

## H4 receptor and human neutrophils

COST BM0806 STSM in Centre for Infection and Immunity,  
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**Timeframe:** 2-3 months

Although eosinophils are usually associated with asthma, in some severe forms of the disease the predominant cell is the neutrophil. Polymorphonuclear neutrophils (PMNs) are recruited to the airways by a complex series of regulated events including chemotactic stimuli such as IL-8, and LTB<sub>4</sub>, transendothelial migration through selectin-mediated cell arrest, and subsequent  $\beta$ 2 integrin-dependent firm adhesion. Chemoattractants drive the recruitment of PMNs to the site of inflammation by controlling the change of conformation of  $\beta$ 2 integrins, the dominant adhesion receptors expressed by PMNs. Chemoattractants bind to receptors expressed on the membrane surface of PMNs, resulting in intracellular signalling. These signals trigger both expression of  $\beta$ 2 integrins on the membrane surface, and switch the  $\beta$ 2 integrins into a high affinity ligand binding conformation. Thus, in response to chemoattractants,  $\beta$ 2 integrins are expressed in abundance on the membrane surface of PMNs, and can bind endothelial ligands, which is a prerequisite for firm adhesion and migration to the site of inflammation.

Histamine, produced by mast cells and basophils, is a well recognized inflammatory mediator, causing for example contraction of bronchial and tracheal smooth muscle. However, studies using traditional anti-histamines (H1 receptor antagonists) have suggested that these compounds are of little or no value in treating asthma. Histamine is also a chemoattractant for eosinophils, an effect mediated through the relatively newly discovered histamine H4 receptor (H4R). This is illustrated by the fact that H4R knockout mice treated with ovalbumin to induce asthma, have reduced eosinophils and cytokines (IL-4, IL-5, IL-13) in their bronchoalveolar lavage fluid (BAL). A similar effect is seen with the use of the H4R antagonist JNJ 7777120. In zymosan murine models of peritonitis, JNJ 7777120 reduced the number of PMNs in the lavage fluid and the concentration of MPO, indicating that the H4R may also control PMN migration. However, the effect of histamine on human PMNs has been little studied. Indeed, it is not known whether the H4R is expressed on PMNs and whether histamine is a chemoattractant for PMNs.

**Our preliminary data** indicate that human PMNs from healthy volunteers express the HR4. Furthermore, histamine changes profoundly the shape of PMNs, and switches the  $\beta$ 2 integrins into a high affinity ligand binding conformation.

**Aim:** The aim of this STSM is investigate the expression and function of the H4 receptor on neutrophils from healthy volunteers.

### **Plan of investigation**

**Aim 1:** To compare levels of active  $\beta 2$  integrins and histamine receptor 4 in PMN

Blood (50 mL) will be collected by venipuncture. PMNs will be isolated by Ficoll-Hypaque sedimentation as described by us. Flow cytometric histograms depicting mean fluorescence intensity of active  $\beta 2$  integrins on PMNs will be generated], using the monoclonal antibody 24 which recognizes the high affinity conformation of  $\beta 2$  integrins. Confirmation of these data will be obtained by comparing  $\beta 2$  integrin-dependent adhesion of PMNs to ICAM-1-coated surfaces, in response to histamine and bacterial fMLP. Since PMNs express 2 types of histamine receptors, H1R, H2R and possibly H4R, we will investigate which receptor is involved in the activation of  $\beta 2$  integrins and chemotaxis (see aim 2). Selective receptor antagonists will be used such as loratadine (H1R), cimetidine (H2R) and JNJ7777120 (H4R). We will also, in parallel, measure by flow cytometry, the level of HR4 in PMNs from healthy controls.

**Aim 2:** Does histamine induce migration of neutrophils ? Histamine has been shown to be a potent chemoattractant for eosinophils. However, it is not known whether histamine plays a similar role in PMNs. To address this question, PMN chemotaxis will be measured using an under agarose assay. The advantage of this assay is that random and directed migration can be distinguished and chemotaxis can be accurately measured by time lapse microscopy. We will also test how antagonists of H1R, H2R and H4R affect PMN migration in response to histamine. Overall, we will test whether histamine is a potent chemoattractant for PMN.