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Decreased Expression of Membrane IL-5 Receptor α on Human Eosinophils: I. Loss of Membrane IL-5 Receptor α on Airway Eosinophils and Increased Soluble IL-5 Receptor α in the Airway After Allergen Challenge

Lin Ying Liu,* Julie B. Sedgwick,* Mary Ellen Bates,‡ Rose F. Vrtis,* James E. Gern,§ Hirohita Kita,⁰ Nizar N. Jarjour,¶ William W. Busse,* and Elizabeth A. B. Kelly²†

IL-5 is a key cytokine for eosinophil maturation, recruitment, activation, and possibly the development of inflammation in asthma. High concentrations of IL-5 are present in the airway after Ag challenge, but the responsiveness of airway eosinophils to IL-5 is not well characterized. The objectives of this study were to establish, following airway Ag challenge: 1) the expression of membrane (m)IL-5Rα on bronchoalveolar lavage (BAL) eosinophils; 2) the responsiveness of these cells to exogenous IL-5; and 3) the presence of soluble (s)IL-5Rα in BAL fluid. To accomplish these goals, blood and BAL eosinophils were obtained from atopic subjects 48 h after segmental bronchoprovocation with Ag. There was a striking reduction in mIL-5Rα on airway eosinophils compared with circulating cells. Furthermore, sIL-5Rα concentrations were elevated in BAL fluid, but steady state levels of sIL-5Rα mRNA were not increased in BAL compared with blood eosinophils. Finally, BAL eosinophils were refractory to IL-5 for ex vivo degranulation, suggesting that the reduction in mIL-5Rα on BAL eosinophils may regulate IL-5-mediated eosinophil functions. Together, the loss of mIL-5Rα, the presence of sIL-5Rα, and the blunted functional response (degranulation) of eosinophils to IL-5 suggest that when eosinophils are recruited to the airway, regulation of their functions becomes IL-5 independent. These observations provide a potential explanation for the inability of anti-IL-5 therapy to suppress airway hyperresponsiveness to inhaled Ag, despite a reduction in eosinophil recruitment. The Journal of Immunology, 2002, 169: 6452–6458.

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3 Abbreviations used in this paper: BAL, bronchoalveolar lavage; βc, βc chain; cPCR, competitive PCR; EDN, eosinophil-derived neurotoxin; ERK, extracellular signal-regulated kinase; m, membrane form; NCS, newborn calf serum; r, Spearman's correlation coefficient; s, soluble form; SBP, segmental bronchoprovocation.

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functions (17). In vitro studies have demonstrated that sIL-5Rα prevents the association of IL-5 with mIL-5Rα (18), and thus acts as an antagonist to inhibit IL-5-mediated signal transduction (18), survival (18), and maturation/differentiation of eosinophils (19). In asthma, mIL-5Rα and sIL-5R mRNA-positive cells are present in the bronchial mucosa. Airway obstruction is associated with increased numbers of mIL-5Rα mRNA+ cells and decreased numbers of cells expressing sIL-5Rα mRNA (20). Consequently, it has been suggested that sIL-5Rα may have a protective role in asthma. However, to date, the presence and biological importance of sIL-5Rα in the airway or any human fluids have not been explored.

The following studies were performed to compare mIL-5Rα expression in human airway and circulating eosinophils and to explore the possibility that the IL-5 antagonist, sIL-5Rα, is present in the airway following airway Ag challenge. To accomplish these goals, airway eosinophilia was induced by segmental bronchoprovocation with Ag (SBP-Ag) in atopic subjects. Eosinophil cell surface expression of mIL-5Rα was determined by flow cytometric analysis; steady state levels of soluble and membrane IL-5Rα transcripts were determined by RT-PCR with Southern blot analysis; and the concentration of sIL-5Rα in BAL fluid was evaluated by ELISA.

Materials and Methods

Subjects and bronchoscopic procedures

To determine levels of IL-5 and sIL-5Rα in BAL fluid, 25 atopic subjects were recruited for analysis of membrane expression of IL-5Rα. In addition, in subjects 14–25, BAL eosinophils were obtained for analysis of mIL-5Rα and mGM-CSFRα by flow cytometry. Abs became available for the β-chain (βc) after the completion of the mIL-5Rα studies; therefore, an additional six subjects were recruited for analysis of membrane expression of βc in relation to mIL-5Rα and mGM-CSFRα. Studies were approved by the University of Wisconsin Health Sciences Human Subjects Committee, and informed consent was obtained from all subjects before participation.

