Apical Membrane Antigen-1 of *Falciparum* Malaria Parasite (Pf83/Ama1): Efficacy as a Malaria Vaccine, Uncertainties and Attempts at Improving Efficacy

By

Ozurumba L.N.
Research Article

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Ozurumba L.N.

Cellular Parasitology Programme, Department of Zoology, University of Ibadan, Ibadan, Nigeria.

Email: leon_ozurumba@yahoo.com, Phone: +2348094870562.

ABSTRACT

Apical membrane antigen (called AMA) found in *Plasmodium falciparum* malaria parasite, has been one of the leading malaria vaccine candidates and a subject of study for its potentials as an effective vaccine. The burden posed by malaria over the decades, more so in the underdeveloped tropical world, re-emergence of malaria in regions it has been perceived to have once been eradicated, problem posed by resistance to effective drug by the parasite are among other reasons for spirited attempts by researchers to come up with good products from their efforts to support malaria control programmes.

Most of the discovered and developed vaccines have protected animal models. Immunity in humans seem short-lived, ineffective and results in milder, sometimes asymptomatic infections in spite of the parasites’ persistence. It has been suggested that cell and antibody mediated immunities jointly confer the protection observed in animal models and this may have important implications for developing human vaccines.

The vital role played by AMA1 during the process of merozoite invasion of red blood cells by the parasite, a key event needed by the parasite to complete its life cycle has been part of the major reasons for considering this antigen for candidacy as a vaccine either as monovalent or polyvalent (some of which have been multi-stage or multi-antigen) AMA1 based or incorporating vaccines. So far, the most successful vaccine candidate to get to the highest level of clinical trials has been the RSS,S/AS02A, the first vaccine to pass through the phase II clinical trials.

The polymorphic nature of AMA1 and some other malaria vaccine candidates such as MSP1 among other issues highlighted in this article have been part of the major problems that appeared to have limited its success in humans, despite successes in certain animal models in clinical trials.

Thus, for AMA1 based malaria vaccines, the tasks for the future has been to seek to improve its immunological responses irrespective of the major bio-technical problems inherent in the parasite’s features at levels good enough to confer protection in both animals and adult humans and able to translate into efficacy when attempted in children recipients.

As man, through vaccine scientists seek to declassify the classified perceived elusive myth that has surrounded successful boding of malaria vaccines for paediatric immunization over the decades, human subjects known to be at highest risks of infection and experiencing clinical diseases, may be near breathing sigh of relief in the event of eventual success of the most clinically advanced form of a malaria vaccine, the RSS,S/AS2OA or the other vaccine candidates being actively worked on to support man’s fight back against the tiny parasite which has boxed its comparatively much bigger man to a tight corner for scores of decades.

Keywords: Antigen, polymorphic, haplotypes, merozoite, antibody, immunization, adjuvant.

INTRODUCTION

Almost all vaccines in use today are against bacterial and viral infections. In the history of vaccine research, no vaccine has been developed for an organism with the complexity close to that of malaria parasites. No safe, effective vaccine for malaria exists. Developing a vaccine is a priority because of one especially exacerbating problem: the malaria parasite and the insects that carry it are becoming resistant to existing drug treatments and therapeutic-control measures while it could also greatly reduce the effects of the disease in terms of suffering and lives lost (Shortwell, 2007). However, development of a vaccine is considered feasible because infection with malaria parasites induces protective immune responses, which include antibodies to a range of proteins on the parasite surface (Coley, et al, 2007). Notably, in ancient times, those who survived an infection seldom suffered a second, similar sickness. Thus, the mildness of some infections in some diseases like Small Pox which was once really
dreaded to have terribly afflicted notable world figures and leaders, led to the deliberate induction of the disease by a process called variolation, first in China and India, then in Europe. Thereafter, Edward Jenner made his landmark contribution to a new concept of vaccination when he used Cow Pox material and later Small pox material to treat small pox in a child in 1796 (Ada, 1992). Louis Pasteur then greatly contributed to vaccinology when he developed a means of changing the properties of microbes to reduce their potentials which was significantly reduced by the idea he bought into play called attenuation, a method engaged by Dr. Sabin in creating and developing his live Polio vaccine that was a far less harmful version of the Polio virus. Other concepts have since followed in vaccinology, moreso in the "prankish and difficult to deal with malaria parasite".

Malaria parasites have an intracellular and an extracellular phase (Ukoli 1990; Ozurumba, 2012a). These makes the immune responses required to control infection differ from that required for other parasites such as viruses and bacteria which tend to produce life long resistance to re-infection. Thus, at each transitional stage of development, the malaria Plasmodium parasite presents potential targets to the host immune system through an array of antigens derived either from the parasite or through representatives of the host cell components altered by the parasites’ actions.

