Life cycle of malaria parasite

The life cycle of malaria provides the basis for understanding malaria vaccines. There are many strategies for developing malaria vaccines, each targeting different stages of the parasite’s development. Life cycle of all malaria parasites is more or less the same. It consists of 1) an exogenous sexual phase (sporogony) with parasite multiplication in Anopheles mosquitoes and 2) an endogenous asexual phase (schizogony) with parasite multiplication in the gut wall of vertebrate host. The latter phase includes 3) two endogenous asexual phases: the phase taking place in the liver cells (pre-erythrocytic schizogony) and the development cycle in red cells (erythrocytic schizogony).

When female Anopheles mosquito carrying malaria parasites feeds on a human, it injects the parasites in the form of elongated sporozoites into the bloodstream of the human. The sporozoites travel to the liver where they enter liver cells and rapidly divide asexually. This asexual division is known as schizogony. Over about 1 to 2 weeks, the sporozoites develop, divide, and produce thousands of haploid forms, known as merozoites, for each liver cell. A number of parasites stay dormant for longer periods of time within the liver, resulting in relapses several days later. Merozoites leave the liver cells and invade other liver cells and re-enter the host’s bloodstream. Once inside the erythrocyte, the merozoite begins to enlarge as a uninucleate cell known as a ring trophozoite. The trophozoite’s nucleus then divides asexually to produce a schizont which contains several nuclei. The schizont then divides and produces mononucleated merozoites, the erythrocyte ruptures and releases toxins throughout the body of the host repeatedly over 1-3 days. This asexual multiplication can lead to numerous parasites infected cells in

the host bloodstream, resulting in fever, chills and other complications which can persist for many months if left untreated.

Instead of replicating, few merozoite-infected erythrocytes exit the cycle of asexual multiplication and develop into sexual forms of the parasite, known as gametocytes (cells capable of producing male and female gametocytes) which circulate within the bloodstream. Erythrocytes containing gametocytes do not rupture. When biting an infected human, the mosquito ingests these gametocytes. The infected human erythrocytes burst within the mosquito's gut, releasing the gametocytes which grow further into mature sex cells known as gametes. Female and male gametes are then fused to form diploid zygotes, which develop into actively moving ookinetes within the mosquito's intestinal wall and differentiate into oocysts. Within the oocytes, repeated mitotic divisions take place, producing large number of active haploid forms called sporozoites. The oocyst bursts after about 1-2 weeks, which leads to the release of sporozoites into the mosquito's body cavity, from which they migrate to and invade the mosquito's salivary glands. From there the sporozoites are injected into the bloodstream of a human, thus starting the life cycle of the malaria parasite again. The time from infection to development of the disease usually takes about 10 to 15 days. This incubation period can last for longer depending on whether the human host has taken any antimalarial drugs.

Targets for malaria vaccines

There are three distinct stages of the parasite's life cycle which are potential targets for both subunit and whole-organism vaccines: a) pre-erythrocytic

stage, b) asexual erythrocytic or blood stage and c) sexual or gametocyte stage.

Pre-erythrocytic vaccines

After inoculation, the first stage in the parasite's life cycle is a fairly short pre-erythrocytic phase. A vaccine at this stage must be able to stimulate an immune response that completely prevents the infection from developing within the human host. Pre-erythrocytic vaccines may be designed to act at two separate stages during the parasite's life cycle. A sporozoite-stage vaccine can prevent sporozoites from invading hepatocytes, while a liver-stage vaccine targets the parasite's development within hepatocytes. These vaccines inhibit the release of merozoites from infected liver cells. The basic aim of sporozoite-stage vaccine is to generate humoral immune response where antibodies will neutralize the sporozites and prevent infection by blocking their ability to move and migrate through tissues. Liver stage is the next stage in the parasite's life cycle in which the sporozoites invade hepatocytes. This will give rise to a disease preventing liver-stage vaccine, which will result in sterile immunity. It has been established in vitro that CD8+ T-cells kill hepatocytes that have been invaded by the malarial parasite. Small peptides or antigens may be processed and displayed with MHC class I molecule, for recognition by CD8+ T-cells, resulting in a cell-mediated response that would kill infected hepatocyes.

Blood stage vaccines

These vaccines are aimed to mainly protect against malaria disease, but not against infection. The specific targets of blood-stage vaccines can vary, they could either destroy the merozoites in the short time before they invade red

blood cells or target malarial antigens expressed on erythrocyte surface by invading parasites. These vaccines will suppress the continuous growth of dividing merozoites, thereby reducing the disease.

