

## **Change in expression of basophil activation markers CD63 and CD203c upon incubation with histamine**

Rita Seguro Dias, Lars K. Poulsen, Bettina M. Jensen  
Laboratory of Medical Allergology, Allergy Clinic, Herlev and Gentofte Hospital, Denmark

### Introduction

Allergy diagnosis can be confirmed, among other techniques, by cellular tests. Currently, two of these in vitro tests rely on basophil activation: the histamine release assay and the basophil activation test (BAT). In both, basophils are activated and release histamine. Since basophils themselves express histamine receptors, we speculated whether this released histamine would have an effect on the activation of basophils and consequently influence the tests results.

### Aim

The aim of this project was to confirm the expression of histamine receptors in basophils and further evaluate the effect of histamine on the expression of CD63 and CD203c using BAT.

### Methods

Basophils were purified from buffy coat blood using negative selection. Purity was 91%. RNA was purified using a TRIzol-phenol-chloroform technique and cDNA was synthesized. PCR analyses of histamine receptor 1-4 were performed and results were expressed as Ct values. Basophil lysates were investigated for histamine receptor expression using SDS-PAGE followed by Western Blot (WB) using specific antibodies for the histamine receptors 1, 2 and 4. Proteins were visualized on x-ray films. BAT was performed on whole blood pre-incubated with histamine at different concentrations (0; 0,001; 0,01; 0,1; 1 µg/ml) for 1 hour at 37°C. Basophils were stimulated with anti-IgE (0, 4, 16, 63, 250, 1000, 4000 ng/ml) and a mix containing antibodies for CD63, CD203c, HLA-DR, CD193 and CD123 was added. Samples were incubated at 37°C for 30 minutes and analysed in a BD LSRFortessa™. The gating strategy was as follows: FSC-A vs. time; FSC-A vs. FCS-H; SSC-A vs. SSC-H; HLA-DR vs. CD123; CD203c vs. CD193. Basophils were then plotted for CD63 and CD203c expression. CD63 upregulation was analysed by means of percentage while CD203c was analysed using median fluorescence intensity (MFI).

### Results

Histamine receptor 1, 2 and 4 expression was confirmed in basophils both at mRNA and protein levels, with histamine receptor 1 being the least expressed. However, mRNA expression did not fully match protein content. PCR showed expression in the following order: histamine receptor 2>4>1 with Ct values of 27, 30 and 37, respectively. Band intensity in WB showed expression of histamine receptor 4>2>1. Histamine dose-response study (histamine concentrations: 0,001; 0,01; 0,1; 1 µg/ml) showed that histamine dampen the basophil activation in a dose-dependent way with 1 µg/ml as the optimal concentration. The effect of histamine was confirmed in 4 additional subjects where both activation markers (CD63 and CD203c) were found to be downregulated after histamine challenge. Downregulation ranged between 14-71% for CD63 and from 20-77% for CD203c.

### Conclusion

Basophils express histamine receptors 1, 2 and 4 at mRNA and protein levels. CD63 and CD203c are downregulated upon pre-incubation with histamine. This finding could mean that histamine can influence in vitro basophil studies. Further investigations should be made to access the impact of histamine on the release of late-phase mediators.