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Short Communication

Design of Nanoparticle-Based Antigens as Multivalent, Multivariant, Low-Polymorphism Blood-Stage Malaria Vaccine Candidates

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Abstract

Several protein-specific domains, such as intrinsically disordered domains and α-helical segments have been described as promising targets for malaria blood stage vaccine candidates. Used alone or in combination, these protein domains have been shown in numerous studies to be antigenic/immunogenic and correlated with protection against clinical malaria. However, their potential as part of an approach to develop a single multivalent, multispecific vaccine antigen based on self-assembled protein nanoparticle (SAPN) delivery system is less explored. Here, using a designed SAPN model, we discuss the relevance of a P27/P27A nanoparticle-based blood stage antigen with other blood stage antigens, such as the dimorphic and C-terminal domain fragments of the two PfMSP2 allelic families. Thus, the goal of this work is to promote studies investigating the stability, safety, and protective properties of multivalent nanoparticle malaria vaccines against the two major malaria pathogens. Therefore, we postulate that the combination of the α-helical coiled-coil and unstructured antigens into SAPN constructs will yield a promising blood stage malaria vaccine candidate to protect against both *P. falciparum* and *P. vivax* infections.

Keywords: Blood-stage antigen; Malaria vaccine, Nanoparticle system, Plasmodium sp

Introduction

Advanced bioinformatic analyses of the Plasmodium genome and proteome have led to the identification of Plasmodium proteins containing α-helical coiled-coil segments and intrinsically disordered regions. We and others have shown that these domains are antigenic/immunogenic, and immune responses against these proteins are correlated with protection against clinical malaria. Their potential, however, has not been fully explored. Their use in multivalent, Self-Assembling Protein Nanoparticle (SAPN) constructs is still largely understudied. These scaffolds can present a variety of antigenic fragments at once, enabling vaccines to generate immunity against multiple species simultaneously [1]. As a result, this strategy is attractive for targeting malaria in regions where Plasmodium species-such as *P. falciparum* and *P. vivax*-coexist [2].

Here, we discuss the relevance and feasibility of synthesizing SAPN vaccine candidates against blood-stage malaria which leverage?? α-helical coiled-coil regions and intrinsically disordered domains. Specifically, we aim to use the non-polymorphic domains P27 and P27A derived from blood-stage protein 1, in conjunction with antigens from the dimorphic and C-terminal fragments from the two PfMSP2 allelic families. The final goal is thus to create multivalent SAPN blood stage malaria vaccines against the two primary malaria pathogens, *P. falciparum* and *P. vivax*.

Current Malaria Vaccines-Limitations and Challenges

So far, only two *P. falciparum* vaccines-RTS, S/AS01/Mosquirix and R21/Matrix-M-are recommended by the World Health Organization (WHO) for widespread use for children in sub-Saharan Africa [3,4]. Both vaccines are pre-erythrocytic, virus-like particles which include the same immunizing antigen: the P. falciparum Circumsporozoite Protein (PfCSP). RTS, S/AS01/ Mosquirix affords modest protection against the disease, with a ~39% efficacy rate in children in Sub-Saharan Africa after administering four doses [3]; however, available supply of RTS, S is limited. Furthermore, R21/Matrix-M reaches a 75% efficacy rate against clinical malaria during the 12 months following a 3-dose series [4]. Despite these advances, the development of efficient and robust malaria vaccines is far from finished. This is especially true for vaccines based on blood-stage malarial antigens, and those that would create immunity against multiple species simultaneously. Promising immunogenic antigens have been identified as ideal for multi-antigenic construct designs. This offers the opportunity to select effective blood-stage antigen candidates targeting both major malaria pathogens [22].

SAPN Delivery System-Relevance in Vaccine Development

Nanoparticles (NP, 1-100 nm in diameter) containing immunogenic antigens are advantageous vaccine candidates. These constructs function both as a delivery system (carrier) of antigens and as an immune-stimulating or immunomodulatory agent (adjuvant), making them attractive research targets compared to conventional vaccines [5,6]. Furthermore, NP protect their corresponding antigens against proteolytic cleavage, prolonging their half-life and increasing the duration of antigen exposure to immune cells [6,7]. As a result, there is great interest in this technology for developing vaccines and drugs against infectious diseases, including malaria [8].

Various NP delivery systems have been explored, including liposome NP, polymeric and inorganic NP, self-assembled protein NP (SAPN), virus-like particles and virosomes, and self-amplifying RNA vaccine deliveries [6,7]. SAPN are well-explored particles, which result from oligomerization of several monomer subunits [9,10]. Indeed, these constructs enable the incorporation of multiple protein domains into a stable complex and present antigenic / immunogenic epitopes on their surface. In addition, SAPN allow for B- and T-cell epitopes to be inserted into the N- or C-termini of the monomer-encoding gene, resulting in more effective immune responses which trigger both cellular and humoral immunity [9,10]. Furthermore, it may be possible to obtain a strong immune response in humans without the addition of antigens as seen in a mouse model with coiled-coil domain [9].

