

# Interleukin-2 inhibits eosinophil migration but is counteracted by IL-5 priming

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## Summary

**Background** Conflicting data on the role of interleukin-2 in the recruitment of eosinophil granulocytes (EOS) to sites of inflammation have been presented. The objective of the present study was to investigate the effect of recombinant human IL-2 and anti-IL-2 on the migration of purified blood EOS.

**Methods** Neutralizing antibodies to IL-2 were added to a cytokine mixture with significant eosinophil chemotactic activity (ECA), and afterwards the ECA was tested on EOS from both normal and allergic donors. EOS migration was measured by a modification of the Boyden technique, using a 48-well microchemotaxis chamber. Recombinant human IL-2 was either added to the lower compartment of the chemotaxis chamber, or to the EOS for a pre-incubation period of 20 min, before migration assays towards the chemotaxins were performed.

**Results** Anti-IL-2 caused a significant increase of EOS migration towards the cytokine mixture. Pre-incubation of the EOS with rhIL-2 inhibited the chemotaxis towards RANTES, PAF, IL-8 and eotaxin, and EOS migration towards IL-2 was lower than that towards buffer. These effects were more pronounced on EOS from normal than from allergic donors. Priming of the EOS with IL-5 prevented the inhibitory effect of IL-2.

**Conclusion** We hypothesize that IL-2 acts as an autocrine regulator of EOS migration, and that this inhibitory effect may be downregulated in allergy, allowing an increased migration of EOS towards chemotactic factors.

**Keywords:** eosinophil, migration, interleukin-2, allergy

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## Introduction

The accumulation of eosinophil granulocytes (EOS) is an important feature of the inflammatory reactions that occur in asthma, allergic rhinitis and eczema [1]. The EOS are assumed to cause damage to the airway epithelium in asthma, and a positive correlation has been found between the number of EOS in bronchoalveolar lavage (BAL) fluid and blood and the severity of the symptoms [2,3]. The accumulation of EOS to the focus of inflammation

comprises several steps, involving a network of different cell types, adhesion molecules and mediators. A number of cytokines play important roles in this process. They prime EOS in the circulation, upregulate adhesion molecules on different cell types, and act as chemotactic factors, locally produced at the inflammatory site. Chemotactic activity for EOS has been detected in BAL fluid from allergic asthmatic patients after allergen challenge and during pollen season, and also from patients with intrinsic asthma [4].

Interleukin (IL)-2 is a cytokine mainly released from activated T cells, and acts as a T cell growth factor. It also stimulates growth and differentiation of B cells and natural

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killer (NK) cells [5]. In addition, IL-2 has been suggested to be a chemoattractant for EOS [6]. However, our preliminary results indicated that the effect of IL-2 on EOS migration might be the opposite. In our hands, antibodies to IL-2 enhanced the migration of EOS towards other cytokines. We therefore hypothesized that IL-2 may inhibit EOS migration, and the aim of the present study was to investigate the effect of recombinant IL-2 on the migration of EOS from both normal and allergic blood donors.

## Materials and methods

### *Blood donors*

Blood from either normal, healthy donors or from allergic donors was used. The normal group consisted of 10 men and eight women, mean age 36 years (range 22–62 years). The allergic group included four men and 17 women, mean age 33 years (range 21–50 years). Blood from nine pollen-allergic individuals was obtained during birch pollen season. Six of the allergic patients were at the beginning of a hyposensitization treatment and were receiving allergen injections, and the remaining six had perennial allergy with rhinoconjunctivitis, asthma or atopic eczema. None of the asthmatic patients received inhaled corticosteroids at doses higher than 800 µg Budesonide daily. The study was approved by the local ethics committee.

### *Cytokines and anticytokines*

Recombinant human IL-2, IL-5, IL-8, eotaxin and RANTES were purchased from R & D Systems Europe Ltd (Abingdon, UK). Neutralizing antihuman antibody to IL-2 and mouse IgG<sub>1</sub> isotype control antibody were purchased from R & D Systems Europe Ltd and from PharMingen (San Diego, Ca, USA). PAF (L-α-Phosphatidylcholine, β-Acetyl-γ-o (Octadec-9-cis-Enyl)) was purchased from Sigma Chemical Company (St Louis, USA).

### *Purification of eosinophils*

Eosinophils were isolated using an immunomagnetic bead technique as previously described [7]. Briefly, 80 mL of heparinized blood was obtained from normal, healthy blood donors or allergic donors, and granulocytes were isolated by Percoll (1.082 g/mL) gradient centrifugation. Red blood cells were removed by hypotonic lysis, and EOS were subsequently isolated using a method based on the fact that EOS, in contrast to neutrophils (NEU), lack the cell surface antigen CD16. As a result, highly purified EOS can be isolated by removing NEU bound to immunomagnetic beads coated with anti-CD16. EOS purity was 99 ± 1%,

and viability 99 ± 1%, as determined by staining with Trypan Blue.

### *Cell counting and quantification*

Total blood cell counts were made by Technicon H1 cell counter (Tournai, Belgium). Cells stained with Türk's dye were counted under the light microscope. Differential counts were obtained using a cytopsin preparation (Cytopsin, Shandon Southern Instruments, Sewickley, USA) stained with May Grünwald and Giemsa, and examined under light microscope.

