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A brief go-to on the epidemiology of Malaria and the usage of virus like particles (VLPs)as a novel immunogenic vaccine platform

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Abstract :

VLPs are non-infectious, self- assembling nanoparticles that have the potency to stand out as an attractive tool in medicine and nanotechnology. They do enable surface display as well as payload encapsulation, proving their versatility in the preparation of subunit vaccines, which otherwise poses as a low immunogenic aid. They do consist of viral structural proteins, which assemble into icosahedrons, mostly in the range of 20-200 nm range. Their repetitive molecularly portrayed architecture and further, the decoration of the VLPs with target-antigens by fusion techniques aspire to trigger various potential immunogenic arms like B cell receptor clustering, drainage to lymph nodes, heightened APC uptake, co-stimulatory responses such as the TLR responses etc.

Keywords: VLPs, Malaria, RTS,S/AS01, Plasmodium sp. , nanoparticles, multimericity

1. Introduction :

Globally, Malaria cases are estimated to be around 229 million across the 87 malaria endemic countries (2019), while the deaths are assumed to be around 409,000 in 2019, with 67 % attributed solely to children under 5 years (World Health Organization, 2019). There are 3 broad strategies for malaria vaccines: the pre-erythrocytic vaccines, which must aim to target the infected hepatocytes, sporozoites, next, the erythrocytic or blood stage vaccines that must target surface antigens on infected RBCs or Merozoites, and finally the transmission blocking vaccines, that should target the further downstream development of sexual stages inside the mosquito vector (Beeson et al., 2019).

The development of an efficient malaria vaccine remains a priority, for even the most advanced one RTS,S/AS01 has shown merely modest activity. The potency of protein subunit vaccines always remain poorly immunogenic. VLPs present

an unique, novel platform in heightening the immunogenicity of vaccines. VLPs are viral structural proteins, that upon recombinant expression, self assemble into non-infectious nanoparticles (Zinkhan et al., 2021). Overall, the multimericity, size, RNA and it's numerous potencies, pose VLPs as a an attractive candidate for Vaccine production. Their activities to induce both humoral and cellular responses have already been reported (Raghunandan, 2011). They can intake larger subunits of proteins to be displayed on their surface by various conjugation techniques like Genetic Fusion. Catcher/Tag, Sortase A Tag, Intein Tag, SNAP Tag or non-covalent ones, exploiting the affinity potential of Biotin/Avidin, His Tag-Ni-NTA.

2. Biology and Life cycle of Plasmodium sp. :

Plasmodium sp. are obligate unicellular, intracellular parasites in the phylum of Apicomplexa, portraying both sexual and an asexual stage, parasitizing in an invertebrate host i.e., Anopheles female mosquitos and a vertebrate mammalian host (Su et al., 2020) ;(Bettencourt, 2020). Five species known to infect human are P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi, wherein P. falciparum is deadliest to human and P. vivax is the most widespread.

Whilst taking blood meal, different species of Plasmodium infected female Anopheles mosquito injects about hundred sporozoites into mammalian skin (Antinori et al., 2012); (Medica & Sinnis, 2005). Sporozoites soon makes it's way to the circulatory system through the capillaries in the skin and finally infect the cells in the liver(Hopp et al., 2015; Shortt et al., 1948), where it travels through several hepatocytes, before selecting one convenient, wherein it resides in it's parasitophorous vacuole, possessing added specialized functionality (Mota et al., 2001);(Mota et al., 2002).During the pre-erythrocytic stage, hepatic infection that proceeds is asymptomatic and often takes about 7 days to complete, during which the parasites go through asexual schizogony to form tens of thousands of merozoites(Antinori et al., 2012). This pre-erythrocytic or exo-erythrocytic phase however represents the bottleneck stage of the disease where it can be easy to block using immunoprophylactic or chemotherapeutic knocks. RTS,S/AS01 is the only malaria vaccine to have passed the phase 3 clinical trials and is made of CSP antigen presented on surface of HBsAg VLPs and coupled with AS01 adjuvant, from GSK (RTS, 2015).

3. Malaria Vaccines : Challenges and Progresses, so far:

There are 3 broad strategies for malaria vaccines: the pre-erythrocytic vaccines, which must aim to target the infected hepatocytes, sporozoites, next, the erythrocytic or blood stage vaccines that must target surface antigens on infected RBCs or Merozoites, and finally the transmission blocking vaccines, that should target the further downstream development of sexual stages inside the mosquito vector (Beeson et al., 2019).

