Spotlights on new publications

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New vaccine candidates

In the present issue, spotlights are focused on vaccine candidates investigated in recent publications. Although inactivated and live-attenuated vaccines are excellent strategies for vaccine development, the most widely investigated vaccine formulations are recombinant DNA and protein subunit vaccines.

Malaria

In the last five years, several anti-malaria vaccine studies were conducted with the investment of ~15 billion $US. However, few reached clinical trials due to obstacles that include short duration of protection, complexity of clinical trial performance, and inefficient vaccine production technology. Mosquirix™ (RTS,S/AS01 malaria vaccine) was designed similar to the Energix-B™ vaccine that was originally developed against hepatitis B virus. Accordingly, the hepatitis B virus surface antigen (HBsAg) was used as a carrier matrix for circumsporozoite protein (CSP). The Mosquirix™ is in the form of a lyophilized injection administered intramuscularly. In its phase III clinical trial, sub-Saharan African children were administered four doses; the first three doses were administered monthly starting from the 5th month of age, while the last dose was administered at 15–18 months. Results revealed only 36% protective immunity against malignant malaria. However, Mosquirix™ represents the only malaria vaccine that advanced to phase III clinical trial. Other vaccine candidates in advanced development include R21, and *Pf*SPZ. Both vaccines are undergoing evaluation for their safety and efficacy in malaria-naïve and exposed children and adults. While R21 is the next-generation of Mosquirix™ (virus-like particle-based vaccines), *Pf*SPZ is composed of radiation-attenuated live sporozoites (intravenous injection of 3–5 doses). Similar to Mosquirix™, both vaccine candidates were designed to target pre-erythrocytic sporozoite stage to prevent human infection.

Compilation No. (1)

In December 2021, the WHO Global Alliance for Vaccines and Immunizations approved introduction of the anti-*P. falciparum* circumsporozoite RTS,S/AS01 vaccine, in a multi-site phase III clinical trial. Unfortunately, its efficacy yielded 36.3% when evaluated in children of several endemic African countries, and malaria developed in a number of the vaccinated children. Two other reasons encouraged the collaborative work undertaken in the present compilation. Results from previous epidemiological studies revealed that antibodies against SE47 and SE36 domains of *P. falciparum* serine repeat antigen 5 (SERA5), showed inhibitory activity against erythrocytic stages through exertion of antibody-dependent complement-mediated schizonts death. Notably, *Pf*SERA5 is a cysteine protease expressed in all erythrocytic stages and is abundantly secreted into the parasitophorous vacuole to contribute to several essential processes such as control of erythrocytes membrane disruption and host immune evasion. Additionally, it interacts with calcium dependent protein kinase 1 to increase cytosolic calcium, the initial trigger for merozoite egress. In another phase I clinical trial, the efficacy of a recombinant SE36 domain formulated with aluminum hydroxyl gel was evaluated in naive Japanese adults, and in another exposed population of Ugandan volunteers (6-32 years). The obtained results provided promising data regarding *Pf*SE36 safety, immunogenicity and reactogenicity, which fortified **Edith Christiane Bougouma** and her colleagues from Burkina Faso, Germany, UK and Japan to investigate its efficacy in protecting healthy Burkinabe children (1-5 years). The investigators conducted a double-blind randomized and controlled phase I clinical trial on three groups (54 children each). Two groups received three doses (100 µg r*Pf*SE36 with aluminum hydroxyl gel as an adjuvant) by either intramuscular or subcutaneous routes on the 1st day then after one and six months. The third control group received Synflorix®, an approved pneumococcal polysaccharide conjugate vaccine, intramuscularly on the first day, and after 6 months, and a physiological saline dose was given after a month. Only 96.3% (104 children) received the three r*Pf*SE36; the majority of them (91.7%) developed local mild reactions. However, the total number of children (11) who developed grade 3 adverse reactions was similar in the three groups. They were hospitalized for malaria infection and treated in less than two weeks. The most frequently reported adverse effect was induration and mild to moderate pain at the site of the injection. Other adverse effects included mild to moderate urticaria, pruritus, diarrhea, and vomiting. Two issues were observed, intramuscular administration yielded lower

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risk at the site of injection than the subcutaneous route, and younger children (1-2 years) showed higher antibody titers.