Blood stage parasites are surrounded by several membrane systems and develop within the erythrocytes (Haldar and Mohandas, 2007; Ozurumba, 2012a). However, some of the antigens of these blood stages (such as AMA1) have conferred protective immunity in primate models against Plasmodium parasite challenge (Sim et al, 1994).

Apical membrane antigen-1 (AMA1) is one of the blood stage proteins and is an apically concentrated antigen. It has been reported to be clearly associated with the surface of the merozoite after schizont rupture (Narum and Thomas, 1994). It is an asexual blood stage protein expressed in the invasive merozoite form of Plasmodium species - the causative agent of malaria. All members of the AMA-1 family are expressed during merozoite formation in maturing schizonts and are initially routed to the rhoptries. However, the processed forms may subsequently be associated with the merozoite surface. AMA1 is encoded by a single copy gene and has been characterized in a number of malaria species as either an 83-kDa de-novo product (P. falciparum AMA1: PF83/AMA1) or a 66kDa product in virtually all the other species (Narum et al, 1993; Silvie, et al., 2004).

Also, in some other Apicomplexans, antibodies to AMA1 have been found to directly interfere with invasion by Toxoplasma sp. tachyzoites (Hehl, et al, 2000) and as already discussed for Plasmodium falciparum merozoites (Narum and Thomas, 1994), suggesting a key role in the invasion process. A similar function for the P. falciparum AMA1 protein (PIAMA1) has been described during sporozoite invasion of hepatocytes (Silvie, et al, 2004)), indicating PIAMA1 might be an effective vaccine target for both the pre-erythrocytic and the asexual blood stages (Kocken, et al, 2002, Stowers, et al, 2002 and Alexander et al, 2006).

AMA1 has also been found on the surface of sporozoites and on hepatic merozoites (Silvie, et al., 2004). This feature makes the antigen a possible target for protective immune responses against both sporozoite and liver stage of the parasite.

Apical membrane antigen1 (AMA1) was first identified in Plasmodium knowlesi, and since then homologues have been seen in all Plasmodium species so far examined as well as at least two other Apicomplexan genera, Toxoplasma and Babesia (homologues have not been detected in organisms outside Apicomplexa (Hehl, et al, 2000). This essential membrane protein is stored in the micronemes of the asexual stages and transported to the parasite surface prior to and during host cell invasion (Bannister, et al, 2003).

AMA1 and merozoite surface proteins (MSP) have been among the antigen vaccine candidates which have shown promise from laboratory in-vivo studies, tests on animal models and field clinical evaluations in humans which are periodically conducted based on extent of successes recorded and new findings that appear hopeful to try; bearing in mind that the malaria vaccine and a vaccine against HIV-AIDS appear to be the major headaches faced in vaccinology of parasitic and microbial infections partly due to a major similar reason of extensive polymorphism of the antigens involved.

A variety of the features of the PF83 (an AMA1 antigen of the human P. falciparum model) suggests that it is a potential candidate in the development of a malaria vaccine. Observations by researchers has indicated that despite the fact that AMA1 has shown levels of efficacy as a vaccine in eliciting immunologically functional levels of antibody responses, particularly more functional in animal models (Anders et al, 1998); however, making this work also in humans has over the years been a bottleneck for vaccine experts. Successes recorded on adults, with some encouraging levels of response of anti-AMA1 antibodies (Polhemus, 2007) has not been translated into success in children (Thera et al, 2008). The extensive polymorphic nature of the antigen in many Plasmodium falciparum proteins may limit the efficacy of vaccines based on just one or two allelic variants that are not broadly cross-protective against diverse antigens found in natural infections (Ouattara et al, 2010) while skirmishes of observed strain specificity in its evoked immunological responses, antigenic diversity and halotypes, and failure of some of its polyvalent forms (such as the bivalent AMA1 based AMA1 - C 1 ) to confer
protection on recipients during clinical trials (Druihlhe et al, 1991; Spring et al, 2009; Drew et al, 2012), have been some of its shortcomings that have hindered success over years of attempts.

Notably, the design of an effective vaccine, requires a clearer understanding of the biological functions of antigen processing and genetic diversity in AMA1 (Harum and Thomas, 1994, Water et al, 1990), and a detailed understanding of how antibodies interact with their target parasite antigens, of which includes the structural outlook of the interaction between a growth-inhibitory monoclonal antibody and the promising vaccine candidate, AMA1

An important presentation from studies in this area shows that mutations of polymorphic AMA1 residues within an epitope called the 1F9 epitope disrupt 1F9 inhibitory monoclonal antibody 1F9 binding and dramatically reduce the binding of affinity-purified human antibodies. Moreover, 1F9 binding to AMA1 is competed by naturally acquired human antibodies, confirming that the 1F9 epitope is a frequent target of immunological attack while antibodies targeting the 1F9 epitope are a component of the human antibody response to AMA1 (Coley et al, 2007).

