Interleukin-13 but Not Interleukin-4 Prolongs Eosinophil Survival and Induces Eosinophil Chemotaxis

Shiro HORIE, Yoshio OKUBO, Mahboob HOSSAIN, Etsuro SATO, Hiroshi NOMURA, Sekiya KOYAMA, Jun-ichi SUZUKI, Mitsuaki ISOBÉ and Morie SEKIGUCHI

The effects of recombinant human (rh) interleukin (IL)-4 or rhIL-13 on survival, and chemotactic activity of human eosinophils were examined. Only rhIL-13 prolonged eosinophil survival in a dose-dependent manner above 3 ng/ml. Eosinophil survival induced by rhIL-13 was inhibited by monoclonal antibodies (mAbs) against IL-3 (p<0.01) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (p<0.05), suggesting that rhIL-13 induced IL-3 and GM-CSF production from eosinophils and an autocrine mechanism is responsible for the eosinophil survival. The effects of rhIL-13 on eosinophil chemotactic activity were also examined. rhIL-13 showed chemotactic activity for eosinophils in a dose-dependent manner. Checkerboard analysis revealed that eosinophil migration was dependent on the concentration gradient, confirming that rhIL-13 is a chemotactic factor. rhIL-4 showed no effects. IL-13 may play an important role in the survival and recruitment of eosinophils in allergic diseases.

Key words: cytokine, eosinophil recruitment

Introduction

Eosinophils play important roles as effector cells in allergic diseases including bronchial asthma (1). To carry out their effector function at the site of inflammation, a series of events including eosinophil activation, adhesion and transmigration through endothelial cells, chemotaxis, and interaction with target cells such as bronchial epithelial cells are subtly regulated by various cytokines. The cytokines interleukin (IL)-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF) participate in eosinophilopoesis and differentiation (2–4), prolonged eosinophil survival (5), and enhancement of adhesion molecule CD11a, CD11b, and CD18 expression (6). In addition, these cytokines enhance (7) or directly induce (8) eosinophil degranulation or superoxide production.

In addition, IL-4 has been shown to be involved in B cell proliferation and isotype switching (9), and in IgE synthesis (10). These cytokines have been referred to as Th2-like cytokines and have been reported in bronchoalveolar lavage fluid in patients with bronchial asthma (11). A positive correlation was observed between Th2-like cytokines and the infiltration of EG2+ eosinophils and activated (CD25+) T lymphocytes (12), suggesting that these molecules are responsible for the recruitment of eosinophils. Furthermore, IL-4 is also involved in eosinophil recruitment by inducing vascular cell adhesion molecule-1 (VCAM-1) expression on endothelial cells (13). However, the effects of IL-4 on the biological activities and/or effector functions of eosinophils have yet to be clarified, although some investigators have observed HLA-DR expression on human eosinophils (14), or reduction of arylsulfatase and β-glucuronidase secretion induced by IgG-coated Sepharose beads (15) when human eosinophils were exposed to IL-4.

Recently, a new T cell-derived cytokine IL-13, is reported to share approximately 30% structural homology with IL-4 and shows effects on B cells and monocytes was reported (16, 17). However, the biological functions of IL-13 on human eosinophils are uncertain. To determine whether recombinant human (rh)IL-13, as well as rhIL-4, exerts specific biological effects on normal human eosinophils, eosinophil survival, degranulation, and chemotactic activity were examined.

Materials and Methods

Reagents

The reagents employed in this study were as follows: rhIL-13 (R&D Systems, Minneapolis, MN); rhIL-4 (Genzyme Corp., Minneapolis, MN); human IL-3, IL-4, IL-5, GM-CSF (Genzyme Corp.); anti-IL-3 and anti-GM-CSF mAbs (Genzyme Corp.); rhIL-1β, rhIL-8, rhIL-12, sphingosine-1-phosphate (S1P, Sigma Chemical Co., St. Louis, MO); [3H]-thymidine (Amersham Life Science, Arlington Heights, IL); 3′-0-(4-chloromethyl)fluorescein diacetate (CMFDA, Molecular Probes, Inc., Eugene, OR); and rhIL-4 (Genzyme Corp., Minneapolis, MN).
Cambridge, MA); rhIL-5 (Genzyme); rhGM-CSF (Genzyme); and platelet-activating factor (PAF) from BIOMOL Research Laboratories (Plymouth Meeting, PA). These cytokines were diluted in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) to 10 μg/ml and stored at -80°C. PAF was dissolved in absolute ethanol to 40 mM and stored at -20°C and then diluted in reaction medium to the desired concentrations immediately before the experiments. Purified human IgG was purchased from Cappel-Organon Teknika Co. (Durham, NC) and dissolved in distilled water at a concentration of 1 mg/ml.

