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Review

Viral vectors for malaria vaccine development

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Abstract

A workshop on viral vectors for malaria vaccine development, organized by the PATH Malaria Vaccine Initiative, was held in Bethesda, MD on October 20, 2005. Recent advancements in viral-vectored malaria vaccine development and emerging vector technologies were presented and discussed. Classic viral vectors such as poxvirus, adenovirus and alphavirus vectors have been successfully used to deliver malaria antigens. Some of the vaccine candidates have demonstrated their potential in inducing malaria-specific immunity in animal models and human trials. In addition, emerging viral-vector technologies, such as measles virus (MV), vesicular stomatitis virus (VSV) and yellow fever (YF) virus, may also be useful for malaria vaccine development. Studies in animal models suggest that each viral vector is unique in its ability to induce humoral and/or cellular immune responses. Those studies have also revealed that optimization of *Plasmodium* genes for mammalian expression is an important aspect of vaccine design. Codon-optimization, surface-trafficking, de-glycosylation and removal of toxic domains can lead to improved immunogenicity. Understanding the vector's ability to induce an immune response and the expression of malaria antigens in mammalian cells will be critical in designing the next generation of viral-vectored malaria vaccines.
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1. Introduction

Malaria is a significant cause of morbidity and mortality, affecting billions of people worldwide. It is estimated that malaria is responsible for the annual deaths of more than one million people, mostly children under the age of five [1]. An effective malaria vaccine is urgently needed to control the disease. Major obstacles to developing an effective malaria vaccine include the complex life cycle and antigenic diversity of the parasite. Despite the challenges, encouraging results have been reported recently for RTS,S, a pre-erythrocytic, circumsporozoite protein (CSP)-based vaccine. This leading candidate has demonstrated protection against clinical malaria in malaria-naïve adults in Phase 2a experimental challenge studies [2] and in African children in a Phase 2b field trial [3,4].

The traditional approach for malaria vaccine development is based on recombinant proteins. However, one of the main challenges for a recombinant protein-based vaccine is that the protein itself is poorly immunogenic. As such, many recombinant protein-based vaccines require the addition of an adjuvant to boost immune responses, especially when a Th1-biased immune response is desired. Most of the experimental malaria vaccines are currently using adjuvants that have yet to be used in licensed vaccines. In addition, many antigenic targets are highly structured and proper conformation seems to be required to generate a functional immune response. This leads to challenges in developing the purification process where refolding and isolation of proper conformers often results in complex manufacturing processes and low yields. Another challenge is the long-term stability of recombinant proteins when they are formulated in adjuvants at ambient temperatures. Viral vectors, on the other hand, appear to be capable of inducing both antibody and T-cell-mediated immunity in the absence of an adjuvant. Furthermore, complex process development may not be required for viral-vectored vaccines, which usually have a consistent purification process, irrespective of the transgene they express. As these vaccines use the eukaryotic cellular machinery to generate the antigenic targets, it may be possible to generate antigens with native conformation. Finally, some of the viral vectors have the capacity to deliver more than one gene. Thus, a single viral vectored construct may contain multiple antigens from the different parasite life stages and would have the potential to induce a broad protective immunity. Significant manufacturing cost savings could also be realized.

Viral vectors have been used in vaccine development for decades. Although earlier studies have generated mixed results, recently there have been substantial advances in applying viral vectors towards malaria vaccine development, and such vaccines have progressed to through Phase 2b efficacy testing in African children. Viral-vectored vaccines have shown some efficacy in malaria vaccine trials in humans but not so far in other diseases. At the same time, significant progress has been made in developing new viral-vector technologies, and some of them are being used for the development of HIV vaccines. To facilitate the application of viral vectors for malaria vaccine development, the PATH Malaria Vaccine Initiative (MVI) organized a workshop on “Viral Vectors for Malaria Vaccine Development.” The purpose of the workshop was to share experience in vector technologies and in malaria vaccine development, which could be very valuable in designing the next generation of effective viral-vectored malaria vaccines.

2. Poxvirus-vectored malaria vaccines

Poxviruses are known to induce long-lasting immunity. The large expression capacity of poxviruses makes them a popular choice for malaria vaccine development. Vaccinia virus expressing CSP has been studied in the ortholog *Plasmodium berghei* rodent model [5]. Although the vaccine reduced parasite load in the liver, its ability to confer sterile protection was modest [6]. However, immunization protocols using recombinant influenza virus as primer and recombinant pox virus as booster in the *Plasmodium yoelii* rodent model induced large CD8+ T-cell responses and a high degree of sterile immunity, demonstrating the high efficiency of heterologous prime-boost immunization protocols for the first time [6].

