

# Novel cooperation between CX3CL1 and CCL26 inducing NK cell chemotaxis via CX3CR1: a possible mechanism for NK cell infiltration of the allergic nasal tissue

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**Background:** Recent data indicated that natural killer (NK) cells and chemokines could play a pivotal role in nasal inflammation. CX3CR1, the only receptor for fractalkine/ CX3CL1, is abundantly expressed by NK cells, and was recently shown to also be a receptor for eotaxin-3/CCL26. However, no reports explored the NK cells-CX3CL1-CCL26 axis via CX3CR1 in allergy.

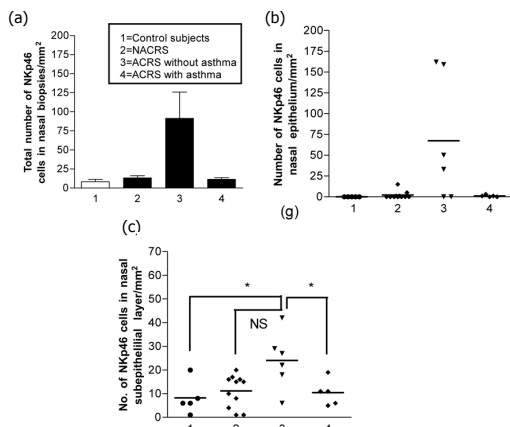
**Objective:** Our goals were first to determine specifically NK cell recruitment pattern in nasal tissue of allergic chronic rhinosinusitis (ACRS) and non-allergic chronic rhinosinusitis (NACRS) patients in comparison with healthy controls, and secondly, to investigate the function of CX3CR1 in NK cell migration.

**Methods:** Immunohistochemistry, microchemotaxis chambers, flow cytometry and confocal microscopy were used in this study.

**Results:** Herein, we showed that NK cells infiltrated the epithelial layers of nasal tissue only in ACRS patients and not in NACRS patients or controls. NK cells were also more numerous in the stroma of the nasal tissue from ACRS patients compared with NACRS patients or controls. This migration could be mediated by both CX3CL1 and CCL26, as these two chemokines induced NK cell migration. Moreover, both molecules also stimulated cytoskeleton changes and F-actin reorganisation in NK cells. Chemotaxis and cytoskeleton changes were sensitive to genistein, a tyrosine kinase inhibitor. By flow cytometry, we demonstrated that a single antigen nasal provocation challenge increased the expression of CX3CR1 on NK cells in allergic rhinitis (AR) patients. The function of this receptor was associated with a significant augmentation of NK cell chemotaxis against the optimal doses of CX3CL1 and CCL26.

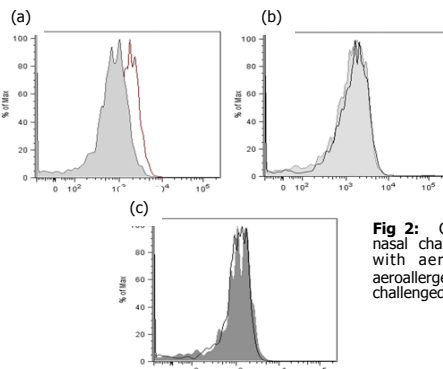
**Conclusions and Clinical Relevance:** Our results highlight a novel role for CX3CR1 in NK cell migration that may contribute to the NK cell trafficking to the allergic upper airway. This could be mediated largely by CX3CL1 and CCL26 stimulation of the tyrosine kinase pathway.

## 1. NK cell recruitment to nasal tissue in CRS



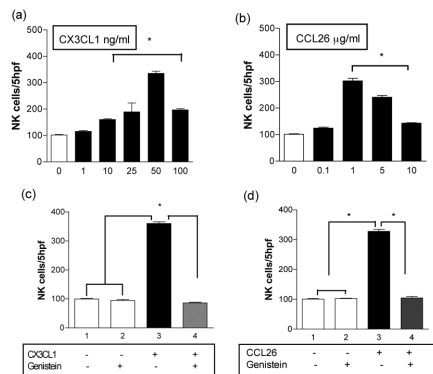
**Fig 1:** Natural killer (NK)p46+ cell infiltration in nasal biopsies. NKp46+ cells (a) in nasal biopsies (means ± SEM), (b) in nasal epithelium and (c) in subepithelial stroma (bars represent the mean values). Control subjects (n = 5), NACRS subjects (n = 11), CRS subjects without asthma (n = 6), ACRS subjects with asthma (n = 5). NS = not significant. Asterisk indicates P < 0.0001 and P = 0.0236 by paired t-test, for bars 1 & 3 and bars 3 & 4 respectively.

## 2. Expression pattern of CX3CR1 by NK cells in AR

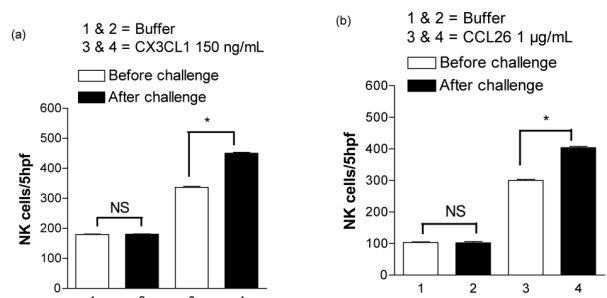


**Fig 2:** CX3CR1 expression on NK cells after nasal challenge from AR patients challenged with aeroallergen (a), challenged with aeroallergen diluent (b) and healthy control challenged with histamine (c).

## 3. CX3CL1 and CCL26 induced-NK cell chemotaxis is tyrosine kinase dependent and is increased after nasal allergen challenge



**Fig 3.1:** Chemotactic activity of CX3CL1 and eotaxin-3 on human natural killer (NK) cells, dose-response experiments. (a) CX3CL1 and (b) CCL26 in inducing NK cell chemotaxis. (c and d) Blockade effect of 1 µg/mL genistein in NK cell chemotaxis against the optimal doses of CX3CL1 and CCL26. Asterisk indicates P < 0.05 (a and b) and P < 0.0001 (c and d), by paired t-test & ANOVA. Results are means ± SEM of 12 independent experiments performed in triplicate.



**Fig 3.1:** Effect of nasal allergen challenge with HDM on (a) CX3CL1- and (b) CCL26-induced natural killer (NK) cells chemotaxis. Results are means ± SEM of six independent experiments performed in triplicate. Asterisk indicates P < 0.0001 by paired t-test & ANOVA.