Malaria Animal Research

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Malaria is not a disease that developed countries worry about because the risk of infection is so low that even if one is infected there are available and affordable medications to treat this disease. On the other hand, people who live in third world countries, such as sub-Sahara Africa and parts of Asia, deal with continuous infection that often kills many of their children. Malaria has been a big public health issue because it kills about 1-2 million people per year, and about 40% of the people living in these endemic areas are at risk of getting infected (Lamb et al., 2006). This infection is a specie-specific disease, which means that different malaria species infect only a given host specie. Even though there are multiple malaria species that infect animals, they all belong to the same genus, *Plasmodium,* including the two that infect humans, *Plasmodium falciparum and P. vivax* (Lamb et al., 2006). Malaria is transmitted via a vector-the mosquitoes-when the female takes a blood meal from an infected host (Burns et al, 1999). After the mosquito bites the host, the gametocytes-which are the non-proliferating forms of the parasite found in the blood meal-start to infect the mosquito. The gametes will fertilize and develop into zygotes within the vector, where the parasite will then establish infection and begin to proliferate (Kaushal et al., 1983). When an infected mosquito takes a blood meal from a host that is not infected, it injects the sporozytes into the host's blood stream which then migrate to the liver where the parasite uses the hepatocytes to multiply and form the merozoites (Lamb et al., 2006). The merozoites are responsible for rupturing the infected hepatocytes as they exit the liver cells to migrate into the blood stream where the asexual cycle can continue, which is the stage responsible for the clinical symptoms seen in patients (Lamb et al., 2006 and Kaushal et al., 1983). The continuous flow of parasites in the host's blood stream initiates the immune response and can sometimes go into over-drive as a response to the parasitemia which can result in worsening of the infection instead of clearing it (Lamb et al., 2006). This disease is very complicated since it has a complex life cycle that requires both the vector and the host in order to reproduce.

Since malaria continues to be a major health problem in third world countries and it highly infects and kills children, it has become a major interest of research. Malaria research has been focused in trying to find a way to control the disease, prevent transmission, and create a vaccine in order to eradicate this disease. This research is trying to attack different aspects of the parasite life cycle such as the pre-erythrocytic stages of asexual parasites to target the sporozoites in the liver using anti-malaria vaccines, and also looking at antigens from the sexual stages to stop transmission from mosquito to the host using transmission blocking vaccines (Burns et al., 1999 and Good, 2011). This paper will provide a summary of the research that has been done in three malaria animal models: birds, mice, and monkeys. They all have made unique contributions to malaria research considering that one of the major problems with malaria is that the parasite is specie-specific. However, the positive aspect of having a specie-specific infection is that these three animals do not have to be manipulated in order to allow infection to take place; they are all natural host to their specie-specific malaria strain. These three animals have helped scientist to better understand the malaria parasite and disease.

One of the birds used in malaria research is the White Leghorn chicken, specie G*allus domesticus*, which can be infected with *Plasmodium gallinaceum*. This animal model has been mainly used to identify monoclonal antibodies that can be potential targets for a transmission blockage vaccine (Carter 2001). Carter's lab showed that malaria transmission blocking immunity can be achieved in the avian model of malaria. Their research showed that inoculation of White Leghorn chickens using gametes of malaria parasites induced an immune response that could protect the host from a subsequent malaria infection of the same malaria strain (Carter 2001). Carter also looked into the possible mediators of immunization-induced malaria transmission to test the blocking effects using the serum of infected birds (Carter 2001). The results showed that the blockage was mediated by antibodies against the surface protein antigens that were present in the midgut of the mosquito after taking the blood meal and infecting the bird (Carter 2001). The antigens that were found to be present in the zygotes of the malaria parasite were two proteins Ps25 and Ps28, which are unique to *Plasmodium* and have analogous proteins in the one of the malaria specie that infects humans, *P. falciparum* (Carter 2001).