The investigated vaccine, whether *via* intramuscular or subcutaneous route, exhibited more than 2-fold change in antibody titers assessed before the second dose. Moreover, the level increased to 4-fold higher after the third dose. In spite of two important limitations of small sample size and absence of a comprehensive assessment of the cell-mediated immune response, the investigators confirmed safety and immunogenicity of r*Pf*SE36 in children from Burkina Faso, noting that this vaccine was not previously investigated in a vaccine clinical trial. Compiled from **"Safety and immunogenicity of BK-SE36 in a blinded, randomized, controlled, age de-escalating phase Ib clinical trial in Burkinabe children. Front Immunol 2022 Aug 31; 13:978591."**

Compilation No. (2)

Since carbonic anhydrases (CAs) catalyze reversible CO_2 hydration to bicarbonate and proton, they are essentially required for several cellular physiological processes such as biosynthetic reactions, pH regulation, and CO_2 transport. Therefore, CAs are promising drug targets, as confirmed by development of selective inhibitors in treatment of several diseases including tuberculosis, cholera, and typhoid fever. Eight genetic families were recognized, alpha (α), beta (β), gamma (γ), delta (δ), zeta (ζ), eta (η), theta (θ), and iota (ι). They vary in amino acid sequence, kinetics, inhibition, and activation profiles. While only α-CA isoform is identified in humans, most pathogens possess genes encoding members of all eight families. All CAs contain metal ion cofactors, as zinc, cadmium, iron, and cobalt. In the present compilation, the used CAs included α-CA involved in membrane transport and η-CA that is essentially required for pyrimidine biosynthetic pathway. Due to the surface accessibility of both CAs, they were selected to identify alternative antigens or peptide sequences for development of a malaria vaccine. On the other hand, both MHC-l and MHC-II are essential proteins for regulation and control of the functions of both T cells ($CD8^+$-MHC-l and $CD4^+$-MHC-II). Both CD cells proved potent inhibitors for growth and development of hepatic and erythrocytic stages, respectively. Accordingly, assessment of both CD cells is widely used for the evaluation of efficacy of a malaria vaccine candidate.

Utilizing immunoinformatics and *in silico* biological tools, **Reza Zolfaghari Emameh** and his colleagues from Iran and Finland analyzed two *P. falciparum* tmCA (α, and η) for their stability, localization, accessibility, and proteasomal cleavage. To assess tmCAs capability to induce protection against malaria, tmCAs' peptides antigenicity, B cell epitopes, and their cell type-specific expression were also investigated. Results revealed identification of two α-CAs and one η-CA in tmCA sequences that possessed high stability, strong antigenicity, and robust affinity towards MHCs. Sequence analysis revealed three conserved histidines located in α-CAs catalytic active site, whereas four conserved histidines and a single phenylalanine were located in the η-CAs. Interestingly, no significant similarity was observed between the identified *Plasmodium* tmCAs and human CA crystal structures, presenting potential specific malaria vaccine targets without any adverse immune response against human membrane α-CAs.

Moreover, their antigenic epitopes included extracellular peptide sequences identified in two *P. falciparum* α-CAs, and in *P. yoelii* η-CA. Being surface accessible, they hold an amino acid sequence for proteasomal cleavage in the antigen presenting cells with the highest binding affinity to MHCs and B cell epitopes; again, presenting potential targets for protective vaccination against malaria. Additionally, the study demonstrated that the intramolecular disulfide-bonds between cysteine residues have an essential role in tmCAs stability and immunogenicity. The investigators concluded that synthetic peptide vaccines technology is the safest strategy for malaria vaccine development since it eliminates transmission risk rather than traditional approaches for vaccine development. As described in other vaccines against HIV, hepatitis B, murine leukemia virus, and Ebola virus, the investigators hypothesized that immunization using *Plasmodium* tmCAs would produce neutralizing antibodies that protect against malaria infection. Accordingly, they recommended further *in vitro* and *in vivo* studies to assess *Pf*tmCAs protective potentiality against malignant malaria. Compiled from **"A reverse vaccinology approach on transmembrane carbonic anhydrases from *Plasmodium* species as vaccine candidates for malaria prevention. Malar J 2022 Jun 15;21(1):189."**

