

Short communication

Demonstration of mast-cell chemotactic activity in nasal lavage fluid: characterization of one chemotaxin as *c-kit* ligand, stem cell factor

Nilsson G, Hjertson M, Andersson M, Greiff L, Svensson C, Nilsson K, Siegbahn A. Demonstration of mast-cell chemotactic activity in nasal lavage fluid: characterization of one chemotaxin as *c-kit* ligand, stem cell factor.

Allergy 1998; 53: 874–879. © Munksgaard 1998.

Mast cells are known to accumulate in tissue during allergic inflammation. However, the chemotaxins responsible are undefined. Using a modified Boyden chamber and the human mast-cell line HMC-1, we first identified mast-cell chemotactic activity in nasal lavage fluid collected before the pollen season after allergen provocation of allergic patients ($n=29$) (mean migratory response compared to medium control was 121%, range 85–198%). Mast-cell chemotactic activity was also detected in lavage fluid collected after allergen provocation at the end of a Swedish birch-pollen season from three different treatment groups: topical steroid treatment with budesonide; the topical antihistamine, levocabastine; and placebo. There was no significant difference in mast-cell chemotactic activity between nasal lavage fluid collected from the placebo group (mean=102%), the budesonide-treated group (mean=114%), or the levocabastine group (mean=125%). Stem cell factor (SCF), a known mast-cell chemotaxin, was present in the nasal lavage fluids from all three groups, and correlated with the mast-cell chemotactic activity ($r=0.67$, $P<0.01$). The mast-cell chemotactic activity was inhibited (range 5–100%) in some, but not all, nasal lavage fluids by a polyclonal antibody directed against SCF. This report describes the presence of mast-cell chemotactic activity in nasal lavage fluid during an allergic reaction. These findings show that SCF may play a pivotal role in the recruitment of mast cells in allergic rhinitis.

Mast cells are the principal effector cells in IgE-dependent hypersensitivity reactions. They are normally distributed throughout connective tissues and are particularly numerous beneath the epithelial surface of the skin, in the respiratory system, in the gastrointestinal and genitourinary tracts, and adjacent to blood or lymphatic vessels (reviewed by Nilsson & Schwartz [1]). Mast cells live in tissues for several months, and their numbers under normal conditions are relatively constant. However, mast cells accumulate during certain pathophysiologic conditions, such as asthma (2), allergic rhinitis (3), scleroderma (4), and rheumatoid arthritis (5).

In allergic rhinitis, an accumulation of mast cells within the epithelial layer of the mucosa, which normally has very few, if any, mast cells, after natural exposure to pollen or after nasal provocation tests has been reported by several groups (3, 6–8). Provocation studies showed that this increase occurs within hours after allergen challenge (9–11). One possible cause of this increase is proliferation and maturation of resident immature mast-cell precursors already present in the tissue (12). However, *in vitro* maturation of mast cells requires at least a few weeks (13, 14). It is therefore likely that a direct migration of mast cells within tissues is an

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Key words: allergy; chemotaxis; mast cell; migration; mucosa; stem cell factor.

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Accepted for publication 1 April 1998

important mechanism for the increase in mast-cell numbers seen in allergic rhinitis.

To begin to define chemotaxins involved in recruitment of mast cells *in vivo*, we examined the ability of nasal lavage (NAL) fluid, collected after allergen provocation of subjects with allergic rhinitis, to induce mast-cell migration *in vitro*. As responder cells, we used a human mast-cell line (HMC-1) (15). Phenotypic characterization of HMC-1 revealed considerable similarities with normal human mast cells (16). On the basis of their neutral protease contents, HMC-1 cells resemble the MC_T type of human mast cells, which corresponds to the human mast cell found in the epithelium of nasal mucosa (17). In this study, we report that NAL fluid contains mast-cell chemotactic activity (MCCA) in part due to the presence of stem cell factor (SCF).