### *Incubation of eosinophils with IL-2 and anti-IL-2*

Purified EOS diluted in Gey's solution (137 mM NaCl, 5 mM KCl, 0.8 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.2 mM KH<sub>2</sub>PO<sub>4</sub>, 11 mM glucose, 10 mM Tris(hydroxymethyl)-aminomethan, 1 mM MgCl<sub>2</sub> and 2 mM CaCl<sub>2</sub> with pH 7.4) with human serum albumin (1 g/L) to a concentration of 1 × 10<sup>9</sup>/L, were incubated with recombinant human IL-2 at the concentrations of 10<sup>-11</sup> or 10<sup>-10</sup> mol/L for 20 min at room temperature. The cell suspensions were then transferred to chambers for measurement of migration towards PAF, IL-8 or eotaxin. EOS pre-incubated with IL-2 at concentrations from 10<sup>-18</sup> to 10<sup>-11</sup> mol/L were used for the migration assay towards RANTES. In additional experiments with RANTES, rhIL-2 was pre-incubated with anti-IL-2 (0.001 µg/mL) or mouse IgG<sub>1</sub> isotype control antibody (0.001 µg/mL) for 5 min at room temperature before addition to the cell suspension.

### *Priming of eosinophils with IL-5*

In three experiments, purified EOS were incubated for 30 min at 37 °C with IL-5 (10<sup>-10</sup>M) or Gey's solution before incubating the cells with IL-2 (10<sup>-11</sup>M).

### *Chemotaxis assay*

Eosinophil chemotaxis was measured by a modification of the Boyden chamber technique [8] using a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, MD). Chemotaxins or Gey's solution (28 µL) were added to the lower compartments. A nitrocellulose filter (Millipore Corporation, Bedford, Ma, USA) with 5 µm pore size was placed between the upper and lower compartment. The cell suspensions, 50 µL per well, were added to each upper well. All tests were performed in duplicates. The incubation time was 60 min and the temperature 37 °C. Migration was assayed according to the leading front technique [9].

The cytokine mixture consisted of IL-5 (10<sup>-9</sup>M), IL-8 (10<sup>-8</sup>M) and RANTES (10<sup>-8</sup>M) mixed together in Gey's

buffer. Anti-IL-2 (0.001  $\mu\text{g/mL}$ , according to the recommendations from the manufacturer), was added to the mixture, and the ECA was measured.

#### Adherence assay

The adherence of eosinophil granulocytes to E-selectin and ICAM-1 was measured according to a recently described method [10] utilizing cell lines of hamster fibroblasts that selectively express E-selectin and ICAM-1. As a reference the adhesion to the hamster fibroblasts without any transfected adhesion molecules was also measured, and referred to as basal adhesion.

#### Statistics

Paired non-parametric statistics were used to evaluate statistical differences in chemotactic activity. Correlation coefficients were obtained by Spearman's rank correlation

test.  $P < 0.05$  was considered significant. All calculations were performed on a personal computer by means of the statistical software, Statistica (Statsoft Inc., Tulsa, OK, USA).

#### Results

##### *The effect of anti-IL-2 on EOS migration towards the cytokine mixture*

The ECA of the mixture of IL-5, IL-8 and RANTES was significantly higher compared to buffer ( $P = 0.001$ ). The difference was also significant in the separate groups of normal ( $P = 0.03$ ) and allergic donors ( $P = 0.02$ ). Anti-IL-2 added to the mixture caused a further increase of the migration of EOS ( $P = 0.006$ ). Separate analyses on the two groups showed that the increase was significant in the case of the normal donors ( $P = 0.04$ ). A similar

