths.\(^1\) Of these 438,000 deaths, 78% of them occurred in children under the age of 5.\(^3\) Although the death rate from malaria is steadily decreasing (Figure 1), malaria is still the leading cause of death and disease in many developing countries, with young children and pregnant women being the most affected groups due to their immunocompromised status.\(^2\)

![Percentage decrease in malaria death rate since 2000](Image)

**Figure 1.** Percentage decrease in malaria death rate between 2000 and 2015.\(^9\)
Of the countries affected, Sub-Saharan Africa carries a high share of the malaria burden. In 2015, 88% of all malaria cases and 90% of malaria deaths were in this area alone. Though malaria is still such a large health burden to half of the world's population, it is a preventable and curable disease. Many countries have eradicated or significantly reduced the incidence of malaria, which can be seen in Figure 2.

**Figure 2.** Estimated number of malaria cases in 2000 and 2015 by region. As shown here, most continents have significantly reduced their malaria incidence rates. Africa still carries a very large portion of the malaria burden, followed by South-East Asia.
Why Malaria is Still a Problem:

Though malaria is preventable and curable, it still remains a large global health problem for many reasons. Most affected areas include poor and underdeveloped regions. Though malaria is treatable, an infected patient would need to be diagnosed and begin treatment very quickly, which is nearly impossible in many remote areas. Even if the affected patient had access to local healthcare, the cost of visiting a physician, diagnostic tests, and medication is likely to be too high for the patient's family due to the poverty-stricken areas in which malaria is endemic.

In addition to these barriers, the symptoms of malaria greatly resemble many other sicknesses including the common cold. This causes a high level of misdiagnosis, which in the rapidly-progressing case of malaria, can easily result in death. Finally, malaria still remains a large problem due to the scarce availability of drug treatments and other preventative measures in the affected areas.

Life Cycle of Parasite:

Malaria is caused by the Plasmodium parasite which is spread to humans through infected female Anopheles mosquitoes, serving as malaria vectors. There are five species of Plasmodium known to cause malaria in humans. The first is P. falciparum, which is very prevalent in Sub-Saharan Africa, can cause severe malaria due to its rapid multiplication in the blood. P. vivax, found mainly in Asia, has a dormant liver stage that can reactivate months or years after inoculation in the body. P. ovale is found in Africa and western Pacific islands, and is similar to P. vivax. P. malariae has a three-day life cycle and can cause chronic infection that can last an entire lifetime. Finally, P. knowlesi is found in Asia and has a 24-hour replication cycle, making it rapidly progress to severe infection.
The parasite acts on two hosts: humans and the mosquitoes. Once the parasite has infected the mosquito, the mosquito transmits the parasite to humans through a bite in the form of sporozoites. In humans, parasites grow and multiply in hepatocytes before moving to erythrocytes. While inside erythrocytes, parasites continue to grow and begin destroying the erythrocytes they occupy, releasing daughter parasites, or merozoites, that then continue with this cycle. A detailed representation of the *Plasmodium* life cycle can be seen in Figure 3.

As seen in Figure 3, the first step of infection takes place when a parasite-infected female Anopheles mosquito bites a human, injecting the parasite's sporozoites into the human host. This then begins the exo-erythrocytic cycle, where the sporozoites travel to the liver and infect hepatocytes. Inside hepatocytes, the sporozoites mature into schizonts. After fully maturing, the
schizonts cause the hepatocyte to burst, releasing merozoites into the blood stream. *Plasmodium vivax* and *Plasmodium ovale* have the special ability of keeping some of the parasite, known here as hypnozoites, in the liver cells which can cause a relapse by rupturing and releasing into the bloodstream weeks or years later. This is known as a dormant stage.\(^9\)

This then begins the erythrocytic stage of the parasite life cycle. In this stage, merozoites undergo asexual replication inside erythrocytes.\(^9\) As shown in step 5, merozoites are infecting red blood cells, where they become immature trophozoites. This is referred to as a ring stage because the parasite takes the shape of a ring inside the erythrocyte. The immature trophozoite now has two options, it can become a mature trophozoite or a gametocyte. Mature trophozoites turn into schizonts, which cause the erythrocyte to rupture, releasing more schizonts into the bloodstream. The schizonts then begin the erythrocytic stage again in a cyclical fashion as long as there are still erythrocytes to infect.\(^9\)