Eosinophil isolation

Eosinophil isolation was performed essentially by the method described by Hansel et al with minor modifications using a magnetic cell separation system (Becton Dickinson, Lincoln Park, NJ) (18). Briefly, heparinized venous blood was obtained from normal volunteers and diluted with PIPES buffer (25 mM PIPES/50 mM NaCl/5 mM KCl/25 mM NaOH/5.4 mM glucose, pH 7.4) at a ratio of 1:1. Diluted blood was layered over isotonic Percoll solution (density 1.082 g/ml) (Sigma Chemical Co., St. Louis, MO) and centrifuged at 1,000 × g for 30 minutes at 4°C. The supernatants and mononuclear cells at the interface were carefully removed, and erythrocytes in the sediment were lysed by two cycles of hypotonic lysis in distilled water. Isolated granulocytes were washed twice with PIPES buffer supplemented with 1% inactivated fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT), and an approximately equal volume of anti-CD16 monoclonal Ab (mAb) conjugated with magnetic particles (Miltenyi Biotec GmbH, Sunnyvale, CA) was added to the cell pellet. After 60 minutes of incubation on ice, 5 ml of PIPES containing 1% FBS was added to the cell/mAb mixture. Resuspended cells were loaded onto the separation column positioned in the magnetic cell separation system’s strong magnetic field. Cells were eluted three times with 5 ml PIPES buffer containing 1% FBS. The purity of eosinophils as counted using Randolph’s stain was >98%. Purified eosinophils were washed twice with PIPES buffer containing 1% FBS and suspended in reaction medium.

Eosinophil survival assay

Purified eosinophils were suspended in Hybri-Care medium (American Type Culture Collection, Rockville, MD), supplemented with 50 μg/ml gentamicin (Sigma), 0.1% Human serum albumin (HSA) (Sigma, A3782), 1.5 g/l NaHCO3, and 10% FBS, (Hybri-Care complete medium) at a cell concentration of 0.2×106/ml. Fifty μl aliquots of eosinophil suspension were incubated with 50 μl of cytokine solution diluted in the same medium for 4 days at 37°C and 5% CO2 in 96-well half area flat-bottomed tissue culture plates (#3696, Coaster, Cambridge, MA). After incubation, 50 μl of supernatant was carefully removed from each well and 10 μl of fluorescent diacetate in PBS at a 1:24 ratio was added onto the cell suspension. After 15 minutes incubation on ice, 10 μl eosinophil suspensions were placed on a Neubauer hemocytometer. The number of viable cells, showing green fluorescence on nine large squares of the hemocytometer was counted using an epifluorescence microscope. The total number of eosinophils on the same area was counted under a light microscopic condition. Eosinophil viability was determined as percentage of viable eosinophils divided by total eosinophil number. In some experiments, eosinophils were incubated with rhIL-13 in the presence of neutralizing mAb against IL-3, IL-5, or GM-CSF (donated by Dr. Gerald J. Gleich, Mayo Clinic, Rochester, MN). Ten μg of anti-IL-3, anti-IL-5, and anti-GM-CSF mAbs completely neutralized 200 pg of IL-3, 300 pg of IL-5, and 20 pg of GM-CSF, respectively (data not shown). Each antibody is specific for its respective cytokine and with no cross-reactivity with other cytokines (data not shown). The purity of rhIL-13 was 97%, and at the maximum concentration used in this study contamination of endotoxin was 42.6±0.6 pg/ml. When eosinophils were cultured in the presence of LPS (serotype: 055: B5 from E.coli, Sigma), even 10 ng/ml LPS did not induce significant prolongation of eosinophil survival.