Compilation No. (3)

In an attempt to develop next-generation malaria vaccines, Walter Reed Army Institute of Research developed a circumsporozoite (CSP)-based soluble antigen with a plant extract (QS-21) as a novel adjuvant and termed it army liposome formulation-QS-21 (ALFQ). This soluble antigen (FMP013) was designed from CSP including its C and N-terminal regions, two junctional repeats (3xNPNV and 3xDPNA), and 15 copies of the major NPNA repeats. Additionally, the ALFQ adjuvant was prepared by combining anionic liposomes formed by a synthetic monophosphoryl-lipid-A analog with QS-21. Previous toxicity studies conducted in mice and rabbits encouraged American investigators (**Jack N Hutter *et al***.) to evaluate its safety (adverse effects) and immunogenicity (immune responses). In a phase I clinical trial, ten healthy non-immune adults received three low and high doses. The former was composed of 20 μg FMP013/0.5 ml ALFQ, and the latter 40 μg FMP013/1.0 ml ALFQ, administered intramuscularly on days 1, 29, and 57. Results revealed acceptable adverse effects, and tolerability profile, with robust humoral and cellular immune responses. Although it exhibited

a Th1-biased cytokine response, it produced significant levels of antibodies that bound with high affinity to the C-terminal region and the major NPNA repeats. Low level of affinity was observed towards the N-terminal region, and the junctional repeats. According to the reaction profile, the investigators recommended using the low dose regimen for further clinical trials. Compiled from **"First-in-human assessment of safety and immunogenicity of low and high doses of *Plasmodium falciparum* malaria protein 013 (FMP013) administered intramuscularly with ALFQ adjuvant in healthy malaria-naïve adults. Vaccine 2022 Sep 22; 40(40):5781-5790."**

Toxoplasmosis

Several vaccine candidates using different formulations were evaluated including *T. gondii* microneme proteins (MICs 6, 8, 11, and 13), MIC 2-associated protein (M2AP), apical membrane antigen 1 (AMA1), secreted protein with an altered thrombospondin repeat (SPATR), and perforin-like protein 1 (PLP1). None of them succeeded to achieve complete protection in addition to several obstacles that affected the outcome of experimental studies preceding progress to clinical trials. These include vaccine formulation and regimen, variable immune responses in animal models, inadequate evaluation parameters, and variable *T. gondii* strains. Accordingly, it became clear that optimizing the immunization protocol, and using a suitable delivery system and molecular adjuvant would initiate progress in development of safe potent immunogenic vaccine.

The most commonly investigated vaccine candidates for *T. gondii* were the MICs, rhoptries, and dense granules antigens expressed on tachyzoites surfaces. Although bradyzoites (the long-lasting intracellular forms) are responsible for latent toxoplasmosis, their antigens attracted few attentions. A Chinese study (**Xiaowei Tian *et al.***) focused on *T. gondii* bradyzoite-formation deficient 1 (*Tg*BFD1) for several reasons. First, accumulated *Tg*BFD high level was reported in stress conditions due to increased tachyzoites → bradyzoites conversion rate, indicating that tachyzoites lacking *Tg*BFD1 are unable to transform into bradyzoites. Second, *Tg*BFD1 was a master regulator of chronic toxoplasmosis. Third, it has critical roles during cyst formation, i.e., *Tg*BFD1-deficient bradyzoites were unable to generate tissue cysts in mice in comparison to wild type. Therefore, the present compilation evaluated *Tg*BFD1 protective efficacy in experimentally infected BALB/c mice. To test its immunogenicity, the investigators used epitope analysis by DNAstar software and Western blots. Results revealed potent immunogenicity that was confirmed by ELISA. Mice were infected by RH and PRU strains to produce acute and chronic toxoplasmosis, respectively. For active immunization, the study used subcutaneous boosting with recombinant *Tg*BFD1 emulsified with ISA 201 as an adjuvant due to its high safety profile, and long-term protective immunity. In chronic toxoplasmosis, recombinant *Tg*BFD1 exhibited strong protective humoral and cellular immune responses, evident by high production of specific IgG and its isotypes with increased IFN-γ and IL-10 cytokine levels. In addition, the study observed an obvious reduction in number and size of brain cysts. In contrast, no protection was observed in acute toxoplasmosis infected mice, a result that was attributed to the unsatisfactory adjuvant selection for improving vaccine efficacy in acute toxoplasmosis. Therefore, they recommended further studies using a more efficient delivery system. Compiled from **"Vaccination with recombinant *Toxoplasma gondii* bradyzoite-formation deficient 1 (r*Tg*BFD1) antigen provides partial protective immunity against chronic *T. gondii* infection. Front Vet Sci 2022 Sep 12; 9:957479."**