Material and methods

Design of the provocation study

The patients and the design of the provocation study have been described in detail elsewhere (18). The patients had a clinical history of birch-pollen-induced allergic rhinitis and a positive skin prick test to birch pollen. Informed consent was obtained from all subjects, and the study was approved by the ethics committee at the University of Lund and by the Medical Products Agency in Sweden. None of the patients had symptoms or signs of perennial allergic rhinitis, nor did they have structural abnormalities of the nose or chronic disease. The provocation study was designed in a single-blind, placebo-controlled fashion. The patients were randomized into three different treatments: 100 µg of levocabastine (Livostin, Janssen Pharmaceutica, Belgium), 100 µg budesonide (Rhinocort aqua, Astra Draco AB, Sweden), or placebo was administered in each nasal cavity twice daily. The treatment started on 15 April and continued to the end of May.

Nasal allergen challenges were performed before the pollen season (February), when the patients had not been exposed to any pollens, and at the end of the pollen season (end of May) after approximately 6 weeks of pollen exposure when the patients had developed allergic rhinitic symptoms. After an initial baseline wash, nasal washings were performed to reduce cell-free mediators and establish a stable baseline. Thereafter, increasing doses of pollen allergen (with a mechanical pump) were given in the nasal cavity (100, 1000, 10000 units of birch-pollen allergen) (ALK, Copenhagen, Denmark). Ten minutes after each challenge, NAL was performed with a nasal pool device (14 ml of

saline; 10-min duration) (19). The NAL fluid was then immediately centrifuged (3000 rpm; 10 min), and the cell-free NAL fluid was frozen at -20°C. The baseline lavage and the lavage after the strongest allergen dose (10000 SQ units) were used for further analysis.

NAL fluid was also collected from 10 subjects, healthy and with no history of allergy, after histamine provocation. After an initial baseline wash with saline, 400 µg/ml of histamine was given, and the NAL fluid was collected as described above.

Chemotaxis assay

HMC-1 (15, 16) (obtained from Dr J. H. Butterfield, Mayo Clinic, Rochester, MN, USA) was cultured in Iscove's modified Dulbecco's medium supplemented with 10% FCS, 2 mM L-glutamine, 100 IU/ml penicillin, 50 µg/ml streptomycin, and 1.2 mM α-thioglycerol. The cell line was passaged every 3–4 days.

The migration of HMC-1 cells was assayed in a modified Boyden chamber by means of the leading front technique as applied to the measurement of human mast-cell chemotaxis, as previously described (20). Micropore filters (150-µm-thick nitrocellulose) with a pore size of 8 µm were coated with fibronectin at a concentration of 100 µg/ml at room temperature overnight. The assay was performed in a 48-well micro-Boyden chemotaxis chamber (Neuroprobe Inc., Cabin John, MD, USA). Thirty microliters of the NAL or other chemoattractants tested was added below the filter, and 50 µl of the cell suspension (1.5×10^6 cells/ml) was added above the filter. For inhibition of the SCF-induced migration, we used a goat-anti-human rSCF antibody (British Bio-Technology Products Ltd, Oxon, UK) (20). After 150 min at 37°C and 5% CO₂, the filters were fixed, stained with Mayer's-Hemalum solution, and mounted. The migration of HMC-1 cells suspended in medium with 10% FCS, and with the same medium below the filter in the Boyden chamber, served as a normal control reference and was referred to as 100% migration. Cell chemotaxis was assayed as the migration distance of the two furthest migrating cells visible in focus of one high-power field (12.5×24) (21). The migration distance in each filter was calculated as the mean of the readings of three to six different areas of the filter. The assay was always done in duplicate.

Stem cell factor (SCF) measurements

SCF concentrations in NAL fluids were measured by an enzyme-linked immunoassay (ELISA) kit purchased from R & D Systems (Minneapolis, MN, USA) (sensitivity: 3 pg/ml).