DNA fragmentation assay

DNA fragmentation assay was performed using a Cell Death Detection Kit ELISA (Boehringer Manheim, Germany). One ml of purified eosinophil suspension in Hybri-Care complete medium at a concentration of 0.1×106 cells/ml was cultured in the presence or absence of cytokines at 37°C and 5% CO2 for 2 days. After incubation, cell suspensions were centrifuged at 500×g for 5 minutes, and supernatants were carefully removed. Cell pellets were lysed with 500 μl of the incubation buffer provided by manufacturer for 30 minutes at 4°C. The lysates were then centrifuged at 20,000×g for 10 minutes, and 400 μl of supernatants containing the fragmented DNA were carefully collected and stored at -20°C until assayed. Fragmented DNA was determined by the sandwich ELISA method from absorbance at 405 nm with a reference wavelength of 492 nm.

Measurement of eosinophil chemotactic activity

To examine the effect of rhIL-13 or human rhIL-4 on eosinophil chemotactic activity, purified eosinophils were suspended in Hanks’ balanced salt solution (HBSS) with 2% BSA at a concentration of 2×106 cells/ml. The chemotaxis assay was performed in 48-well microchemotaxis chambers (Neuroprobe, Cabin John, MD) as described elsewhere (19). The bottom wells of the chambers were filled with 25 μl of HBSS containing various concentrations of rhIL-13 or rhIL-4, and then they were covered with polycarbonate filters with a pore size of 5 μm (Nucleopore Corp., Pleasanton, CA). The silicon gasket and upper piece of the chamber were applied, and upper wells were filled with 50 μl of eosinophil suspension. The chambers were incubated in humidified air at 37°C for 3 hours. After incubation, the chambers were disassembled, and the filters were removed. The filters were then fixed, stained with Diff-Quik (American Scientific Products, McGraw Park, IL), and mounted on glass slides. Eosinophils which completely migrated through the filter were counted in five random high-power fields (×1,000) per well. Chemotactic activity was expressed as percentage of migrated eosinophils with each stimulus divided by the number
of migrated eosinophils induced by $10^{-7}$M PAF as a positive control. To determine whether the migration of eosinophils was due to movement along a concentration gradient (chemotaxis) or stimulation of the eosinophils to migrate randomly (chemokinesis), checkerboard analysis was performed. In this experiment, various concentrations of rhIL-13 were placed both above the membrane with the eosinophils and below the membrane.

**Eosinophil degranulation as monitored by eosinophil-derived neurotoxin (EDN) assay**

Eosinophil degranulation was induced by immobilized IgG, PAF, or rhGM-CSF and the modulatory effects of rhIL-4 and rhIL-13 were examined. For eosinophil degranulation by immobilized human IgG, 96-well flat-bottomed tissue culture plates (Falcon #3072, Becton-Dickinson, Lincoln Park, NJ) were coated with 5 μg of purified human IgG which is the optimal dose for induction of eosinophil degranulation at 37°C overnight. The plates were then washed with PBS three times and wells were blocked by 50 μl of 2.5% HSA dissolved in PBS (pH 7.4) at 37°C for 2 hours. For eosinophil degranulation induced by soluble stimuli, PAF and rhGM-CSF, 96-well flat-bottomed tissue culture plates were coated with 50 μl of 2.5% HSA at 37°C for 2 hours to avoid nonspecific degranulation (11). The purity of the HSA was certified by manufacturer, and the product was globulin-free. After blocking with HSA, wells were washed three times with PBS and stored at 4°C until use. Freshly isolated eosinophils were washed twice with RPMI 1,640 medium (RPMI 1,640, 1.5 g/l NaHCO3 and 10 mM HEPES (Sigma), pH 7.2), and resuspended in the same medium at 5x10^5 cells/ml. The soluble stimuli were also diluted in the same medium. Subsequently, 100 μl aliquots of cell suspension were added onto the HSA-coated tissue culture plates, and incubated with 50 μl aliquots of rhIL-13 or rhIL-4 for 10 minutes before adding stimuli or medium alone. Eosinophil degranulation was initiated by adding 50 μl of rhGM-CSF or PAF at final concentrations of 10 ng/ml and 1 μM, respectively. After incubation at 37°C in a 5% CO2 atmosphere for 4 hours, the supernatants from wells were collected carefully and kept at −20°C until assayed. All experiments were carried out in duplicate.