The immature trophozoites that mature into gametocytes enter into a sexual erythrocytic stage.\(^9\) These gametocytes are either male, microgametocytes, or female, macrogametocytes. If an infected human host receives a second bite from a female *Anopheles* mosquito, these male and female gametocytes will be ingested by the mosquito and will enter the sporogonic cycle, part C in Figure 3, inside the mosquito's stomach.\(^9\)

To begin this stage, microgametes enter into the macrogametes, which generates a zygote.\(^9\) The zygote becomes elongated and gains the ability to move on its own. These qualities give the zygote the ability to carry out its next function. At this point, the zygote is referred to as an ookinete. This ookinete inserts into the midgut wall of the mosquito, where it develops into an oocyst. An oocyst becomes much more round in shape than an ookinete. The oocyst then grows
and ruptures, which releases sporozoites into the mosquito host. These sporozoites enter into the mosquito's salivary glands, where they are primed and ready to enter a human host with the mosquito's next bite.\(^9\)

**Where Malaria Occurs:**

Malaria transmission is found in tropical and subtropical regions of the world.\(^{12}\) Many of these places are poor and underdeveloped communities.\(^2\) The main geological factor affecting malaria transmission is temperature.\(^{12}\) *Anopheles* mosquitoes require a relatively warm environment to survive and multiply.\(^{13}\) Plasmodium cannot complete its growth cycle in temperatures below 20° C.\(^{12}\) Even in countries where malaria is endemic, transmission does not occur or significantly decreases during colder seasons, at high altitudes, and in deserts.\(^{12}\) Regions closer to the equator have more intense transmission and longer transmission seasons.\(^{12}\)

As mentioned before, Sub-Saharan Africa experiences the bulk of the malaria burden, with 88% of all malaria cases in 2015 occurring there.\(^4\) In temperate areas of western Europe and the United States, public health measures and economic development have eliminated malaria.\(^{12}\) However, these areas do have *Anopheles* mosquitoes, so reintroduction of malaria is always a risk.\(^{12}\) Areas where malaria transmission occurs can be seen in **Figure 4.**
Drug Treatments

**Drugs Against Plasmodium:**

Drugs used to treat *P. falciparum* malaria target the intraerythrocytic stage of the parasite.\(^1\)\(^4\) This stage of the parasite life cycle is where symptoms first present in an infected patient.\(^1\)\(^4\) Three broad classes of drugs are used are quinolines, antifolates, and artemisinin-combination therapies.\(^1\)\(^4\) Quinolines act as hemozoin inhibitors.\(^1\)\(^5\) Heme is toxic to *Plasmodium*, so the parasite must use hemoglobin proteolysis for nutrients.\(^1\)\(^5\) The *Plasmodium* food vacuole lyses hemoglobin, producing hemozoin.\(^1\)\(^5\)

![Figure 4. Map of malaria transmission.\(^1\)\(^2\)](image-url)
Antifolates block the folic acid synthesis pathway by binding to tetrahydrofolate dehydrogenase more tightly than to the host enzyme. This prevents the parasite from using pyrimidines synthesized in the host, forcing it to create its own. Artemisinin-combination therapies are widely the most commonly used treatment for *P. falciparum* infection. Artemisinins come from a Chinese herb called *Artemisia annua*, and are suspected to work through a mechanism similar to that of quinolines, reversing the conversion of heme to hemeozoin. They carry out their functioning in the parasite mitochondria by forming peroxide bridges. Peroxide bridges can be cleaved by iron ions to form free radical oxygen species. These species would then go on to attack the parasite merozoites. Though the three broad classes of antimalarial drugs were discussed, there are seven more specific classes, which are seen in Figure 5.