It is envisaged by scientists that since AMA1 is expressed on pre-erythrocytic and erythrocytic stages of the parasite, it appears to be a promising vaccine antigen to induce malarial protection by targeting both the humoral and cell-mediated arms of the immune system (Spring et al, 2009). More importantly, by introducing specific anti-AMA1 antibodies into infected cell cultures, studies have shown that malaria parasites are unable to infect red blood cells and thus, unable to reproduce and spread disease (Haas and Fulling, 2008).

However, the success from the attempts by Dr. Cohen and his research team, scientists who hold the patent for the promising RSS,SAO2A malaria vaccine, which has virtually almost crossed the difficult large scale incorporating Phase-3 clinical trials (Heppner et al, 2005; Shotwell, 2007; Navarro, 2011) appear to be inspiring researchers working on various antigen candidate forms of malaria vaccines such as AMA1, MSP1 and other antigen stage - based vaccines, alongside multi-stage, multi-antigen and multi-species based malaria vaccines, such as AMA1/MSP1 recombinant based malaria vaccine (Malkin et al, 2008) on their projects and efforts. RSS,SAO2A malaria vaccine targets the liver stage of the parasite (Mackenzie, 2012) while AMA1 malaria vaccine targets one of the blood stages of the malaria parasite which invades the erythrocytes (Spring et al, 2009). Currently, there are well over ten active malaria vaccine projects worldwide.

The need to develop an effective malaria vaccine has been compelled by issues related to drug resistance by the parasite, therapeutic chemical resistance by the transmitting insect vector cost of effective antimalarials and the cost of implementing the myriad of various control methods geared towards reducing morbidity and mortality; bearing in mind that majority of world’s malaria population may not be able to afford them and generally the burden inflicted on man by the diseases (Haas and Fulling 2008; Ozurumba, 2012b).

Generally, in vaccine science, designing one has involved several approaches, some of which includes: two traditional methods of live attenuated organisms and inactivated whole organisms (such as irradiated sporozoites) and the sub-unit preparations of which includes refolded recombinant fragments and homologous constructs (Kocken et al, 2000).

Malaria is an infectious disease which is responsible for up to 2% of global mortalities. It kills up to 2.7 million people each year, most of whom are children in Sub-Saharan Africa (Ozurumba and Ogundinigi, 2011). Moreso, in the tropics where the disease is far more burdensome, the most responsible protozoan parasite, Plasmodium falciparum causes the most deadly form of malaria in humans. Notably, malaria parasites are transmitted to humans via female Anopheles mosquitoes (Haas and Fulling, 2008). Severe and potentially fatal malaria may occur in children between 6months and 3years in endemic and non-endemic areas and in naïve adults in endemic areas with passive immunity being a feature in children above 3years and adults from endemic areas (Ozurumba, 2008). It is worth bringing to fore, the fact that despite the eradication of malaria in a good number of developing countries, through stemming down of the environmentally related capacity for the mosquito to breed, and the use of effective drugs and good hygiene, malaria seem to be re-emerging (Ozurumba, 2011, Global Health Council, 2011) in some of these areas.

What makes this review on AMA1 different from others is the fact that it tries to briefly present some of the various brands of AMA1 attempted through various techniques in vaccinology, the shortcomings, challenges posed and problems encountered with these vaccines. This review searched for reasons behind these problems, while creating avenue to alongside, chip in vital facts on the presently only malaria vaccine which has crossed the difficult Phase III clinical trial for malaria, in relation to efforts in attempts to engage AMA1 through a myriad of technologies.

**Difficulties With The Ama1 As A Successful Malaria Vaccine**

Over the scores of decades of search and research, it had been difficult developing and coming out of complete clinical evaluation of candidate malaria vaccines successfully. Some of the reasons for this difficulty have been adduced to the existence of many stages of the parasite in the course of its going through and completing its life cycle (mentioned as part of findings in the study by Ozurumba et al, 2006), the parasite’s spending of part of its life cycle inside the erythrocytes (and possibly the hepatocytes) where targeting of the parasite with an effective vaccine...
had proved tasking and difficult, the existence of various species of the parasite found to be endemic in different geo-locations, the existence of strain-specific species in these various regions which seem to compound problems engaging antigen based -vaccines such as AMA1 and MSP1 and the polymorphic nature of most of the antigens of the parasite with the associated genetic diversity of parasite antigens of several halotypes of these antigens found in screened subjects (Druilhe et al, 1991; Ouattara, 2010). Recent findings reveal that about six or more species may have been confirmed to actually exist. These include P. falciparum, P. malariae, P. vivax, P. ovale curtisi (classic type) and P. ovale wallikeri (variant type) with the variant types of P. ovale found in Africa and Asia. Recently, it has been observed that P. knowlesi cause quotidian malaria in south-east Asia (WHO, 2012; van Hellemont et al, 2009).