To overcome the shortfall of a single, antigen-expressing vaccinia virus, NYVAC-Pf7, a poxvirus-vectored, multi-antigen, multi-stage vaccine candidate was developed. NYVAC is a highly attenuated vaccinia virus strain derived from the Copenhagen vaccine. NYVAC-Pf7 expresses *Plasmodium* (*P.*) *falciparum*-derived CSP, trombospondin-related adhesive protein (TRAP), liver-stage antigen 1 (LSA-1), merozoite surface protein 1 (MSP-1), apical membrane

antigen 1 (AMA-1), serine repeat antigen (SERA) and Pfs25 [7]. The vaccine candidate was tested in a Phase 1/2a human trial [8]. Immune responses to all antigens except SERA were detected, although the titers were low. Vaccine doses of 10^8 plaque forming unit (PFU) appeared to be more immunogenic than 10^7 pfu. Pre-existing immunity against vaccinia virus appeared capable of modulating immunogenicity. The immunized subjects were subsequently challenged with *P. falciparum*-infected mosquitoes. One of the 35 immunized patients was fully protected from the challenge. An average of a 2-day delay in the pre-patent period was detected in the vaccinees.

In addition to NYVAC, other attenuated poxviruses such as modified vaccinia virus Ankara (MVA) and fowlpox strain FP9 have been used to deliver malaria antigens. MVA is another highly attenuated vaccinia virus strain that undergoes limited replication in human cells [9]. FP9 is an attenuated avipoxvirus that is particularly effective in inducing CD8+ T-cell responses. MVA expressing CSP and TRAP induced only a modest level of protection in the rodent malaria model. However, in combination with DNA vaccines in a heterologous prime-boost regimen, the recombinant MVA expressing CSP or TRAP induced high levels of CD8+ T-cell responses and provided protection against challenge with sporozoites of rodent malaria *P. berghei* [10].

ME-TRAP, a malaria vaccine candidate encoding TRAP and multiple malaria epitopes derived from different pre-erythrocytic stage antigens, was expressed in MVA and FP9. The vaccine candidates have been tested in humans in Phase 2a challenge trials, in both homologous and heterologous prime-boost regimens. Heterologous prime-boost was more effective in inducing T-cell-mediated immunity and in reducing parasite load, representing a 92% calculated reduction in liver-stage parasites [11–13].

Memory T-cells, as assessed by a cultured ELISPOT assay, were more strongly associated with the delay in pre-patent period than effector cells measured in an ELISPOT assay. Two of the five volunteers who received the heterologous regime with challenge less than a month after the final vaccination were fully protected against sporozoite challenge. One of the two volunteers was fully protected from a second sporozoite challenge 6 months post-immunization and a third challenge 20 months after the final vaccine [12]. This FP9-ME-TRAP and MVA-ME-TRAP regime was safe and immunogenic in West African adults [14] and Kenyan children [15]. A Phase 2b efficacy trial of this heterologous prime-boost regimen in Kenyan children started in early 2005 (A.V.S. Hill, personal communication).

In contrast to ME-TRAP, CSP expressed in MVA and FP9 was poorly immunogenic in humans. No protection was detected in heterologous prime-boost regimen. A polypeptide L3SEPTL, composed of LSA-3, sporozoite threonine, aspargine-rich protein (STARP), exported protein 1 (Exp-1), Pfs16, TRAP and LSA-1 has also been expressed in MVA and FP9 [16] and is expected to enter Phase 1 studies in 2006. Studies in an animal model demonstrated that all six antigens in the candidate were immunogenic. In addition to its utility as a vector, data in mice indicate that poxvirus can enhance the immunogenicity of recombinant protein-based vaccines through co-administration, indicating that the virus may be capable of acting as an adjuvant [17]. This finding raises the intriguing prospect of using a vectored vaccine to deliver malaria antigens while simultaneously serving as an immunostimulator for recombinant proteins.