Pre-erythrocytic vaccines (PEV) target the clinically silent forms of the parasite and must have the ability to induce antibodies that can erase surface antigens in blood, skin and prevent from infecting more hepatocytes, or stimulate T cell responses that can resist infected Hepatocytes. RTS,S/AS01 is a PEV that houses a P. falciparum CSP fragment, a major surface antigen of sporozoites and was fused to HBsAg , co-assembled with mixed lipoprotein particles and formulated with GSK's licensed AS01 adjuvant(*US20060073171A1 - Vaccine Composition against Malaria - Google Patents*, n.d.). After 3 doses, at a month's interval, and a booster 18 months after the latter dose, clinical malaria episodes subsided by about close to ~36% in young children and ~26% among infants, but the efficacy subjugated by about ~68% in first 6 months(Agnandji et al., 2014). Also that the primary hepatocytes are difficult to cultivate in vitro, ex vivo, as compared to their regenerative potential, in vivo, and are phenotypically instable, exhibiting greater variability, lack several maturity markers viz; Cytochrome P450 gene expression, thereby making it difficult for a coherent understanding of liver stage malaria (Castell et al., 2006; Gomez-Lechon et al., 2008; Hu & Li, 2015) .

Blood Stage Vaccines (BSV) target the rapidly multiplying asexual developing stages in the erythrocytes, which cause in result in disease and death. After completion of each cycle, one to two dozen of merozoites egress from erythrocytes and within seconds infect fresh erythrocytes. However challenges in developing anti merozoite vaccines lie in the brief time period between successive cycles, when they are accessible to antibodies, polymorphism of antigens, and the larger niche of parasites that are to be targeted, as compared to bottlenecks targeted by TBVs and BSVs(Duffy &

Patrick Gorres, 2020). MSP1, AMA1, EBA-175, MSP3 are some of the blood stage antigens that were targeted(Sirima et al., 2011). AMA-1 is an essential protein for blood stage parasite development, it binds with rhoptry neck protein RON2, at the merozoite-erythrocyte interjunction. AMA-1 though could induce high titre antibody in in vitro trial assays, but failed to elicit any efficacy against controlled infection with homologous parasite. AMA1-RON2 when coupled together showed greater protection and passed on sterility to half the animals (Srinivasan et al., 2017)

Transmission Blocking Vaccines (TBV) incorporate surface antigens of mosquito/sexual stages, either gametes or zygote. Among the four leading candidates, Pfs230 and Pfs48/45 are gamete surface proteins, produced on gametocytes in human blood, and Pfs25 and Pfs28 are zygote surface proteins, expressed in mosquito vector, post fertilization (Carter & Kaushal, 1984; Duffy et al., 1993; Grotendorst et al., 1984). Both Pfs25 and Pfs230 have elicited poor immunogenicity as monomers. Pfs48/45 expression occurs during later stages of gametocyte development in human blood, and with Pfs230, via complex formation, play a role in male gamete motility. However, issues have occurred in Pfs48/45 vaccine development in prop, because of improper folding of recombinant protein (Kocken et al., 1993; KUMAR, 1987).

4. Virus like particles (VLPs), as a novel Immunogenic Platform :

The development of an efficient malaria vaccine remains a priority, for even the most advanced one RTS,S/AS01 has shown merely modest activity. The potency of protein subunit vaccines always remain poorly immunogenic. VLPs present an unique, novel platform in heightening the immunogenicity of vaccines. VLPs are viral structural proteins, that upon recombinant expression, self-assemble into non -nfectious nanoparticles (Zinkhan et al., 2021). Their small size ranging from 20 - 200 nm make it handy enough for drainage to the subcapsular area of lymph nodes and easy uptake by the APCs (Cubas et al., 2009; Manolova et al., 2008). AP205 conjugated vaccines have shown Antigen specific IgA antibodies and strong enhancement of class switching to IgG1 and IgG2a antibodies in BALB/c mice, and IgG2a is well known for it’s role in promoting opsonization, inducing the complement system and roles in promoting immune effector function (Liu et al., 2021). RNA packed inside VLPs is also

believed to promote class switching to Ig2a and in initiating TLR 3/7/8/9 signalling in B cells (Bessa et al., 2008) ;(Hua & Hou, 2013).

VLPs are naturally biocompatible. Their dense geometric structure, repetitive antigen display, small size does together help trigger B cell receptor clustering (Bachmann & Jennings, 2010; Hua & Hou, 2013) . After entering the lymphatic vessels and subcapsular sinus of lymph nodes, the VLPs can get across the B cells and Dendritic Cells, and initiate an humoral immune response. Because of it's small size, proper drainage of VLPs becomes feasible, and generation of higher affinity containing antibodies stand in chance. Overall, the multimericity, size, RNA and it's numerous potencies, pose VLPs as a an attractive candidate for Vaccine production. Their activities to induce both humoral and cellular responses have already been reported (Raghunandan, 2011).