These hindrances led to the notion that different populations may need different vaccine products, a precept that is uncertain in vaccinology of malaria and outcome of clinical evaluations may likely release more insights on this idea. It may be worth noting that in HIV-induced immunosuppressed asymptomatic individuals, who are non-immuned for malaria but infected with the parasites and recrudescing for malaria, under a subsequent re-challenge by malaria parasites, they are likely to have their ability to develop effective degree of protection against homologous and heterologous Plasmodium malaria parasites in doubt and hampered. This could enhance progression of malaria, especially when not well treated (Ozurumba, 2008).

### Some Clinical Trials With Ama1 Based Vaccines And Advances In Malaria Vaccinology

Interesting as it may be, pre-clinical trials of AMA1 based vaccines in lower animals has shown protection characterized by survival with reduced parasitemia against the rodent parasite P. chabaudi by active immunization with adjuvantned homologous recombinant AMA1 protein; and by passive transfer of immunoglobulin from vaccinated rabbits while Aotus monkeys immunized with P. falciparum complete Freund's adjuvantned recombinant AMA1 showed significant delays in parasitemia after homologous blood stage challenge were seen as compared to monkeys immunized with a similarly adjuvantned control malarial antigen (Stowers, et al, 2002).

However, some of the vital clinical trials conducted with prototypes of monovalent AMA1 based vaccines have revealed the following:

In a phase-I clinical trial conducted by Thera et al, (2008) on children, in a region endemic for Plasmodium falciparum and intense seasonal transmission in Mali, there was a significantly increased anti-AMA1 antibodies which reached 100fold in the three groups that were administered with the doses of the vaccine, an immune response that appeared sustained the following year. Two Phase-1 dose-escalation adult vaccine trials have been completed, one by a team based at Walter Reed Army Institute of Research (WRAIR) and one by a team in Mali, evaluating FMP2.1 in Adjuvant System AS02A. It was found to be immunogenic, of good safety level and tolerated by the recipients (Thera et al, 2008).

In a Phase I clinical trial of FMP2.1/ASO2A AMA1 based malaria vaccine candidate in North America volunteers, the vaccine evoked potent humoral and cellular responses, while immunofluorescence assay revealed recognition of sporozoites and merozoites stages of the parasite in the immune sera of the volunteer recipients who were naïve North Americans (Polhemus et al, 2007).

In a Phase 1/2a clinical trial of AMA1/AS01A and AMA1/AS01B in healthy malaria-naïve adults (aged 18–50 years in the metropolitan Washington DC area) employing a primary sporozoite challenge model, high Antibody titre manifested immune response (and all three vaccine candidates induced cellular responses as measured by IFN-γ production) did not translate into significant vaccine efficacy but a partial biological vaccine effect and it was found to cause moderate reactogenicity, manifested specifically by local erythema and swelling (Spring et al, 2009).

It should be noted that other monovalent AMA1 based vaccines, adjuvantned but derived from other clones of P. falciparum exist, an example being that derived from FVO clone of P. falciparum.

In one of the clinical trials conducted with one of the prototypes of a bivalent vaccine, this category of AMA1 vaccine named AMA1-C1 on Malian children between 2006 and 2007 in a Phase II clinical trials of this vaccine, the researchers found no evidence of vaccine selection or strain specific efficacy, though it failed to confer protection on the recipients. No effect on parasite density nor on clinical malaria was observed. The researchers recounted that the vaccine only appeared to provide protection against parasites with AMA1 halotypes (Ouattara et al, 2010).

The malaria vaccine candidate RTS,S/AS02A, having a team of scientists led by Dr. Cohen as its patent holders and inventors, in partnership with GlaxoSmithKline appear to be presently in the fore-front, in terms of success for a candidate malaria vaccine, following its progress through the phases of clinical evaluations in the field (Navarro, 2011). RTS,S/AS02A is a vaccine directed against the pre-erythrocytic circumsporozoite protein CSP of Plasmodium falciparum malaria parasite and it was found not to appear to result in selection or allelic specific efficacy. It proved to be the first candidate malaria vaccine to pass Phase II clinical trials when it was successfully engaged in adults and later in children in Africa.

In a phase III clinical trial to monitor safety and potential side effects, evaluate efficacy on a large scale, RTS,S/AS02A prevented clinical malaria in 56% of children between 5months to 17 months old, prevented severe
malaria in 475 of the cases after 3doses of the candidate vaccine, in a trial that was done in 11sites in 7countries across sub-sahara Africa (GSK and PATH MVI, 2009). Significantly as it may be, this success appear to be a milestone achievement, considering the fact that Phase III clinical trials is one of the final stages in the field evaluation of a vaccine candidate for safety, which is done on a large scale before the submission of the candidate vaccine to regulatory authorities for Phase IV which involves post-marketing safety monitoring and determination of duration of protection conferred by the vaccine candidate, alongside its compliance as a vaccine. The patent holders envisage that RTS,S could be available for use first in infants aged 6-12weeks and later in children aged 5-17months older than the prior group of potential recipients. Success at the last stage (a phase IV clinical trials) holds the key to unlock its capacity to be licensed by GSK. Thereafter, it has been envisaged that it could be possibly available for routine immunization of children. These successes with the RTS,S/AS02A potentially could inspire work and efforts on the AMA1 prototype vaccine types being developed and re-engineered by vaccinologists to be more effective.