Antigenic-SAPN As Stable, Multivalent Vaccine Candidates Against Blood-Stage Plasmodium

P27, P27A And MSP2 Characterized Motifs as Promising Antigen Candidates

We in conjunction with others have demonstrated that α-helical coiled-coil regions and intrinsically unstructured domains present in the blood-stage proteins P27 and P27A, as well as MSP2, are promising vaccine candidates that warrant further exploration [9,11-13]. P27 (27 aa:845-871) and P27A (104 aa:223-326) fragments are derived from TEX1/PFF0165c. Our work demonstrated that immune responses against these proteins is associated with partial protection from Plasmodium [14]. P27 and its extended segment Ext-P27 (Pf-27-NC; 49 aa:846-893) are α-coiled coil domains with antigenic properties that can be safely coupled with adjuvants. We also found that P27 and its extension Pf-27-NC and P27A are suitable for inclusion into self-assembling protein nanoparticle (P27/P27A-SAPNs, P27A/Pf-27-NC-SAPNs, P27A/Pf-27-NC-SAPN), with SAPN monomers effectively displaying each antigen at their N and C termini [9]. We showed that these constructions had high immunogenicity in murine models without the use of

an adjuvant. The Pf-27-NC fragment is highly conserved among different Plasmodium species and therefore has been recommended for use in constructs aimed at providing cross-protection against P. falciparum and P. vivax [9].

In addition, we have shown that the intrinsically unstructured fragments in the PfMSP2 allelic family proteins 3D7 and FC27, such as their dimorphic (D) regions (88aa:111-198 and 48 aa:143-191, respectively) and the constant C-terminal (40 aa:198-238; 3D7 numbering) were antigenic and immunogenic, both alone and in combination with various adjuvants in both mice and humans [11,15]. Those studies also revealed significant cross-reactivity of natural antibodies for both PfMSP2 allelic families over time, which is strong evidence that including both allelic family fragments in a vaccine construct will further enhance efficacy.

SAPN Antigen Construct Containing P27, P27a and PfMSP2 Motifs

Here we illustrate the design of P27/P27A-based SAPN constructs using the antigen described above. In addition, recombinant antigen (FusN) comprising the two allelic D and C fragments of PfMSP2 and P27A [16] proved to be a better antigen/immunogen, with specific antibodies strongly associated with protection. This SAPN was further modified by changing P27 with the extended Pf27-NC. The 3D arrangement of the second construct was modeled using AlphaFold [17] (Figure B). Only one designed construct out of many is shown within the spherical core of the SAPN. As reported above, the three antigens P27, P27A and MSP2 were tested in mice with conventional adjuvants and were found to be highly immunogenic and superior to a mixture of individual ones [15,16].

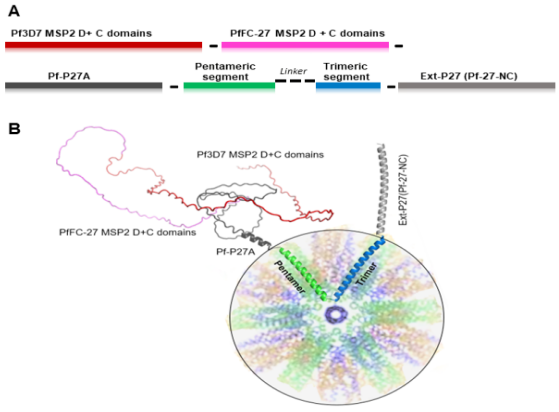

Figure: Self-assembling protein nanoparticle construct containing Plasmodium falciparum α-helical coiled-coil region and intrinsically unstructured domain derived from TEX1 and PfMSP2 Self-assembling protein nanoparticle (SAPN) construct includes the dimorphic D and C-terminal domains of the two allelic families of PfMSP2 (3D7 and FC27), Pf-P27A and designed pentameric and trimeric α-helical coiled-coil segments as a core for the construction of SAPN, and extended Pf-P27 (Ext-P27) fragment, i.e., Pf-27-NC; NC, N- and C-terminal. **(A)** Schematic representation of the SAPN construct sequence and **(B)** its 3D arrangement, modelled with AlphaFold [119]. Only one designed construct of many is shown within the spherical core of the SAPN; the results are not sufficiently accurate for unstructured regions. TEX1, trophozoite exported protein 1; MSP2, merozoite surface protein 2; D, dimorphic region of each allelic family of MSP2; C, C-terminal or common region for the two allelic MSP2 families, Pf, *Plasmodium falciparum*.

In addition, the novo-designed pentameric and trimeric oligomerization domains present in the SAPN constructs lack homology with human proteins, which minimizes the possibility of inducing immune responses that could be detrimental to the host. At the same time, our strategy emphasizes the potential for including both *P. falciparum* and *P. vivax* antigens in a single antigen, thereby promoting cross-protective candidates. Indeed, we have shown a high degree of cross-reactivity between *P. falciparum* and *P. vivax* coiled coil orthologs [18]. Thus, fragment Pf-27-NC, which is highly conserved among Plasmodium species, may be an effective antigen for multivariant vaccines to generate cross-reactive protective immunity. This is similar to the inclusion of both allelic family MSP2 protein fragments as described above.

