Self-Assembling Protein Nanoparticles: implications for HIV-1 vaccine development

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“This technology could be adapted for HIV-1 vaccine development to present the V1V2 loop in a native-like conformation to induce high titers of binding, neutralizing and functional Abs, T-cell responses targeting multiple epitopes and subdominant epitopes, all of these will hopefully expand upon and improve the results of RV144.”

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Self-Assembling Protein Nanoparticles: a modern platform to increase the immunogenicity of vaccines

Subunit vaccines are one of the major focuses of modern vaccine development. They tend to be more tolerable than whole organism vaccines, however, they also tend to be less immunogenic. To address this problem, different approaches such as delivery of the antigen on a particulate carrier, displaying multiple copies of an antigen and/or use of a modern adjuvant system can lead to stronger immune responses [1]. Nanoparticles can be designed to combine all of these approaches allowing for the development of effective vaccines against various pathogens [2].

One such nanoparticle platform, the Self-Assembling Protein Nanoparticles (SAPNs), have been used to develop vaccines for different infectious diseases [3–13]. SAPNs are designed using coiled-coil protein folding motifs. Each SAPN monomer is composed of two different oligomerization domains connected by a short linker. On either or both termini of these protein chains antigens can be engineered to be surface exposed on the refolded particle [14]. To increase cellular immune responses, CD4⁺ T_H and CD8⁺ epitopes can also be engineered into both the surface exposed and core regions of the SAPNs [3–5]. Monomers spontaneously self-assemble into a three-dimensional particle that can be decorated with multiple copies of the antigens [14].

Modern vaccines contain adjuvant formulations that can enhance, modulate and prolong the immune response. SAPNs can be used in combination with a built-in adjuvant system [5,6], a traditional adjuvant [7,8] or a modern adjuvant system [9]. Thus, SAPN-based vaccines may increase not only the magnitude, but also the durability of the immune responses.

Previous SAPN applications

Malaria

The first application of the SAPN technology was the development of a malaria vaccine. The initial candidate targeted the asexual pre-erythrocytic stage of the disease by presenting 60 copies of the immunodominant repeat region of the circumsporozoite protein antigen (CSP) from rodent malaria species *Plasmodium berghei*. This vaccine resulted in long-lasting protection in multiple mouse strains without the presence of an adjuvant [10]. A more advanced candidate for the human malaria species *Plasmodium falciparum* was further developed that also included the immunodominant repeat region along with the addition of multiple CSP CD8⁺ epitopes. It induced high antibody (Ab) titers that again led to long lasting protection in a murine model without the addition of an adjuvant [3]. The current vaccine candidate (FMP014) included the thrombospondin Type-1 repeat (αTSR)

domains of the CSP protein, as well as multiple CD4⁺ epitopes to the core. This candidate was combined with two different adjuvant systems, admixed liposome adjuvants, Army Liposome Formulations (ALF) [9] or a built in TLR5 agonist adjuvant, flagellin [6]. Immunization of C57BL/6 mice resulted in very high immunoglobulin G (IgG) Ab titers and T-cell responses. FMP014 adjuvanted with ALF containing QS-21 (ALFQ) has been used in rhesus macaques and will be used in a controlled human malaria infection Phase I/IIa clinical trial.

SAPNs were also used to target the asexual blood stage of the parasite's life cycle with a recently identified antigen, Tex1. Two previously established immunogenic regions of the Tex1, P27 and P27A were engineered into the particle. P27A is predicated to be an intrinsically unstructured region of the protein, however, P27 is a trimeric coiled-coil. The SAPN allowed P27 to be presented in its native trimeric conformation, which led to an increase in immunogenicity in mice. The P27/P27A-SAPN reacted with sera from individuals from a malaria endemic region and these novel SAPNs are predicted to provide cross-protection between *P. falciparum* and *Plasmodium vivax* [4].

SARS

One of the other initial attempts to develop a SAPN based vaccine targeted the coronavirus causing severe acute respiratory syndrome. This SAPN presented 60 copies of the SARS B cell epitope from the C-terminal heptad repeat of the virus' spike protein. Using the SAPN scaffold, the vaccine was capable of mimicking the native trimeric coiled-coil structure of the spike. Immunization of BALB/c mice with this SAPN candidate resulted in the development of high titers of conformation specific Abs that were capable of neutralizing the SARS virus *in vitro* [11].

Toxoplasmosis

The primary effector response against the causative parasite *Toxoplasma gondii* is mediated by cytotoxic T-lymphocytes necessitating a vaccine candidate that utilizes CD8⁺ and CD4⁺ epitopes. Initial designs focused on a single antigen (dense granule 7 [GRA7₂₀₋₂₈ LPQATTAAT]) presented on a SAPN that contained the universal CD4⁺ epitope PADRE. This candidate was capable of eliciting antigen-specific CD8⁺ responses in a transgenic mouse strain for human HLA challenge model and significantly reduced *T. gondii* cysts in the brain [12]. A more advanced candidate was designed to contain CD8⁺ epitopes from the surface antigen-1 (SAG1), the dense granule proteins (GRA5 and GRA6), and the SAG1-related sequences (SRS52A). In addition, this candidate with a built in flagellin adjuvant was admixed the TLR4 agonist GLA-SE. The construct also contained multiple universal CD4⁺ epitopes. As predicted, this vaccine elicited specific CD8⁺ responses and it significantly reduced the *T. gondii* cysts in the mouse brain [7].