Ag dose for SBP was defined as previously described (3). Briefly, consecutively higher concentrations of Ag were inhaled until forced expiratory volume in 1 s fell by 36% from baseline (21) and then the provocative dose of Ag resulting in a 20% drop in lung function was calculated (PD20; Madison Scientific Software, Wexford, PA). SBP and BAL were performed, as previously described (22), with the following modifications. Two different bronchopulmonary segments were identified for SBP-Ag. For each segment, a wedge position was achieved by the fiberoptic bronchoscope and a baseline BAL was performed. Ag was instilled into each segment through the wedged bronchoscope. One segment received a dose equal to 10% of the provocative dose of Ag resulting in a 20% drop in lung function. If this dose was well tolerated, a second segment was challenged with a dose of 20%. After 48 h, bronchoscopy was repeated and BAL was performed on each of the two previously challenged segments. BAL fluid from the two Ag-challenged segments was pooled for analysis of fluid and cells. Heparinized venous blood (120 ml) was drawn into 60-cc syringes immediately before each bronchoscopy.

Analysis of BAL fluid

BAL cells were recovered from the lavage fluid by centrifugation at 400 × g for 10 min at 4°C, then washed twice with HBSS containing 2% newborn calf serum (NCS). Total BAL cell numbers were determined by hemacytometer using Turk’s counting solution containing acetic acid and methylene blue. For differential cell counts, cytospin preparations of BAL cells were stained with the Giemsa–based Diff-Quik stain (Baxter Scientific Products, McGaw Park, IL). BAL cells were used for flow cytometric analysis and to obtain purified eosinophils. BAL fluids were stored at −70°C until analyzed.

Table I. Subject characteristics

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<tr>
<th>Subjects</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Meth PC20 (mg/ml)</th>
<th>Ag PD20 (CBU)</th>
<th>FEV1 (% Pred)</th>
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<td>341.1</td>
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aBAL fluid was analyzed in all subjects. BAL eosinophils were evaluated in subjects 14–25.

bConcentration of methacholine that caused a 20% fall in forced expiratory volume in 1 s (FEV1).

cProvocative dose of Ag resulting in a 20% drop in lung function.

dCumulative breath units.

εPercentage of predicted value.

fRagweed Ag.

gCat dander.

hHouse dust mite.

iMedians with 25 and 75 percentiles.
Protein measurements
A sensitive two-step sandwich-type ELISA was developed to measure sIL-5Rα in BAL fluids. Horseradish peroxidase-conjugated anti-human IL-5Rα (clone A17; BD Pharmingen, San Diego, CA) was coated overnight at 4°C with a predetermined optimal concentration of purified monoclonal anti-human IL-5Rα (clone A17; BD Pharmingen, San Diego, CA). Non-specific binding sites were blocked with 10% diazylated NCS. Test samples were incubated overnight at 4°C on Ab-coated plates, and sIL-5Rα was detected with a biotinylated goat anti-human IL-5Rα polyclonal Ab (R&D Systems, Minneapolis, MN). Streptavidin conjugated to a HRP polymer (POLY-HRP-40; Research Diagnostics, Flanders, NJ) was used to increase assay sensitivity. A one-component substrate, 3,3',5'-tetramethylbenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD), was used for color development, and data were analyzed with Biolink Software (Dynametech Laboratories, Chantilly, VA). The concentration of sIL-5Rα in BAL fluids was calculated by comparison with a standard curve generated with known amounts of human rIL-5Rα (Sigma-Aldrich, St. Louis, MO). The assay sensitivity for sIL-5Rα was ±12 pg/ml. IL-5 was also measured by a two-step ELISA, as previously described (6). The coating Abs and biotinylated detection Abs were purchased from BD Pharmingen. The sensitivity for IL-5 was ±3 pg/ml.

