Details of Research for claim of award:

The main focus of my research has been in the area of Immunology of Infectious diseases, mainly the HIV, HCV, HIV-TB co-infection and experimental leishmaniasis. It is in the area of development of a vaccine against visceral leishmaniasis that we have made a good headway in terms of designing of multi-epitope constructs recently that induced a sterile immunity in an animal model of experimental visceral leishmaniasis. So, I think I would like to share our consistent efforts made in this particular area to claim an award.

I have been involved in this field since 1982, when I got registered for my PhD program and was asked by my mentor Prof Shobha Sehgal in PGIMER Chandigarh to start the axenic culture of Leishmania parasite and also make an animal model. The main target was to identify relevant antigens of leishmania parasite that could be used as a prophylactic vaccine against visceral leishmaniasis, a tropical disease which is also termed as kala azar in India. The disease is more prevalent in many tropical countries in the world including some Eastern states of India, where it is endemic. The disease is caused by a parasite, *Leishmania donovani*, which is transmitted through sand fly, an insect vector. In early 1980's we had some chemotherapeutic options for treatment of this infection using antimony-based chemo agents like sodium stibogluconate and amphotericin B, which are very toxic and prone to development of drug-resistance. Subsequently, many new and safer agents have been developed including the oral Miltefosine and lesser toxic Liposome encapsulated amphotericin B (AmBisome), which is an anti-fungal drug but found to be highly efficacious against leishmania infection also. The immunology of infection indicated that there is some resistance to reinfection in the individuals who recover from active disease and so we thought a vaccine approach would be feasible. The infection induces a strong polyclonal B-cell activation leading to hyper-gammaglobulinemia but antibodies are not found to be protective. Cell mediated immune responses are generally suppressed in active disease and the recovered patients showed development of delayed type hypersensitivity (DTH) to leishmania soluble antigens. Further reports also indicated a Th1 biased immune-response to be protective in mice model and so, we started with identification of leishmania antigens which could induce a Th1 biased immune response with the expression of high level of interferon gamma (IFNγ). we searched through literature to find a good lab maintaining the *L.donovani* parasite and found one in National Institute of Communicable diseases (NICD) at New Delhi. I obtained a culture of the Leishmania promastigotes in the NNN medium. Although the promastigotes lived happily in NNN medium, yet for antigenic analysis we wanted large number of parasites, so we designed a biphasic medium containing brain-heart infusion and defibrinated rabbit blood in the agarose slants overlayed with Hank's balanced salt solution. This medium offered a very high expansion of promastigote growth. We used the parasite for preparing whole leishmania promastigote soluble antigen (LPSA) as well as created an *in vitro* infection model of peritoneal macrophages drawn from the Balb/c mouse in which the parasite transformed into amastigotes and offered a good model for screening of novel drugs *in vitro*. Many of these applications were published (1-7, 12). We even used the excreted factors of Leishmania parasite released in the

culture supernatant as antigen to devise diagnostic assays including the counter-current immune-electrophoresis (CIEP) and Enzyme-linked immunosorbent assays (ELISA) (8, 9). Then we tried raising an animal model of visceral leishmaniasis and obtained Golden Syrian hamsters (*Mesocricetus auratus*) to develop this model, as the hamster is reported to make the best model for visceral leishmaniasis, simulating many clinical features of the disease closer to human kala azar. On many trials, we could not develop a visceral disease in these animals, but on a longer wait in few hamsters we observed lesions in foot pads and scrotal sacs. On slit smear staining we saw millions of parasites growing in these lesions. We got the parasite strain characterized from London School of Tropical Medicine and found it to be *Leishmania Mexicana* instead of *L. donovani*. Though disappointed, we used the *ex vivo* and *in vivo* models successfully to test the receptor-mediated drug delivery of a cytotoxic drug, methotrexate conjugated to methylated albumin, which is supposed to bind specifically to scavenger receptors on infected macrophages, developed in the laboratory of Prof Sandeep Basu at Institute of Microbial Technology, Chandigarh. The interesting results showed a significant increase in the efficacy of drug with reduced toxicity (10).