Leishmaniasis

There are three generations for protective vaccines against leishmaniasis: live-attenuated or killed fractions, recombinant proteins, and naked DNA vaccines. Due to standardization and safety issues, results of the first-generation vaccines were not satisfactory in phase III clinical trials. In the last two decades, vaccine trials included several antigens such as *Leishmania*-derived recombinant polyprotein (*Leish*-111f), sterol 24-c-methyltransferase (SMT), *Leishmania* receptors of activated C kinase (LACK), and Kinesin motor domain. While *Leish*-111f is a single polyprotein composed of three fused molecules: *L. major* homologue of eukaryotic thiol-specific antioxidant, *L. major* stress-inducible protein-1 and *L. braziliensis* elongation and initiation factor, the SMT enzyme is the major membrane sterol in *Leishmania* spp. that is essentially required for ergosterol biosynthesis. The protective efficacy of both vaccines in addition to LACK, protected mice challenged with *L. major* infection, but failed to protect against visceral leishmaniasis. On the other hand, Kinesin is a major protein in microtubule filaments, and its active movement is essential for several cellular processes, i.e., mitosis, meiosis, and transport. Additionally, ribosomal proteins protected susceptible mice against primary *L. major* infection and exhibited effective protective results for long-term maintenance of immunity against cutaneous leishmaniasis. Surface glycoproteins (GPs) are established virulence factors in pathogenic trypanosomatids, and the Leishmania lipid-anchored-GPs possess a highly conserved glycosylphosphatidylinositol (GPI)-anchor motif that utilizes several GPIs to be conjugated in the surface membrane complex. Parasite surface antigen (PSA) is also a vaccine candidate because it is involved in macrophage invasion through the interaction of its leucine rich repeats with complement receptor 3. Additionally, HSPs 70, 83, and 100, alone or combined, were investigated either as vaccine candidates or adjuvants, and exhibited satisfactory protective potency. Hence, the recombinant proteins and DNA vaccines are the most widely investigated nowadays due to their effective induction of $CD8^+$ and $CD4^+$ T cells activity in

addition to their ability to produce long-lived antigens and folded polypeptides. However, these trials are still in the early phases of clinical trials.

Moreover, histone and its variants (H2A, H2B, H3, and H4) are essential components of the nuclear chromatin that prevent DNA damage during cell division, and regulate DNA replication and gene expression. Notably, histone variants are substitutes for the established core histone in nucleosomes. Since H2B has a limited number of variants, and conserved sequence among several *Leishmania* spp., it was previously investigated for its potential immunogenicity and its protective role. The present compilation is a collaborative work by **Sana Ayari-Riabi** and her colleagues from Tunisia, Bahrein and France. In an attempt to design a protective vaccine against cutaneous leishmaniasis, they utilized poly (D,L-lactide) nanoparticles (PLA-NPs) as a delivery system for recombinant *L. major* histone H2B (*Lm*H2B). The researchers preferred using PLA-NPs because it was approved in human applications due to its biocompatibility and lack of toxicity. The investigators succeeded to produce well-formed H2B-adsorbed NPs (*Lm*H2B/PLA) without a surfactant to avoid the inherent cutaneous toxicity reported in several previous studies. The *Lm*H2B/PLA showed a narrow size distribution (287 nm) and a positive zeta potential (30.9 mV). The *in vitro* study showed a release pattern at predetermined time intervals for 30 days. Results revealed that the recombinant H2B started desorption on the 7th day and was followed by continuous release for the next 3 weeks.

