

SCIENTIFIC REPORT

Short-Term Scientific Mission (STSM)

COST Action BM0806

Host Institute: Swiss Institute for Allergy and Asthma Research (SIAF), Davos, Switzerland

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Introduction:

I have spent two months at SIAF within a Short-Term Scientific Mission funded by COST action BM0806 under the supervision of Dr. Liam O'Mahony. Our aim was to develop a method for the isolation of plasmacytoid dendritic cells (pDCs) with high purity from human peripheral blood. In addition, we examined histamine receptor expression on these highly purified pDCs and the effect of histamine co-incubation on pDCs following *in vitro* activation (e.g. in case of TLR ligands).

Background and description of the work carried out:

Plasmacytoid dendritic cells are one of the two principal subsets of human dendritic cells (DCs). They play a central role in the regulation of immune responses and link innate and adaptive immunity since they sample environmental antigens (such as microbes) and produce large amounts of cytokines which can activate many other cell types e.g. monocytes, B cells, T cells and NK cells. In allergic diseases, the activated immune response results in a chronic pro-inflammatory state characterized by antibody secretion (IgE) and T cell activation to normally well-tolerated antigens. The role played by microbial and allergen activated dendritic cells and especially pDCs in these phenomena is not well understood. In addition, the influence of histamine signaling on dendritic cell responses to TLR ligands is not well characterised.

Methods used:

We examined a number of different experimental conditions in order to develop a method for isolating highly purified pDCs. The final protocol which we generated involved the direct isolation of pDCs from human peripheral blood using BDCA-4 (CD304) beads followed by positive selection via the MACS system. Since the purity of the cells was not more than 80% after magnetic selection (as determined by CD123 and CD303 positivity), cells were further purified using a BD FACS Aria flow cytometric cell sorter resulting in >99% pure pDC populations. Cells were cultured *in vitro* with IL-3 and stimulated with the TLR9 ligand PTO-CpG2006 with or without histamine costimulation at 1×10^{-5} M. RNA was isolated from the cells after 4 hours stimulation and mRNA expression was tested by qRT-PCR.

Main results obtained:

We optimised a protocol for the successful isolation of highly purified pDCs from human peripheral blood. This method can now be applied to patient populations in order to determine the biological alterations that may occur in the pDCs compared to healthy volunteers. In the small number of healthy individuals that we isolated pDCs from, we confirmed that pDCs express histamine receptors and following CpG stimulation, pDCs express high levels of IFN- α and TNF- α . The expression of histamine receptors (HR) by myeloid dendritic cells has been well described. However, HR expression by pDCs is less well understood that is why we investigated pDC expression of histamine receptors by qRT-PCR.

Future directions:

As this method was developed using pDCs from healthy volunteers, it would be really interesting to investigate and to know how pDCs behave in allergic and in asthmatic patients. In particular, it will be exciting to examine pDC histamine 4 receptor expression and function on cells isolated from an inflamed environment.