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ScienceDirect
European Journal of Pharmacology 566 (2007) 200–205
www.elsevier.com/locate/ejphar

Interactions between eotaxin and interleukin-5 in the chemotaxis of primed and non-primed human eosinophils

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Received 20 April 2006; received in revised form 8 September 2006; accepted 11 September 2006
Available online 17 February 2007

Abstract

This study was designed to understand the relationship between interleukin-5 and eotaxin in modulating the chemotaxis of eosinophils obtained from healthy subjects and subjects with allergic rhinitis. Chemotaxis of eosinophils from patients with allergic rhinitis toward interleukin-5 (0.25 ng/ml) was 78% higher than that of healthy subjects. Incubation of eosinophils with eotaxin (100 ng/ml) did not change the interleukin-5-induced chemotaxis of eosinophils from healthy subjects, but it reversed the enhanced chemotaxis seen in eosinophils from allergic patients. Chemotaxis of eosinophils from patients with allergic rhinitis toward eotaxin (100 ng/ml) was 65% higher than that of eosinophils from healthy subjects. Incubation of eosinophils with interleukin-5 (100 ng/ml) significantly increased the eotaxin-induced chemotaxis in both subject groups, but such increases were markedly higher for cells from patients with allergic rhinitis. Our finding that eotaxin inhibits the enhanced eosinophil chemotaxis toward interleukin-5 in primed cells suggests that this chemokine may downregulate eosinophil accumulation in the nasal mucosa of allergic patients.

Keywords: Allergic rhinitis; Eosinophil chemotaxis; Interleukin-5; Eotaxin; Fibronectin

1. Introduction

Allergic diseases of the airways, such as asthma and allergic rhinitis, are inflammatory disorders that differ mainly in the sites of the inflammatory reaction and clinical manifestations. Allergic rhinitis is an inflammatory disease of the upper airways that is characterized by the accumulation of eosinophils in the nasal mucosa of patients (Quraishi et al., 2004). Eosinophils interact in a sequential order with endothelium and interstitial extracellular matrix components, such as fibronectin, resulting in their extravascular migration and accumulation at inflammatory sites (Kuna et al., 1998; Hanazawa et al., 2000). The early steps in the movement of eosinophils from the peripheral blood to tissues take place via the binding of the integrin family of adhesion molecules, the β_1 subfamily of very late antigen (VLA) 4 ($\alpha_4\beta_1$; CD49d/CD29) as well as the Mac-1 ($\alpha_M\beta_2$, CD11b/CD18) member of the β2 subfamily, with the vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1), respectively (Wardlaw, 2001).

A number of chemokines have been shown to attract eosinophils with variable degrees of selectivity, including the subfamily of CC-chemokines, amongst which eotaxin (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26) play important roles (Shahabuddin et al., 2000; Teran, 2000; Kaplan, 2001). Increased expression of eotaxin has been demonstrated in the airways of subjects with asthma and in the nasal mucosa of subjects with allergic rhinitis, suggesting an important role in allergic diseases (Lamkhioued et al., 1997; Minshall et al., 1997). In addition, increased levels of eotaxin have been detected in the serum/plasma (Jahnz-Ro et al., 2000; Tateno et al., 2004) and sputum (Yamamoto et al., 2003) of asthma patients. Eotaxin contributes to selective eosinophil chemotaxis and transendothelial migration via the eotaxin receptor CCR-3, responses which are primarily mediated by β_2-integrins (Tachimoto et al., 2002). For instance, in eosinophils prestimulated with interleukin-5, eotaxin upregulates the CD11b/CD18

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doi:10.1016/j.ejphar.2006.09.075

molecule, leading to increased adhesion to fibronectin in vitro (Lundahl et al., 1998).

In healthy individuals, eosinophils adhere to activated endothelial cells, but do not transmigrate through this layer. Prior treatment with a cytokine such as interleukin-5 enables the eosinophils to migrate through the endothelium. In contrast, in allergic individuals, eosinophils not only adhere to but pass spontaneously through the endothelial cell layer, suggesting that priming of circulating eosinophils is a fundamental prerequisite for endothelium transmigration (Lampinen et al., 2004). Moreover, in guinea pigs and mice, eotaxin and interleukin-5 act co-ooperatively to promote the recruitment of eosinophils into tissues (Mould et al., 1997; Palframan et al., 1998; Foster et al., 2001). It has been suggested that interleukin-5 participates in eosinophil mobilization from bone marrow, whereas eotaxin induces their trafficking to tissues (Palframan et al., 1998). The present study was designed to further understand the relationship between interleukin-5 and eotaxin in modulating eosinophil chemotaxis in vitro, comparing this functional response in cells from healthy individuals (non-primed) and from subjects with allergic rhinitis (primed).