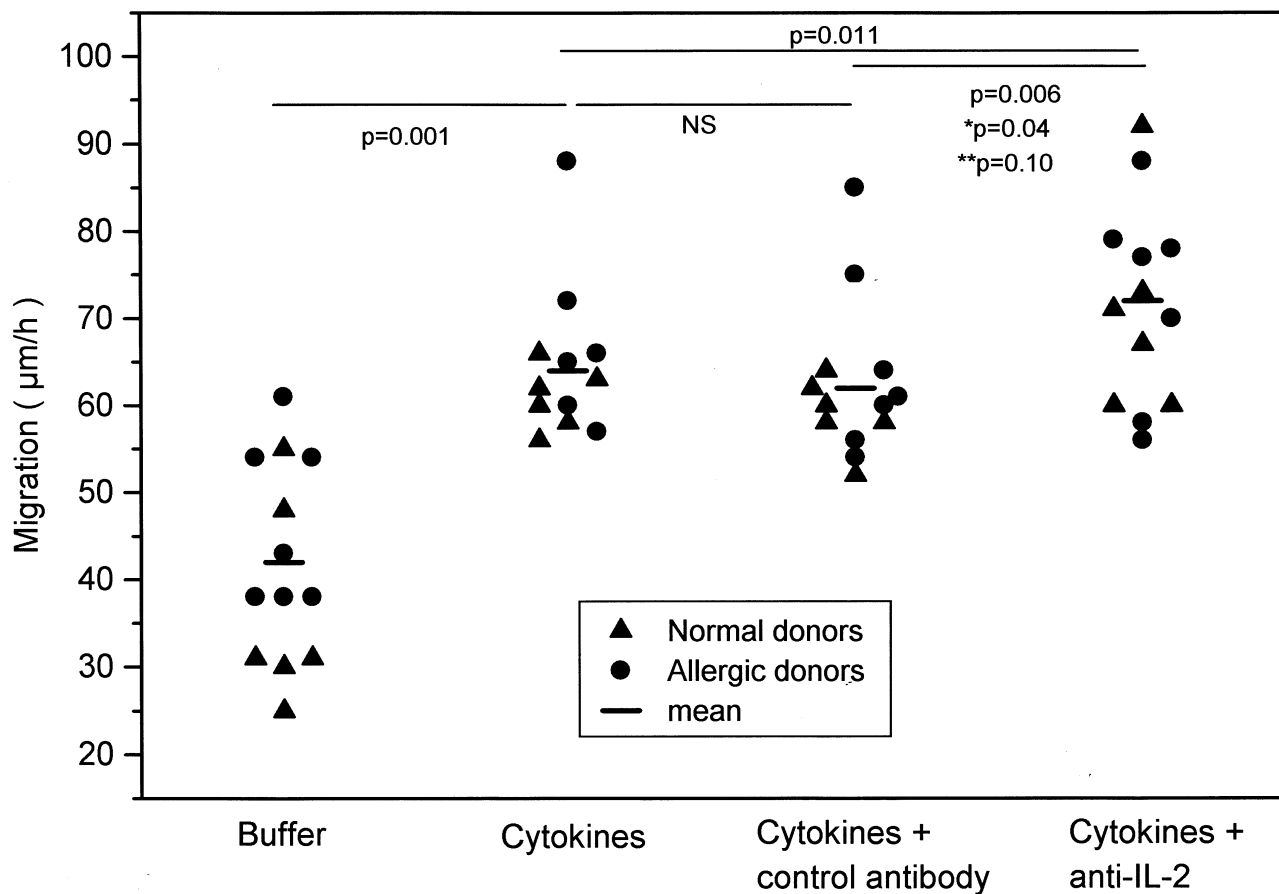


**Fig. 1.** Effect of anti-IL-2 on EOS migration towards a cytokine mixture. EOS migration towards a mixture of IL-5, IL-8 and RANTES ( $10^{-9}$  M,  $10^{-8}$  M and  $10^{-8}$  M, respectively) with or without anti-IL-2 (0.001  $\mu\text{g/mL}$ ). The respective means and the significant differences between the different migratory responses, determined by Wilcoxon matched pairs test, are indicated. The separate statistical evaluations on eosinophils from normal donors (\*) ( $n = 6$ ) and eosinophils from allergic donors (\*\*) ( $n = 6$ ) are indicated.

relationship was found in the allergic group, even though the difference did not reach significance. (Fig. 1.)

#### *EOS migration towards IL-2*

The migration of EOS towards IL-2 at different concentrations was found to be lower than migration towards buffer. The difference was significant at concentrations of  $10^{-11}$ – $10^{-8}$  on EOS from normal donors, and at concentrations of  $10^{-10}$  M and  $10^{-9}$  M for EOS from allergic donors ( $P < 0.05$ ). (Fig. 2 A, B.)

#### *Pre-incubation of EOS with IL-2*

As previously described, RANTES ( $10^{-7}$  mol/L) was chemotactic for EOS [11–13]. Pre-incubation of the EOS with IL-2 ( $10^{-11}$  mol/L) significantly inhibited their migration towards buffer or RANTES ( $P = 0.027$  and  $P = 0.002$ , respectively) (Fig. 3). Pre-incubation of IL-2 with anti-IL-2 completely abolished this inhibition, while mouse IgG1 isotype control had no effect (Fig. 3). The effect of IL-2 on EOS migration was concentration dependent, and the response towards RANTES was significantly inhibited at concentrations of  $10^{-16}$  mol/L and above (Fig. 4.). A negative correlation was found between the chemotactic response of normal EOS to RANTES ( $10^{-7}$  M) and the inhibitory effect of IL-2 on migration towards buffer ( $r = -0.82$ ,  $P = 0.021$ ,  $n = 7$ ). Thus, cells with a high response to RANTES were less inhibited by IL-2.

PAF ( $10^{-8}$  mol/L) and eotaxin ( $10^{-7}$  mol/L) induced migration of EOS from both normal and allergic donors, while IL-8 ( $10^{-7}$  mol/L) only induced migration of EOS

from the latter group (Table 1). Pre-incubation of the cells with IL-2 ( $10^{-11}$  or  $10^{-10}$  mol/L) inhibited both the basal EOS migration ( $P = 0.007$ ) and the migration towards PAF ( $P = 0.007$ ), IL-8 ( $P = 0.010$ ) and eotaxin ( $P = 0.011$ ) (Table 1). The chemotactic response to the optimal concentrations of PAF, IL-8 and eotaxin was, however, not completely inhibited by IL-2. The inhibitory effect of IL-2 on migration towards buffer was more pronounced on EOS from the normal than from the allergic donors ( $P = 0.028$ ) (Fig. 5.). No difference was found between the groups concerning the influence of IL-2 on the migration towards eotaxin ( $10^{-7}$  mol/L) (Fig. 5.), or towards PAF ( $10^{-9}$  and  $10^{-8}$  mol/L).

Priming, *in vitro*, with IL-5 changed the sensitivity of the EOS to IL-2. Pre-incubation of IL-5-primed EOS with IL-2 ( $10^{-11}$  mol/L) did not influence the chemotactic response to RANTES ( $10^{-7}$  mol/L) or buffer. In contrast, pre-incubation of unprimed EOS with IL-2 resulted in inhibition of the migration towards both RANTES and buffer (Table 2).

#### *Effect of IL-2 on EOS adhesion*

The presence of IL-2, at molar concentrations between  $10^{-12}$  and  $10^{-8}$ , during EOS adhesion to E-selectin and ICAM-1 did not induce any alterations in EOS adhesion. Furthermore, IL-2 did not influence the basal adhesion of EOS (results not shown).

