Allergology International 65 (2016) 280–285

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Eosinophil chemotaxis assay in nasal polyps by using a novel optical device EZ-TAXIScan: Role of CC-chemokine receptor 3

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ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form
19 December 2015
Accepted 9 January 2016
Available online 10 February 2016

Keywords:
Asthma
Chronic sinusitis
Eosinophils
Eotaxin
Nasal polyps

Abbreviations:

CCR3	CC-chemokine receptor 3
CRS	chronic rhinosinusitis
CRTH2	chemoattractant receptor-homologous molecule expressed on Th2 cells

ABSTRACT

Background: The chemokine receptor, CC-chemokine receptor 3 (CCR3), and its major ligands, eotaxin, RANTES, and MCP-4, are involved in eosinophil chemotaxis. It is thought that CCR3 plays an important role in the recruitment and activation of eosinophils in nasal polyposis. We examined nasal polyp extract-induced eosinophil chemotaxis and the effect of a CCR3 antagonist using EZ-TAXIScan, a novel real-time chemotaxis assay device.

Methods: Nasal polyps were obtained from chronic rhinosinusitis (CRS) patients during surgery. The polyps were homogenized and eotaxin levels in the extracts were measured. Eosinophils were purified from human peripheral blood by the CD16 negative selection method. Nasal polyp extract-induced eosinophil chemotaxis, with or without CCR3 antagonist, was assessed by EZ-TAXIScan.

Results: There was a significant positive correlation between the eosinophil counts in nasal polyp and eotaxin levels in the nasal polyp extracts. Using EZ-TAXIScan, eosinophil chemotactic responses were observed following stimulation with nasal polyp extracts. There was a significant positive correlation between the chemotactic index toward the nasal polyp extracts and their eotaxin levels. Nasal polyp extract-induced chemotaxis was completely inhibited by CCR3 antagonist but not by chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) antagonist which inhibited PGD_2-induced eosinophil chemotaxis.

Conclusions: The CCR3 pathway may play an important role in the pathogenesis of eosinophil recruitment in nasal polyps through selective eosinophil chemotaxis.

Introduction

Nasal polyps occur more commonly in asthmatic patients, and there are greater densities of activated eosinophils in nasal polyps from asthmatic patients compared to non-asthmatics.[1–3] Eosinophils play an important role in the pathogenesis of allergic airway disease by secreting a wide variety of cationic proteins, lipid mediators, and cytokines/chemokines that mediate terminal effector functions and the innate immune response.[4] CC-chemokine receptor 3 (CCR3) belongs to a family of seven transmembrane-spanning G protein-coupled receptors and binds three eotaxin family proteins, eotaxin-1/CCL11, eotaxin-2/CCL24 and eotaxin-3/CCL26, that are potent chemoattractants for eosinophils.[5–7] The eotaxin concentrations of nasal polyp extracts are significantly increased in eosinophilic chronic rhinosinusitis (CRS) patients compared to the non-eosinophilic CRS group.[8,9] CCR3 plays an essential role in eosinophil recruitment to the skin and the lung and in the development of airway hyperresponsiveness in a CCR3-deficient mouse model.[10] Because CCR3 expression is associated with Th2 lymphocytes, eosinophils, and mast cells, CCR3 may be central to the induction of the inflammation associated with allergic disease.[11,12] However, the role of CCR3 in eosinophil recruitment in nasal polyps is not clear. To address this issue, we employed a nasal polyp extract-induced eosinophil chemotaxis assay with a novel real-time chemotaxis assay device, EZ-TAXIScan, which can monitor and record horizontal cell migration.[13] We found that nasal polyp extract-induced eosinophil chemotaxis was correlated with the eotaxin levels of nasal polyps, and addition of a CCR3 antagonist completely inhibited chemotaxis. On the other hand chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) antagonist which inhibited PGD_2-induced eosinophil chemotaxis did not inhibit nasal polyp extract-induced

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Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2016.01.001

eosinophil chemotaxis. These findings suggest that the CCR3 pathway may play an important role in the pathogenesis of eosinophil recruitment in nasal polyps through selective eosinophil chemotaxis, raising the possibility that CCR3 antagonists may be effective for the treatment of eosinophilic nasal polyps.

Methods

Tissue collection

Nasal polyps were obtained from 12 patients with CRS (five male and seven female) during endoscopic sinus surgery. There were six asthmatic and six non-asthmatic patients. Systemic corticosteroids were discontinued at least one month prior to surgery. The nasal polyp samples were divided in half, and one specimen was immediately snap-frozen in liquid nitrogen and stored at −80 °C until processed. The other was fixed in a 10% neutral formaldehyde solution and embedded in paraffin. Counting of the number of eosinophils in the nasal polyps was performed at high magnification (400×). In each patient, 5 visual fields were randomly selected and the mean eosinophil count per field was calculated. The study was approved by the ethics committee of Akita University School of Medicine. Informed consent was obtained from each patient before collecting samples.

