Summary

DCs are the sentinels and key regulators of T and B cell immunity, owing to their superior ability to take up, process and present antigens compared with other APCs. They were introduced as adjuvants in vaccination strategies that aimed to induce antigen-specific effector and memory cells. DCs are pivotal for the activation of immune responses, bringing about effector functions of T lymphocytes and maintenance of tolerance. DCs are essential for the induction of adaptive immune responses against malignant cells by virtue of their capacity to effectively cross-present exogenous antigens to T lymphocytes. The standard treatment procedures utilize PBL monocyte-derived DCs. We wanted to validate the potency of UCB-derived DCs as an allogenic source for clinical grade DCs. Thus an in depth study on the functional aspect of DCs derived from both these sources was essential.

The first part of our study was dedicated to this aspect. Maturation status plays a decisive role in antigen presentation, costimulation and ultimately decides whether the outcome will be immunogenic or tolerogenic. In vitro manipulation of cellular vaccines is crucial for their successful use in clinics. In the second part of our study we tried this manipulation by adding arachidonic acid at the terminal differentiation step of DCs.

Functionality of DCs i.e. priming of naïve CD8+ T cells to give rise to the effector functions depends upon the expression of receptors and costimulatory molecules on the mature DCs. Our data showed that typical DC specific markers along with the costimulatory molecules, associated integrins and adhesion molecules were expressed on the cells from both the sources and were comparable. DCs have the characteristic property to activate naïve T cells and this functional aspect depends upon their efficiency of antigen uptake, processing and presentation. Immature DCs capture antigens by phagocytosis, macropinocytosis or via interaction with a variety of cell surface receptors and endocytosis. Antigen uptake capacity of UCB-DCs was comparable to PBL-DCs. MLR measures early events in the sensitization phase of antigen-specific cell-mediated immune responses, aiming at the efficiency of the most immunostimulating population of DCs. Allogeneic MLR assay also indicated that UCB-DCs and PBL-DCs were comparable in their immunostimulatory ability. The migration and accurate positioning of DCs is indispensable for immune surveillance. Before DCs can perform their major functions i.e.
to initiate the immune response it is preceded by two major events, migration and maturation. Migration of DCs toward a CCL-19 gradient is an important functional property in the immunotherapy protocols as they migrate from the site of injection towards the nearest lymph nodes, where they interact with the T cells and initiate the immune response. UCB and PBL-DCs showed similar pattern of in vitro migration towards the gradient of chemokine CCL-19. IL-12 is essential for ontogenesis of effector functions in T cells as well as in the establishment of functional memory. In vitro generated pulsed UCB-DCs were able to secrete significantly higher levels of IL-12p70 as compared to PBL-DCs. On other hand, levels of IL-10 secretion by PBL-DCs were higher than those of UCB-DCs. It is clearly evident that the UCB-DCs had a more favorable Th1 cytokine profile, as compared to PBL-DCs. In order to make it a readily available source, we next addressed the question, whether in vitro generated pulsed DCs can be cryopreserved? We observed that long term freezing of mature pulsed DCs was possible and thus it is feasible to freeze them for future use.

CTLs are generated by activation of naïve CD8+ T cells against the antigen presented by mature DCs through MHC class I. CTLs generated by pulsed DCs from both the sources were evaluated for granzyme A, granzyme B and IFN-γ. Granzymes are serine proteases that are released by cytoplasmic granules present within cytotoxic T cells. They induce programmed cell death in the target cell, thus eliminating cells that have become cancerous or are infected with viruses or bacteria. CTLs generated from both the sources showed the presence of cytosolic granzyme A and B in activated CTLs. The presence of these serine proteases underscores the killing potential of the cells as they are now armed as effector cells designated to find and kill the target cells. IFN-γ regulates multiple aspects of CD8+ T cell homeostasis as it promotes expansion and formation of memory cells. DCs from both sources were able to generate CTLs against the MUC1peptide after a secondary stimulation. The specificity and enumeration of CTLs was further fortified by MUC1-peptide specific tetramer staining. The ability of
antigen specific CD8^+ T cells to seek out pathogen-infected cells or tumor cells and to efficiently kill them in a highly specific manner constitutes a powerful immune effector function. They protect the host against intracellular pathogens, viral infections and cancer. CTLs generated from lysate pulsed DCs from both the sources were able to kill the target cells in vitro at different effector ratios. In vivo potency of the CTLs generated from HLA-A^*0201 positive samples was evaluated by a xenograft model of MCF-7-luc-F5 in female NOD/SCID mice. We found that UCB/CTLs and PBL/CTLs exhibited immunotherapeutic effects against MCF-7-luc-F5 solid tumors. It was evident from the IVIS data that there was a significant regression in tumors, ten days after CTL infusion in the test groups. The H&E and immunofluorescence staining of tumour sections further revealed substantial homing and infiltration capability of infused CD8^+ T cells. Ex vivo CTLs generated from UCB pulsed DCs have antitumor activity similar to their PBL counterpart and therefore can be administered in adoptive T cell therapy.

In the second part of the thesis, we made attempts to increase the efficacy of in vitro generated DCs by addition of arachidonic acid (AA) during the differentiation step. The AA^+DCs showed higher antigen uptake along with significantly higher T cell stimulatory capacity and in vitro CTL activity than the AA^−DCs. A more favorable Th1 cytokine profile and significantly higher transcript levels of COX-2 were exhibited by AA^+DCs, making them more proficient in functional attributes. Moreover, AA^+DCs exhibited an enhanced in vitro migration towards the chemokine CCL-19 as well as in vivo migration in NOD/SCID mice as compared to AA^−DCs. Exogenous addition of AA to the DC culture restored the levels of eicosanoids in a COX-2 mediated pathway thereby enhancing the functionality of these cells to be used as a potent cellular vaccine.

With the advancement in technologies our perspective on the role of DCs as adjuvant in cancer immunotherapy has stretched out remarkably. We are at the door step to enter a new era, in which immunotherapy is going to revolutionize the treatment of almost every kind of cancer. Thus these findings will be helpful in the better contriving of DC based vaccines for cancer immunotherapy, leading a step further in the field of translational medicine.