3. Adenovirus-vectored malaria vaccine

Replication-deficient adenovirus vectors have been widely used in the development of vaccines against a variety of infectious diseases. There has been significant advancement in using adenovirus vectors for the development of HIV vaccines, in particular the T-cell-based vaccines [18]. In rodent malaria models, with *P. yoelii*, it has been shown that adenovirus vectors expressing CSP are excellent in inducing both T-cell and antibody responses, and in inducing protection against sporozoite challenge in the *P. yoelii* model [19,20]. A combination of experimental adenovirus-vectored malaria vaccines expressing CSP and AMA-1 of *P. falciparum* are scheduled for testing in humans in both homologous and heterologous prime-boost immunization regimens with DNA vaccines (D.L. Doolan, personal communication).

Adenovirus vectors are also currently being used to develop two second-generation, multi-antigen, multi-stage malaria vaccines. One of the vaccines contains blood-stage antigens AMA-1 and MSP-1, the other contains three pre-erythrocytic antigens CSP, LSA-1 and TRAP. Each of the malaria antigens is being optimized for expression in mammalian cells and for immunogenicity in animal models. It is known that there are significant differences in protein synthesis between protozoan and mammalian cells. For example, codon-usage of the *Plasmodium* gene is different from the mammalian gene. Codon-optimization significantly increases the expression level of *Plasmodium* antigen in mammalian cells. In addition, other than the glycosylphosphatidylinositol (GPI) anchor that attaches some of the antigens to the parasite surface, native *P. falciparum* proteins are not glycosylated [21]. However, AMA-1 and MSP-1 of *P. falciparum* contain multiple potential N- and O-linked glycosylation sites. It has been reported in one study that N-glycosylation of MSP-1 in mammalian cells decreased protective immune responses in non-human primates [22], whereas O-glycosylation of AMA1 did not appear to have an effect on the functionality of the antigens [23]. Studies are therefore being conducted to determine whether the removal of N-glycosylation sites leads to increased immunogenicity of the adenovirus-vectored, blood-stage vaccines.

Many Plasmodial surface proteins are anchored by GPI that appear to be poorly processed in mammalian cells. For

example, the GPI of MSP-1 does not function well in eukaryotes and this may be one of the main reasons that native MSP-1 is transported poorly to the surface of mammalian cells [24]. Attempts are being made to replace the Plasmodial GPI signal sequence with the mammalian sequence to improve the immunogenicity of the adenovirus-vectored MSP-1 vaccine. Also, the presence of GPI signal sequences appear to interfere with protein expression. For example, GPI signal sequence from CSP has been shown to affect protein expression in vitro and immunogenicity in a rodent model [25]. Site-directed mutagenesis is being conducted to reduce antigen-related toxicity and to improve the immunogenicity of the pre-erythrocytic stage vaccines. In addition, the use of a single adenovirus vector for expression of multiple antigens using different promoters is being studied. Preliminary studies suggest that the RSV promoter exhibits reduced gene expression and immunogenicity in animal models as compared to the cytomegalovirus (CMV) promoter (J. Bruder, personal communication). These data suggest that selection of a particular promoter will play an important role for adenovirus-vectored malaria vaccines.

A major challenge of adenovirus vectors for vaccine development is pre-existing immunity against adenovirus type 5 (Ad5), the prototype adenovirus vector. Pre-existing immunity has been shown to reduce the immunogenicity of these adenovirus-vectored vaccines in animal models [26–28]. Seroprevalence for Ad5 is as high as 95% in Africa [29,30]. To overcome the pre-existing immunity against Ad5, a new adenovirus vector based on serotype 35 (Ad35) has been developed [30–35]. The seroprevalence against Ad35 is significantly lower than that for Ad5. In Africa for example, Ad35 seroprevalence is only about 20%. [29,30] The Ad35 vector can be produced in PER.C6/55 K cells, a PER.C6 cell line that has been modified to express the Ad35-E1B 55 K protein [31], or in 293ORF6 cells, a cell line that is currently used for cGMP manufacturing of experimental vaccines. Ad35 can efficiently transduce human antigen-presenting cells. An Ad35 expressing CSP of *P. yoelii* has been tested in mice and shown to be highly immunogenic and protective [36]. Pre-existing immunity to Ad5 virus did not inhibit the immunogenicity of Ad35-derived vaccines. An Ad35 vector expressing CSP of *P. falciparum* (Ad35-CS) was tested in rhesus macaques. Ad35-CS induced a strong T- and B-cell-immune response. Interestingly, in contrast to Ad5-based vectors, Ad35 vectors are efficient in homologous prime-boost studies in primates. This construct is scheduled for testing in humans. In addition, a new candidate, Ad35.LCS, expressing a polypeptide containing LSA-1, CSP and STARP of *P. falciparum*, is being constructed. Future work will determine whether the three malaria antigens act synergistically in inducing protective immunity.