Jasinskiene's lab also studies birds and malaria. They focus in a single chain antibody that can bind to the avian parasite *P. gallinaceum* and has been shown to reduce the intensity of sporozoite infection in the mosquito's salivary gland (Jasinskiene et al., 2007). The monoclonal antibody recognizes the circumsporozoite protein (CSP) of *P. gallinaceum* and blocks invasion of sporozoites in the salivary gland after the mosquito takes a blood meal (Jasinskiene et al., 2007). Transgenic mosquitoes have been created to express the monoclonal antibody (MAbs) by injecting a transformation vector and helper plasmid into the mosquito embryos, these are then heat shocked for 24 hours to stimulate the over-expression of MAbs (Jasinskiene et al., 2007). After the mosquitoes are engineered, the infected chickens are used to feed the mosquitoes to test if the over-expression of the MAbs can inhibit the development of the parasite within the mosquito. In conclusion, all mosquitoes showed a reduced number of parasites after they took a blood meal from an infected host, suggesting that the malaria parasite development was successfully blocked in the transgenic mosquitoes that expressed the MAbs (Jasinskiene et al., 2007).

Shahabuddin et al. targets parasite development and possible transmission inhibition. They use defensin, an antibacterial peptide in chickens, infected with *P. gallinaceum*, to see if this peptide can have an effect on the development of the malaria parasite (Shahabuddin et al., 1998). Their results show that expression of defensin does not affect the zygote stage of the parasite, but it does affect the matured stages such as the oocysts (Shahabuddin et al., 1998). The 7 day consecutive injection of defensin treatment into the mosquito showed that the sporozoites and older parasites in the mosquito can be killed, stopping the life cycle of the parasite in the vector. Results showed abnormalities in the oocysts found in the mosquitoes after they fed from infected chickens, suggesting a possible inhibition in the parasite cycle once it enters the vector, supporting the idea that defensin can potentially be used to inhibit transmission (Shahabuddin et al., 1998). Another group, Kokoza's lab, studies defensin as well as cecropin to create a transgenic mosquito to over-express both peptides (Kokoza et al., 2010). Kokoza et al. uses the transgenic mosquito to see if the overproduction of these two peptides can decrease parasitemia after they are fed from infected chickens. Results showed that after the mosquitoes had a blood meal, a decrease number of oocysts where seen in the midgut of the mosquito, suggesting that the over-expression of both

peptides can indeed inhibit the development of oocysts. The results also showed that the number of sporozoites decreased in the mosquito's salivary gland (Kokoza et al., 2010). In conclusion, the use of peptides such as defensin and cecropin, which are produced during the infection of chickens with *P. gallinaceum*, can be over-expressed in order to block the transmission of the parasite from mosquito to host by limiting proliferation and development of the parasites within the mosquitoes (Kokoza et al., 2010).

Most of the research using avian malaria focuses on looking at the antigens produced during the different stages of this parasitic infection to find stage-specific antigens for vaccine candidates. Since it is difficult to study the sexual stages of malaria in any animal model due to the parasite life cycle in the mosquito, birds are used to study malaria gametes and the antibodies produced against the surface proteins of these gametes. The avian malaria model is mainly used for the transmission blocking vaccine studies and to challenge the mosquitoes to test the immune response after infection. The disease seen with avian malaria is not as closely related to the one seen in humans; therefore, using the avian malaria model to understand the disease course is not ideal. On the other hand, the similarity between the two malaria species and the homology of parasite surface antigens between *P. falciparum* and *P. gallinaceum* has played a crucial role in testing and developing monoclonal antibodies for vaccine development. Chickens are not the ideal animal model to study disease symptoms or process, but it has helped in creating promising vaccines that can inhibit transmission of this disease.

The second animal model used to study malaria is the mouse, strains CBA and C57BL/6 which can be infected with *Plasmodium berghei*, *P. yoelii* or *P. chabaudi* and shows similar symptoms to those seen with *Plasmodium falciparum* in humans. Mice are a natural host when infected with their specie-specific malaria strain so they do not have to be altered or immunocompromised in order to be used for malaria research. Mice are the only animal model that has reproduced the cerebral malaria (CM) symptoms similar to those seen in humans infected with *P. falciparum* (Lamb el al., 2006). The immune response against malaria parasites during CM shows a measureable pro-inflammatory response in both mice and humans (Lamb el al., 2006). CM causes sequestration of infected red blood cells on the brain's

