Activation of human skin mast cells by serum from chronic spontaneous urticaria patients – an *ex vivo* skin microdialysis study

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Background
Chronic spontaneous urticaria (CSU), a disabling disease characterized by itchy wheals and/or angioedema, does not have definite eliciting factors, and the exact pathogenesis remains to be characterized in detail. However, it is now widely accepted that CSU, in most patients, is an autoimmune disorder and tissue-resident mast cells are believed to be major effector cells. When activated by cross-linking of the high affinity IgE-receptor, either by IgG autoantibodies against IgE/FcεRI or by IgE against autoallergens, mast cells give rise to CSU symptoms by releasing inflammatory mediators, including histamine. Through passive transfer to human skin mast cells (either *in situ* or purified), we studied the mast cell activating effects of sera from ten CSU patients. Released histamine was sampled from *ex vivo* human skin after injection of the sera using microdialysis and measured in supernatants from purified skin mast cells after incubation for comparison.

Methods
Sera from ten CSU patients enrolled in the CORSA study were selected based on their ability to release histamine from basophils in the basophil histamine release assay (BHRA, RefLab ApS, Denmark). Abdominal skin was obtained from three anonymous donors undergoing cosmetic surgery and used for *in situ* activation of skin-resident mast cells: Twelve microdialysis probes (3000 kDa molecular weight cut-off, EP Medical, Denmark) were inserted into each skin specimen, and the ten sera, a positive control (anti-IgE) and serum from a healthy control were injected around individual probes. Microdialysis sampling was carried out in 10-minute intervals for 1 hour, and histamine was subsequently quantified in the dialysates using the HR Assay (RefLab ApS, Denmark). The total area under the curve (AUC) was calculated for each serum. Mast cells were isolated from breast skin obtained from three additional donors and incubated for 1 hour with 20% patient serum before histamine was measured in the supernatants and expressed as % release of the total histamine content, which was determined by lysing the cells.

Results
CSU patient sera can induce histamine release from skin mast cells *in situ*, and this response correlates with the results of the basophil histamine release assay (BHRA, P=0.0005, Pearson correlation). Purified skin mast cells were also found to release histamine upon incubation with sera from CSU patients. The amount of histamine released from skin mast cells *in situ* was highly correlated with the histamine release measured in supernatants from purified skin mast cells *in vitro* (P<0.0001, Pearson correlation).

Conclusion
The human *ex vivo* skin model is a new tool to study mast cell activation *in situ* by injecting CSU patient sera into excised skin followed by sampling of histamine using microdialysis. The histamine recovered from mast cells *in situ* was found to be significantly correlated with histamine release from purified skin mast cells after incubation with the CSU patient serum, however, the latter is a more simplified model to study mast cell activation due to the *in vitro* setting.