Scientists at the University of Edinburg in the United Kingdom developed a malaria vaccine, which the team envisage could have a unique ability to inhibit different forms of the parasite that cause disease, while also assisting those that are most vulnerable to infection. Antibodies at levels and range that is adequate to fight these different forms of the parasite is a feature of its qualities. The group of scientists led by Dr. David Cavanagh identified and obtained multiple versions of a key protein found in many types of malaria parasite which are vital for conferring immunity and they exploited this factor to create and develop this vaccine (Cavanagh, 2011). Also, there are now some multi-antigen peptide vaccines (MAP vaccines) designed against Plasmodium falciparum which are in process of undergoing field clinical evaluations. In another attempt, malaria antigens from P. vivax and P. falciparum and from multiple phases of the parasite life cycle have been used (WHO, 2013) in one of the most advanced vaccine development projects.

A multi-antigen blood stage malaria vaccine evaluated in Papua, New Guinea reduced parasite density and pre-clinical infection in a strain-specific manner, suggesting selection of non-vaccine variants (Genton et al, 2002). This is one of its major differences with the second generation RTS,S/AS02A vaccine that does not exhibit this attribute. Currently, over 30 other malaria vaccine projects have reached the clinical evaluation stage (WHO, 2013).

**Types Of Ama1 Based Malaria Vaccine Candidates In Vaccinology**

A school of thought in malaria vaccinology has categorized AMA1 based malaria vaccines into the following groups:

1. Monovalent AMA1 based vaccine, a typical example of which is FMP2/ASO2. This vaccine comprises a recombinant protein FMP2.1 which is obtained from the 3D7 clone of P. falciparum and adjuvanted with ASO2A – an adjuvanted system of which GSK Biologicals holds the manufacturing proprietary (Spring et al, 2009). Typical examples include WEHI/LaTrobe/WRAIR - AMA1 (Loucq, 2011) and PfAMA1 Pastoris pichia FVO allele malaria vaccine with DDA/TDB vaccines.

2. Bivalent and recombinant AMA1 based vaccines, protein adjuvanted, and derived from both 3D7 and FVO clones of P. falciparum versions of AMA1. One of the adjuvants is Aluminium (III) hydroxide. A version (prototype) of a bivalent AMA1 malaria vaccine, in a Phase II clinical trial in Mali failed to confer protection on children against malaria after it had passed the Phase I clinical trial also in Mali, with observations of safety, modest immunogenicity and tolerability from outcome of the trials (Ouattara et al, 2010). The recombinant/DNA based multi-antigen AMA1 on development added to the groups of vaccines are highlighted here, with the various references stated below:

Recombinant AMA1 vaccines requiring adjuvants (such as TLR agonist CpG 7079, alum, ISA 720) are being explored to improve immunogenicity and persistent high antibody levels. AMA1–C1/Alhydrogel contain an equal mixture of AMA1 from two different clones of Plasmodium falciparum (FVO and 3D7) both produced separately as recombinant proteins expressed by Pichia pastoris (PpAMA1-FVO and PpAMA1 3D7);AMA1-C1 which is a mixture of 3D7 and FVO clones with adjuvant ISO720; AMA1 of the 3D7 allele and PfAMA1 3D7 plus KI Pf CP Montanide ISA720. The efficacy of protein adjuvanted AMA1 malaria vaccines are being explored and developed.

3. Virally vectored and delivered AMA1 malaria vaccines. Some of these include:

AMA1 delivered using vector platforms such as Pox vectored multi-antigen candidate vaccine NYVACPi7, DNA based plus virally vectored combination AMA1 vaccines; ChAd63 (in Adenovirus- Ad) vectors encoding AMA1, MVA vectors encoding AMA1, ChAd63 and MVA vectors encoding AMA1 (ChAd63 and MVA AMA1), GenVec Ad5-AMA1: MSP1, ChAd63 and MVA vectors encoding AMA1 formulated with Alhydrogel ± CpG adjuvant, ChAd63-MVA AMA1 combined with AMA1-C1 protein vaccine formulated with Alhydrogel ± CpG adjuvant, ChAd63 - MVA PvDBP - RII ;
ChAd63-MVA AMA1 ± MSP1 vaccine, AMA1 DIII peptide on Influenza virosomes linked to CSP expressed in Hepatitis B surface antigen, Pf25 AMA1 fused to Neisseria meningitidis outer membrane complex, and Pf AMA1 Escherichia coli 3D7 FMP2.1 ASO2A or ASO1B.