Eotaxin assay

Nasal polyp samples were homogenized in 500 μl of buffer containing 1% NP-40, 150 mM NaCl, 50 mM Hepes, and centrifuged at 13,000 rpm for 10 min. The concentration of eotaxin in the nasal polyp extracts was measured by an ELISA kit (R & D Systems, Minneapolis, USA), according to the manufacturer's instructions.

Eosinophil preparation

Eosinophils were purified from normal donor blood by negative selection, as previously described.[14] Briefly, eosinophils were isolated by sedimentation with 6% dextran saline solution followed by centrifugation on 1.088 Percoll (Pharmacia, St. Louis, USA) density gradients. The cells were further purified by negative selection using anti-CD16 immunomagnetic beads and a MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany). Eosinophil purity of >98% was routinely obtained as determined by microscopic analyses.

Chemotaxis assay

Eosinophil chemotactic responses were measured using the real-time chemotaxis assay device, EZ-TAXIScan (Effector Cell Institute, Tokyo, Japan). The EZ-TAXIScan chamber was assembled with a 260 μm wide × 4 μm thick silicon chip on a 2 mm untreated glass base, as described by the manufacturer, and filled with RPMI medium containing 1% FBS. Eosinophil suspension (1 μl containing 2×10^6 cells/ml) was injected into one side of the EZ-TAXIScan chamber. Chemoattractant solution (1 μl eotaxin, PGD_2 or nasal polyp extract) was injected into the opposite side of the chamber to initiate chemotaxis. Migration of eosinophils over the glass surface was recorded with a CCD camera located beneath the glass every minute for 60 min at 37 °C. At the end of the assays, we calculated the chemotactic index by dividing the number of eosinophils that had migrated over the halfway line towards the chemoattractants by the number of eosinophils remaining at baseline.

CCR3 or CRTH2 antagonist TREATMENT

Eosinophils (2×10^6 cells/ml in RPMI medium containing 1% FBS) were incubated for 30 min with or without CCR3 antagonist, SB328437 (1000 nM) (R & D Systems, Minneapolis, USA) and CRTH2 antagonist, CAY10471 (1000 nM) (Cayman Biochemical, Michigan, USA).

Statistical analyses

Data are presented as mean ± SE. Comparisons of two groups of data were performed using the Wilcoxon single rank test, and simple regression analysis was performed. Significance was established at the $p < 0.05$ level.

Results

Eosiniphil count and eotaxin level in nasal polyp

There was a significant positive correlation between the eosinophil counts in nasal polyp and eotaxin levels in the nasal polyp extracts (Fig. 1).

Eosinophil chemotaxis induced by eotaxin

Migration of human eosinophils from normal donor blood toward a concentration gradient of eotaxin (1000 nM) was recorded using EZ-TAXIScan. Images were recorded every minute for 60 min; images at 0, 10, 20, 40, 60 min tracing the trajectory of eosinophil chemotaxis are presented (Fig. 2). The chemotactic index with 1000 nM eotaxin was significantly higher than that of the control (Fig. 3).

Effect of CCR3 antagonist on eosinophil chemotaxis

We investigated the effect of the CCR3 antagonist, SB328437 or CRTH2 antagonist, CAY10471 on eotaxin (1000 nM) or PGD_2 (1000 nM)-induced eosinophil chemotaxis to establish whether the CCR3 antagonist directly modulated the eosinophil chemotactic response. Dose-dependent inhibition of eotaxin-induced chemotaxis of eosinophils by CCR3 antagonist and inhibition of PGD_2-induced chemotaxis of eosinophils by CRTH2 antagonist were observed. CCR3 antagonist did not inhibit PGD_2 -induced chemotaxis of eosinophils (Fig. 4). Our results indicated that the CCR3 antagonist inhibited the eotaxin/CCR3 chemotactic response directly.

Eosinophil chemotaxis induced by nasal polyp extracts

The numbers of eosinophils in nasal polyps were significantly higher in asthmatic patients than in non-asthmatic patients (data not shown). Migration of human eosinophils toward nasal polyp extracts was recorded and images at 0, 10, 20, 40, 60 min tracing the trajectory of asthmatic patients' nasal polyp extract-induced chemotaxis are presented (Fig. 5a–d). Inhibition of nasal polyp extract-induced chemotaxis of eosinophils by CCR3 antagonist was observed (Fig. 5e–h). There was a significant positive correlation between the chemotactic index toward the nasal polyp extracts and their eotaxin concentration (Fig. 6). In addition, CCR3 antagonist (1000 nM) inhibited nasal polyp extract-induced eosinophil chemotaxis completely. On the other hand CRTH2 antagonist (1000 nM) did not inhibit nasal polyp extract-induced chemotaxis of eosinophils (Fig. 7). These results indicated that the CCR3 pathway has an important role in eosinophil recruitment in nasal polyps.