Eosinophils are multifunctional leukocytes that act as modulators of host immunity and play an important role in host defence against bacteria and parasitic worms. Eosinophils develop and mature in the bone marrow from multipotent hematopoietic stem cells under the influence of cytokines like IL-3, IL-5 and GM-CSF and various transcription factors like GATA-1, PU.1 and C/EBP. Upon stimulation by various allergens, they get activated and migrate to the site of action and secrete granule proteins ECP, EDN, MBP and EPO, pro-inflammatory cytokines, chemokines, lipid mediators and neuromediators. The granule protein ECP is known to be cytotoxic to various mammalian cell lines and non-mammalian species such as bacteria, helminths and protozoa. The eosinophil count rises significantly under various inflammatory reactions indicating a role of these cells in asthma, allergic-inflammatory diseases, and parasitic infections.

In this thesis, we have investigated the modulation of various cellular genes
and proteins involved in the differentiation of eosinophils from their progenitor cells using a model cell line HL60 clone15. Treatment of these cells with butyric acid resulted in differentiation of the parent cells to eosinophil like cells. The levels of ECP and EDN were found to increase upon differentiation while MBP levels were decreased. An increased expression of mRNA of GATA-1, GATA-2, CCR-1 and CCR-3 in BA differentiated cells was found in accordance with earlier reports. Study of the transcriptome profile of these cells before and after differentiation showed modulation of a number of biological pathways. These majorly included signalling pathways like MAPK, JAK-STAT, Insulin, VEGF, Wnt, TLR chemokine signalling pathways. A significant upregulation was also observed in cytokine-cytokine receptor interaction and cell adhesion molecules. We found a significant upregulation in the transcription levels of genes for various interleukins and their receptors upon differentiation. These included IL-1 and IL-8 and receptors for IL-10, IL-2, IL-3, IL-4, IL-5 and IL-18. All these interleukins have been known to play an important role in the eosinophil maturation and differentiation. In addition, the expression levels of mRNA of MAPK regulators; DUSP 4 and DUSP 6 were significantly increased while that of pro-apoptotic markers such as Bag-1, and Cytochrome C was found to be lowered upon differentiation. Thus, the differentiation of eosinophils from progenitor cells involves a major interplay of cytokines, chemokines and cell adhesion molecules. The mechanism involves modulation of a number of signalling pathways especially the MAPK and JAK-STAT pathways.

We have also conducted a detailed study on the role of eosinophil granule proteins in pathogenesis. The precise mechanism of involvement of these cells in various diseases is not clearly understood, however the action of eosinophils is believed to be mediated via these granule proteins. ECP cytotoxicity on mammalian cells was studied by analyzing their transcriptomic and proteomic profiles. Treatment with ECP induced apoptosis in the target cells via free radical generation. It resulted in the downregulation of cellular antioxidants like TPx and PPIases and derangement of glycolysis and other metabolic pathways. The actin cytoskeleton was also modulated and expression of genes involved in endocytosis such as HS6ST1, EPN3, ACVR1B was significantly upregulated. Thus, treatment of cells with ECP may result in its uptake via endocytosis following which ECP may trigger oxidative stress and free radical generation which in turn would induce apoptosis in the cells.
We also studied the modulation of genes and proteins by transfecting genes for the eosinophil granule proteins, EDN and ECP in a cell line which inherently does not express these granule proteins. We found changes in metabolic pathways especially carbohydrate metabolism. The proteome profile showed an upregulation of GAPDH, aldolase A, and phosphoglycerate kinase upon ECP transfection and GAPDH and aspartate aminotransferase upon EDN transfection. Analysis of the transcriptome profile showed an upregulation of genes belonging to various pathways like MAPK, Wnt, and TGF-β signalling pathways. The expression of mRNA for genes belonging to endocytosis and heparin sulphate biosynthesis and apoptosis was observed upon ECP transfection.

In conclusion, we have identified intracellular targets for the eosinophil granule proteins at the time of maturation as well as activation. Further biochemical studies based on the leads generated are needed to establish the precise mechanism of eosinophil differentiation and eosinophil granule protein toxicity.