endothelium, damaging and swelling the brain vessel walls causing vascular hemorrhage which results in major organ failure and eventually death (Lamb el al., 2006). Lamb et al. studies the immunopathology of mice infected with malaria during this process. They used mice infected with *P. berghei* to see how the immune system reacts to defend the host from infection and observed that there is an increased pro-inflammatory immune response (Lamb el al., 2006). This immune response has the ability to go into over-drive which increases the pathology and symptoms during infection. The results from blood taken from infected mice, showed that there is an acute immune phase with increased levels of interleukin 1 and 6 (IL 1, IL6), and tumor necrosis factor α (TNF- α), and this innate immune response can trigger the activation of natural killer cells (NK) (Lamb el al., 2006). Lamb's lab suggested that the increased of TNF- α is implicated in the pathogenesis of malaria infection and is associated with the susceptibility of the host for cerebral malaria (Lamb el al., 2006). Another major finding from Lamb's lab was that the mice that were more resistant to CM showed to have a different immune response where they had a type 1 response involving IL2 and IFN γ followed by a type 2 response after 3-4 weeks of the type 1 response (Lamb el al., 2006). These results showed that the pro-inflammatory and cytokine response play a crucial role in controlling parasitemia in the infected host (Lamb el al., 2006). In conclusion, these results suggest that inadequate pro-inflammatory response leads to uncontrolled parasite multiplication rates which lead to damaging immunopathology and severe symptoms (Lamb el al., 2006).

Another laboratory that researches malaria in the murine model is Alaro et al. They use the malaria specie *P. yoelii* to infect mice, which show a similar immune response when compared to the human immune response to malaria infection (Alaro et al., 2010). They focus on vaccine development using the merozoite surface protein 1 and 8 (MSP1/8) to create a chimeric *Py*MSP1/8 vaccine. The subunit vaccine is designed to block merozoites from invading and replicating in the red blood cells because it can potentially limit the pathology caused by infection (Alaro et al., 2010). Blood samples from the mice were used to analyze the protective immune response after the mice where immunized twice at a 3 week intervals with the vaccine and the adjuvant Quil A (Alaro et al., 2010). After the mice were immunized they were challenged intraperitoneal with blood stage parasites of *P. yoelii*, taken from an

infected mouse donor (Alaro et al., 2010). The results showed that the mice vaccinated had a protective response of T cells and B cells and showed an antigen-specific response by producing high levels of protective antibodies that were sustained after infection (Alaro et al., 2010). Immunized mice also developed a lower grade parasitemia and were able to clear the infection when compared to control mice, which succumbed to the infection by day 12 (Alaro et al., 2010). In conclusion, the results showed that *Py*MSP1/8 vaccine induces a long-lasting protective response, mice cleared the blood-stage parasite from circulation, and infection was not detected for 2.5 months after it was cleared (Alaro et al., 2010).This vaccine shows to be promising because it has an efficient immune response and MSP8 protein is conserved in *P. falciparum*, which can be beneficial when taking the vaccine into human trials (Alaro et al., 2010).

Burns' lab studies the protective immune response using the liposome encapsulated vaccine (pAg-1), which is the blood-stage antigen pypAg-1 protein from *P. yoelii* associated with the erythrocyte membrane of the parasites (Burns et al., 1999). The mice were immunized three times at 3 week intervals using the adjuvant Quil A and blood was collected 1 week following the second immunization and 2 weeks following the last immunization, but before mice where challenged (Burns et al., 1999). After last blood collection, mice were challenged with *P. yoelii* parasitized erythrocytes and blood parasitemia was monitored; the blood results showed that vaccinated mice were able to reduce parasitemia levels after they peaked and successfully cleared the infection (Burns et al., 1999). Overall, the results showed that the pAg-1 vaccine contributed to the protection against infection by inducing an immune response against blood-stage parasites by producing the antigen pypAg-1 associated with infected erythrocytes (Burns et al., 1999). This vaccine could be another potential candidate to use against *P. falciparum* since this antigen has a known homologous in this malaria strain (Burns et al., 1999).