Eosinophil purification
BAL eosinophils were purified by a modified Percoll gradient. BAL cells (50 × 10^6) were treated with DNase (RQ1 RNase-free DNase; Promega, Madison, WI) to degrade DNA, and cDNA was synthesized, as previously described (26). PCR was conducted with the forward and side scatter plot was established by backgating on electronically gated (R2) within this forward and side scatter gate are eosinophils. Nonetheless, all test samples contained a FITC- or PE-labeled anti-CD16 and anti-CD14 cocktail, which allowed for further electronic exclusion of any contaminating neutrophils and monocytes, respectively. To determine the percentage of positive cells, dot plots were created based on R1, and a tight electronic gate (R2) was set to encompass only the eosinophils in the isotype control sample. A larger region, R3, was drawn to include the isotype control and all positive eosinophils. Cells were considered positive if there was an electronic shift out of the R2 isotype control region and into R3. Thus, the percentage of positive eosinophils was determined as (1 - (R2/R3)) × 100.

RT-PCR for detection of IL-5Rα mRNA
Total RNA was extracted from eosinophil cell pellets using a one-step phenol/chloroform extraction reagent (Tri Reagent; Sigma-Aldrich). The total RNA was treated with DNase (RQ1 RNase-free DNase; Promega, Madison, WI) to degrade DNA, and cDNA was synthesized, as previously described (26). PCR was performed by transferring 4 μl of cDNA to a 650 μl thin-walled PCR tube along with 2.5 U Platinum Taq (Invitrogen Life Technologies, Carlsbad, CA), 5 μl of PCR buffer, 0.1 μM dNTPs, 50 mM MgCl₂, and 0.2 μM of primer in a final volume of 50 μl. A forward primer specific for mIL-5Rα, position 1033-1056, and two different reverse primers, sIL-5Rα at position 1270-1298 and mIL-5Rα at position 1542-1564, were constructed using published sequences (17). Primers for amplification of GAPDH mRNA were as previously described (26). Upstream and downstream primers were separated by introns so that any genomic DNA amplified by these procedures could be discriminated from cDNA based on size. The predicted size of cDNA fragments was 266 bp for mIL-5Rα and 527 bp for sIL-5Rα. PCR was conducted with the following protocol: 94°C for 2 min, then 24 cycles of 98°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The number of PCR cycles (24) was optimized to maintain a linear relationship between mRNA and PCR products. Controls included in each PCR run included samples containing reagents with no cells and samples that had not been transcribed. A DNA probe (205 bp, position 1033-1337) for both forms of IL-5Rα was synthesized and labeled with HRP, and PCR products were detected by Southern blot analysis (ECL system; Amersham, Piscataway, NJ).

GAPDH mRNA was analyzed in parallel with IL-5Rα mRNA to assess consistency of RNA preparations. GAPDH mRNA levels were quantitated by a competitive PCR (cPCR) ELISA. cDNA competitors consisting of nonsense DNA flanked by GAPDH-specific primers were added to test samples in graded quantities to compete with native cDNA for primer binding (27). cPCR was then performed with a biotinylated forward primer to end label the PCR product for analysis by ELISA. To perform the DNA ELISA, the biotinylated PCR product was denatured and added to a 96-well streptavidin-coated plate. PCR products were detected by hybridization with a fluorescein-labeled Ab (BD PharMingen, San Diego, CA) followed by incubation with an anti-fluorescein Ab conjugated to aequorin (Aqualite; Chemicon, Temecula, CA) (29). Upon addition of calcium ions, blue light is emitted and detected in a microplate luminesimeter. The concentration of the sample is calculated by plotting the sample/competitor signal ratio against the concentration of the competitor: when the ratio equals one, then the concentrations are equal. The RT-cPCR ELISA was found to be linear and reproducible, with a coefficient of variation of 10%.

The relative amount of IL-5Rα mRNA was normalized to GAPDH. The band intensity of the IL-5Rα mRNA species was determined from the Southern blots and given as arbitrary units. These units were then divided by the amount of GAPDH mRNA calculated from the RT-cPCR ELISA to give the ratio of sIL-5Rα/GAPDH or mIL-5Rα/GAPDH.

Statistical analysis
Statistical analysis was performed using the SigmaStat software package (Jandel Scientific Software, San Rafael, CA). Data are expressed as medians with 25 and 75 interquartiles (or the mean ± SEM for normally distributed data). The Wilcoxon signed rank test (or a paired t test for normally distributed data) was used to compare different time points to 0 h or to compare data obtained at baseline and 48 h after SBP-Ag. Correlations were made using Spearman rank order correlation test. A p value of <0.05 was considered significant.