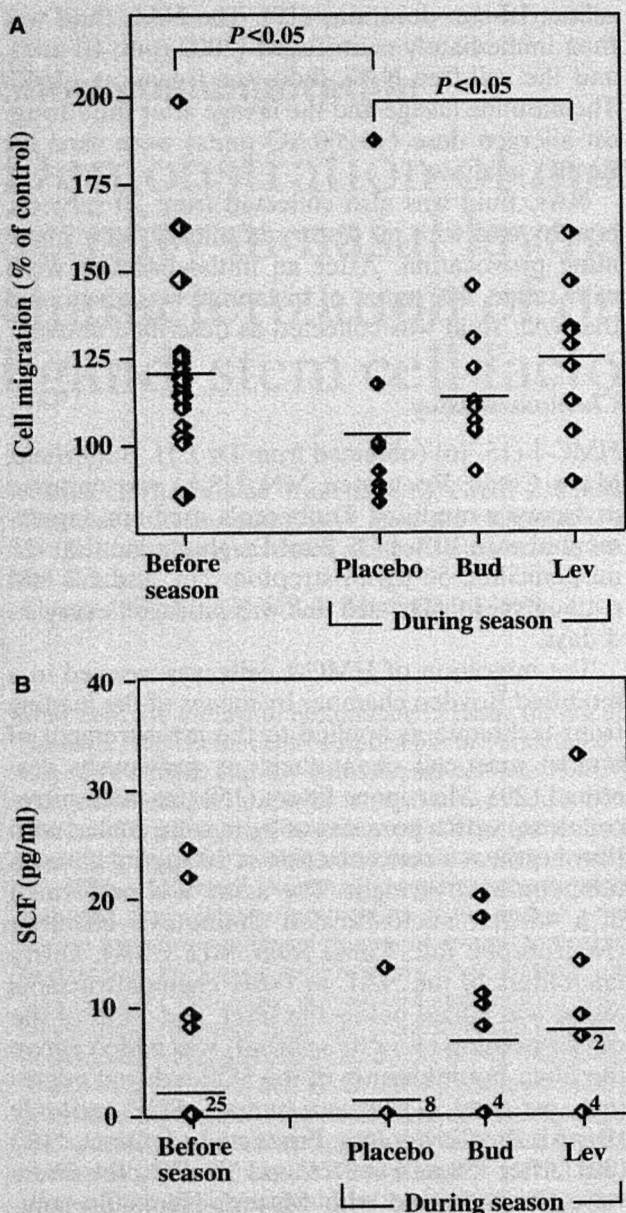


Fig. 1. NAL fluid was collected either before pollen season ($n=29$) or during season. During season, subjects were divided into three groups receiving, respectively, placebo ($n=9$), budesonide (Bud) ($n=9$), or levocabastine (Lev) ($n=9$). A) Mast-cell migration toward NAL fluid collected after allergen provocation of patients with allergic rhinitis. Migratory response is expressed as percentage of response to medium control. Horizontal bars represent means. B) SCF levels in NAL fluid collected after allergen provocation of patients with allergic rhinitis. Levels below detection limit were set to 0 pg/ml. Horizontal bars represent means. Number on right shows number of samples with same SCF concentrations.

Statistical analysis

For analysis of significant differences, analysis of variance (ANOVA) was used. The correlation coefficient was calculated to measure the relations between two parameters, and the t -test for corre-

lation was used for calculation of significance. Differences were considered significant at $P<0.05$.

