Vaccine 30 (2012) 5191–5198

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Malaria vaccines: Focus on adenovirus based vectors

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ARTICLE INFO

Article history:
Received 14 March 2012
Received in revised form 9 May 2012
Accepted 19 May 2012
Available online 7 June 2012

Keywords:
Adenovirus
Malaria
Circumsporozoite protein
Vaccine
Review

ABSTRACT

Protection against malaria through vaccination is known to be achievable, as first demonstrated over 30 years ago. Vaccination via repeated bites with *Plasmodium falciparum* infected and irradiated mosquitoes provided short lived protection from malaria infection to these vaccinees. Though this method still remains the most protective malaria vaccine to date, it is likely impractical for widespread use. However, recent developments in sub-unit malaria vaccine platforms are bridging the gap between high levels of protection and feasibility. The current leading sub-unit vaccine, RTS,S (which consists of a fusion of a portion of the *P. falciparum* derived circumsporozoite protein to the Hepatitis B surface antigen), has demonstrated the ability to induce protection from malaria infection in up 56% of RTS,S vaccinees. Though encouraging, these results may fall short of protection levels generally considered to be required to achieve eradication of malaria. Therefore, the use of viral vectored vaccine platforms has recently been pursued to further improve the efficacy of malaria targeted vaccines. Adenovirus based vaccine platforms have demonstrated potent anti-malaria immune responses when used alone, as well when utilized in heterologous prime boost regimens. This review will provide an update as to the current advancements in malaria vaccine development, with a focus on the use of adenovirus vectored malaria vaccines.

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1. Natural infection and hopes for a malaria vaccine

Recently the numbers of malaria cases and malaria deaths have decreased worldwide in large part due to use of pesticides and bed nets that together kill or prevent mosquitoes from biting susceptible humans. In 2009, 225 million people were infected with malaria, down from 244 million in 2005 [1]. While this is an encouraging trend, there were still 781,000 malaria deaths worldwide, indicating new preventions must be developed and employed if malaria is to be eradicated. Malaria has been considered "eliminated" in the United States of America since 1970 and there were no locally acquired cases of *P. falciparum* reported in the European region in 2009 [1]. However, malaria still remains a prominent threat in areas of South America, Sub-Saharan Africa and Southeast Asia placing roughly one third of the world's population at risk of contracting malaria [1].

Five protozoan parasites are known to cause malaria in humans, *P. falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium*

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http://dx.doi.org/10.1016/j.vaccine.2012.05.048

vivax, and *Plasmodium knowlesi*, with *P. falciparum* being the most deadly accounting for 80% of all malaria cases, and 90% of all malaria deaths [2]. Sadly, the majority of malaria related deaths occur in children, since many adults have acquired immunity to malaria "naturally" over time as a result of surviving repeated malaria infections [3]. While it appears natural immunity to human malaria is largely IgG antibody mediated, it has proven difficult to pinpoint the specific antigens these antibodies target [3–5]. Antibody responses to malaria antigens are generally short-lived, possibly because natural malaria infections hinder the development of B cell memory [3–7]. For example, *P. falciparum* infection can induce expression of a T cell inhibitory receptor called Programmed Death-1 (PD-1), leading to poor $CD4^+$ T cell responses. Simultaneous blockade of both PD-1 ligand and Lymphocyte Activation Gene-3 (LAG-3: a negative regulator of T cell function) together can allow for more rapid clearance of blood stage infections in mouse models, and has been recently targeted as a strategy to treat active malaria infection in humans [8].

2. Previous examples of putative malaria vaccines

Other malaria prevention methods attempt to proactively vaccinate individuals from malaria infection (i.e. "unnatural" immunity). Potent humoral and/or $CD8^+$ T cell responses against multiple malaria antigens have been identified to be partially responsible for protection from malaria infection [9]. Based upon these findings, it has been postulated that preemptive induction of adaptive immune responses to malaria derived antigens may be of benefit in preventing the symptoms of subsequent malaria infection, if not protection from malaria infections in general.

We know that artificial or "unnatural" inductions of immunity to malaria are achievable. In 1975 mosquitoes infected with *P. vivax* or *P. falciparum* were irradiated (preventing live parasite transmission) and then used to bite a human volunteer, as a result the volunteer was protected from natural malaria infections for a short period of time [10]. Although the experiment was subsequently validated in larger groups of human volunteers, the approach was not practical, as many hundreds of bites were required, and the resulting protection was equally short lived [11,12]. Despite this disadvantage, the approach still remains one of the most protective malaria vaccine platforms to date, as protection rates approached over 90%.

