Designing and testing of an epitope-based protective vaccine against visceral leishmaniasis:

Visceral leishmaniasis is a tropical disease caused by protozoa *Leishmania donovani*. During the course of infection in human, the parasite resides inside the macrophages. To achieve this possibilty the parasite has evolved strategies to down-modulate the effector functions of macrophages e.g. oxidative burst, antigen presentation and Th1 cytokine IL-12, & IFN-γ generation(31). Reason for down modulation of proinflammatory responses are the activation of negative regulators of NFκB pathways like SHP-1. The activators of PTNP are early virulence factors present on surface of parasite itself and secreted via exosomes(32). These virulence factors include KMP-11, HSP-60, 70, 90, GP63, lipophosphoglycan, cystein proteases, etc.(33). Due to inhibition of inflammatory responses a large surge of IL-10 and IL-4 is observed, causing humoral response to mount up which contains non-specific IgG and form hypergammaglobulinemia, while at the same time suppresses cell mediated immunity (34). IFN-γ has been proposed as a fulcrum point in immunity against *Leishmania* (3). Presence of IFN-γ is associated with activation of macrophages against the pathogen, production of IL-12 besides the development of memory Th1 cells and CD8+ T cell proliferation (35). Till now vaccine trials with whole antigen peptides have had limited success owning to the fact that they contained both Th1 as well as Th2 cytokine inducing domains. A vaccine which can target the virulence factors released early through exosomes and contain multiple proinflammatory cytokine specific epitopes would provide a better protective response, where fusion of exosomes with neutrophils and macrophages could be inhibited and cell mediated immunity is activated (36).

The new developments in data processing and better algorithms can map the cytokine specific epitopes for Th1 development, IFN-γ induction, and CD8 cell response from the known recombinant peptide sequences (37–39). Prediction of B cell epitopes for antibody generating domains has significantly improved over the years (39). A utility of these prediction capabilities is in the development of epitope based vaccines. Using the mapped epitopes, a multi-epitope construct can be designed, which would be rich in Th1 epitopes and also carry antibody generating domains against Leishmania secreted virulence factors or exosomes. This approach will likely be able to induce a very strong protective response against Leishmania infection by inhibiting the exosome fusion with the macrophage and induce the T cell activation in Th1 cytokine specific manner. The epitope mapping can be applied to translate peptide sequences of 3 novel *Leishmania* genes already cloned in the lab, and used in designing a multi-epitope cocktail vaccine. The proposed genes have previously been tested for their protective efficacy and were found to be capable of inducing production of IFN-γ, IL-12 and lethal free radicals (20,21). A more refined and specific form composing of selective epitopes can elicit better response and generate higher strength of immunity against *Leishmania*.

We hypothesize that utilization of selective T cell inducing epitopes from different peptides can evoke an enhanced cytokine environment which can favor the development of resistance to infection and facilitated parasite clearance. An antibody response against exosome secreted by Leishmania can provide protection against early virulence factors contributing to the anergy experienced by macrophage upon Leishmania infection. By administration of B cell epitopes against surface proteins of exosomes, and Leishmania epitopes specific for IFN-γ, Th1 and CTL, a cytokine-cellular synergy can be induced which would likely provide a sterile immunity (40). With the incorporation of epitopes from 3 different genes, a multi-epitope chimeric vaccine can provide enhanced protection than epitopes from a single protein alone(40). Recently T-cell inducing epitopes based vaccine approach has been used by researchers (Agallou et al 2014, Das Shantanabha et al 2014) using *Leishmania infantum* proteins CPA, KMP-11, LeIF & Histone H1 (41) and KMP-11, CPA, CPB &P74 (42) respectively.

Our work:
T-cell epitope mapping: a useful approach to generate a potentially protective vaccine against Leishmania donovani:

In the direction of a prophylactic and therapeutic vaccine development our lab has screened out three novel genes from Leishmania cDNA library. The three identified novel genes A2/1(accession No. AY377788), F2/1 (Accession NO. AY180912) and B4/1 (Accession NO. AY161269) were isolated from a cDNA library of Leishmania. They were screened along with other candidates for IFN-γ induction from an in-house established Leishmania specific cell line(13). The recombinant proteins from these genes showed various levels of protection in a hamster model of visceral leishmaniasis(13,20,21). The peptide formulation of rA6 and rF14 showed a large increase in IFN-γ production. The DNA vaccine formulation of recombinant B4/1 showed increased surge of IL-12. All three recombinants have shown potential Th1 type immune response and the activation of macrophage to produce high levels of NO and ROS. One of the recombinant (rA6) has recently been shown to have an immunomodulatory potential, as it activated the macrophages with enhanced oxidative burst leading to parasite clearance ex-vivo. It also facilitated early parasite clearance when used along with known anti-leishmanial chemo-agents even at sub-optimal therapeutic doses, suggesting a synergistic effect(22). Currently, we designed three multiepitope vaccine candidates by the selection of multiple IFN- inducing MHC-I and MHC-II binder T-cell specific epitopes from three previously identified antigen genes of Leishmania donovani from our lab by an immuno-informatic approach using IFNepitope, the Immune Epitope Database (IEDB) T cell epitope identification tools, NET-MHC-1, and NET MHC-2 webservers. We tested the protective potential of these three multiepitope proteins as a vaccine in a hamster model of visceral leishmaniasis. The immunization data revealed that the vaccine candidates induced a very high level of Th1 biased protective immune response in-vivo in a hamster model of experimental visceral leishmaniasis, with one of the candidates inducing a near-sterile immunity. The vaccinated animals displayed highly activated monocyte macrophages with the capability of clearing intracellular parasites due to increased respiratory burst. Additionally, these proteins induced activation of polyfunctional T cells secreting INF-γ, TNF-α, and IL-2 in an ex-vivo stimulation of human peripheral blood mononuclear cells, further supporting the protective nature of the designed candidates.

Some references from lab:

1. Lack of serological specificity of recombinant leishmania hsp70. Arora SK, Melby PC and Sehgal S. Immunol Cell Biol 1995; 73:446-451.
2. Recombinant heat shock protein is recognized from individuals with prior *L.donovani* infection. Arora SK, Sehgal S, Tryon VV and Melby PC. Immunol Infect Dis 1995; 5:282-286.
3. Heterogeneity in the heat shock protein gene of leishmania isolates. Arora SK, Singh G and Sehgal S. Immunol Cell Biol 1998; 76:186-189.
4. Identification of sero-specific epitope of recombinant hsp70 of *leishmania donovani*. Arora SK, Kaur D, Kapoor GS and Sehgal S. J Parsit Dis 2000; 24:19-22.
5. Recognition of *Leishmania donovani* by CD4+ T-cells of naïve healthy uninfected individual, Pal N and Arora SK. Submitted to J PARASIT DIS, 2004; 28:11-16.

6. Recombinant antigens of *Leishmania donovani* inducing IFN-g release from Leishmania specific cell line, Arora SK, Pal NS & S.Mujtaba. EXP PARASIT 2005; 109:163-170.
7. *Leishmania donovani*: identification of novel vaccine candidates using human reactive sera and cell lines. Arora SK, Pal NS, Mujtaba S. Exp Parasitol. 2005 Mar;109(3):163–70.
8. Efficacy of *Leishmania donovani* ribosomal P1 gene as DNA vaccine in experimental visceral leishmaniasis. Masih S, Arora SK, Vasishta RK. Exp Parasitol. 2011 Sep;129(1):55–64.
9. Vaccination with a novel recombinant Leishmania antigen plus MPL provides partial protection against *L. donovani* challenge in experimental model of visceral leishmaniasis. Bhardwaj S, Vasishta RK, Arora SK. Exp Parasitol. 2009 Jan;121(1):29–37.
10. Leishmania recombinant antigen modulates macrophage effector function facilitating early clearance of intracellular parasites. Ratna A, Arora SK. Trans R Soc Trop Med Hyg. 2016 Dec 1;110(10):610–9.
11. Epitope based vaccine designing- A mini review. Arora SK, Arya A. Epitope Based Vaccine Designing- A mini review. J Vaccines Immunol 2020; 6(1): 038-041. DOI: https://dx.doi.org/10.17352/jvi.000036.
12. A T-Cell Epitope-Based Multi-Epitope Vaccine Designed Using Human HLA Specific T Cell Epitopes Induces a Near-Sterile Immunity against Experimental Visceral Leishmaniasis in Hamsters. Arya A, Arora SK. Vaccines 2021, 9, 1058. https://doi.org/10.3390/vaccines9101058.