Results
Detection of cell surface IL-5Rα on BAL eosinophils following airway Ag challenge
Forty-eight hours after SBP-Ag challenge, both eosinophil percentage (Table II) and number in BAL fluids were markedly increased (54.4 ± 3.8% and 131.5 ± 29.9 × 10³/ml BAL fluid, mean ± SEM) compared with baseline (1.2 ± 0.4% and 0.01 ± 0.00 × 10³/ml BAL fluid, n = 25, p < 0.001). The expression of mIL-5Rα on peripheral blood (0 and 48 h after Ag challenge) and BAL (48 h) eosinophils was determined by flow cytometry. Analysis of mGMP-CSFRA was also performed because IL-5 and GM-CSF have overlapping functions and the βc subunit of their receptors is identical. Both the percentage of mIL-5Rα-positive eosinophils was significantly increased (54.4 ± 3.8% and 131.5 ± 29.9 × 10³/ml BAL fluid, mean ± SEM) compared with baseline (1.2 ± 0.4% and 0.01 ± 0.00 × 10³/ml BAL fluid, n = 25, p < 0.001). The expression of sIL-5Rα on peripheral blood (0 and 48 h after Ag challenge) and BAL (48 h) eosinophils was determined by flow cytometry. Analysis of sGMP-CSFRA was also performed because IL-5 and GM-CSF have overlapping functions and the βc subunit of their receptors is identical. Both the percentage of sIL-5Rα-positive eosinophils was significantly increased (151.3 ± 56.2% and 321.1 ± 56.2 × 10³/ml BAL fluid, mean ± SEM) compared with baseline (1.2 ± 0.4% and 0.01 ± 0.00 × 10³/ml BAL fluid, n = 25, p < 0.001).

<table>
<thead>
<tr>
<th>Table II.</th>
<th>BAL cell numbers and differential*</th>
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<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cells, × 10⁶</td>
<td>151.3 ± 56.2</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>8.5 ± 1.2</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>87.3 ± 1.5</td>
</tr>
</tbody>
</table>

* Data are depicted as means ± SEM.
of eosinophils (Fig. 1A) and the amount of cell surface mIL-5Rα (Fig. 1, B and C) were significantly diminished on BAL (48 h) compared with peripheral blood eosinophils obtained either before (0 h) or 48 h after Ag challenge. In addition, βc, which was expressed on 100% of circulating eosinophils both at 0 and 48 h after Ag challenge, was markedly reduced on BAL eosinophils (Fig. 1, D–F). mGM-CSFRα was expressed on nearly 100% of peripheral blood and BAL eosinophils (Fig. 1G), and, in contrast to mIL-5Rα and βc, the relative intensity of mGM-CSFRα staining was significantly augmented on BAL compared with peripheral blood eosinophils (Fig. 1, H and I). BAL eosinophils could not be evaluated at baseline due to the small numbers of available cells.

Detection of sIL-5Rα in BAL fluid and the relationships between BAL fluid levels of sIL-5Rα, IL-5, and eosinophil numbers 48 h after Ag challenge

Concomitant with the reduction of cell surface mIL-5Rα (Fig. 1A), BAL fluid concentrations of sIL-5Rα were significantly elevated 48 h after Ag challenge when compared with baseline values (Fig. 2A). Moreover, there was a strong positive correlation (Spearman’s correlation coefficient ($r_s = 0.741, p < 0.001$)) between the levels of sIL-5Rα in BAL fluid and the number of BAL eosinophils 48 h after Ag challenge (Fig. 2B). The levels of sIL-5Rα also correlated ($r_s = 0.731, p < 0.001$) with IL-5 concentrations in BAL fluid (Fig. 2C).