Another non-irradiated, mosquito bite based vaccine platform utilizes chloroquine to control parasite infection, in which *P. falciparum* infected mosquitoes are allowed to bite volunteers while chloroquine is administered to prevent blood stage infection by the live parasites. Chloroquine controlled infection has shown high rates of effector memory mediated protection upon rechallenge that lasted for up to 2 years, a significant increase over the few months observed in irradiated mosquito platforms [13,14]. Chloroquine controlled infection also decreased the amount of mosquito bites required for protection from many hundreds to 10–15 [13]. Notably, chloroquine controlled infection also decreases PD-1 expressing $CD4^+$ T cells improving the $CD4^+$ T cell exhaustion phenotype [8]. However, due to extensive use of chloroquine in East Africa, some strains of *P. falciparum* have developed resistance to the prophylactic [15]. Since non-chloroqine resistant strains must obviously be used in this method, further research must be conducted to ensure that chloroqine controlled vaccination with non-chloroquine resistant parasites can protect against challenge with chloroquine resistant parasites in order to prevent the rapid spread of the resistant strain.

The use of purified radiation attenuated sporozoite's is another method attempted for use as a prophylactic malaria vaccine. In this method sporozoites are harvested from the salivary glands of irradiated mosquitoes and injected with a needle rather than via bites from the irradiated mosquitoes. Analysis of immune correlates of protection performed after the use of irradiated sporozoites suggest that $CD8^+$ T cell responses against the liver stage of the parasite appear to be more important for protection than generating a potent antibody response [16–19]. Further supporting this correlation, studies where $CD8^+$ T cells specific for liver stage malaria antigens were passively transferred into naive mice demonstrated protection from intravenous (IV) sporozoite challenge [20].

Unlike natural infections with malaria carrying mosquitoes, or use of irradiated mosquitoes, experiments with vaccines composed of irradiated sporozoites isolated from the salivary glands of infected mosquitoes attempt to provide protection from malaria without the unpleasant and cumbersome necessity for multiple mosquito bites. Subcutaneous injections of irradiated sporozoites were proven capable of protection in mouse models [17,21]. Unfortunately the method requires higher doses of sporozoites and was demonstrated to be poorly immunogenic in human trials [17,21]. More recent animal studies confirmed that protection was improved by IV injection of irradiated sporozoites, and future human trials will be required to assess the immunogenicity of IV injected irradiated sporozoites [17]. The complexities of harvesting, storing, and transporting purified irradiated sporozoites is a concern as well, as sporozoites are very fragile outside of their mosquito host. Attempts to cryopreserve irradiated sporozoites demonstrated that sporozoites do not survive the freeze thaw process well. Inoculations with cryopreserved irradiated sporozoites also required fourfold more sporozoites than fresh sporozoite preparations to achieve the same effectiveness in animal models [17].

Use of live (non-irradiated) but genetically attenuated sporozoites to increase the immunogenicity and therefore decrease the number sporozoites required to achieve protection has also been recently described. In a mouse model of malaria, attenuated *P. yoelli* sporozoites that have been genetically engineered to arrest late in the liver stage were capable of stimulating broader and more potent $CD8^+$ T cell responses to malaria antigens (including blood stage antigens) than what was observed using irradiated *P. yoelli* sporozoites [22]. Mice vaccinated with lower numbers of the genetically attenuated sporozoites displayed a wider range of antigen responses and were also protected against blood stage challenge as compared to purified radiation attenuated sporozoites injected IV [22]. Whether these results translate to *P. falciparum* and human malaria infections remains to be seen.

The use of attenuated sporozoites, whether purified and injected, or administered through mosquito bites, has shown promising results in the laboratory. However, the necessity for multiple bites from infected mosquitoes and the inability to mass produce and preserve purified sporozoites according to regulatory standards for human use, has prompted the development of alternative, subunit based vaccines targeting specific malaria parasite antigens.

3. RTS/S

CS protein is the most abundantly expressed protein during the sporozoite stage and is found both on the surface of sporozoites and in the plasma membrane and cytoplasm of infected hepatocytes during early liver infection [23]. CS protein has been repeatedly shown to be an immunodominant protective antigen [16,20,24]. In fact, when transgenic mice were altered to express and therefore tolerate CS protein, the absence of a CS protein specific B and T cell response dramatically decreased the ability of irradiated sporozoites to protect the transgenic mice from malaria challenge [24]. Conversely, mice vaccinated with irradiated