Inhibition of SHIP-1 with 3-α-AC affects the reactivity of human blood basophils and cultured human mast cells differently

Authors
Pernille Rasmussen[1,2], Edzard Spillner[2], Hans Jürgen Hoffman[1]

[1] Department of Respiratory Diseases and Allergy, Aarhus University Hospital
[2] Department of Immunological Engineering, Aarhus University

Introduction
IgE-mediated activation of mast cells and basophils follows a bell-shaped dose-response curve. The decreased activation at supraoptimal allergen stimulation is thought to be associated with SH2-containing inositol-5'-phosphatase 1 (SHIP-1) and its down regulatory role in PI3K signaling. SHIP-1 dephosphorylates phosphatidylinositol-3,4,5-trisphosphate (PIP_3), which is an important immediate product involved in degranulation of mast cells and basophils. It is found that SHIP-1 phosphorylation is inversely related to IgE-mediated releasability of basophils and it is suggested that high SHIP-1 activity is distinctive in basophils from non-responder patients. A selective chemical SHIP-1 inhibitor could increase the IgE-mediated response of mast cells and basophils and potentially facilitate a basophil response in non-responder patients.

Objective
This study sought to clarify the regulatory role of SHIP-1 in IgE-mediated upregulation of CD63 in mast cells and basophils by suppressing SHIP-1 activity with a selective inhibitor called 3-α-aminocholestane (3-α-AC).

Methods
Ten grass pollen allergic patients were recruited and six different cultured human mast cell lines were included. Basophils were challenged with increasing concentrations of grass pollen extract while the cultured human mast cells sensitized against Der p 2 were challenged with natural Der p 2 allergen. Reactivity and sensitivity were assessed by flow cytometry with CD63 as activation marker. The effect of 3-α-AC (0.1-60 µM, 0-45 min) was analyzed at individual suboptimal, optimal and supraoptimal allergen concentrations. The measured CD63 upregulation at different conditions was compared with CD63 upregulation of untreated cells to evaluate the maximal effect of SHIP-1 inhibition.

Five non-responder patients were included. Basophils form non-responder patients were treated with 3-α-AC at optimal conditions (10 µM, 30 min) and challenged with anti-IgE antibodies.

Results
At high concentrations (>60 µM) of 3-α-AC, cells appeared to enter apoptosis which is a known feature of 3-α-AC. For basophils, the reactivity was unaffected by 3-α-AC at optimal and supraoptimal allergen stimulation, whereas a minor increase was observed at suboptimal stimulation. The highest increase was achieved at 10 µM of 3-α-AC incubated for 30 minutes and it increased the median reactivity from 27.5 % to 44.9 % $CD63^+$ basophils (IQR: 34.7-68.2; $p = 0.0313$). Mast cells did not show a supraoptimal response. At both suboptimal and optimal allergen stimulation, the median reactivity of mast cells were decreased. At suboptimal stimulation and 10 µM of 3-α-AC, the median reactivity decreased from 32.85 % to 16.5 % (IQR: 7.08 – 20.03; $p = 0.0465$) $CD63^+$ mast cells. The negative effect mediated by 10 µM of 3-α-AC on mast cell reactivity was significantly different from the positive effect mediated in basophils ($p = 0.0043$).

No increased response was measured in basophils from non-responder patients.

Conclusion
SHIP-1 inhibition with 3-α-AC induced different effects in mast cells and basophils suggesting that there exist some differences in the regulatory mechanisms between the two cell types. This provides new insights into the regulation of IgE-induced mast cell and basophil degranulation. Further experiments are needed to confirm these controversial findings.

SHIP-1 inhibition with 3-α-AC did not stimulate a basophil response in non-responder patients suggesting that SHIP-1 is not the only enzyme responsible for non-releasability of basophils within non-responder patients.