Some studies suggested that Ad35 is less immunogenic than Ad5 vector in animal models. This may be due to the lack of the high affinity receptor for Ad35 vector, the human CD46 protein, in the animal systems. In an effort to improve the immunogenicity of Ad35-based vaccines, one approach has been to exchange the fiber structures between Ad5 and Ad35. This has resulted in improved immunogenicity of Ad35-based vaccines in a rodent model [37]. In addition to Ad35, simian adenoviral vectors have been studied to overcome pre-existing immunity against Ad5. Preliminary studies using ME-TRAP suggests that simian adenovirus vectors are very efficient in inducing CD8+ restricted T-cell responses.

Intranasal administration of Ad5-based vaccine appears to be immunogenic and offers the potential of overcoming pre-existing immunity against the Ad5 vector. An influenza vaccine based on an adenovirus vector has been studied in humans [38]. This route appeared to be effective in inducing antibody responses at an inoculation dose of 5×10^8 particle units. It would be very interesting to see whether a higher immunization dose results in improved immunogenicity.

4. Alphavirus vectors

The alphavirus is a positive sense RNA virus. Replication-defective vectors, also referred to as replicon particles, have been developed using three different viruses: Semliki Forest virus (SFV), Sindbis virus and Venezuelan Equine Encephalitis (VEE) virus [39–42]. Replicon particles are excellent for induction of humoral and cellular immune responses. Double-stranded RNA generated during the replication of the vector may act as an immunostimulatory adjuvant. Alphavirus vectors have been used to deliver antigens for vaccines against a variety of diseases, including malaria [43,44]. In an animal model, a homologous immunization regimen with SFV replicon particles was very efficient in inducing antibody and T-cell-mediated immunity [45]. However, a challenge for alphavirus replicon particle technology is the lack of a cGMP-grade production cell line. Although the current electroporation-based manufacturing process is capable of producing cGMP-grade vaccine for human trials, technical hurdles have to be overcome before this method will be suitable for commercial manufacturing.

In addition to the replicon technology, alphaviruses can be used as vaccine vectors in the form of naked RNA and DNA. Plasmid DNA that expresses alphavirus replicase induces significantly higher levels of immune responses as compared to plasmid DNA without the replicase [46]. Similar to poxvirus, SFV particles can also provide adjuvant effects when co-administered with other vaccines.

5. Comparison of vectored malaria vaccines

The ability of different vectors to induce cell-mediated immunity (CMI) and antibody responses has been studied in a rodent malaria model. Adenovirus, alphavirus, poxvirus and DNA vectors expressing *P. yoelii* CSP as a model antigen were evaluated in mice in homologous and heterologous prime-boost regimens. In homologous regimens, ade-

novirus was most efficient in inducing cell-mediated immunity, whereas poxvirus was the least efficient vector for priming CMI. In heterologous regimens, adenovirus was very efficient in priming and boosting CMI responses. The poxvirus vector was excellent in boosting CMI but was very poor in priming the CMI response. Alphavirus replicons were efficient in priming CMI but less effective in boosting CMI (D.L. Doolan, personal communication).

The ability of each vector to prime and boost antibody responses was similar to that described for CMI. A notable difference was that the alphavirus replicons were excellent in priming and boosting antibody responses. In fact, homologous alphavirus immunization was as efficient as any heterologous immunization regimen with regard to induction of antibody responses. Protective immunity was also studied in the *P. yoelii* rodent malaria model. Immunization regimens including a poxvirus vector as a boost resulted in the highest level of protective immunity. However, this protective immunity appeared to be short-lived, as there was a significant reduction in protection 6 months post-immunization. In contrast, priming with adenovirus and boosting with an alphavirus vector resulted in long-term protective immunity. There was a strong correlation between CD8+ interferon γ responses and protection against sporozoite challenge (D.L. Doolan, personal communication).

In another study, different vectors expressing a CTL epitope, SYVPSAEQI, which is derived from CSP of *P. yoelii*, were compared in a rodent model. Adenovirus vector was excellent in induction of CD8+ T-cell responses. Alphavirus replicons were also very efficient in inducing $CD8^+$ T-cell responses, whereas vaccinia virus was a poor inducer of CMI. These results were in agreement with the data obtained using CSP described above. The ability of different vectors to boost the immune response was evaluated in mice primed with γ-irradiated sporozoites. Vaccinia vector was excellent in boosting CD8+ T-cell responses. Adenovirus vector was less effective in boosting CD8+ T-cell responses induced by γ-irradiated sporozoites (F. Zavala, personal communication).