To monitor eosinophil degranulation, concentrations of eosinophil protein X (EPX) usually referred to as EDN in the sample supernatants were measured by radioimmunoassay using an EPX kit (Kabi Pharmacia Diagnostics, Tokyo, Japan). To measure the total content of EPX, eosinophils were lysed with 0.5% Nonidet P-40 (NP-40). All assays were carried out in duplicate.

**Statistical analysis**

Statistical significance of the differences between various treatment groups was assessed with paired Student’s t-test.

**Results and Discussion**

**rhIL-13 prolongs eosinophil survival**

We examined the effect of rhIL-13 on eosinophil survival. In the absence of cytokines such as IL-3, IL-5, or GM-CSF, most eosinophils die within 96 hours (5). As shown in Fig. 1, the survival of the eosinophils cultured with rhIL-13 for 4 days was significantly prolonged at 3 ng/ml and was enhanced in a dose-dependent manner; 50% survival was achieved at 100 ng/ml, and more than 80% of eosinophils were viable at a concentration of 500 ng/ml. Although rhIL-13 showed prolonged eosinophil survival at high concentrations, rhIL-4 did not prolong eosinophil survival. Receptors of IL-13 and IL-4 are heterodimeric complexes. IL-13 and IL-4 share a common receptor component and that is now considered to be IL-4α chain which induces activation of STAT 6 (20). One possible explanation for the failure of rhIL-4 to prolong eosinophil survival is that the number or affinity of IL-4 receptors on eosinophils may be different from those of IL-13. In fact, prolonged eosinophil survival induced by rhIL-13 was not inhibited by pretreatment with rhIL-4, suggesting that rhIL-13 binding to eosinophils may not be inhibited competitively by rhIL-4 (Fig. 2).

**rhIL-13 prolongs eosinophil survival by abrogating apoptosis**

IL-3, IL-5, and GM-CSF have been shown to prolong eosinophil survival by preventing apoptosis which is characterized by DNA fragmentation (21). Based on the hypothesis that
Figure 2. Effects of preincubation with rhIL-4 on eosinophil survival induced by rhIL-13. Eosinophils were incubated with various concentrations of rhIL-4 for 2 hours before addition of rhIL-13 (250 ng/ml). The results (mean±range) represent one of 2 experiments performed in duplicate.

Figure 3. a) Kinetics of eosinophil survival cultured with rhIL-4 and rhIL-13. Eosinophils were incubated with rhIL-4 (250 ng/ml), rhIL-13 (250 ng/ml), or rhIL-5 (10 pg/ml) up to 4 days. The results (mean±range) represent one of 2 experiments performed in duplicate. b) Effects of rhIL-4 and rhIL-13 on DNA fragmentation in eosinophils. Eosinophils (1×10^5) were cultured with rhIL-4 or rhIL-13 for 2 days and DNA fragmentation was determined by ELISA. The results are presented as mean±SEM from 4 experiments performed in duplicate. Significant differences are **p<0.01 from values obtained with medium alone.

the same mechanisms may be involved in the rhIL-13-induced eosinophil survival, DNA fragmentation assay was performed. As shown in Fig. 3a, eosinophils cultured with medium alone, or rhIL-4 showed less viability on day 2 compared with the viability of human rhIL-13. Therefore, DNA fragmentation assay was carried out on day 2. The absorbance of the lysates of freshly isolated eosinophils was less than 0.01. When eosinophils were cultured for 2 days with rhIL-4, rhIL-13, or medium alone, eosinophils cultured with rhIL-13 at concentrations of 100 ng/ml and 250 ng/ml showed significantly lower absorbance values, suggesting less fragmentation of DNA (Fig. 3b). On the other hand, rhIL-4 did not inhibit DNA fragmentation. These results show that rhIL-13-induced eosinophil survival is dependent on the inhibition of apoptosis.

Eosinophil survival induced by rhIL-13 was inhibited by mAb against IL-3 and GM-CSF

For prolongation of eosinophil survival, extremely high doses of rhIL-13 were required. The effect of 100 ng/ml rhIL-13 was almost equivalent to that of 1 pg/ml rhGM-CSF, or 10 pg/ml rhIL-5. This leads to the question of whether rhIL-13 prolongs eosinophil survival directly or through induction of release of other cytokines from eosinophils. It is well known that eosinophils are capable of producing cytokines such as IL-3, GM-CSF, and transforming growth factor-β, in the peripheral blood obtained from patients with eosinophilia (22). Furthermore, calcium ionophores and adhesion of eosinophils to the extracellular matrix protein fibronectin also induce IL-3 and GM-CSF production by eosinophils (23, 24). Production of these cytokines particularly IL-3 and GM-CSF, by eosinophils can prolong their survival by autocrine or paracrine mechanisms.