(Li et al, 2007; PATH MVI 2007; Remarque et al, 2008; NLM, Clinical trial data, 2008; Draper and Heeney, 2010; Loucq C., 2011; Sean et al, 2013; Sheehy et al, 2013).

Certain Institutes and organizations have been really active and prominent in malaria vaccine research of which include – WEHI, La Trobe, WRAIR, Gennova, John Hopkins University, Sabin Research Institute, New York University, Merck, ICGB India, Liquida, Aeras, Inovio, Sanaria Inc, University of Pennsylvania, Prefectus, Monash University, QIMR Australia, Oxford University, Edinburg University, Apovia ICC, GenVec, Emory University, Cracell, NIAID Malaria Vaccine branch at NIH, GSK, Tulane University, VRC, Oncovir and IDRI among other malaria research centres. Various methods and prototype malaria vaccines created and developed by the researchers are on investigation in their laboratories with some on clinical trials, using recombinant AMA1 peptides, Pf CSP, EBA/Rh, adjuvanted CSP, virally vectored platforms, adjuvanted nanoparticles, CSP RI conjugates, among other malaria vaccine constructs in novel approaches.

**Some Major Methods Engaged For Developing Ama1 Based Malaria Vaccines**

1. Approaches that produce parasite derived AMA1 vaccines.
2. Approaches that produce novel AMA1 peptide analogue based vaccines.
3. AMA1 peptide construct delivered with a bacterium vector, such as in *E. coli*.
4. AMA1 peptide construct delivered with a fungus vector such as Yeast *Pichia pastoris*.
5. AMA1 peptide construct delivered with a viral vector, such as in Virosomes and Simian Adenoviruses,
6. Recombinant DNA based AMA1 vaccines such as those produced from recombinant FMP2.1/ASO2A or FMP2.1/ASO1B AMA1 protein constructs made from 3D7 *Pf* clone (for instance by using refolding strategies to change the conformation of the conserved cysteines which contribute to the disulfide bonding in the ectodomain of AMA1 protein) and the combination of different clones of *Plasmodium falciparum* embodied in one vaccine formulation, like the FVO and 3D7 clones of *Pf* in one formulation.
7. Multi-stage/multi-component AMA1 malaria vaccines delivered without or through vector.
8. Recombinant DNA plus viral based delivery systems of AMA1 vaccines.
9. Then, the adjuvanted AMA1 vaccines in which any of these vaccines above are formulated or adsorbed in various types of adjuvants and media geared towards enhancing immunogenicity and persistence of antibody levels. (Li et al, 2007; Draper and Heeney, 2010; Loucq, 2011).

**Uncertainties In Efficacy Of Ama-1 Based Malaria Vaccines**

AMA1 vaccines could be made up of one or a few alleles due to the extreme genetic diversity of the AMA1 antigen, related to the high polymorphic nature of AMA1 antigen. A source revealed that over 300 unique halotypes of AMA1 have been identified worldwide (Thera et al, 2010).

About 62 polymorphic amino acids have been identified in AMA1, which represented over 15% of the amino acid sites distributed over 3 domains of the protein (Drew et al, 2012). Thus, it was suggested that the broad stretching polymorphism in many *P. falciparum* proteins may have been contributing to the hitherto restrictions placed on the efficacy of vaccines based on one or two allelic variants which are not extensively cross-protective against diverse antigens found in natural infections.

This has rekindled commitment by some researchers to step-up attempts at creating and developing multi-antigen blood stage malaria vaccine candidates. One of such vaccine candidates had undergone clinical trials in Papua New Guinea where the vaccine reduced parasite density, prevalence of infection in a strain specific manner (Ouatarra et al, 2010).

Thera et al. (2010) remarked that the strain specific immunity, with respect to AMA1 antigen may be a feature in host-parasite interactions involving AMA1 antigen and the associated anti-AMA1 antibodies.

The most pronounced polymorphism in AMA1 was found in its domain1, occurring in a cluster of amino acids near a hydrophobic pocket and this is believed to be actively involved in the process of red blood cell invasion by the parasite (Haas and Fulling, 2008), with extreme diversity, potentially limiting its efficacy against infection and disease caused by *Plasmodium falciparum* parasites with diverse forms of AMA1.

Following the introduction of attenuated vaccine technology by Louis Pateur in 1992, various other technologies have been developed and engaged over the years which resulted in the other methods such as use of toxins from pathogens (tetanus toxoid vaccine), then in malaria vaccinology research, we had adjuvanted antigen vaccine systems (engaged at John Hopkins University in collaboration with research partners), recombinant and sub-unit vaccines, adjuvanted nanoparticles created and developed by researchers at Emory University and by Liquida.
Incorporated, recombinant vaccines as created and investigated by Cracell Incorporated and WRAIR in partnership with a Pharmaceutical firm, and antigen based conjugates which scientists at the New York University in collaboration with Merck have been studying (Ada, 1992; PATH MVI, 2007; Loucq, 2011; Hill, 2011; Vannice et al, 2012).