![Figure 5. Seven classes of antimalarial drugs and the structures of the compounds in each class.](image)
**Occurrence of Antibiotic Resistance:**

The first occurrence of antibiotic resistance in malaria drugs occurred in 2000, when mutations within *P. falciparum* conferred resistance to chloroquine in Colombia and Thailand.\(^1\)\(^8\) Before this resistance, quinolines had been used to fight malaria since the 17th century.\(^1\)\(^8\) Chloroquine-resistant mutations have been spreading throughout most endemic countries since this first occurrence.\(^1\)\(^9\) In response to this resistance, sulfadoxine-pyrimethamine largely replaced chloroquine in areas with chloroquine resistance.\(^2\)\(^0\)

Unfortunately, resistance to sulfadoxine-pyrimethamine rapidly evolved and now also occurs in most endemic regions.\(^2\)\(^0\) To counter this resistance, artemisinin-based combination therapies began to be largely used.\(^2\)\(^1\) These have higher production costs, which is a significant barrier to translation into endemic areas.\(^2\)\(^2\) Antibiotic resistance in parasite DNA evolves very quickly, so development of affordable and effective drugs is a major problem in fighting the spread of the disease.\(^2\)\(^3\)

**The Search for a Vaccine Candidate**

**Overview of Vaccine Research:**

In 2007, an official research and development agenda for malaria eradication was established, with the goal of completely eradicating the disease.\(^2\)\(^4\) One large strategy to achieve this was to support the development of a vaccine. Much research has been put into the search for a malaria vaccine, and the vaccine RTS,S/Mosquirix has been the most advanced thus far.\(^2\)\(^5\) This vaccine protects against *P. falciparum* and has been approved by the European Medicines Agency (EMA) for children aged 6 weeks to 17 months.\(^2\)\(^6\)
In the development of a vaccine, finding the best target, a good production and delivery method, and appropriate formulation and adjuvants are very important and has proven difficult.\textsuperscript{27,28} Achieving large-scale production of protein antigens with the correct folding is necessary for achieving highly specific high titres in humans, which has proven to be another difficult task.\textsuperscript{29-31} 

*Escherichia coli*, *Lactococcus lactis* bacterium models, *Baculovirus*, yeast, plant-based systems, and algae are among the many expression systems being used for recombinant antigens.\textsuperscript{32-37} Another delivery route being researched is particle-delivery technology.\textsuperscript{38} This would include the use of virus-like particles and nanoparticles.\textsuperscript{39} An emerging field in vaccine research is using DNA vaccine technology, which is still very early in its development.\textsuperscript{40,41} Another route being researched is providing immunity through the use of different antigens. Most of these are still in the preclinical stage but are being considered for their first test in humans.\textsuperscript{42} Two different recombinant strategies targeting Pfs25 antigen are in phase I clinical trials and are the only candidates targeting sexual, sporangonic, or mosquito-stage antigens.\textsuperscript{43,44} Even so, these are only a small portion of the many malaria vaccines being tested.\textsuperscript{45} 

**RTS,S:**

RTS,S is a vaccine made of a liposome-based adjuvant (AS01) and hepatitis B virus surface antigen (HBsAg) virus-like particles that incorporate a portion of the *Plasmodium falciparum*-derived circumsporozite protein (CSP) genetically combined with HBsAg.\textsuperscript{62} If approved by regulatory authorities, this vaccine would be used for infants between 6 weeks and 17 months old. It also will have been in-the-making for more than 30 years. A timeline listing the major events in the creation of RTS,S can be seen in Figure 6.
RTS,S targets the circumsporozite protein, which plays a large role in maturing the *Plasmodium* oocyst in the midgut of the mosquito. Only a small portion of sporozoites in the salivary glands of the mosquito are inoculated into the bloodstream of the human. Due to this parasite numerical bottleneck, it is thought that the parasite is most vulnerable to immune attack as it switches between the mosquito host and human host. This provides a good opportunity to induce a novel immune response through active immunization. The circumsporozite protein is located on the surface of the parasite sporozoite and is responsible for forming a coat on the parasite surface.