Results

Mast-cell chemotactic activity (MCCA) in NAL fluid from allergic rhinitis

To address the issue of migration of mast cells into the intraepithelial layer in allergic rhinitis, the presence of MCCA in NAL fluid was analyzed with HMC-1 as a bioassay. Patients were challenged with allergen before or at the end of the pollen season. For the NAL fluid collected during season, the subjects were divided into three groups; one receiving placebo, one treated with budesonide, and one treated with levocabastine. MCCA could be demonstrated in NAL fluid collected both before season (mean=121%, range 85–198%) and among subjects in all three groups collected during the pollen season (placebo mean 102%, range 83–187%; budesonide mean 114%, range 92–145%; levocabastine mean 125%, range 89–160%) (Fig. 1A). The migration to NAL fluids from placebo-treated patients was significantly different compared to before season and levocabastine-treated patients ($P<0.05$).

Detection of SCF in NAL fluid

We have earlier shown that SCF is a potent chemotaxin for human mast cells (20). We therefore hypothesized that the MCCA in NAL fluid, at least in part, could be attributed to SCF. The presence of SCF in NAL fluid was analyzed by ELISA, and samples with no detectable SCF were set to zero. As shown in Fig. 1B, SCF could be detected in 15/57 samples analyzed. There was no significant difference between the different groups.

For determination of whether the levels of SCF in NAL fluid correlated with the MCCA found, all samples collected both before and during season, and from placebo-treated subjects, as well as subjects receiving treatment, were used for calculation of the correlation coefficient (Fig. 2). A positive, significant correlation between SCF levels in NAL fluid and induced migration of HMC-1 cells was found ($r=0.67$; $P<0.01$).

Blocking of MCCA with an antibody against SCF

To test the involvement of SCF in the migration of HMC-1 cells toward the MCCA in NAL fluid, we used a goat-anti-SCF antibody, as described previously (20). The antibody was able to block the MCCA in some of the samples (5–100% inhibition in 17/20 samples tested), whereas some samples

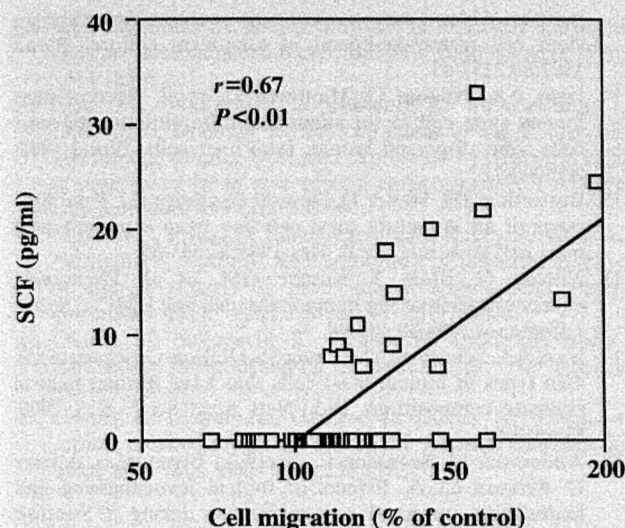


Fig. 2. Correlation between SCF in NAL fluid and migratory response to NAL fluid of subjects from all groups studied. Correlation coefficient was calculated for relation between SCF and cell migration, and *t*-test for correlation was used for calculation of significance ($r=0.67$, $P<0.01$).

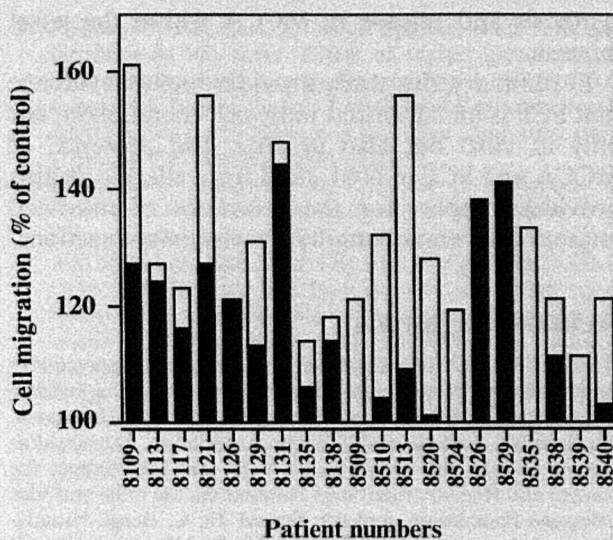


Fig. 3. Blocking of MCCA by Ab against SCF. Migratory response to NAL fluid (open bars) or NAL fluid plus anti-SCF Ab (filled bars) is shown. In comparison, migratory response to 20 ng/ml of SCF (201%) was completely blocked by Ab.

were unaffected by pretreatment with the antibody (Fig. 3).