Validation of ELISA for detection of sIL-5Rα and IL-5 in BAL fluid and flow cytometric assay for detection of cell surface mIL-5Rα

Because IL-5 is present in BAL fluid following Ag challenge (6), it was necessary to determine whether IL-5 interferes with the ability to accurately measure mIL-5Rα and sIL-5Rα. For the sIL-5Rα ELISA, addition of high concentrations of IL-5 (10 ng/ml) to sIL-5Rα standard had no effect on sIL-5Rα detection (Fig. 3A), suggesting that IL-5 in BAL fluid did not interfere with measurement of sIL-5Rα. Furthermore, a high concentration of sIL-5Rα...
(10 ng/ml) had no effect on the detection of IL-5 in the IL-5 ELISA (Fig. 3B). For flow cytometric analysis, it was also necessary to exclude the possibility that prior binding of endogenous IL-5 in BAL fluid to airway eosinophils blocked the detection of mIL-5Rα. To evaluate this possibility, increasing concentrations of sIL-5 were added to peripheral blood eosinophils before staining of mIL-5Rα. Even at concentrations of 10 ng/ml IL-5, there was no significant effect on detection of mIL-5Rα by flow cytometric analysis (Fig. 3C).

**Comparison of IL-5Rα mRNA in BAL and peripheral blood eosinophils**

The regulation of IL-5Rα and IL-5Rα mRNA in human blood eosinophils by the IL-5-family cytokines was previously reported by Wang et al. (16). However, the expression of mRNA for these receptor subunits in airway eosinophils has not been studied. Because there were not sufficient numbers of BAL eosinophils present in the airway before Ag challenge, mRNA levels in purified BAL eosinophils obtained 48 h after Ag challenge were compared with peripheral blood eosinophils obtained from the same subject immediately before the post-Ag BAL. sIL-5Rα and mIL-5Rα mRNA were measured by RT-PCR, and the identity of PCR products was confirmed by Southern blot analysis using sIL-5Rα- and mIL-5Rα-specific probes. The housekeeping gene, GAPDH, was determined by an RT-PCR competitive ELISA-type assay. No enhanced expression of IL-5Rα mRNA was discernible in any of the four BAL samples relative to the expression in the peripheral blood samples (Fig. 4). In fact, when the levels of IL-5Rα mRNA were expressed as a ratio to GAPDH (as indicated by the number below each blot), there was a noticeable decrease in both sIL-5Rα and mIL-5Rα in BAL compared with blood eosinophils in two of the four subjects.

**FIGURE 3.** Validation of assays. A, Detection of sIL-5Rα by ELISA in the presence (●) and absence (○) of 10 ng/ml IL-5. B, Detection of IL-5R by ELISA in the presence (●) and absence (○) of 10 ng/ml sIL-5Rα. C, Detection of mIL-5Rα by flow cytometric analysis following preincubation of blood eosinophils with increasing concentrations of IL-5. Data for each graph represent mean ± SE of three different experiments.

**FIGURE 4.** Expression of mRNA for IL-5Rα in blood and airway eosinophils. mRNA was extracted from 4 × 10⁶ eosinophils and amplified by RT-PCR, and PCR products were confirmed by Southern blot analysis. Data show a comparison of sIL-5Rα and mIL-5Rα mRNA from BAL or blood (BLD) eosinophils obtained from four individual subjects 48 h post-SBP-Ag. The double bands present for mIL-5Rα most likely result from secondary structure in these transcripts. GAPDH was analyzed by a RT-PCR competitive ELISA-type assay. The ratio of sIL-5Rα/GAPDH or mIL-5Rα/GAPDH is indicated at the bottom of each respective lane.

**Comparison of IL-5 and GM-CSF for ex vivo degranulation of BAL eosinophils**

Eosinophil degranulation was used to assess the potential functional significance of the reduction of mIL-5Rα on BAL eosinophils. Isolated blood or BAL eosinophils were cultured with medium alone or 1 ng/ml IL-5 or GM-CSF, and released EDN was determined. Blood eosinophils showed significant cytokine-induced EDN release in response to either IL-5 or GM-CSF (Fig. 5A). In contrast, BAL eosinophils responded to GM-CSF, but were refractory to stimulation by IL-5 (Fig. 5B). These data indicate that a reduction in mIL-5Rα expression, which is seen on BAL eosinophils, is associated with a decrease in the ability of these cells to respond to IL-5 with the release of EDN, i.e., there was a demonstrable functional correlate for the observed change in receptor expression.