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**Figure 6.** Timeline of the development of RTS,S spanning more than 30 years. GlaxoSmithKline (GSK) and the Walter Reed Army Institute of Research (WRAIR) initiated this development in 1984, and the PATH Malaria Vaccine Initiative (MVI) was established in 2001. RTS,S entered clinical trial Phase III in 2009 and was completed in 2014.
In its most recent Phase III clinical trials, RTS,S was evaluated in eight different African countries.\textsuperscript{62} Vaccine efficacy has proven to be modest with varying ranges of efficacy depending on the trial site tested. Some sites showed up to 87.6\% efficacy, while others showed close to 0\% efficacy.\textsuperscript{62}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7.png}
\caption{Vaccine efficacy and impact against clinical malaria for Phase III trial in eight African study sites.\textsuperscript{62} (A) represents children aged 5-17 months, and (B) represents infants aged 6-12 weeks. The R3C group received three doses of RTS,S and a control booster dose, while the R3R group received three doses and a booster dose of RTS,S.}
\end{figure}
Figure 7 shows final results for the phase III efficacy and safety trial MALARIA-055. This was a double-blind, randomized, controlled trial where participants ages 5-17 months or 6-12 weeks received either three doses of RTS,S and a booster, three doses of RTS,S and a control vaccine, or only control vaccines throughout. It is noted that the number of cases of clinical malaria averted had a greater impact in sites with higher malaria burden. Figure 7 shows vaccine efficacy and impact against clinical malaria. A Forest plot is shown of efficacy with 95% confidence intervals for the group that received four doses of RTS,S (R3R). A bar graph is shown for the number of cases averted for groups that received three doses (R3C) and four doses (R3R) of RTS,S by site with A representing the 5-17 months age group and B representing 6-12 weeks age group. Although the vaccine efficacy at some sites is promising, there is still a great deal of uncertainty about just how effective RTS,S would be overall due to the large discrepancy in efficacy rates across all testing sites. Thus, it remains necessary to explore more vaccine candidates.

Experimental Design

**Background:** Epidemiological studies have shown that immunity against severe malaria is acquired by the age of 5 years in areas with intense malaria transmission. In these same areas, immunity against uncomplicated malaria is usually achieved by adulthood, while immunity against asymptomatic infection is never achieved. The rapid acquisition of immunity against severe malaria supports the claim that a malaria vaccine targeting young children could be very effective. Antigens found on the surface of infected erythrocytes (iRBC) inhibit sequestration of iRBCs, and promote opsonization of iRBCs for phagocytic attack. It was found through epidemiological data that antibodies to apical membrane antigen 1 (AMA1) significantly reduce the odds of developing severe malaria in children 0-5 years old, suggesting
that it is a viable vaccine candidate for young children.\textsuperscript{54} It is also suggested that passively transferred maternal IgG contributes to protective immunity.\textsuperscript{54}

The mechanism through which many antibodies mediate immunity to malaria has long barred vaccine development. It has recently been found that acquired human anti-malarial antibodies promote complement deposition on the Plasmodium merozoite.\textsuperscript{55} This plays a large role in inhibiting erythrocyte invasion through C1q fixation and activation of the classical complement pathway.\textsuperscript{55} Epidemiological data suggests that inhibitory activity was mainly mediated by C1q fixation with merozoite surface proteins 1 and 2 (MSP1 and MSP2) being the major targets.\textsuperscript{55} This suggests that antibody-mediated complement-dependent inhibitory activity could be induced by immunization with a merozoite surface-protein vaccine.\textsuperscript{55}

\textit{Apical Membrane Antigen 1:}

Apical membrane antigen 1 (AMA1) is a transmembrane protein in the Apicomplexa phylum and is thought to help shape the tight junction formed between the \textit{Plasmodium} parasite apex and the host cell.\textsuperscript{67-73} This tight junction formed between the \textit{Plasmodium} parasite and host erythrocyte can be seen in \textbf{Figure 8}. AMA1 has a cytoplasmic tail which is reported to bind to aldolase. The ectodomain of AMA1 binds in parasite extracts to the rhoptry neck 2 (RON2) protein.\textsuperscript{70-72} RON2 proteins localize at the tight junction where the parasite inserts into the host cell membrane.