Discussion

This study has demonstrated that

- 1) MCCA can be detected in NAL fluid collected in allergic rhinitis
- 2) SCF can be detected in NAL fluid
- 3) SCF contributes to the MCCA found in the lavage.

Mast cells have been implicated in the pathogenesis of allergic diseases ever since histamine was localized to these cells, and the role of mast cells as effector cells in allergic rhinitis is now well established (reviewed by Sulakvelidze et al. [22]). During natural pollen exposure (6, 7, 23, 24) or after experimental allergen challenges (9–11, 25), an increase in mast-cell numbers has been reported. This increase in mast-cell numbers is directly correlated with symptoms (23). Of special interest is the finding that intraepithelial mast cells mostly increased (3, 26). The nasal epithelial mast cells, as opposed to lamina propria mast cells, are believed to be the major effector cells of allergic rhinitis (7, 24). The recruitment of mast cells into the intraepithelial cell layer is therefore likely to be of great significance for the pathophysiology of allergic rhinitis. This study shows that MCCA can be detected in the NAL fluid from some allergic subjects, a finding which may account in part for the attraction of mast cells into the intraepithelial cell layer.

The presence of SCF in NAL fluid was not surprising since SCF is a known chemoattractant *in vitro* for mouse mast cells (27), HMC-1 cells and cord blood-derived mast cells differentiated *in vitro* (20), and purified pulmonary mast cells (28). It is not possible from our present findings to identify the cell type(s) responsible for the secretion of SCF. However, several cell types have been shown to express and secrete SCF, such as fibroblasts (29), endothelial cells (30), bone-marrow stromal cells (31), and keratinocytes (32). Of particular interest for this study is the recent finding that nasal epithelial cells express SCF (33) and that expression of SCF is more frequent in patients with allergic rhinitis than in controls (34). Local production of SCF by epithelial cells may therefore contribute to the accumulation of mast cells within the intraepithelial cell layer.

Topical administration of corticosteroids is an established treatment for allergic rhinitis (35). However, publications on the effect of steroid treatment on mast-cell numbers have reported varying results (36–41). Antihistamines have not been reported to change the numbers of mast cells but may reduce the appearance of mast-cell mediators in NAL fluid after allergen challenge (42). One explanation of the unexpected effect with no apparent decrease in MCCA after medication compared to controls may be that higher doses of allergen, which was used for the provocation in this study, override any effect by corticosteroids or the antihistamines.

However, more studies need to be done on the effect of corticosteroids and antihistamines on the

synthesis and release of MCCA within the nasal mucosa.

In summary, this study provides further evidence that SCF is an important mast-cell chemotaxin, not only *in vitro* but also *in vivo*. The presence of MCCA and SCF in NAL fluid from allergic rhinitis provides support for the relevance of mast-cell migration in immediate hypersensitivity reactions.

Acknowledgments

We thank Matilda Johnell for excellent technical assistance with the chemotaxis assay. This work was supported by the Swedish Foundation for Health Care Sciences and Allergy Research, the Swedish Asthma and Allergy Association, the Swedish Cancer Society, the King Gustav V 80-year Foundation, the Torsten and Ragnar Söderbergs Foundation, the Ollie and Elof Ericssons Foundation, and the Konsul Th. C. Bergh Foundation, and also partly by the Swedish Medical Research Council (project no. 8308) (M.A.).

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