In 2006, MVI highlighted about three specific findings of which included - the protective role of infected sporozoite model in conferring protection on human volunteers infected via irradiated sporozoites present in mosquitoes, and the onset of some level of natural protection in humans living in malaria endemic areas which indicate feasibility for developing a malaria vaccine (PATH MVI, 2006). The WHO in 2006, after some concerted and careful re-appraisals on efforts made thus far, developed and published a roadmap for achieving success in eventually having a functional malaria vaccine of at least average level of efficacy at around 50%. RTS,S/AS02 appear to have been the vaccine to fit into the attainment of this initial goal by the WHO which projected the period between 2006 and 2015 for its Malaria Vaccine Technology Roadmap in which it was envisaged that the 1st generation functional malaria vaccine of around 50% efficacy would have been developed, tested in filed trials and be on its deck of successful vaccine portfolio, approved and made available for use.

Thus, the subsequent effort of various vaccinologists which ought to be encouraged when noticed to have real potentials to enhance the chances of uplifting the efficacy form 50% to above 80% in the next successful malaria vaccine that would likely meet up with the goals of the 2nd generation malaria vaccines projected by the WHO to be available by 2025, which would prevent clinical disease and last longer than 4 years in its protection duration on vaccinated humans. Success with RTS,S/AS02 had been attributed partly to the vaccine’s capability to make antibodies against the intravascular CSP which enters the blood stream soon as an individual is inoculated by the vaccinated humans. Success with RTS,S/AS02 had been attributed partly to the vaccine’s capability to make antibodies against the intravascular CSP which enters the blood stream soon as an individual is inoculated by the vaccinated humans. Success with RTS,S/AS02 had been attributed partly to the vaccine’s capability to make antibodies against the intravascular CSP which enters the blood stream soon as an individual is inoculated by the vaccinated humans.

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On the idea of- “which antigen combination with CSP may prove highly efficacious for the 2nd generation malaria vaccines projected by the WHO in 2006?” The other question may also be – “which nature of recombinant vaccine constructs or multi-component malaria vaccine incorporating a CSP could best achieve the WHO set goal of, a push up in efficacy of the 2nd generation malaria vaccine to a range above 80% while adding at least a four year protection duration for vaccinated humans to its qualities?”

Attempts Made To Improve Ama-1 Malaria Vaccines’ Efficacy

Could approaches that make-up or cover-up for antigenic diversity in AMA1 and other malaria antigens with such polymorphic natures work for the good of success in creating and developing such a malaria vaccine that could be actually effective, not only in animal models, but also bode well in clinical evaluations in children? The outcome of such an advanced stage clinical evaluation could determine if such a vaccine could step up into the elusive stage of being made available for routine paediatric immunization. So far, only one vaccine candidate has gotten close to the later, which is RTS,S/AS02A, which appear close to this stage in clinical trials.

AMA1 is perceived as one of the candidate antigens in amalgams or cocktails of the multi-stage, multi-antigen malaria vaccines. The active involvement of AMA1 in merozoite invasion of erythrocytes, an event crucial for the parasite to complete its life cycle, has been a key factor for its consideration in multi-stage multi-antigen based vaccine believed to be possibly counterable through the use of related inhibitory antibodies.

Thus, such a multi-stage and antigen based vaccine candidate, which could attain rigorously boosted levels of anti-AMA1 antibodies, is perceived as one of the steps forward in the quest and attempts at not only creating, but developing an effective AMA1 incorporating or based malaria vaccine.

Molecular interactions that govern this process such as those involving host cell binding, merozoite reorientation, junction formation, parasitophorous vacuole formation and host cell entry appear to be only some of the areas scientists have just started to examine (Howell et al. 2001). It is envisaged that future research may concentrate on finding compounds that can fit inside this trough and where it could block the parasite from attaching to red blood cells (Haas and Fulling, 2008).

A school of thought opined that their AMA1-based malaria vaccine development strategy could require that two sequential milestones be accomplished prior to incorporation of one or more AMA1 antigens into a multi-stage, multi-component vaccine (Heppner et al, 2005). First, an AMA1 vaccine must confer significant clinical benefit in either a Phase 2a malaria challenge or in an endemic population. Second, the AMA1 vaccine must be sufficiently active against diverse AMA1 alleles such that the risk of allelic escape is very low. In anticipation of the potential requirement for inclusion of additional AMA1 alleles in a multi-antigen vaccine, the team produced GMP AMA1 representing the FVO allele and anticipate potentially combining the two FVO and 3D7 antigens in a vaccine, while current research efforts at Walter Reed Army Institute of Research (WRAIR) seek to identify a consensus AMA1 molecule/chimera that would elicit a broad immunity active against multiple P. falciparum AMA1 phenotypes (Spring et al, 2009).