Antibodies that inhibit the AMA1-RON2 interaction reduce host cell invasion by \textit{Plasmodium} merozoites.\textsuperscript{74-76} It was shown that AMA1-deficient merozoites displayed a three- to five-fold decrease in overall invasion efficiency in penetrating host cell erythrocytes.\textsuperscript{77} Also, a large body of work shows that antibodies to AMA1 are effective in blocking erythrocyte invasion.\textsuperscript{78-80} This
effect may also reduce sporozoite invasion of hepatocytes.\textsuperscript{81} A study found that strategies targeting only AMA1 would not be effective.\textsuperscript{77} Thus, a vaccine targeting a combination of AMA1 and another target may prove to be effective in every stage of the parasite life cycle.

\textbf{Figure 8.} A \textit{Plasmodium} merozoite (Mz) forming a tight junction (TJ) with a host red blood cell (RBC) in order to invade the RBC and continue its parasitic life cycle.\textsuperscript{82}

\textit{Merozoite Surface Protein 1:}

Merozoite surface protein 1 (MSP1) is found on the surface of the merozoite in the \textit{Plasmodium falciparum} parasite and is implicated in the process of merozoite invasion of the erythrocyte.\textsuperscript{83,84} Among the several events driving erythrocyte invasion is merozoite binding to sialic acid residues on erythrocyte receptors.\textsuperscript{117} MSP1 is required for attachment of merozoites to the
specific host receptor named Band 3. MSP1 is also required for normal parasitic
development. MSP1 is synthesized as a precursor and then processed in two steps. The
primary step produces a complex of four fragments present on the merozoite surface, and the
secondary step at invasion results in the shedding of the complex from the surface. After this
shedding, only the C-terminal domain remains on the parasite surface by a
glycosylphosphatidylinositol moiety. This C-terminal domain remaining is well conserved and
contains two epidermal growth factor-like (EGF-like) domains that play a large role in merozoite
invasion in erythrocytes. Thus, antibodies that target this EGF-like domain on the C-terminal
motif of MSP1 would prevent host invasion.

A ribbon diagram of the C-terminal domain containing EGF-like domains from Plasmodium
falciparum MSP1 can be seen in Figure 9. Several studies show that the protective immune
responses against P. falciparum MSP-1 in mice are directed against the C-terminal domain. A
possible mechanism for inhibition of merozoite invasion has been proposed to be through the
inhibition of MSP-1 processing by conformation-specific antibodies.
Merozoite Surface Protein 2:

Merozoite surface protein 2 (MSP2) is a blood-stage protein that is essential for completion and viability of the *Plasmodium* life cycle.\(^{94-97}\) It has been found to induce specific antibodies that are active against parasite merozoites and are associated with protection in malaria endemic areas.\(^{98-100}\) It is a glycosylphosphatidylinositol-anchored protein found on the merozoite surface and has 200-250 amino acids, all encoded by one exon on chromosome 2. MSP2 has conserved N and C terminal (C) regions flanking a polymorphic central repeat region.\(^{101}\) A non-repeat semi-conserved dimorphic (D) region defines two allelic families of MSP2. These families are 3D7 and FC27.\(^{102}\)

A specific semi-immune antibody against MSP2 is cytophillic IgG3. IgG3 along with IgG1 antibodies are thought to play a role in antibody-mediated mechanisms of parasite clearance.\(^{103-106}\)

In a phase I clinical trial, a recombinant vaccine candidate containing both 3D7 and FC27
forms of MSP2 showed that subjects elicited antibodies that were specific for both forms of MSP2 and active in inhibiting parasite growth in antibody-dependent cellular inhibition. Thus, both allelic forms of MSP2 remain a possible vaccine candidate in a combination vaccine. The crystal structure of anti-MSP2 fragment complexed with both allelic forms of MSP2 can be seen in Figures 10 and 11.

Specific Aim 1:

The first specific aim of this study is to determine whether independently increasing each antibody identified will decrease the incidence of malaria. We will begin with four groups of healthy humanized female mice. Female mice will be used because they are less aggressive, smaller, and less expensive than male mice. Mice used will be three weeks old. By this age, mice can open their eyes, have mobility, and can be successfully removed from their mothers. Female mice reach sexual maturity by the age of six weeks. Therefore, female mice between
the ages of three weeks to six weeks will closely represent the target age group for a vaccine in humans.\textsuperscript{115} Purchasing three week old mice will allow them to be viable for this study for three weeks. This will ensure that data can still be accurately collected and trials can be repeated if necessary.