2. Materials and methods

2.1. Patients

All subjects gave their written consent for participation in the study. The protocols were approved by the local Ethics Committee and were conducted in accordance with the Declaration of Helsinki. Briefly, peripheral blood leukocytes were obtained from two groups of volunteers grouped primarily on the basis of their atopic status: (1) 33 normal healthy non-atopic subjects with peripheral blood eosinophilia ranging between 2% and 5% of total leukocytes; and (2) 54 patients with allergic rhinitis with a peripheral blood eosinophilia ranging between 5% and 19% of total leukocytes who had been pre-screened for the presence of allergen-induced rhinitis symptoms (sneezing, rhinorrhea, and nasal congestion). Atopy was defined on the basis of positive skin prick test reactions to a panel of common aeroallergens, including house dust mite, animal danders, and grass pollen. None of the patients were receiving antihistaminics, steroids, or nonsteroidal anti-inflammatory drugs at the time of the study. For the chemotaxis and adhesion assays, 30 normal healthy non-atopic subjects and 20 patients with allergic rhinitis were selected. Serum interleukin-5 levels were measured in all the subjects.

2.2. Eosinophil isolation

Eosinophils were isolated from peripheral blood as described by Hansel et al. (1991), with minor modifications. Briefly, 60 ml of heparinized (20 U/ml) blood from healthy or allergic subjects was diluted 1:1 with phosphate-buffered saline (PBS) and 35 ml of diluted blood was overlaid onto a 15 ml Percoll gradient (1.088 g/ml, pH 7.4, 340 mosmol/kg H_2O). Gradients were centrifuged at 700 *g* for 20 min, 4 °C (Jouan, Saint-Herblain, France) and the red cell pellet was collected. Red cells in the granulocyte pellet were lysed with lysis buffer (155 mM NH4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA). Washed granulocytes were incubated with anti-CD16 immunomagnetic microbeads and then passed over a steel-matrix column in a magnetic field (Miltenyi Biotec Inc., Aubum, USA); CD16-negative eosinophils were collected. The final suspension contained 97–99% eosinophils and contaminating cells were neutrophils. Cell viability (>93%) was assessed in the trypan blue dye exclusion test. Before use, eosinophils were resuspended in Eagle's minimum essential medium (MEM), pH 7.2.

2.3. Chemotaxis assays

Eosinophil migration was measured using a 96-multiwell ChemoTx 101–5 chamber. The wells in the microplate (e.g., bottom compartment) were filled with 29 μl of chemotactic agent (eotaxin or interleukin-5) diluted in MEM. A polycarbonate filter (5 μm pore size) was positioned on the loaded microplate and secured in place with corner pins. Next, eosinophils (25 μl; 4×10 cells/ml) treated with either interleukin-5 (0.2 ng/ml) or eotaxin (100 ng/ml) stet placed directly onto the filter sites. Untreated eosinophils (incubated with MEM) were used as controls. The chamber was then incubated for 2 h at 37 °C in a humid atmosphere with 5% CO_2. After incubation, the non-migrating cells on the origin side (i.e., top) of the filter were removed by gently wiping the filter with a tissue and the chamber was centrifuged at 200 *g* for 5 min at 20 °C. The filter was then removed and the number of cells that had migrated into the bottom compartment was determined by measuring residual eosinophil peroxidase (EPO). Each experiment was carried out in triplicate.

2.4. Cell adhesion assays

The adhesion assay was performed as previously described (Conran et al., 2001). In brief, 96-well plates were prepared by coating individual wells with 60 μl of fibronectin (20 μg/ml in PBS) overnight at 4 °C. Wells were then washed twice with PBS before blocking non-coated sites with 0.1% (w/v) bovine serum albumin (BSA) for 60 min at 37 °C. Wells were washed twice again with PBS and the plates were allowed to dry. Eosinophils were added in a volume of 50 μl of MEM/ovalbumin (7×10^4cells/ml) to the coated wells of a 96-well plate. Cells were allowed to adhere to wells for 15 min at 37 °C in a humid atmosphere with 5% CO_2. After incubation non-adhered cells were removed and the remaining cells were washed twice with PBS. Fifty microliters of MEM was added to each well and varying concentrations of the original cell suspension (in MEM) were added to empty wells to form a standard curve. Eosinophil adhesion was calculated by measuring the residual eosinophil peroxidase (EPO) activity of adherent cells. Eosinophils were pre-incubated with eotaxin (100 ng/ml) or interleukin-5 (0.25 ng/ml) for 15 min at 37 °C, in a humid atmosphere with 5% CO_2 before the cells were added to fibronectin-coated wells.