The surface proteins of merozoites can be the vaccine target by enhancing antibody production against these surface proteins which can prevent infection of red blood cells by stimulating increased humoral immune response to merozoites circulating in the blood. However, this approach is made difficult due to the lack of Major histocompatibility (MHC) molecules expressed on the surface of erythrocyte.

Instead of MHC antigens, potential blood-stage vaccines can target specific ring-infected surface antigens (RESA) that are expressed on the surface of infected erythrocytes. There is less possibility of damaging already infected erythrocytes by increasing a cellular immune response than by targeting circulating merozoites, but effective blood-stage vaccines that are currently in development include a combination of both categories of antibodies. Thus the goal of blood-stage vaccines is to reduce the parasitic load in the blood after infection.

Hence, protection offered by these vaccines will be both antibody dependent and cell mediated immunity.

Anti-disease vaccines:

Anti-toxin vaccines

During the asexual blood stage when the schizont ruptures, a number of malarial toxins such as glycosylphosphatidyl inositol (GPI) are released, a key mediator of malaria pathogenesis. Studies have shown the GPI anchor, which

binds numerous Plasmodium antigens to the membrane, are highly toxic in mouse models. Administeration of GPI into mice formed features of severe malarial infection, e. g. hypoglycaemia and severe anaemia. The damaging effects of malarial GPI are associated with its ability to stimulate a pro-inflammatory response through cytokines such as TNF-alpha. Hence, malaria toxins represent another possible target for anti-disease vaccines, where these vaccines may induce immune response by the production of human antibodies which neutralize harmful soluble parasite toxins.

Anti-cytoadhesion vaccines

P. falciparum is the only species associated with cytoadhesion, the binding of infected erythrocytes to vascular endothelium. This process is involved in the pathogenesis, virulence and survival of P. falciparum, and is induced by several adhesins that are encoded by the parasite, P. falciparum erythrocyte membrane protein 1 (PfEMP1) family members. PfEMP1 binds to CD36 receptor on endothelial cells and are the only surface antigens on infected erythrocytes that present the parasite with its ability to adhere and remain sequestered. Cytoadhesion can cause parasitic sequestration in brain microcapillaries, which leads to neurological deficits or even coma. Studies have shown that naturally acquired antibodies against PfEMP1 during malarial infection seem to be protective. Thus these molecules are another potential target for anti-disease vaccines, where the antibodies act against surface antigens on the infected erythrocytes and may agglutinate the erythrocytes and prevent cytoadherence by inhibiting receptor-ligand interactions (CD-36 receptor), thus preventing any deadly outcome.

Transmission-blocking vaccine (TBVs)

These vaccines can be used to block transmission of the parasite to the mosquito by targeting against the sexual-stage Plasmodium gametocyte or ookinete. Unlike other classes of malaria vaccines, the aim of transmission-blocking vaccines is to prevent onwards transmission of the parasite through infected mosquito vectors. These TBVs are termed altruistics, because they mediate their action within the mosquito and would not offer any protective benefits to the vaccinated individual, but would prevent that individual from transmitting the infection to malaria vectors, i. e. prevent the next person from being beaten by that mosquito.

TBVs can inhibit the development of gametocytes by inducing an immune response to the surface antigens of gametocytes by the use of human antibodies that the mosquito takes up when it bites, thereby neutralizing the sexual stages. The anti-gametocytes antibodies block the development of zygote, whereas anti-ookinete antibodies inhibit the ability of the ookinete to migrate. This type of vaccine is potentially very important as it can be used to locally eliminate the parasite from low endemic transmission regions or for preventing the spread and development of vaccine-resistant parasites.

	Pre-erythrocytic	Erythrocytic	Transmission-blocking
Vaccine Stage	Protect against infection	Protect against disease	Prevent onwards transmission

Target	Sporozoite stage Liver stage	Merzoite Malarial toxins Malarial adhesions	Gemtocyte Ookinete
Goal	Prevent sporozoites from invading hepatocytes by inducing antibody-mediated immune response.	Decrease parasite density; Prevent disease pathology	Inhibit zygote development
Activity	Prevent parasite’s development within hepatocytes by inducing T-cell-mediated immune response	Inhibit cytoadhesion by inducing antibody-mediated immune response	Inhibit ookinete movement by inducing antibody-mediated immune response

Table 1. Comparison of the different strategies which can be used by potential malaria vaccines to target each of the different stages of the malaria life cycle.