#### **Discussion**

The recruitment of eosinophil granulocytes to sites of inflammation is a multistage process that is regulated by cytokines released by different cell types. We investigated

**Table 1.** Effect of pre-incubation of EOS with IL-2 on the migration towards PAF, IL-8 and eotaxin

| Concentration of chemotaxins (mol/L) | Normal donors ( $n = 6$ ) |                          |                          | Allergic donors ( $n = 9$ ) |                          |                          |
|--------------------------------------|---------------------------|--------------------------|--------------------------|-----------------------------|--------------------------|--------------------------|
|                                      | IL-2 (0)                  | IL-2 ( $10^{-11}$ mol/L) | IL-2 ( $10^{-10}$ mol/L) | IL-2 (0)                    | IL-2 ( $10^{-11}$ mol/L) | IL-2 ( $10^{-10}$ mol/L) |
| 0                                    | 57 ± 13                   | 25 ± 5*                  | 24 ± 9*                  | 52 ± 11                     | 34 ± 11*                 | 32 ± 11*                 |
| PAF, $10^{-9}$                       | 66 ± 16                   | 32 ± 17*                 | 28 ± 15*                 | 62 ± 13                     | 32 ± 13*                 | 32 ± 12*                 |
| PAF, $10^{-8}$                       | 80 ± 12 †                 | 44 ± 16*†                | 45 ± 26*†                | 82 ± 20†                    | 48 ± 18*†                | 36 ± 13*                 |
| IL-8, $10^{-8}$                      | ND                        | ND                       | ND                       | 58 ± 12                     | 36 ± 13*                 | 34 ± 13*                 |
| IL-8, $10^{-7}$                      | ND                        | ND                       | ND                       | 67 ± 15†                    | 44 ± 22*†                | 42 ± 17*†                |
| Eotaxin, $10^{-8}$                   | 47 ± 12                   | 22 ± 6*                  | 19 ± 4*                  | 62 ± 21                     | 44 ± 29*                 | 41 ± 27*                 |
| Eotaxin, $10^{-7}$                   | 97 ± 13 †                 | 61 ± 25*†                | 48 ± 22*†                | 106 ± 9†                    | 66 ± 28*†                | 66 ± 29*†                |

Eosinophil migration towards PAF, IL-8 and eotaxin expressed as  $\mu\text{m/h}$  (mean ± SD). Effect of pre-incubation of EOS with IL-2 ( $10^{-11}$  or  $10^{-10}$  mol/L). The statistical differences between EOS with and without IL-2 are indicated by \* ( $P < 0.05$ ) (Wilcoxon matched pairs test), and that between migration towards buffer and chemoattractants by † ( $P < 0.05$ ) (Wilcoxon matched pairs test).