To analyze the mechanisms of rhIL-13-induced eosinophil survival, concentrations of IL-3, IL-5, and GM-CSF in the culture supernatant were measured by ELISA. As these cytokines were below the detectable range (data not shown), a more
sensitive bioassay using specific mAb was carried out. Eosinophils were incubated with rhIL-13 (250 ng/ml) in the presence of 10 μg of mAb against IL-3, IL-5, or GM-CSF for 4 days (Fig. 4). rhIL-13-induced eosinophil survival was significantly inhibited by anti-IL-3 (p<0.01) and anti-GM-CSF mAbs (p<0.05), while the anti-IL-5 mAb showed no effect. Combination of anti-IL-3 and anti-GM-CSF mAbs showed further inhibition of eosinophil survival compared with anti-IL3 mAb alone or anti-GM-CSF alone. These results suggest that rhIL-13 prolongs eosinophil survival through an autocrine mechanism. Furthermore, IL-3 production from eosinophils may play the main role in rhIL-13-evoked eosinophil survival, and GM-CSF production may also be partially involved. Combination of the three mAbs was still insufficient to inhibit eosinophil survival to the baseline which was given by medium alone. These results imply the involvement of other eosinophil survival-enhancing factors. The present results, however clearly showed that IL-3 and GM-CSF production and/or release from eosinophils induced by rhIL-13 are responsible for prolonged eosinophil survival.

**rhIL-13 is a novel chemotactic factor for eosinophils**

The effect of rhIL-13 on eosinophil chemotactic activity was examined. As shown in Fig. 5, rhIL-13 induced obvious chemotactic activity when it was added to the lower compartment of the chambers at a concentration of 30 ng/ml; this activity

![Figure 4. Blocking of rhIL-13-induced eosinophil survival by mAbs against cytokines. Purified eosinophils were cultured with rhIL-13 (250 ng/ml) in the presence of mAbs against IL-3, IL-5, GM-CSF, or various combinations of these mAbs. The results are presented as mean±SEM from three experiments performed in duplicate. Significant differences are **p<0.01 and *p<0.05 from values obtained without mAb.](image)

![Figure 5. Eosinophil chemotactic activity of rhIL-4 and rhIL-13. Purified eosinophils were incubated in the upper compartment of chemotactic chambers with various concentrations of rhIL-4 (open circle) or rhIL-13 (closed circle) in the lower compartment for 3 hours. The results are presented as mean±SEM from five experiments performed in duplicate. rhIL-13 revealed significant eosinophil chemotactic activity (**p<0.01) in comparison with medium alone.](image)

<table>
<thead>
<tr>
<th>Concentration of human rhIL-13 (Upper Chamber) (ng/ml)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>125</th>
<th>250</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.71±1.83</td>
<td>21.54±2.04</td>
<td>20.43±1.45</td>
<td>16.44±2.33</td>
<td>13.39±0.62</td>
<td>19.00±0.55</td>
</tr>
<tr>
<td>30</td>
<td>34.01±5.69</td>
<td>21.91±2.66</td>
<td>21.12±2.79</td>
<td>17.71±0.98</td>
<td>13.98±2.35</td>
<td>15.33±0.51</td>
</tr>
<tr>
<td>60</td>
<td>45.25±3.83</td>
<td>33.35±3.02</td>
<td>24.33±2.36</td>
<td>20.72±1.75</td>
<td>11.83±1.83</td>
<td>15.99±2.39</td>
</tr>
<tr>
<td>125</td>
<td>45.35±2.99</td>
<td>43.94±4.52</td>
<td>40.34±4.87</td>
<td>23.91±2.27</td>
<td>11.54±0.95</td>
<td>21.40±0.62</td>
</tr>
<tr>
<td>250</td>
<td>53.11±4.93</td>
<td>47.93±2.68</td>
<td>52.04±3.01</td>
<td>40.72±5.80</td>
<td>18.84±0.07</td>
<td>17.89±1.03</td>
</tr>
<tr>
<td>500</td>
<td>63.40±3.76</td>
<td>59.80±2.30</td>
<td>58.94±1.96</td>
<td>49.32±11.73</td>
<td>40.42±10.20</td>
<td>21.60±1.42</td>
</tr>
</tbody>
</table>

