Advanced basophil activation test combining histamine release with surface marker expression

Ewa Bartko, Lars Blom, Lars K. Poulsen and Bettina M. Jensen

Allergy Clinic, Herlev and Gentofte Hospital, University of Copenhagen, Denmark

Background
Predictions estimate that by the year 2025 half of the Europeans will be affected by at least one kind of allergy. Thus, new diagnostic tools or development of already existing methods could ease and fasten diagnosis. Nowadays, the gold standard for allergy diagnosis is skin prick test and specific IgE level. Occasionally unique and challenging allergy cases require more specific and sensitive diagnostic assays, such as the basophil activation test (BAT), which utilizes one of the effector cells of an allergic reaction, basophils. Accessibility and the specific activation profile of basophils contributed to their use as a diagnostic tool. Recently, it was reported that the increased expression of CD63 upon basophil activation does not always reflect the pattern of histamine release. To address this issue an enzyme-affinity-method has been established. It combines the basophil activation test with intracellular detection of histamine using fluorophore-labelled diamine oxidase, an enzyme binding histamine with high affinity.

Objective
Firstly, to develop an in-house flow cytometry-based technique that allows for intracellular detection of histamine with simultaneous identification of activation markers. Secondly, to broaden description of basophil activation profile, by evaluating connection between activation markers, CD63 and CD203c and release of histamine.

Methods
The activity of DAO and DAO-PE were assessed by efficacy to degrade histamine. Intracellular histamine was detected by a flow cytometric method, that combines the basophil activation test with histamine release test. It allows for simultaneous detection of intracellular histamine using fluorophore-labelled diamine oxidase and activation markers, CD63 and CD203c. To define population of basophils the following gating strategy was applied: FSC-A vs. time; FSC-A vs. FCS-H; SSC-A vs. SSC-H; CD193c vs. CD123 and SSC-A vs FSC-A. Subsequently, activation profile was assessed by gating on CD63 vs. CD203c; CD63 vs. DAO and CD203c vs. DAO. The results were analysed in terms of median fluorescence intensity (MFI) for CD203c and percentage of basophils containing the marker of interest for CD63 and histamine.

Results
Conjugation of DAO with fluorophore does not affect activity of the enzyme. It maintains nearly the same ability to degrade histamine as the unlabelled one. Flow cytometric intracellular staining protocol allows for detection of histamine. Its shows that, expression of activation markers is not affected by additional steps in the protocol. Moreover, activation of basophils with anti-IgE or fMLP triggers histamine release that can be investigated by means of flow cytometry. Concurrent analysis of CD63 reveals that within the basophil population, phenotypically distinguished subgroups of cells can be found. For each donor 4 population of cells are described: $CD63^-$ $histamine^-$, $CD63^+histamine^-$, $CD63^-histamine^+$ and $CD63^+histamine^+$ and the activation profile pattern is defined as follows: unstimulated basophils contain histamine and are negative for CD63, activation of cells induces shift of basophil population that becomes $histamine^-$ and $CD63^+$. Inter-individual comparison of each subpopulation suggests that unless a donor is a nonreleaser the subpopulation of basophils that release histamine and express CD63 can be found in each donor, however the percentage of this subpopulation differs between the donors. Interestingly, the subpopulation that release histamine but do not express CD63 is determined in few donors, proving that degranulation can occur without appearance of CD63, suggesting importance of the piecemeal degranulation.

Conclusion
We have established a flow cytometry-based test which simultaneously detects surface marker expression and intracellular histamine in basophils. It allows for more detailed description of the basophil activation profile, proving that in most cases CD63 expression reflects the histamine release, however, that piecemeal degranulation also seems to contribute to the release of histamine in basophils.