Passively sensitized human organotypic tissue as asthma model to study mast cell-nerve interaction

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Introduction

Mast cells play a key role in allergic asthma. It is well known that mast cell activation is mediated through the aggregation of their high-affinity IgE-receptor (FcεRI) by IgE and antigen. Nevertheless, new evidences suggest that mast cells can also respond to stimuli that are independent of FcεRI, such as tachykinin peptides. Therefore, neurogenic mechanisms may contribute to the pathogenesis of asthma. Additionally, there is a lack of in vitro models to study the local mast cell-nerve interaction. We aimed to investigate if C-fibers activation by capsaicin and the release of neuropeptides lead to mast cell degranulation and bronchoconstriction, using passively sensitized human Precision-Cut Lung Slices (PCLS) as an ex vivo model.

Materials and Methods

Human PCLS were generated using resection material from lung tumor patients. PCLS were passively sensitized with 1% plasma of HDM-allergic donor and incubated +/- the histamine 1 receptor antagonist, the NK1 receptor inhibitor and a recombinant monoclonal antibody against IgE. Sensitized PCLS were stimulated with capsaicin or house dust mite (HDM). Finally, bronchoconstriction was measured by videomicroscopy and reduction of the airway area was calculated as percentage of the initial airway area (Fig. 1). Also, histamine release was detected after capsaicin or HDM stimulation using ELISA. Additionally, cryosection preparations from human bronchi were immunostained after +/- capsaicin stimulation using antibodies against the mast cell tryptase.

- Histamine is released after HDM stimulation in passively sensitized PCLS. Capsaicin induces mast cells degranulation in human bronchi

Capsaicin stimulation showed small amounts of histamine release of ~2 ng/ml. However, HDM stimulation in PCLS treated with 1% allergic plasma reflected ~14 ng/ml (Fig. 2 A). Mast cell degranulation was observed in human bronchus after capsaicin stimulation. Immunostaining of mast cells revealed degranulation as evidenced by cell-stored and appearance of freely located mast cell tryptase (Fig. 2 B).

- Passive sensitization of human PCLS leads to increased bronchoconstriction after stimulation with capsaicin or HDM.

Capsaicin and HDM induced bronchoconstriction leads to a significant reduction of the airway area (RAA) of 47% and 58% respectively, in sensitized PCLS. Capsaicin stimulation in PCLS treated with 1% non allergic plasma showed 15% RAA and PCLS treated with medium showed 18% RAA after two days incubation. HDM stimulation in PCLS treated with 1% non allergic plasma showed 10% RAA (Fig. 3 A, B).

- Bronchoconstriction in passively sensitized PCLS as a consequence of capsaicin stimulation is Histamine 1 receptor, NK1R and IgE dependent.

Capsaicin stimulation leading to bronchoconstriction was totally inhibited by 15 µM IgE antagonist, 100 nM NK1 receptor antagonist or 10 µM of the histamine 1 receptor antagonist (Fig. 4 A, B, C).

Conclusions

We propose a hypothesis to explain these results. Allergic airway microenvironment was mimicked by passive sensitization. Here, IgE binds to its high affinity receptor on mast cells. Mast cell sensitization leads to the upregulation of the NK1 receptor (step 1). Afterwards, a local axon reflex is mimicked by capsaicin stimulation. Here capsacin activates C-fibers via vanilloid receptors (step 2). C-fibers activation release tachykinin peptides such as neuropeptide substance P (SP), which binds to its high affinity receptor NK1R on mast cells. This binding may trigger a secretion of selective mast cells mediators leading to bronchoconstriction (step 3 and 4). Mast cell mediators such as prostaglandins; leukotrienes or even neurotrophins are interesting molecules candidates to be analyzed. In this model we propose that mast cell sensitization was avoided by the IgE antagonist, SP-induced mast cell degranulation was prevented by NK1R antagonist and mast cell mediators released was blocked by HR1 antagonist. We concluded that human passively sensitized PCLS represent a suitable ex vivo model to study mast cell-nerve interaction during allergic pathophysiological responses such as bronchoconstriction.

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