ImmunoTools *special* Award 2013

Chong SHEN, PhD, Postdoc researcher

Pole de Pneumologie, ORL et Dermatologie, IREC, Université Catholique de Louvain, Tour Claude bernard-PNEU, Ave Hippocrate 54, Bte B1.54.04, 1200 Brussel, Belgium

Characterization of dendritic cells in patients with allergic rhinitis and asthma

Allergic asthma and rhinitis are chronic airways diseases representing a major health problem worldwide. In asthma, $CD4^+$ Th2-driven inflammatory responses to allergens play a central role, and result from aberrant signals provided by mucosal epithelial and dendritic cells (DC) that trigger antigen-specific Th2 cells. DCs have the potential to take up, process and present antigens in the cleft of major histocompatibility complex (MHC) I and MHCII molecules to T cell receptors. When an antigen is encountered, the simultaneous triggering of pattern recognition receptors on the surface or within the vacuoles (endosomes) of DCs leads to their increased expression of chemokine receptors such as CCR7 that direct the trafficking of DCs into the T cell zone of the regional draining lymph nodes in responses to chemokine ligands. In the T cell zone, DCs present antigenic peptides of the inhaled allergens to naive CD4 T cells and induce Th1 or Th2 cell differentiation. It is well established that the deleterious allergic response is initiated by T cell recognition of major histocompatibility class II-peptide complexes at the surface of APCs. We speculate an intrinsic and extrinsic (as a result of elevated IL-33 or TSLP, for example) dysfunction of DC leads to Th2 responses in allergy and asthma in human subjects. We showed that tissue and blood mDC from atopic patients are to some extent deficient in expressing IL-10 and that pDC are impaired in their capacity to induce IL-10 in naive $CD4^+$ T cells.

Based on these data, we hypothesize that mucosal factors may, under normal circumstances, act on DCs to keep these antigen presenting cells (APC) in an immature stage that promote immune tolerance at mucosal surfaces. We plan to explore this through the following research project with 3 aims, by utilising intensively the reagents from ImmunoTools.

Aim 1: to assess the effects of soluble factors on differentiation and maturation of DCs:

We will focus on the effects of cytokines and other mediators on human monocyte differentiated DCs - using validated protocols (with IL-4 + GM-CSF or TGF-β) to generate myeloid DC subsets (monocyte-derived DC, MD-DCs; and Langerhans cells, LCs). Effects will also be studied on the activation/maturation of MD-DC and circulating myeloid DCs (mDCs), purified from blood by MACS. Activation will be

carried out by ligation of TLR4 (LPS) for mDCs or TLR7 / TLR9 for pDCs, or by a cytokine combination (IL-1, IL-6 and TNF). Extensive phenotyping of DCs will then be carried out by flow cytometry (CD1a, CD11c, CD33, HLA-DR, CD40, CD80, CD86, CD71). We will also study plasmacytoid DCs.

Aim 2: to determine T cell regulation by conditioned DCs:

Immunoregulation of DCs by soluble factors may result in redirecting T cell responses to antigens following DC/T cell interactions, through cell surface molecules (cell-cell contact via costimulation molecules) and/ or indirectly through soluble factors/cytokines. Whether activation of DCs will result in changes in T cell activation pattern will be evaluated (CD3, CD4, CD8, CD25, CD62L, CD69, CD45RA), as well as the mechanisms involved to generate effector (Th1, Th2 and Th17) or regulatory T cells (Treg), in the models of mixed leukocyte (autologous or allogeneic) reactions.

Comparison of the two datasets (healthy versus allergic patients) in response to these factors will potentially provide functional discrepancy of DC and T cells. Both peripheral blood and mucosal biopsy samples can be analyzed. Correlation of the DC immune phenotype will be tested for clinical features such as age of onset, treatment, disease location and severity. This approach could reveal functional relationships between immunological and clinical phenotypes.