Statement of Research achievements of Dr. Sunil K Arora:

My lab has been interested in the Immunology of infectious diseases with specific reference to development of a vaccine against visceral leishmaniasis (kala azar), cellular immune defects in HIV & HCV and molecular diagnosis of tuberculosis primarily extra-pulmonary TB.

1. Among research achievements from my lab, we started with detailed antigenic analysis of the axenically cultured promastigotes of *Leishmania donovani* virulent strain (Dd8). We identified many immunodominant antigens of leishmania parasite by western blot analysis using immune sera samples from recovered kala azar patients (1).
2. 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 (2).
3. We established an *ex vivo* macrophage cell-based infection model to screen various anti-leishmania drugs (3). In collaboration with Institute of Microbial technology, we tested a novel chemotherapeutic approach using methylated albumin conjugated methotrexate which binds specifically to scavenger receptors on the infected macrophages against leishmaniasis in our *ex vivo* culture model as well as in hamster model *in vivo* for receptor mediated drug-delivery to infected cells (4).
4. We generated a cDNA library of leishmania genes from mRNA isolated from axenically cultured promastigotes of virulent strain of *L. donovani* and for identification of cDNA clones expressing immunoprotective antigens, we screened with immune sera as well as cells from recovered kala azar patients (5, 6).
5. We identified many clones expressing heat shock proteins 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* (7).
6. We generated a Leishmania-specific cell line which came out as a very handy tool to screen a very large number of recombinant clones expressing leishmania antigens (8). We identified three novel clones that could induced high level of Interferon gamma (IFNg) on stimulation *ex vivo* (9). The recombinant peptides expressed by these clones were checked for their protective efficacy in a hamster model of experimental visceral leishmaniasis and found these to have a protective efficacy varying from 70-85% (10, 11).
7. 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. This study has recently been published in Vaccines (12).

8. 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.
9. 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 in *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 (13). 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.
10. In the area of HIV and HCV, our lab has been interested in understanding the immune-defects leading to immunocompromised state in the advanced stage of these two chronic viral infections. These two viral infections have common transmission mode and both lead to chronic infection in the infected individuals. While HIV-1 infection leads to severe immunocompromised state in the individual in due course of time due to gradual loss of CD4+ T-cells, which are the host cells for this virus. The cell death is not only because of infection but also because of the immune response generated against the virus and the CD8+ cytotoxic T-cells kill not only the infected cells but also the by-stander uninfected cells are found to be dying because of strong immune activation. But surprisingly as the disease progresses a state of severe immune-deficiency sets in and the infected individual gets prone to many opportunistic infections like pneumocystis carinii, *Mycobacterium tuberculosis* etc.
11. Very interesting studies from my lab have shown that the *Mycobacterium tuberculosis* (Mtb), the most common opportunistic co-infection among HIV-1 infected individuals, modulates many genes in the co-infected host cells to facilitate the replication and HIV-1, leading to faster disease progression in such individuals (14). Further we delineated the molecular mechanisms in HIV-TB co-infected individuals and observed genetic evolution of HIV in the co-infected individuals in terms of accumulation of many mutations in the RT and PR genes leading to high level of resistance against anti-retroviral drugs (15). Moreover, the HIV-1 isolates from co-infected individuals were also found to acquire additional NF-kB sites in the LTR region, making this virus more replication competent and genetically fit to survive and poor responder to ART (16). These finding have great clinical implications and warrants for

modifying the treatment regimen for better management of co-infected individuals.

12. We reported many years back that HIV-1 subtype C is the predominant virus subtype among patients in India and proposed that all the HIV positive individuals should be genotyped for DR mutations, as the HIV isolates from many ART-naïve patients carry few DR mutations, which may become predominant when put on ART (17, 18)
13. Since the online DR database (e.g. Stanford DR data base) are based on HIV Subtype-B sequences, our lab for the first time reported a unique HIV-1 subtype-C specific drug-docking prediction model for assessment of drug-resistant mutations based on binding energy to RT and PR genes based on sequences from HIV-1 subtype-C isolates (19, 20).
14. In view of the poor handling of opportunistic infections in the advanced stage of HIV, my lab further hypothesized that there may be many other immune defects in the infected individuals than only the depletion of CD4+ T-cell numbers. Since the myeloid dendritic cells (mDC) are the major antigen presenting cells for generation of immune response, we wanted to check the functional state of these cells in infected individuals at different stage of infection. We reported that the mDCs loose their capacity to mature in response to the antigenic stimulation in the advanced stage of disease (when the CD4 count is below 250 cells/cmm) (21). We further showed the increased expression of negative regulatory genes (SOCS1, SOCS3, PD-L1 etc.) as the molecular mechanism for maturation defects in the mDCs in the patients with advanced HIV disease (22)
15. Similarly, we reported the functional and maturation defects in the mDCs in the chronic HCV infection and these mDC regain their maturation potential on successful antiviral treatment (23). The response to treatment in CHC was found to be directly associated with the functional status of mDCs (24). An upregulation of negative factors was found to be the cause of these functional defects which even effects the response to anti-viral treatment in these individuals (25). Although similar maturation defects were also seen in mDC from patients with Non-alcoholic fatty liver disease (NAFLD), the mechanisms were more due to release of inflammatory cytokines in response to endotoxins (26)
16. Recently we have used mesenchymal stem cells isolated from Wharton's jelly of human umbilical cord (hUC-MSC) for treatment of arthritis induced in Rats using chicken collagen in a Collagen induced arthritis(CIA) model. We showed that the hUC-MSCs created a strong anti-inflammatory environment in the arthritic joints by suppressing the inflammatory cytokine expression and inducing the expression of IL-10, TGFβ. Besides, we observed regeneration of the damaged cartilage and bones tissue in the arthritic joints as vouched by histopathological examination, arthritic scoring and radiology. These findings were supported by our *in vitro* evaluation of mono-nuclear cells isolated from synovial fluids and peripheral blood of active arthritis patients when co-cultured with hUC-MSCs. The multiplex cytokine analysis of culture supernatant showed suppressed expression of proinflammatory cytokines and

upregulated expression of anti-inflammatory factors in presence of hUC-MSCs (27). These results strongly supported the hypothesis that the allotransplantation of mesenchymal stem cells can be used as a viable cell-based therapeutic option in severe RA patients.

17. In another study we have reported that the frequency of cancer stem cells is directly associated with the aggressive behavior of human breast carcinoma in female patients. We also observed that many factors released by the breast cancer stromal cells facilitate the expansion of Breast cancer stem cells in the tumor microenvironment (28). The pathway analysis has revealed some signature pathways that may be important and may lead to designing some novel blockers for effective management of metastatic breast carcinoma.

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