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Role of antigen presenting cells in the development inflammatory response: their modulation by neurotransmitters

Parallel advances in neuroscience and immunology established the anatomical and cellular basis for bidirectional interactions between the nervous and immune systems. Acetylcholine (ACh) is the primary parasympathetic neurotransmitter and also a non-neural paracrine factor produced by different cells, included immune cell. Respect of the ACh immunomodulatory action, the study was concentrate in the inflammatory process that happen in the airways, settling down that both an increase of ACh as a dysfunction of the receptors that mediate their action, contribute in the etiopathogenesis of the illness in the airways, such as asthma and chronic obstructive pulmonary disease (COPD).

Dendritic cells (DCs) are highly specialized antigen-presenting cells with a unique ability to activate resting T lymphocytes. They can induce the differentiation of T $CD4^+$ cells into different profiles, T helper 1 (Th1), Th2, Th17 and T Regulatory. This decision depends, of the cytokine production by own CDs.

It is known that exposure to allergens CDs induces the development of a Th2 profile characteristic of the allergic process, and we demostrate that ACh, is able to modulate the function of CDs. Despite the relevance of the CDs to the development of allergic processes, as well as the influence of the cholinergic system in the etiopathogenesis of chronic inflammatory diseases of the airways; there is no previous works in the scientific literature that analyze the immunomodulatory actions produce by ACh on the physiology of the developing CDs allergic processes. Therefore, we propose to evaluate the importance of the cholinergic system in the modulation of the activation of DCs when they are exposed stimuli that induce a Th2 profile, and what would be the participation of receptors muscarinic in this modulation.

Generation of human Dendritic Cells: Peripheral blood mononuclear cells (PBMC) are isolated from peripheral blood by Ficoll-Hypaque (1.077 g) density gradient centrifugation.

$CD14^+$ cells are then isolated by positive selection. The purity is checked by flow cytometry analysis using anti-CD14 monoclonal antibody (mAb) and is found to be 95%. To obtain DCs, monocytes (10^6/ml) are cultured in RPMI 1640 medium supplemented with 10% of heat-inactivated FCS, 50 U/ml penicillin, 50 µg/ml streptomycin, 20 ng/ml IL-4, and 50 ng/ml GM-CSF. On day 5, the cells are analyzed by flow cytometry with anti-CD1a antibody.

To evaluated the activity and the maturity of DCs in presence of absence of the cholinergic agonist and the rhTSLP, we used flow cytometry; for that the cells are washed twice with PBS supplemented with 2% FCS and suspended in PBS supplemented with 10% heat-inactivated FCS. Fluorescein isothyocyanate (FITC) and phycoerythin (PE) conjugated mAbs are added at saturating concentrations for 30 min at 4 °C, and two additional washes are then performed. Human cells are stained with the following mAbs: FITC- or PE-conjugated mAbs directed to CD1a, CD40, CD86, CD80, HLA-DR, and HLA- ABC. We evaluate the apoptosis and viability of the culture with Annexin V.

To evaluate the function of the DCs we use the Mixed lymphocyte reaction (MLR): The DCs are cultured for 24 h with or without cholinergic agonist and with or without rhTSLP. DCs (1×10^4/100 µl) are cultured alone or in the presence of 2×10^5 freshly isolated allogeneic PBMCs during 5 days in 96-well U-bottom plates. Then the proliferation is evaluated with labeled of CFSE and antibody anti-CD4 or anti-CD8. For the last, the cytokine production is measurement, previously and after of the MLR, IL-4, IL-8, IL-12p70, TNF-α and IL-6 in cell supernatants by ELISA.