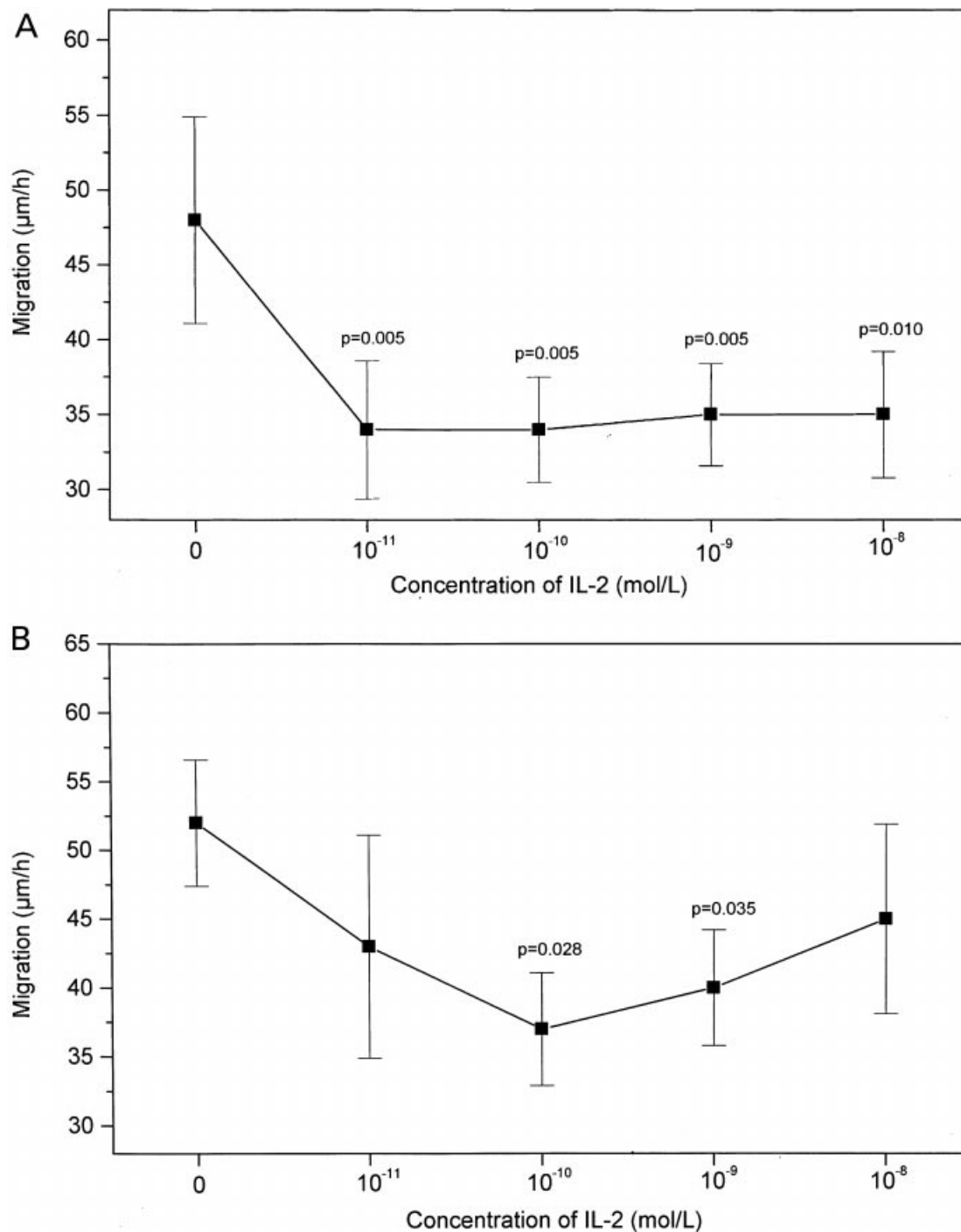


**Fig. 2.** EOS migration towards IL-2 expressed as  $\mu\text{m/h}$  (mean  $\pm$  SEM). The statistical differences between migration to IL-2 and buffer are indicated (Wilcoxon matched pairs test). A: Eosinophils from normal donors ( $n = 10$ ). B: Eosinophils from allergic donors ( $n = 6$ ).

the effect of recombinant human IL-2 on the migration of eosinophils and our results suggest that IL-2 may act as an inhibitory agent on EOS migration. Furthermore, eosinophils from allergic patients were less sensitive to IL-2,

probably due to exposure to IL-5 in the circulation. In addition, eosinophils from allergic donors may also produce less IL-2.