Conclusion

Overall, our proposed strategy will determine the design feasibility and efficacy of multivalent, multispecies, cross-protective malaria vaccine candidates. We have highlighted the advantages of using SAPN delivery systems to design blood-stage malaria vaccines. We will incorporate antigens derived from both *P. falciparum* and *P. vivax* into the N- and C-termini of SAPN to obtain a cross-reactive immunogen. In addition, these strategies will overcome the extensive polymorphism exhibited in many human Plasmodium antigens and avoid time-consuming characterization of the three-dimensional structure of native proteins/domains.

Expert Opinion

The present work outlines the trends and perspectives in developing malaria vaccines using highly conserved α-helical coiled-coil regions and intrinsically unstructured domains present in the asexual blood-stages antigens of both P. falciparum and P. vivax parasites.

Here, we focused on α-helical coiled-coil protein segments suitable for inclusion into malaria vaccine candidates that target blood-stage malaria. Typically, α-helical coiled-coil motifs are composed of 30-50 aa residues containing multiple seven-residue repeat sequences (abcdefg)n, where nonpolar residues are usually present at positions a and d, and polar residues everywhere else in the sequence [13,19]. These α-helical coiled-coil motifs occur frequently on the surface of pathogens and, when isolated, can spontaneously assemble into stable, native folded structures. Their features make them attractive templates for mimicking structural epitopes in vaccine development. The initial study consisted of in-silico identification of these motifs from a large group of proteins from the P. falciparum erythrocytic phase, using the malaria gene bank. Selected sequences were synthesized, chemically characterized and further used to determine their antigenicity using ELISA assessment with sera of individuals from malaria endemic regions in Africa. Circular dichroism studies of these segments showed, in general, the presence of an α-helical structure [20,21].

These studies also indicated that many of the motifs were recognized by semi-immune individuals in an age-dependent manner; of note, the sera reactivity was associated with clinical immunity to P. falciparum in the study populations [20,21]. We assessed the in vitro activity of antibodies against a select set of antigens and found a significant association between in vitro antibody-dependent cell-mediated inhibition (ADCI) assays and clinical immunity of donors [12,21]. Furthermore, we selected three coiled-coil fragments displaying the best performance in the described assays and designed a multi-epitope construct (Pf-181), which was synthesized and tested to determine its immunogenicity in mice [12].

Based on this encouraging data and the conserved nature of these protein fragments, we performed an in-silico analysis to identify ortholog P. vivax coiled-coil sequences. These were synthesized and evaluated for immunoreactivity using sera from P. vivax endemic areas in Papua New Guinea and Colombia [22]. Both the high antigenicity and immunogenicity of the orthologous motifs, their association with clinical immunity, and their cross-reactivity with P. falciparum sera, were confirmed [18,22]. A synthetic P. vivax multi-epitope construct has been recently produced using the selected orthologous sequences and has demonstrated good immunogenicity in rodents.

The α-helical coiled-coil epitopes make these domains prime candidates for incorporation into Self-Assembling Protein Nanoparticles (SAPNs) which our laboratory has developed over the past few years [9]. An early SAPN prototype, displaying both a coiled-coil sequence and the intrinsically unstructured P27A region of the Tex1 protein, demonstrated good immunological properties and served as the basis for engineering several α-helical coiled-coil antigens into one single SAPN to serve as a multi-functional immunogen [9].

We have also investigated so-called "natively unstructured regions" of protein fragments from blood-stage P. falciparum to include them into in malaria vaccine candidates [19]. These unstructured motifs, which are frequent in all genomes (30-40%) and especially common in eukaryotic cells, are most suitable for mimicking native linear epitopes [23]. Indeed, these motifs have highly hydrophilic amino acid sequences that cannot form a hydrophobic core needed to stabilize a globular structure. Furthermore, these regions can be readily identified in bioinformatic analyses, and their corresponding peptides are mostly soluble, unfolded and mimic the native state [19,23-25]. Furthermore, we have investigated peptide fragments and recombinant proteins representative of these intrinsically unstructured domains – such as those of the merozoite surface protein 2 (MSP2) -as vaccine antigens [11,15]. Indeed, these domains were found to be reactive against sera from endemic areas, and immunogenic in mice. In addition, various epitopes from the unstructured domains of MSP2 could be identified, with variations according to the age of the population. We have furthermore reported that antibodies specific to these intrinsically unstructured domains (alone or in combination) had a significant association with protection against clinical malaria and exhibited parasite growth inhibition activity [12,15,16]. Therefore, we are confident that the combination of the α-helical coiled-coil and unstructured antigens into SAPN constructs will yield a promising malaria vaccine candidate to protect against both P. falciparum and P. vivax infections.

Author Contributions

All authors contribute equally in conceiving, writing and revising the manuscript. They all read and approved the last version of the manuscript.

Conflict of Interest

Peter Burkhard is the founder, co-owner and CEO of Alpha-O Peptides AG, a company involved in nanoparticle vaccine design. The other authors declare that the research was conducted without any commercial or financial relationship that could be construed as a potential conflict of interest.

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