Studies on the immunogenicity of different vectors revealed that the magnitude of the CD8+ T-cell response, defined by ELISPOT assay or FACS analysis, does not always correlate with the anti-parasite activity of primed CD8+ T-cells. This may be explained, at least in part, by recent findings indicating that memory CD8+ T-cells are a heterogeneous population consisting of different memory cell subsets with distinct functional properties. In certain viral systems, the "central memory" subset has been identified as the main mediator of protection [47], while studies using the *P. yoelii* system indicate that protective memory CD8+ T-cells belong to the "effector" or "peripheral" cell subset, which resides preferentially in non-lymphoid organs such as liver [48]. Also important is the fact that memory CD8+ T-cells of identical epitope specificity may sharply differ regarding their protective capacity thus emphasizing the urgent need to define new and more relevant criteria for the evaluation of T-cell responses induced by vaccine candidates.

6. Emerging viral vectors

6.1. Flavivirus vectors

The yellow fever (YF) virus is the prototype flavivirus. The YF 17D strain is a licensed, live attenuated vaccine strain that has been shown to be safe in infants older than 9 months of age. [49] A single dose of YF 17D provides lifelong immunity [50]. YF 17D is being used as a vector for the development of vaccines against other flaviviruses such as Japanese encephalitis (JE), West Nile (WN) and dengue viruses [51–53]. The vaccine approach included exchange of coat protein genes of YF virus with the corresponding genes from other flaviviruses. The chimeric vaccines have been shown to be safe in animal models [52,54]. Three of the chimeric vaccines, JE, WN and Dengue-3 are being tested in Phase 2 or 3 human trials [53] (T.P. Monath, personal communication).

The YF virus can be also used to express heterologous (non-flavivirus) genes through insertion in the non-structural region or selected sites of the E gene. A YF 17D virus expressing a CTL epitope of *P. yoelii* has been shown to reduce parasite load in a rodent malaria model [55]. In addition, when the repeat sequence of *P. falciparum* CSP was inserted in the F/G loop of the E protein, the resulting virus was capable of inducing anti-CSP antibodies in a rodent model [56]. The current expression capacity for YF 17D is about 1.5 kb. This limitation may present a challenge in expressing a full-length malaria antigen. Another challenge for using YF virus for malaria vaccine development is its contraindication for use in infants younger than 6 months of age. In this age group, YF 17D might be used as a booster in a heterologous prime-boost immunization regimen.

6.2. Measles virus vectors

Measles virus (MV) is a non-segmented, negative-stranded, RNA virus. The Edmonston-Zagreb strain is a live attenuated MV widely used for vaccination in pediatric populations. It has an excellent safety and efficacy record. The reverse genetics technology for MV has been well established [57]. MV has been used to express antigens derived from SIV, HIV, hepatitis B, mumps, WN virus and SARS-CoV [58–61]. In most of these studies, recombinant MVs that express heterologous antigens appeared to induce specific humoral neutralizing antibodies in a transgenic mouse model [62]. The MV expressing HIV Gag or Env were capable of inducing cellular immune responses to these proteins [58,60]. Moreover, in transgenic mice and macaques, MV expressing gp140 was capable of inducing anti-HIV antibody responses in the presence of pre-existing immunity against MV [58,60,63]. MV expressing *P. falciparum* MSP-1 is being investigated for use in malaria vaccine development (J.-F. Viret, personal communication). Due to the established safety profile of MV vaccine, it is recommended for young infants after the age of 6 months. In addition, several reports

have established the safety of the vaccine in children after an aerosolized application [64,65]. In this context, the approval of MV vaccination as an aerosol would open the way towards its application in younger infants even in the presence of pre-existing maternal antibodies. Therefore, the usefulness of MV in malaria vaccine development should be investigated for its ability to protect infants and children, with the added benefit of a simultaneous immunization against measles, one of the high priorities of WHO's Expanded Program on Immunization [63]. Alternatively, MV may also be used as a booster in a heterologous prime-boost regime for young infants.