In previous studies, we found that antibodies to IL-5,

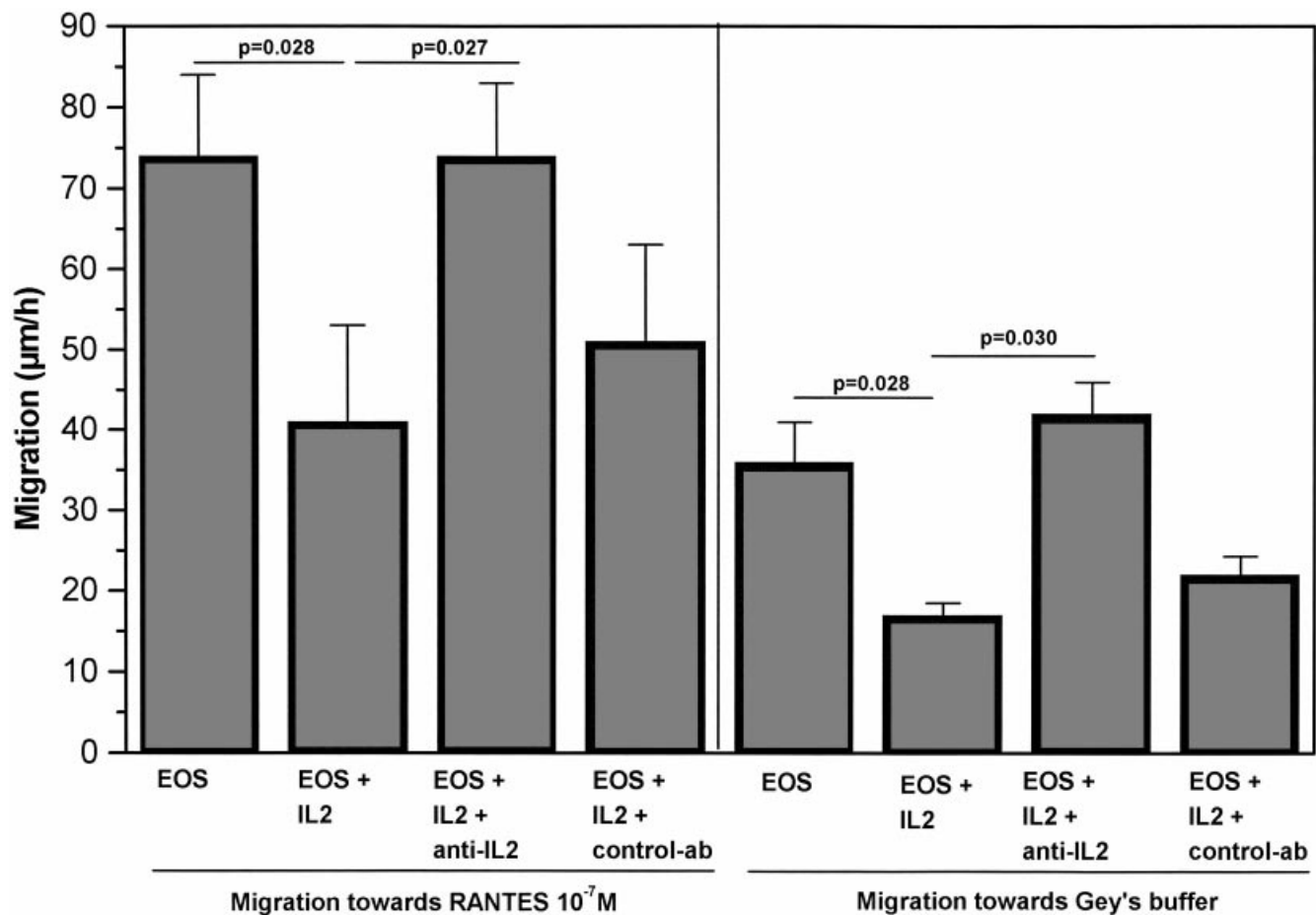


**Fig. 3.** Effect of pre-incubation of EOS with IL-2 with or without anti-IL-2 or mouse IgG<sub>1</sub> isotype control antibody on migration towards RANTES or buffer. EOS migration towards RANTES ( $10^{-7}$  mol/L) expressed as  $\mu\text{m/h}$  (mean  $\pm$  SEM). Effect of pre-incubation of the EOS with IL-2 ( $10^{-11}$  mol/L) and anti-IL-2 (0.001  $\mu\text{g/mL}$ ) or mouse IgG<sub>1</sub> isotype control antibody (0.001  $\mu\text{g/mL}$ ). Eosinophils from normal donors ( $n = 6$ ). Significant differences, determined by Wilcoxon matched pairs test, are indicated.

IL-8 and RANTES inhibited the eosinophil chemotactic activity in BAL-fluid obtained from pollen-allergic patients during birch-pollen season [13,14]. Anti-IL-2 did not influence the chemotactic activity of the BAL fluid when EOS from allergic donors were used. In contrast, a slight enhancement of the migration of normal EOS towards the BAL-fluid treated with anti-IL2 was observed. In an

attempt to reconstruct the situation in the BAL-fluid, recombinant IL-5, IL-8 and RANTES were mixed together, each one in a sub-optimal concentration. The ECA of the mixture was of the same magnitude as that of the BAL-fluid (on average 160% of buffer migration).

In correspondence with the study on BAL fluid, neutralizing antibodies to different cytokines, among others

**Table 2** Effect of priming eosinophils with IL-5 on the influence of IL-2 on eosinophil migratory responses. Median and range of three experiments with similar patterns. The results are expressed as  $\mu\text{m/h}$ .

| Eos pre-incubated with: | IL-5-primed eosinophils          |                     | Unprimed eosinophils             |                     |
|-------------------------|----------------------------------|---------------------|----------------------------------|---------------------|
|                         | Migration to RANTES, $10^{-7}$ M | Migration to buffer | Migration to RANTES, $10^{-7}$ M | Migration to buffer |
| Gey's buffer            | 91 (86–92)                       | 74 (67–86)          | 94 (82–94)                       | 32 (22–88)          |
| IL-2, $10^{-11}$ M      | 96 (94–100)                      | 74 (64–90)          | 78 (72–84)                       | 20 (13–29)          |