5. EPL – Construction of Particulate Vaccines/ VLNPs :

5.1. Genetic Fusion, it's entailing challenges :

Although this might seem robust and might display activities in the first construct, there are several reasons why, the genetic fusion to VLPs have not been successful. Fusion of a peptide/antigen in any of the terminus might destabilize the VLP moiety, and also if the terminus faces inside, the overall structure might quite unlikely induce Antibodies (Brune & Howarth, 2018). VLP assembly is often flickering, for small changes in VLPs, viz; fusion may cause major difficulty. Folding of Chimeric VLP is highly prone to errors such as misfolding, incomplete post translational modifications etc (Zlotnick, 1994), and also that the symmetry of many VLPs range from trimeric to icosahedral, therefore there always lies a greater pressure in matching the intended symmetry. However, host optimal for expression of a VLP might not be identical with that of the protein antigen, and often E. coli host isn't feasible for promoting glycosylation or formation of disulfide bonds (Liew et al., 2010; Nothaft & Szymanski, 2013).

5.2. Modular Vaccine Assembly :

Modular Vaccine Assembly of VLNPs refer to separately expressing and purifying the VLPs and antigens, in separate systems/hosts, whatever gives an optimal yield and takes care of post translational modifications, conformation and then coupling them by an additional

extra step, thereby avoiding the challenges of Genetic fusion. This can actually pave the way for mass production of an easy to couple VLNP Scaffold, share costs across multiple disease and antigens and accelerate the mass production of vaccines against some challenging diseases (Charlton Hume & Lua, 2017; Crosnier et al., 2013; Proietti & Doolan, 2015). In the classical approach of modular VLP decoration , cross linkers are used and interaction is done between nucleophilic amino acid side chains of both VLP and antigen viz; acylation of lysine or N-terminus of VLP by N-hydroxy succinimide arm of cross linker , and then alkylation of cysteine by maleimide arm of cross-linker. Cysteine, if absent can be introduced by site-directed mutagenesis (Engeroff et al., 2018; Leneghan et al., 2017). In the latest approaches of post assembly decoration of VLNPs, lies the non-covalent and the covalent modes. The classical examples of non-covalent mode are His-Tag/Ni-NTA Affinity system, Biotin–Avidin Affinity system, Anthrax Toxin Receptor Decoration. His tag binds with nickel-loaded tris-nitrilotriacetic acid (trisNTA) with affinity, which can however be affected by lower pH(Guignet et al., 2004). Biotin-Avidin/Streptavidin affinity is one of strongest known non covalent interactions, and this is being exploited for the decoration of VLNPs, however to avoid the challenges for cross linking tetrameric Avidin/Streptavidin, monomeric Avidin/Streptavidin is used and there also stays a chance of reshuffling, upon storage(Wu & Wong, 2005). Covalent interactions between VLNPs and Antigens brings in the room for stability. Several studies have reported the use of unnatural amino acids viz; azidohomoalanine in the coat proteins (Patel & Swartz, 2011). Use of Halo Tag which is a modified haloalkane dehalogenase binds irreversibly to haloalkane ligands have been shown and also used to decorate VLNPs. This doesn't even require use of additional co-factors or post translational modifications for coupling(Sun et al., 2015). Use of a similar 20 kDa protein called SNAP tag has also been reported, which also irreversibly conjugates single chain variable fragments of antibodies to VLNPs(Eckhardt et al., 2011). Sortase, an enzyme of Gram-positive bacteria promotes covalent joining of proteins with a C-terminal LPXTGX motif to proteins with a N-terminal oligoglycine motif. Sortase A (Srt A), an enzyme from Gram positive bacteria has also been used to conjugate to a LPETGG motif containing HBV core antigen VLPs. The efficiency of conjugation with this system can be as high as 90 % (Hashad et al., 2019; Reed et al., 2020 Wójcik et al., 2019, 2020). Use of Split Intein method

where N and C terminal Inteins ligate non covalently and splice each other out, leading to extein formation (attached fragments) (Shah & Muir, 2014). Catcher/Tag technology, engineered from cell surface proteins of Gram positive bacteria forms intramolecular iso-peptide bonds, autocatalytically, this covalent interaction confers resistance, grit to the conjugates against the challenge of pH change, chemical, thermal extremities, mechanical stress etc. They can confer upto 99 % efficiency (Brune et al., 2016; Brune & Howarth, 2018; (Thraneet al., 2016).

6. Conclusion :

VLPs are an attractive candidate for therapeutic potential, they are presently being considered for Cancer, Tumour suppression vaccines and many other diseases. We developed a vaccine candidate for Malaria with a transmission stage antigen R0.6C. Characterization was done with DLS, TEM Imaging. In further studies, as planned by our Lab, the immunogenicity of the vaccine candidates will be subject to study, by immunising mice and studying the sera for neutralizing antibodies. There remains a huge prospect for reducing the cost and enhancing the mass production of vaccines, using the VLNP platforms, which are potent enough for stimulating both the humoral and cell mediated arms of immunity, because of it's size, mobilisation,multimeric nature etc.

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