The process of humanizing a mouse to be used as a malaria mouse model can be seen in Figure 12. The specific strain used will be the NOD/SCID/IL2Rγnull strain. This strain has shown to be the most effective in previous malaria studies using mouse models.\textsuperscript{113} In order to replicate the immunodeficiency that young children in malaria-endemic areas will be, the mice will be injected with liposomal-clodronate formulations.\textsuperscript{113} This injection will deplete murine phagocytic cells, and it has been shown that this produces a mouse model of malaria in which 100\% of infected mice show development of \textit{P. falciparum}-infected erythrocytes.\textsuperscript{114}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{humanizing_mice.png}
\caption{Humanizing a mouse to be used as a mouse model for malaria. A humanized mouse is a model where a mouse gene is replaced by the human gene. Then, the human protein domain is expressed while the mouse protein domain is suppressed in all cells and tissues.\textsuperscript{110}}
\end{figure}
One group will receive an injection of anti-AMA 1 antibodies, the second group will receive anti-MSP1 antibodies, the third group will receive anti-MSP2 antibodies, and the fourth group will receive an injection of phosphate buffer solution (PBS) to serve as a control group. All antibody formulations will be diluted in PBS. These groups and their injections are listed in the table below. Each group will include 25 mice, for a total of 100 mice to be used in specific aim 1.

<table>
<thead>
<tr>
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<th>Anti-AMA1</th>
<th>Anti-MSP1</th>
<th>Anti-MSP2</th>
<th>PBS</th>
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<tr>
<td>Group 1</td>
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<td>Group 4</td>
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The mice will then be placed in a large enclosure with four separate sections for each mouse group. The enclosure will be sectioned by a piece of hard plastic containing several small holes. Female *Anopheles* mosquitoes carrying the *Plasmodium falciparum* parasite will then be released into the mouse enclosures. The small holes in the plastic interior walls of the enclosure will be large enough for mosquitoes to pass freely through. A representation of this enclosure can be seen in Figure 13.
After a period of time, the mosquitoes will be collected and the mice will be analyzed for the presence of a malaria infection. Blood smears will be collected from each mouse at three separate time intervals. Blood smears will first be taken immediately after mice are brought out of the enclosure, then again one day later, and finally one week later. Each sample will be stained with Giemsa stain, which will give parasites a distinct appearance, as seen in Figure 14. Samples will then be microscopically analyzed for the presence of *Plasmodium falciparum* parasites.
The DNA from these blood smears will then be extracted and PCR will then be performed to test for the presence of parasite nucleic acids. If a sample shows the presence of a parasite through microscopy or from PCR, that mouse will be considered to have tested positive for malaria infection. These results will then be graphed and analyzed to determine the effect of increased antibody levels on the incidence of malaria infection.

**Specific Aim 2:**

While specific aim 1 determined the effect of the three antibodies independently, specific aim 2 will focus on determining the effect of using a combination of antibodies on malaria incidence. In this experiment, mice will be divided into 5 groups, each receiving a different combination of antibody injections. These combinations will be anti-AMA 1 and anti-MSP1, anti-AMA 1 and anti-MSP2, anti-MSP1 and anti-MSP2, and anti-AMA 1, anti-MSP1, and anti-MSP2. The final

**Figure 14.** Blood smears stained with Giemsa stain. Giemsa stain gives parasites a distinct appearance inside the red blood cells, giving a clear indication of malaria infection.
fifth group will receive an injection of PBS and will serve as the control group. All antibody formulations will be diluted in PBS. Each group and their injections are listed in the table below. Each group will include 25 mice, for a total of 125 mice to be used in specific aim 2.

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<th>Anti-AMA 1</th>
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<th>Anti-MSP2</th>
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<tr>
<td>Group 1</td>
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Mice will then be placed in separate attached enclosures, parasite-carrying mosquitoes will be released in enclosures, and blood samples from mice will then be taken at three separate time intervals as in specific aim 1. Blood smears will then be analyzed using microscopy and PCR as before, and results will be graphed and analyzed.