Influenza

The first attempt at a universal influenza A vaccine utilized the external domains of the viral membrane proton channel M2. By presenting 48 copies of this antigen in its native tetrameric conformation on a SAPN, vaccinated animals developed high Ab titers that were capable of reducing viral shedding during a low pathogenic viral challenge in chickens [13]. A more advanced construct contained not only M2, but also the conserved Helix C of the hemagglutinin stalk, which was presented in its native trimeric conformation. This candidate also had multiple universal CD4⁺ epitopes as well as the immunostimulatory molecule flagellin. Higher titers of Abs were obtained in animals immunized with self-adjuvanted SAPNs than nonadjuvanted constructs. Vaccinated chickens were capable of raising Abs with neutralizing activity against multiple viral subtypes. In a mouse model, animals were completely protected from a high pathogenic influenza challenge [5].

SAPNs for HIV-1 vaccine development

A SAPN-based vaccine may be optimal for the development of a potent HIV-1 vaccine. Previously, we developed a SAPN that contained the membrane proximal external region antigen (MPER) of HIV-1. This candidate was capable of inducing high Ab titers, however, not surprisingly, those Abs were not neutralizing [8]. To date, MPER-based vaccine candidates have been able to generate neutralizing Abs with only low potency and breadth [15]. Recently, a new target was identified in a Phase III clinical trial, RV144. In this trial, modest efficacy against HIV-1 acquisition was demonstrated, and the risk of infection was inversely correlated with the presence IgG Abs raised against the V1V2 loop of the virus envelope protein [16,17].

The V1V2 loop represented only a small fraction of the RV144 immunogens in the vaccination regimen, and a subunit vaccine with increased copies of this region may lead to a better vaccine. To develop a strong Ab response

Figure 1 Computer models of a potential V1V2 Self-Assembling Protein Nanoparticles. The V1V2 loop is highlighted. (A) SAPN monomer design. (B) Side view of the V1V2 trimer on the coiled-coil arm of SAPN. Three SAPN-monomers assembled as a trimer. (C) Top-down view of the V1V2 trimer. (D) The fully assembled SAPN structure. Icosahedral SAPNs display 20 copies of the V1V2 trimer. Color scheme: Grey: HisTag; Green: Pentamer; Blue: Trimer; Red: Stem of the V1V2 loop; Gold: V1 loop; Purple: V2 loop. SAPN: Self-assembling protein nanoparticle.

to viral surface proteins it is known that the spacing and density of these epitopes is important [18]. SAPNs allow for high density presentation of the V1V2 loop and optimal spacing to increase the Ab response. It is also believed that the trimeric presentation of the V1V2 loop is essential for the development of functional Abs [19]. The spontaneous formation of the trimeric coiled-coils of the SAPN allows 20 copies of the V1V2 loop trimer to be presented. By using computer modeling the SAPNs can be optimally designed, so the V1V2 loop can be presented in a native-like trimeric conformation (Figure 1).

An effective vaccine needs to induce functional Abs. Previous studies have indicated that antibodies raised against the V1V2 loop do not function through neutralization, but through other antibody-mediated mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), and antibody-dependent complement-mediated lysis (ADCM) [20–22]. By incorporating universal CD4⁺ epitopes into the SAPN core there should be an increase in the titer of functional Abs to the V1V2 loop as demonstrated with previous SAPN candidates [3,5,7]. Due to the native-like presentation of the V1V2 loop, conformational Abs should be generated, again increasing the functionality of the vaccine.

A major concern with HIV-1 vaccine development is the sequence diversity of the virus. We can address this problem by two different approaches. The first is the choice of the V1V2 loop sequence that is presented on the SAPNs. By identifying loops that contain reduced hypervariable regions we can direct the immune response to the more conserved residues. We have previously demonstrated that the influenza candidates containing highly conserved sequences can result in a vaccine candidate that is cross-reactive between different strains [5]. Another approach is to generate particles that are co-assembled from SAPN monomers that contain different V1V2 loops. A tailored vaccine candidate can be developed for each geographical region taking into account the predominantly circulating strains.

Despite the failure of T-cell vaccines tested to date, cellular immunity may be needed for an efficacious prophylactic vaccine. Non-human primate (NHP) studies support the potential efficacy of a prophylactic T-cell vaccine [23]. In addition to the B cell immunogen, SAPNs can contain multiple CD4⁺ and CD8⁺ epitopes in their cores and universal and/or HIV-1-specific CD4⁺ sequences that may induce a balanced humoral and cellular immune response to the vaccine.

Conclusion

SAPNs are a unique vaccine platform. They can display B cell immunogens, are capable of multivalent presentation of epitopes and have the potential to induce CD8⁺ T-cell responses through cross presentation. The immune response to a SAPN-based vaccine can further be increased by the incorporation of the TLR5-agonist flagellin into the particle [5,6] or by the addition of potent next generation adjuvants such as one of the Army Liposome Formulations [24]. This technology could be adapted for HIV-1 vaccine development to present the V1V2 loop in a native-like conformation to induce high titers of binding, neutralizing and functional Abs, T-cell responses

targeting multiple epitopes and subdominant epitopes, all of these will hopefully expand upon and improve the results of RV144. SAPNs also allow for reasonably low cost and high yield production of the V1V2 loop that provide a very promising platform for HIV-1 vaccine development.

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