Draper (2012) led his research team to develop the simian adenovirus (ChAd63) and MVA viral vectored vaccines and worked on optimisation of antibody induction by subunit vaccines against the blood-stage and more recently the mosquito-stage, of malaria parasite. The vaccine demonstrated potent and effective immunogenicity in pre-clinical models for both major arms of the body’s immune system. They have translated these findings into Phase I/IIa clinical vaccine trials funded by the MRC and EMVDA (Sheehy, et al.,2012).

At this point, it is pertinent to bring to fore the fact that when the Polio virus struck humanity in the 1930s, scientists began with efforts and searches for the solution to the dreaded Polio virus using various control methods directed against the environment and directly on the virus through vaccinology. The Polio virus, often described as the most lethal disease caused by any of the enteroviruses, belong to the Picornaviruses (Brooks et al, 2004) which have clinically manifested symptoms and sub-clinical infection whose etiology appeared more difficult to establish at that period in history and at certain stages it lead to the paralytic form of the disease. Two of the prominent scientists on board in the search and effort for effective vaccines were Dr. Sabin at Cincinnati who engaged a method that involved the stimulation of body to create its own antibodies using a live, less virulent strain of the virus and orally administered (NLM/NIAID, 2013; Wikipedia Free Encyclopedia, 2013b). It elicited production of antibodies in the intestinal linings and cleared the flourishing virus from the gut. Dr. Jonas Salk in 1947 (who was working on his search at Virus Research Laboratory at the University of Pittsburg) (Academy of Achievement, 2005) used a killed form of the virus for the vaccine which provoked the immune system to make antibodies in the blood at very effective levels to prevent paralysis, achieving successes before Dr Sabin. (NLM/NIAID, 2013; Wikipedia Free Encyclopedia, 2013a).

The success of Dr. Salk in the 1950s did not deter Dr. Sabin on his effort in the laboratory at coming up with a successful Polio vaccine. Also, the efforts of both scientists ended up shedding more and vital light on certain areas of technicalities and technologies engaged in vaccinology from which other scientists picked up useful facts.
and step on in their various vaccine research commitments against various other pathogens plaguing humans. The amazing thing is that both scientists followed different ideas and approaches on what a successful Polio vaccine may likely incorporate. Dr. Sabin’s oral Polio vaccine was easy to administer and conferred longer-lasting immunity. Hence, the two scientists had done great works - the first breakthrough for mankind on the Polio vaccine - applauded; then the second breakthrough which came some years after, had been very successful. The malaria Technology road map for the malaria vaccine may also likely achieve a higher level of efficacy and success than the first generation vaccine. Today, only about four countries on earth are yet to be free from the ravages of the Polio virus. The effort of both scientists had been pivotal in this respect.

CONCLUSION

PF83/AMA-1 shows homology with its counterpart - PK66/AMA from which an effective vaccine has been developed in animal models. This is one of the positive features of a vaccine candidate. The particular role of processing of PF83/AMA-1 in orientation of the parasite on the surface of the erythrocytes from its apex seem not yet definitive.

A clearer understanding of the entire biological functions and immunological targets presented by apical/rhoptry proteins of which the PF83/AMA-1 is one of them, may provide vital clues of features of the molecule that could be exploited in new immunological strategies against parasite. The efforts being made to improve on the shortcomings of AMA-1 as a vaccine candidate and consequent levels of improvements recorded by vaccinologists appear to be a compliment to the quest to fight malaria through the immune system by engaging an effective vaccine.

There are numerous candidate vaccine antigens and partial successes have been recorded in the past in attempts to develop vaccines from them. So, while the other approaches involved in controlling malaria are still being engaged, the search for a functional and effective malaria vaccine continues and AMA-1 has been and is one of those seemingly promising vaccine candidates being investigated.

Invariably, with success seemingly close for the RTS,S malaria vaccine to meet the 50% target product profile for the 1st generation malaria vaccine, projected through the commendable efforts by the WHO against the disease, thorough periodic re-appraisals and re-evaluations of the malaria vaccines in various laboratory bench works and on clinical trials worldwide; efforts using other technologies, concepts and malaria antigens of which include AMA1, with observed likely potentials as vaccine candidates, ought not to be totally waved-off or discouraged. It’s a malaria vaccine technology roadmap, there are various types of bends to negotiate, potholes to fill on the road, bumps to climb, but the destination is made clear by the WHO - beyond the success recorded by the 1st generational RTS,S/ASO1; on-towards a 2nd generation malaria vaccine success of much higher totality of efficacy. Eventually, the related and associated scientific community would benefit from the body knowledge and of course, humanity as well, either on the short or long run, as the vaccine research community and related funding agencies and stakeholders, step-up gear to enhance achievement of the goals on the qualities of the futuristically expected 2nd generation malaria vaccine

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