**Specific Aim 3:**

Specific aim 3 will test whether the antibodies analyzed previously can cross the placental barrier of a pregnant mother and induce acquired immunity in her fetus. The most effective antibody combination, as determined in specific aim 2, will be injected into pregnant female NOD/SCID/IL2Rγnull humanized mice. As a control, one group of pregnant mice will be injected with only PBS. Each group will contain 25 pregnant mice, for a total of 50 mice. The pregnant mice will then carry their pups to term and will give birth naturally. The pups will then be permitted to grow and mature normally. At three weeks of age, the pups will be placed in the
same enclosure as specified earlier, and female *Anopheles* mosquitoes carrying the *Plasmodium* parasite will be placed in the enclosure. As before, blood samples will be taken at three different time periods and will be analyzed by microscopy and PCR. Results will then be graphed and analyzed.

**Proposed Results**

In specific aim 1, I hypothesize that we will find a statistically significant difference between the mice with no antibody injection and the mice with anti-AMA1 antibody injection. In addition, I predict that a slightly statistically significant difference will be found both between the control group and the anti-MSP1 group, and between the control group and the anti-MSP2 group. These findings would suggest that anti-AMA1, anti-MSP1, and anti-MSP2 antibodies contributes to malaria immunity in some way.

In specific aim 2, I hypothesize that a slightly significant difference will be found between the control group and each group containing two antibody injections (anti-AMA1 and anti-MSP1; anti-AMA1 and anti-MSP2; anti-MSP1 and anti-MSP2). In addition, I predict that a very large statistically significant difference will be found between the control group and the group receiving an injection of all three antibodies. These findings would suggest that the three antibodies tested not only contribute to malarial immunity, but that they also work synergistically. AMA1, MSP1, and MSP2 are all present of the surface of the parasitic merozoite. By adding antibodies specific for each of these antigens to a person's adaptive immunity arsenal, the immune system will be more than prepared to fight against all merozoites that enter the body if infected by a mosquito.
In specific aim 3, I hypothesize that a statistically significant difference will be found between the group of mice whose mothers were injected with antibody formulation and the control group. This hypothesis is based on the fact that IgG antibodies are capable of passing the placental barrier. This finding would suggest that the antibody formulation has the ability to cross the placental barrier and induce acquired immunity in an unborn fetus. This finding would be consistent with a finding from a previous study that suggests that some children born to mothers infected with *Plasmodium* while pregnant are born with B cells capable of secreting IgM and IgG antibodies to antigens AMA-1, MSP-1, and MSP-2. If these results align with that found from the previous study, it would show that *Plasmodium falciparum* antibodies do cross the placenta and activate fetal B and T cells in utero. This would be promising for the introduction of a malaria vaccine in malaria-endemic countries because the vaccine could target two groups (young children and pregnant mothers) rather than just young children.

**Future Studies**

If the results show that the three antibodies tested reduce the incidence of malaria, then the antibodies will serve as another possible target for a vaccine candidate. Further study into the degree of malaria protection each antibody or a combination of the antibodies produces should be done. Experimentation to determine the most effective dosage and concentration of antibodies should also take place since too low of a dose could produce ineffective results.

Other studies should revolve around testing these antibodies’ efficacy among various age groups, genders, and specific malaria risk groups (such as pregnant women or immunocompromised people). The efficacy rate among these different groups is very likely to differ from one another.
Another important research route to explore is to elucidate the mechanisms through which these antibodies inhibit parasitic infection.

References


26. EMA. First malaria vaccine receives positive scientific opinion from EMA—London, The European Medicines


63. Kappe S., Vaughn A., Boddey J., and Cowman A.: That was then but this is now: malaria research in the time of an eradication agenda. Science 2010; 328: pp. 862-866


110. Humanized Mouse Model | Knockin Engineering | genOway. 


112. Axel G. Griesbeck, Jörg Neudörfl, Achim Hörauf, Sabine Specht, and Angela Raabe. Antimalarial Peroxide Dyads from Natural Artemisinin and Hydroxyalkylated 1,2,4-Trioxanes. Journal of Medicinal Chemistry 2009 52 (10), 3420-3423. DOI: 10.1021/jm9002523