Burns' lab also studies the immunity against *P. chabaudi* using a protein expressed on the surface of the blood-stage merozoite as the vaccine target (Burns et al., 2003). The vaccine uses a recombination of two proteins, the apical membrane antigen 1 (AMA1) and merozoite surface protein 1 (MSP 1) (Burns et al., 2003). The vaccine is designed to prevent the merozoite invasion of erythrocytes (RBCs), and both

AMA1 and MSP1 are known to play a crucial role during this process (Burns et al., 2003). AMA1 antibodies have shown to inhibit merozoite invasion of RBCs in vitro and this antigen is conserved across all *Plasmodium* species (Burns et al., 2003). Mice were immunized subcutaneously with the vaccine and Quil A adjuvant twice at 3 week intervals, and blood was collected at days 8 and 10 post immunization, and mice were then challenged with parasitized erythrocytes (Burns et al., 2003). Results showed that pre-challenge blood samples had a dominant IgG response and after the 2nd and 3rd immunization mice had increased antigen specific levels of IgG supporting an adequate immune response (Burns et al., 2003). In conclusion, immunization of mice with recombinant vaccine of AMA1 and MSP1 induced high level of protection against *P. chabaudi* malaria resulting in a protective antigen response (Burns et al., 2003). This is the next generation for vaccines because it uses two antigens instead of just one, which creates a broader target against the parasite and not a narrow target when using only one antigen to target the parasite. Even though mice are not the closest in relation to humans, they have provided the most understanding about malaria. Mice research has provided the possibility to better understand the disease of cerebral malaria and the host's immune response during this process. Although vaccine results seen in mice is not an accurate expectation of what it could be seen in humans, the results can be used to understand the immune response and determine if they are good candidates to try them in an animal model closer to humans, such as non-human primates.

The third animal model is the Rhesus monkey, specie *Macaca mulatta*. The malaria species that infects this macaque is *Plasmodium knowlesi* and the focus of research is mainly for vaccine safety and efficacy. There are multiple laboratories studying vaccines for the prevention of malaria. Hamid laboratory studies blood stage candidate antigens that can be used to manufacture vaccines (Hamid et al., 2011). One of the potential candidates is the apical membrane antigen 1 (PkAMA1) which has a homologous antigen with *P. falciparum* (Hamid et al., 2011). The PkAMA1 is an invariant merozoite antigen that has been shown to protect rhesus monkeys from asexual blood stage when challenged with *P. knowlesi* (Hamid et al., 2011). In this experiment, the vaccine contained the antigen pkAMA1 and was used with a novel adjuvant to produced higher levels of antigen in the vaccinated macaque (Hamid et al.,

2011). The vaccine was administered three times to sedated monkeys on the upper legs on days 0, 28, and 56 and blood samples from these animals were taken 1, 7, and 14 days after the immunizations (Hamid et al., 2011). In order to see the effects of the vaccine, the monkeys were challenge with *P. knowlesi* and parasitemia blood levels were controlled daily by collecting blood using a finger prick (Hamid et al., 2011). Results from this experiment showed that the rhesus monkeys tolerated the vaccine well, no adverse events or reactions were seen, and white blood cells, basophils, and monocytes increased after vaccination supporting an effective immune response to the vaccine, but they were able to return to baseline levels preventing an over-drive immune response (Hamid et al., 2011). The pkAMA1 vaccine protected the rhesus monkeys from subsequent blood-stage challenges and allowed the immune system to control parasitemia levels (Hamid et al., 2011). The protection seen was antibody mediated, in which high levels of growth inhibiting antibodies were required to achieve protection, and the level of protection increased as the macaques were exposed to multiple doses of the vaccine (Hamid et al., 2011).

Kusi et al. studies vaccines targeting apical membrane proteins but they use the malaria strain, *P. falciparum*. They focus on studying safety and immunogenicity of a multi-allele vaccine formulation using PfAMA1 as the antigen (Kusi et al., 2011). They use rhesus macaques since they are closer related to humans and they have shown to have similar reactions to the vaccine adjuvants as humans have. PfAMA1 Diversity-Covering (DiCo) vaccine is a candidate that targets the blood stages of the malaria parasite, which is the stage responsible for the clinical symptoms of this disease, making it a good stage to target (Kusi et al., 2011). AMA1 is a protein found in the merozoites and sporozoites of malaria, and is translocated to the parasite surface membrane when it invades the red blood cells, and it plays a major role during the process of invasion (Kusi et al., 2011). This experiment used the DiCo vaccine in combination with two different adjuvants: CoVaccine HT or Montanide ISA 51, to try to induce a broad-strain antibody response in non-human primates (Kusi et al., 2011). The macaques were immunized intramuscularly in altering legs on days 0, 28, and 56, and blood was taken at multiple days to run chemistry, serology, and hematology (Kusi et al., 2011). On day 70, two weeks after the last injection, blood was used for in vitro testing to look for anti-AMA1 IgGs and for in vitro growth inhibition assays