Table 1. Checkerboard Assay of rhIL-13 on Eosinophils. Values are Mean±SEM from Three Experiments Performed in Duplicate and Shown as % Chemotactic Activity of PAF-Induced (100 nM) Eosinophil Chemotaxis
increased in a dose-dependent manner and reached a plateau at 125 ng/ml. On the other hand, rhIL-4 did not induce chemotaxis of eosinophils. Checkerboard analysis revealed that rhIL-13 enhanced eosinophil migration along a concentration gradient, suggesting that rhIL-13 is a chemotactic factor for eosinophils (Table 1). Thus, IL-13 is clearly a new cytokine with chemotaxis inducing activity for eosinophils similar to PAF, leukotriene B4, C5a, IL-5, and RANTES.

**Effect of rhIL-13 on eosinophil degranulation**

Degranulation is one of the most important eosinophil effector functions. Our previous study showed that rhGM-CSF, rhIL-5, and PAF induce eosinophil degranulation by themselves (8). We examined the effects of rhIL-13 on the eosinophil degranulation induced by rhGM-CSF, PAF, and immobilized IgG. As shown in Fig. 6, neither rhIL-13 nor rhIL-4 affected eosinophil degranulation in response to these stimuli.

Immunohistochemically, infiltration of CD4+ T cells and eosinophils is characteristic of allergic diseases. Analysis of the cytokines in nasal mucosa after local allergen provocation or in the bronchoalveolar lavage fluid from patients with bronchial asthma indicated predominant Th2-like cytokines such as IL-5, IL-4, IL-3, and GM-CSF at both protein and mRNA expression levels (11, 25). The late phase reaction of bronchial asthma is characterized by the effector function of activated eosinophils, and IL-5 is now widely accepted to play major roles in the infiltration and activation of eosinophils (26). Furthermore, Th2 type cytokines are also highly involved in the immediate allergic response through IL-4-induced IgE synthesis by B cells (10). IL-13 has also been reported to induce IgE synthesis by B cells and to stimulate B cell proliferation (16).

However, the roles of IL-13 and IL-4 in the biological functions of eosinophils are not clear. Previous studies showed that IL-4 induces HLA-DR expression (14), and inhibits the Ig-induced cytotoxicity of eosinophils (15). The results of the present study clearly showed that rhIL-13 but not rhIL-4 prolonged eosinophil survival and induced eosinophil chemotaxis.

In the last decade, the mechanisms of eosinophil recruitment into the sites of inflammation have been investigated. Various adhesion molecules including β2 integrins (CD11b/CD18 or CD11a/CD18) and β1 integrin (CD49d/CD29; VLA-4 or CD49f/CD29; VLA-6) on the eosinophils are involved in eosinophil chemotaxis inducing activity for eosinophils similar to PAF, leukotriene B4, C5a, IL-5, and RANTES.

**Figure 6. Effects of rhIL-4 and rhIL-13 on eosinophil degranulation.** Eosinophil degranulation was induced by immobilized IgG, PAF, or rhGM-CSF in the presence of various concentrations of rhIL-4 or rhIL-13. The results (mean ± range) represent one of three experiments performed in duplicate.
participate in the activation and recruitment of eosinophils in cooperation with other cytokines. In such conditions, much smaller amount of IL-13 may act effectively. For example, it has been reported that IL-4 did not show chemotactic activity for normal human eosinophils; however, it is a potent chemoattractant for eosinophils from patients with atopic dermatitis (30).

In the present study, it was shown that rhIL-13, but not rhIL-4 prolonged survival of normal human eosinophils and induced eosinophil chemotaxis. Furthermore, it was suggested that high doses of rhIL-13 induce the production of IL-3 and GM-CSF by eosinophils.

Acknowledgements: We thank Dr. Gerald J. Gleich of Mayo Clinic (Rochester, MN, USA) for providing monoclonal antibodies against human IL-3, IL-5, and GM-CSF.

References