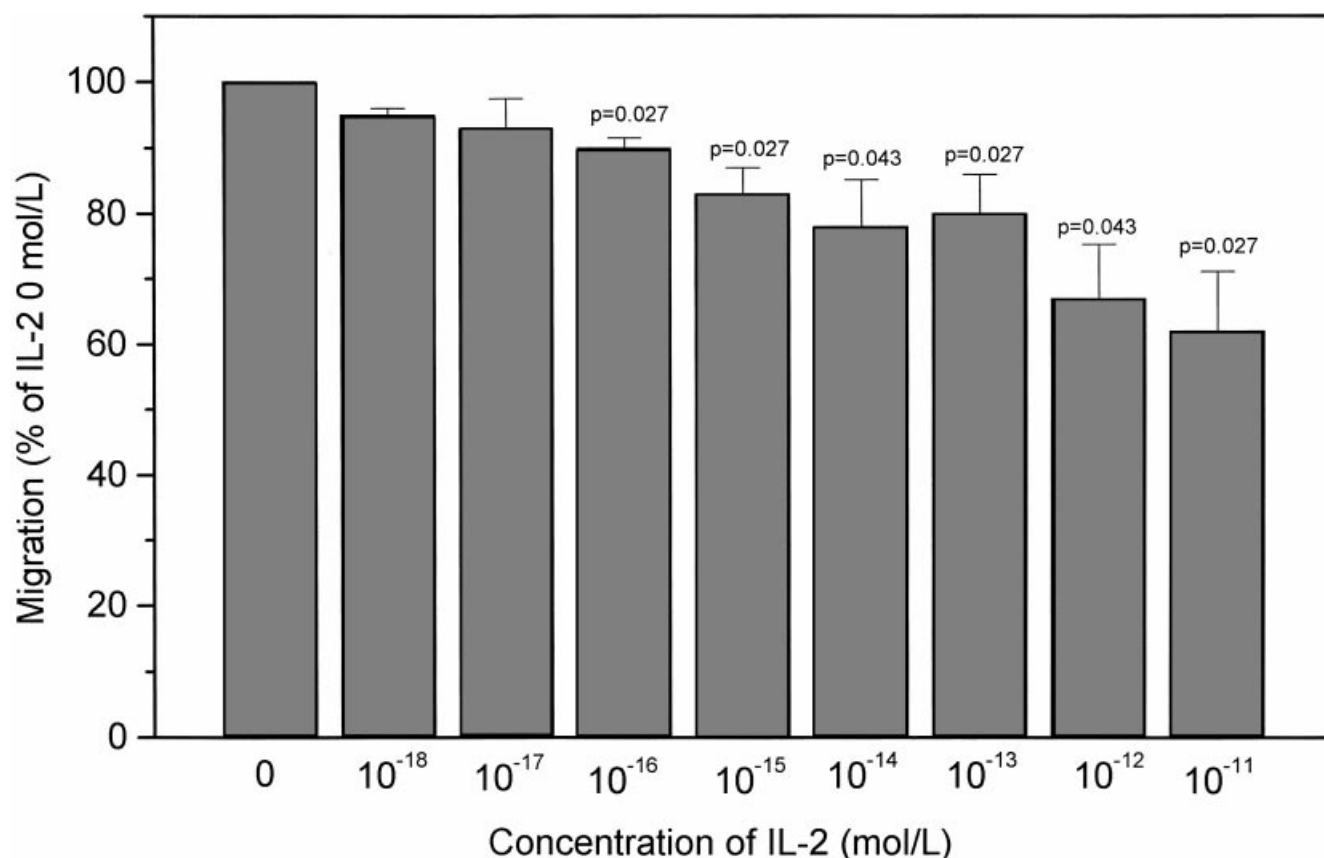


**Fig. 4.** Relationship between concentration of IL-2 and effect on EOS migration towards RANTES. EOS pre-incubated with IL-2 ( $10^{-18}$ – $10^{-11}$  mol/L) before migration towards RANTES ( $10^{-7}$  mol/L). The results are expressed as percentage of the migratory response towards RANTES in the absence of IL-2. The EOS were obtained from normal donors. Significant differences, determined by Wilcoxon matched pairs test, are indicated.

to IL-2, were added to the cytokine mixture, and the ECA was measured. In these experiments, anti-IL-2 enhanced the migration of EOS from normal donors, and had a similar, though not statistically significant, effect on EOS from allergic donors. The fact that no IL-2 was added to the cytokine mixture indicated that this cytokine was present in the suspension of EOS, exerting its inhibitory functions directly on the cells. Indeed, it has been shown that EOS are able to synthesize and release IL-2, and that the synthesis may be stimulated by cytokines, such as GM-CSF [15–17].

Pre-incubation of EOS with recombinant human (rh) IL-2 inhibited the basal EOS migration towards buffer and the chemotaxis towards RANTES, PAF, eotaxin and IL-8, although the chemoattractants still had some effect on the cells. Serum albumin augments the basal level of EOS migration, and the response varies depending on what the EOS have been exposed to *in vivo* [13]. Pre-incubation of eosinophils from normal individuals with IL-2 inhibited their migration down to the level of random migration in

the absence of albumin. Furthermore, albumin-stimulated migration was inhibited almost to the same degree when the cells migrated towards IL-2. Thus, IL-2 inhibits the albumin-stimulated migration of the EOS rather than their response to chemoattractants. The finding that IL-2 is unable to completely block the cytokine-induced chemotaxis may indicate IL-2-independent pathways. A similar effect of rhIL-2 has been shown on CD4<sup>+</sup> and CD8<sup>+</sup> T cells, where pre-incubation of these cells attenuated their migration towards IL-8 and RANTES [18].

In the majority of our experiments we observed that EOS from allergic donors were less sensitive to IL-2 than normal EOS. The present study also showed that priming of EOS with IL-5 *in vitro* made the cells completely insensitive to IL-2. This may explain the lower sensitivity of EOS from allergic donors to IL-2, which previously have been shown to be primed *in vivo* [6,19], presumably by IL-5. Furthermore, we found a relationship between the chemotactic response and the sensitivity to inhibition by IL-2, indicating that the EOS with a high chemotactic response to