Three groups of mice were subcutaneously immunized two times with H2B alone, H2B/PLA-NPs, and H2B with a Th1-activated adjuvant (CpG7909). The study analyzed the results in comparison to the unvaccinated mice. All mice were challenged with a virulent *L. major* strain (GLC94) isolated from a human lesion of cutaneous leishmaniasis. The study parameters included clinical manifestations, mortality rates, and IgG total intensity. Both adjuvant formulations produced similar IgG total intensity higher than that obtained in mice immunized by sole H2B. Mice immunized by H2B formulations did not develop ulcers until the 8th week post infection. Besides, H2B/PLA showed significant reduction in footpad swelling with no mortality within 17 weeks. In spite of the satisfactory results, the investigators recommended further studies to gain more knowledge on *Lm*H2B physicochemical characteristics for use as a suitable adjuvant or delivery system in order to design a safe, steady, and immunogenic vaccine. Compiled from **"Polylactide nanoparticles as a biodegradable vaccine adjuvant: A study on safety, protective immunity, and efficacy against human leishmaniasis caused by *Leishmania major*. Molecules 2022 Dec 8; 27(24):8677."**

Schistosomiasis

The WHO proposed six antigens as promising vaccine candidates to encourage development of an efficient anti-schistosomiasis vaccine. These included glutathione S-transferase (P28/GST), paramyosin (Sm97), IrV-5 (62 kDa), triose phosphate isomerase (TPI), *Sm*23, and *Sm*14. All vaccine candidates are expressed in adult worms, schistosomulae, and eggs; except for *Sm*97 and *Sm*14 which are not expressed in eggs. It is worth mentioning that GST and TPI are enzymes, *Sm*97, and IrV-5 are muscle proteins, *Sm*23 is an integrated membrane protein, and *Sm*14 is a fatty acid-binding protein. Initial experimental studies revealed protection that ranged from 30-70%. Since the *Sm*14 vaccine was developed in 2018, Brazilian scientists (Current status of the *Sm*14/GLA-SE schistosomiasis vaccine: Overcoming barriers and paradigms towards the first anti-parasitic human vaccine. Trop Med Infect Dis 2018; 3:121) reviewed its protective status against schistosomiasis. The vaccine successfully completed phase I and phase IIa clinical trials and underwent phase II/III trials in Africa that will be followed by other trials in Brazil. Recombinant *Sm*14 (50 µg) formulated with a synthetic adjuvant (glucopyranosyl lipid A, GLA-SE) in two dosages (2.5 µg and 5 µg/dose) administered intramuscularly, showed safe adverse effects with both doses. The clinical trials were conducted in Brazil in healthy adults living in a non-endemic area, as well as adults and children living in highly endemic areas for *S. mansoni* and *S. haematobium*.

Hence in the present compilation, **Marília Santini-Oliveira** and her Brazilian and American colleagues conducted an open, non-placebo-controlled, standardized-dose immunization trial to evaluate recombinant *Sm*14+GLA-SE. Phase Ib clinical trial included 10 healthy women (18-49 years old) administered intramuscularly with three doses of 50 µg *Sm*14 plus 10 µg GLA-SE with 30 days intervals. Clinical, biochemical, and immunological parameters were assessed up to 120 days post immunization. No adverse clinical effects were observed, and the vaccine induced high titers of IgG against *Sm*14, but IgE was not produced. Additionally, the vaccine exhibited significant strong broad spectrum immune responses that included increased TNFα, IFNγ, and IL-2 levels after 90- and 120-days post-immunization. The study recommended proceeding with *Sm*14-GLA-SE vaccine to phase II clinical trials in endemic areas. Compiled from **"Development of the *Sm*14/GLA-SE schistosomiasis vaccine candidate: An open, non-placebo-controlled, standardized-dose immunization phase Ib clinical trial targeting healthy young women. Vaccines (Basel) 2022 Oct 15; 10(10):1724."**