Subsequently we obtained a virulent strain of *Leishmania donovani* (Dd8) from IICB Kolkata for further experiments. We started with detailed antigenic analysis of the axenically cultured promastigotes and identified many immunodominant antigens of leishmania parasite by western blot analysis using immune sera samples from recovered kala azar patients (17-20). We made monoclonal antibodies (Mab) of the promastigote soluble antigens and used some of highly reactive Mab for detection of leishmania antigens in the sera of kala azar patients which could be used for diagnosis of active infection (11,13-15). We generated a cDNA library of leishmania genes from mRNA isolated from axenically cultured promastigotes of this virulent strain of *L. donovani* and for identification of cDNA clones expressing immuno-protective antigens, we screened the library with immune sera as well as T-cells from recovered kala azar patients (13, 14). Surprisingly we detected many clones expressing heat shock proteins (hsp70) of leishmania, which though were not found suitable as vaccine candidates, yet the lab was able to identify a leishmania specific sequence in the hsp70 gene and designed primers for a species-specific PCR test for the detection of *L.donovani* (21-22, 25-26). In order to detect T-cell based epitope expressing clones, we generated a Leishmania-specific cell line from PBMCs of a healthy donor, which came out as a very handy tool to screen a very large number of recombinant clones expressing leishmania antigens. Using further analysis and sequencing we identified three novel clones that expressed proteins which induced T-cell activation with high level of Interferon gamma (IFNg) release on stimulation *ex vivo* (23-24). The recombinant peptides expressed by these clones were checked for their protective efficacy in the hamster model of experimental visceral leishmaniasis and found that the animals immunized with these recombinant proteins had significantly lower parasite burden in the spleens and livers. The peritoneal macrophages from these animals could make very high levels of reactive oxygen species and nitric oxide with ability to clear intracellular parasites. But these vaccines were found to have a protective efficacy varying from 60-85% only (27-29).

We even checked the immunotherapeutic potential of one of these antigens and showed that this protein could induce production of high level of ROS and NO when used ex vivo culture of peritoneal macrophages. The macrophages stimulated with this protein cleared the intracellular parasites more efficiently due to increased oxidative burst. We also showed that when used in conjunction with the anti-leishmanial drugs like miltefosine and Ambisome, the protein stimulation facilitated the clearance of parasites with half the therapeutic dose of the drug and at much earlier time point (30). This indicated that the recombinant protein was a potential immunomodulator and when used as a immunotherapeutic agent along with existing anti-leishmania drugs will reduce the toxic effects of the drug due to reduced dose of drugs being effective at shorter time points.

We worked consistently to find ways to improve the efficacy of this vaccine. After years of hard work and scientific enquiry, we proposed 'multi-epitope constructs' as vaccine candidates including the select T-cell based epitopes mapped from our previously cloned genes using immune-informatic tools. We found that one of these multi-epitope peptides was able to induce 'sterile immunity' in the hamster model of experimental visceral leishmaniasis. The animals were nearly 100% protected to a lethal challenge with the virulent parasite. The peritoneal macrophages from the protected animals showed enhanced capacity for producing enhanced oxidative burst and clearing the intracellular parasites. The multi-epitopic vaccine induced a Th-1 skewed immune response with increased expression of inflammatory cytokines and decreased expression of immune-suppressive cytokines like IL-10 and TGFβ. The protein could activate polyfunctional T-cells inducing release of IFNγ, TNFα and IL2 from human healthy donors indicating it's suitability for a protective immune response in humans as well. This study has recently been published in Vaccines (31).

This is a remarkable progress in the field of vaccine development against this tropical disease, which indicates a consistent and focused approach by our group in this area of research. The proposed approach of selective epitope mapping and designing of multi-epitope constructs as vaccine candidate may not remain restricted to leishmaniasis but would be highly translatable to epitope-based vaccine designing for other emerging infections as well.

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