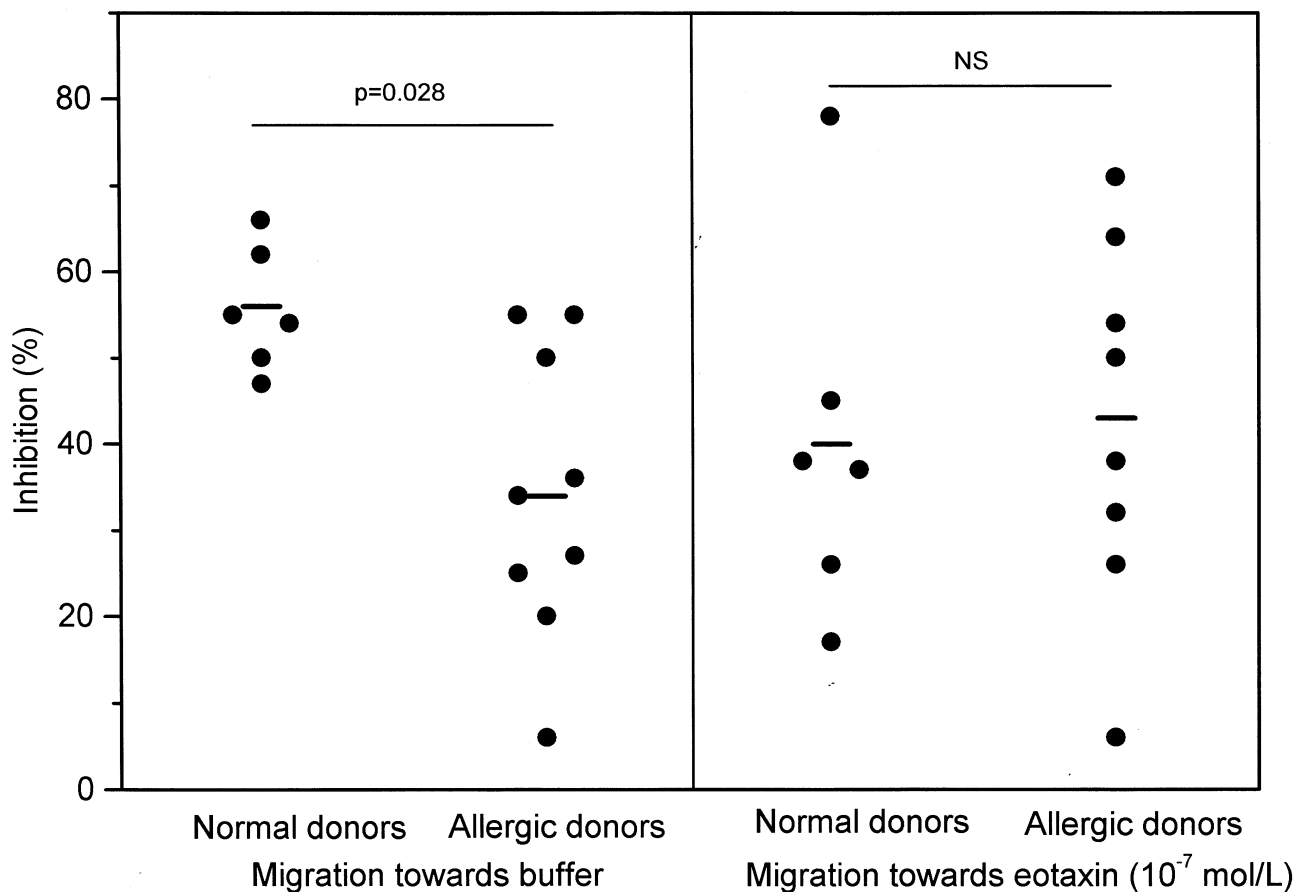


**Fig. 5.** Difference in sensitivity to IL-2-pre-incubation between EOS from the normal and allergic group. The relative (%) inhibition of EOS migration towards buffer (= 100%) or eotaxin ( $10^{-7}$  mol/L) (= 100%) after pre-incubation with IL-2 ( $10^{-11}$  mol/L). The mean values for each group are indicated. The statistical differences between normal and allergic individuals were determined by Mann–Whitney U-test.

RANTES were less sensitive to IL-2. This is in agreement with our previous results showing that exposure of EOS to IL-5 in the circulation leads to an increased ability to migrate towards RANTES [13]. Thus, IL-5 may contribute to EOS accumulation not only by acting as a chemokinetic and chemotactic factor, but also by eliminating the inhibitory effect of IL-2 on EOS migration. In addition to the influence of IL-5, a lower endogenous release of IL-2 of EOS from allergic patients might explain the less pronounced effect of anti-IL-2 on the response of EOS from allergic individuals to the cytokine mixture.

The IL-2 receptor  $\alpha$ -chain (CD25) has been detected on EOS from patients with hypereosinophilic syndrome or normal EOS stimulated with GM-CSF [5,20,21]. No expression of the IL-2R  $\beta$ -chain was found when analysed by flow cytometry. Normal unstimulated EOS demonstrated little or no expression of CD25. In our study, EOS from normal donors were more sensitive to the inhibitory effects of IL-2 than EOS from allergic donors. Differences in the

expression of IL-2-receptors may affect the ability of the EOS to respond to IL-2. We hypothesize that IL-2 normally acts as a regulatory factor on EOS migration through a receptor that becomes downregulated or blocked in allergy giving rise to enhanced EOS migration. IL-2 is mainly produced by T helper cells of the Th<sub>1</sub>-phenotype. In allergy, the predominant T cell is the Th<sub>2</sub>-phenotype, which produces IL-4 and IL-5 but not IL-2 or IFN- $\gamma$  [22]. A lower production of IL-2 by EOS from allergic subjects together with priming by IL-5 would indicate a Th<sub>1</sub> to Th<sub>2</sub> switch also in case of the EOS.

One possible cause of the inhibiting effect of IL-2 on eosinophil migration could be an increased adhesiveness of the EOS, making them stick to the filter, and thereby move more slowly. In our experiments, however, IL-2 did not affect the adhesiveness of the cells, the basal EOS adhesion, nor adhesion to E-selectin or ICAM-1. Consequently, the inhibition of EOS migration by IL-2 could not be explained by an increased adhesiveness of the EOS.

Previous data on the impact of IL-2 on EOS migration are conflicting. One publication, by Rand et al. reported that IL-2 was a potent chemoattractant for EOS [6]. A number of other investigators did not, however, find any direct effect of IL-2 on EOS migration, but showed that it acts by stimulating T cells to produce increased amounts of IL-5 [23–27]. The contradiction between our results and those of Rand et al. could be explained by several different factors; one is the selection of blood donors, and thereby differences in the state of priming of EOS. Their study included two donors with hypereosinophilic syndrome (HES) and six normal donors, however, with slightly elevated EOS counts. The purity of the EOS may also influence the results. Rand et al. principally used EOS with a purity of 70–75%, while 99% pure EOS were used in the present study. Another difference between our studies is the method of measuring EOS migration in the filter, which may not be altogether comparable.

Whether the main action of IL-2 on EOS functions is direct, or indirect through other cells and mediators, still has to be established. IL-2 may contribute to eosinophilia by stimulating the production of IL-5 by T cells. However, we conclude that the direct effect of IL-2 on EOS migration is inhibitory. It may act as an autocrine regulator of migration in normal situations, while in allergy, this inhibitory effect may be downregulated, allowing an increased migration of EOS towards chemotactic factors.

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