6.3. Vesicular stomatitis virus vectors

Vesicular stomatitis virus (VSV) is a non-segmented, negative-strand, RNA virus in the family Rhabdoviridae. In nature, arthropods are the likely reservoir for VSV. However, the virus can also be transmitted to livestock, causing vesicular lesions on the skin around the mouth, nostrils and teats. Human infection with wild type (wt) VSV is rare, and consequently there is a very low VSV sero-prevalence in the human population. When VSV does infect humans, it is either asymptomatic or may result in a mild, self-limited, flu-like illness. When it became possible to recover VSV from genomic cDNA, the potential of recombinant VSV (rVSV) as a vaccine vector was explored. In the SHIV challenge model, it has been demonstrated that rVSV expressing SIV gag and HIV env provides significant protection against development of AIDS in non-human primates (NHPs) [66]. However, results from an exploratory neurovirulence (NV) study in NHPs indicated that rVSV was insufficiently attenuated for human testing. The NV phenotype represented a potential obstacle to regulatory approval of rVSV vaccine vectors. To address this problem, a variety of strategies were developed to generate further attenuated rVSV vectors. As a result of these efforts, two rVSV mutants, N4CT1 and N4CT9, which have the N gene translocated from the first to the fourth position in the genome, and the cytoplasmic tail (CT) of the attachment (G) protein truncated from 29 amino acids to either one or nine amino acids, respectively, were generated and shown to cause very minimal neuropathology in a subsequent NHP NV study. Surprisingly, the highly attenuated N4CT1 and N4CT9 rVSV vectors expressing HIV gag were shown to be more immunogenic than the much more virulent prototypic rVSV-gag vector, following intramuscular inoculation of mice. Both attenuated vectors also elicited cellular immune responses in NHPs that were equivalent in magnitude to responses elicited by the prototypic rVSV-gag vector (D. Clarke, personal communication).

7. Discussion

Viral vectors are excellent delivery vehicles for malaria antigens. Understanding the unique properties of individual vectors to induce humoral and cellular immunity is an important aspect in vaccine design. The classical vaccinia virus vector appears to be less effective in priming immune responses, but is excellent in boosting immune responses. As such, it is likely that a vaccinia virus vector will be useful in heterologous prime-boosting regimens in combination with other vectors or DNA/protein-based vaccines. Replication-deficient adenovirus vectors have been shown to be excellent in priming and boosting immune responses, in particular for generating cellular immune responses. Therefore, it may be the preferred vehicle for T-cell-based vaccines. However, most of the adenovirus-vectored vaccines are based on Ad5. Therefore overcoming pre-existing immunity against the adenovirus vector may be critical for vaccine development. Alphavirus vectors have been shown to be excellent in inducing antibody responses in animal models. They may be useful for blood stage or sexual stage malaria vaccines, where it has been demonstrated that antibody responses are associated with inhibition against parasite growth. However, a cGMP manufacturing production cell line is needed for alphavirus vectors. The current production process using electroporation is unlikely to be suitable for commercial manufacturing.

New emerging vectors provide additional opportunities to design new malaria vaccine candidates. In contrast to the classical replication-deficient viral vectors, vectors such as measles virus, yellow fever virus and VSV are replicating vectors. They have small genomes and therefore the level of antigen expression is likely to be high. Furthermore, vectors such as measles virus and yellow fever virus are based on pediatric vaccines with proven safety and immunogenicity profiles. However, the use of these replicating viral vectors poses some challenges in safety and immunogenicity in the target population of very young infants. Nonetheless, these vectors should be explored for the development of malaria vaccines.

The rational optimization of a vectored malaria vaccine will also be dependent on understanding the processing of particular malaria antigens in mammalian cells. In some cases, the gene may benefit from codon-optimization to mimic that of mammalian usage. Removal of N-glycosylation sites should be evaluated for maintenance of the conformation and antigenicity of the native protein. For proteins that are targeted for antibody responses, there may be a need to exchange signal peptide and anchor sequence to mammalian-like signals to facilitate transportation to the cell surface. Finally, as some of the Plasmodial antigens may be toxic to the vector or mammalian cells, site-directed mutagenesis may be necessary to reduce the toxicity.

The use of viral vectors for the development of multi-stage, multi-antigen vaccines will provide useful information on synergies of protective immunity and/or immunological interferences amongst different antigens. In addition, studying the fundamentals of the immune response induced by viral vectors may also help us to identify correlates of protection and to design the next generation of more effective vaccines.

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