(Kusi et al., 2011). The results showed that IgG levels of AMA1 alleles induced a good level of antibody response against the tested antigen, showing a peak in levels at day 70 (Kusi et al., 2011). The results showed that the multi-allele vaccine using PfAMA1, caused no adverse reactions in the monkeys, both adjuvants were tolerated well, and the levels of IgG against AMA1 increased after the third immunization (Kusi et al., 2011). These results suggest that DiCo is a promising vaccine candidate against malaria blood stages. Even though both adjuvants showed good results, using CoVaccine HT as an adjuvant showed a higher response against the parasites and induced higher titer levels of functional IgGs when compared to the adjuvant Montadine ISA 51 (Kusi et al., 2011).

Lumsden's lab studies a novel recombinant vaccine antigen using the vivax malaria protein (VMP001), originated from the circumsporozite protein of *P. vivax* (Lumsden et al., 2011). They used a second generation vaccine to identify the correct adjuvant that would induce a strong, long lasting immune response against malaria parasites (Lumsden et al., 2011). The two adjuvants studied were stable emulsion (SE) and a synthetic Toll-like receptor 4 (TLR4; named GLA-SE) to see which one would produce a better immune response when combined with the VMP001 vaccine (Lumsden et al., 2011). The rhesus macaques were immunized three times intramuscularly in alternating femoris muscles at weeks 0, 4, and 8, and blood was collected at baseline and 14 days post each immunization to observe the IgG response (Lumsden et al., 2011). The results from the blood samples showed that there was a significant increase in titers of IgG after the second immunization and the levels increased even more after the third immunization, suggesting that the second boost was beneficial and helped to increase antibody levels (Lumsden et al., 2011). The comparison of both adjuvants showed that using the TLR4 adjuvant (GLA-SE) had a better response than the SE adjuvant, which is expected since the TRL4 target stimulates both the innate and adaptive immune responses in the host (Lumsden et al., 2011). Even though, the TLR4 response in rhesus macaques mimics the one in humans, the TLR4 ligand differs from the one in humans, which can be a barrier when the adjuvant is being tested in people (Lumsden et al., 2011). The anti-VMP001 antibodies showed to have the ability to detect naïve protein on the surface of sporozoites after infection with *P. vivax* in vitro (Lumsden et al., 2011). Lumsden et al. also looked at the

immune response and expression of CD4, CD8, IL2, and IFN in the blood samples collected after immunization (Lumsden et al., 2011). This analysis showed that there is an increased levels of IL2 and IFN production, but IL2 and CD4 T cells were also detected after the 1st immunization was administered (Lumsden et al., 2011). The results showed that memory cells were formed and the T cells present had a predominant central memory phenotype after the third vaccination (Lumsden et al., 2011). This suggests that the vaccine antigen and immune response are successfully creating memory cells, which are crucial for protection against later malaria infections. The results from this experiment showed that this vaccine has a safe immunogenicity response in non-human primates and has a safe profile, suggesting that it can move into clinical trials.

The disease caused by *P. knowlesi* is similar to *P. falciparum* since they both result in high parasitemias and can cause death if the infection is left untreated (Hamid et al., 2011). Malaria parasite is specie-specific, but the immune response seen in infected non-human primates has been the closest to the human response when looking at the different animal models. Malaria research using monkeys is very important because they the closest related to humans, making the vaccine results more applicable and predictable to what it could be seen in humans. The continuous testing in non-human primates is important in order to obtain additional information on vaccine safety and efficacy (Kusi el al., 2011). Even though some of the vaccines show promising results when looking at the antibody response against the parasite, there is the problem that scientists continue to face, what is seen in a non-human primate is not always what is seen in humans. Even though the research done with monkeys is very important, it has also brings some challenges to the field because non-primates are the most expensive of the three animal models to use and take care of and they have a limited availability to use as research subjects. Malaria research continues to move forward and the joined effort and research of different animal models will allow for a vaccine and better treatments to be a reality in the near future.

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