**Discussion**

In this study, we established that, following airway Ag challenge of atopic subjects, mIL-5Rα and βc were markedly reduced on airway eosinophils compared with circulating eosinophils. Furthermore, sIL-5Rα protein was detected, and the concentrations were significantly elevated in BAL fluid 48 h after Ag challenge. Although the precise role of mIL-5Rα and sIL-5Rα in the regulation of airway eosinophil functions is unknown, we speculate that these events control IL-5-mediated activation of eosinophils. In this regard, we have demonstrated that, in contrast to their circulating counterparts, BAL eosinophils obtained following SBP-Ag do not release EDN when exposed ex vivo to IL-5. Taken together, these observations further support the notion that the primary function of IL-5 is eosinophil hemopoiesis and release from the bone marrow (30, 31). Moreover, the decrease in mIL-5Rα expression on BAL eosinophils and the lack of responsiveness to IL-5 suggest a switch to IL-5-independent cell function once this cell is in the airway.

The decrease in the expression of mIL-5Rα on human airway eosinophils compared with their circulating counterparts is consistent with the observations by Tomaki et al. (9), who showed that mIL-5Rα was detectable by immunohistochemistry on bone marrow, but not BAL eosinophils following airway allergen challenge in a murine model of allergen-induced eosinophilic airway inflammation. The decrease in mIL-5Rα expression could occur via several possible mechanisms. First, the receptor could undergo proteasome degradation. Recent studies in an IL-5-responsive
cells in the bronchial mucosa from asthmatic subjects correlated with an improvement in pulmonary function, whereas the presence of mIL-5Rα mRNA-positive cells was associated with airflow obstruction. Based on these observations, it is tempting to speculate that sIL-5Rα may serve a protective role in IL-5-mediated airway diseases. However, definitive studies await purification of the protein from BAL fluid.

We have presented compelling evidence that IL-5-mediated eosinophil activation in the airway is controlled at the level of IL-5Rα expression. We recognize, however, that there are certain limitations to our studies of human airway eosinophils. First, we cannot ascertain where the switch to IL-5 unresponsiveness may occur. This is due, in part, to the lack of sufficient numbers of BAL eosinophils for analysis at baseline, and the inability to study the functional capacity of eosinophils in the airway mucosa. Second, we have yet to identify the IL-5-independent factor(s) that controls eosinophil activation in the airway. The role of GM-CSF in this regard is not entirely clear. Although airway eosinophils retain some degree of responsiveness to GM-CSF, as demonstrated by EDN release, the expression of βc is markedly reduced on these cells, and the degree of responsiveness to exogenous GM-CSF is significantly less than that of circulating eosinophils. Whether the differences between blood and BAL eosinophils are due to reduced signaling by GM-CSFR or reflects other differences between these cells is not yet known.

In conclusion, we propose that, following airway Ag challenge, IL-5 is primarily responsible for the release of mature eosinophils from the bone marrow and their subsequent recruitment to the airway. This is based on the high expression of mIL-5Rα on circulating, but not airway eosinophils, and is consistent with a number of animal studies showing IL-5-induced eosinophiliopoiesis and recruitment to the airway. Within the airway, expression of mIL-5Rα on eosinophils is attenuated, and sIL-5Rα is released into the BAL fluid. As a result, the response of the airway eosinophil to IL-5 is ablated, and additional factors may be required for further eosinophil activation. Thus, we postulate that within the airway, the regulation of eosinophil functions may be switched from an IL-5-dependent to IL-5-independent mechanism(s). Although these conclusions are, at present, speculative, we propose that this paradigm may begin to explain why recent studies with anti-IL-5 mAb treatment of mild asthmatic patients reduced circulating and sputum eosinophils, but did not inhibit the airway response to inhaled allergen (38). In support of this possibility, Kay and colleagues (39) have recently reported that a significant proportion of eosinophils is retained within the airway mucosa following anti-IL-5 treatment of patients with asthma. Furthermore, anti-IL-5 had no effect on the detection of eosinophil granule protein present in the bronchial mucosa. Taken together with our observations that airway eosinophils (and presumably tissue-dwelling cells) have reduced expression of mIL-5Rα and do not degranulate to IL-5, it is not surprising that anti-IL-5 did not modulate the airway response to Ag. This possibility emphasizes the need to closely evaluate effects of allergic mediators in various compartments, i.e., circulation, bronchial mucosa, and airway lumen, during allergic inflammation, and suggests that effective treatment may require selective elimination of multiple cytokine pathways.

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FIGURE 5. Functional capacity of blood (A) and BAL (B) eosinophils to release the eosinophil granule protein, EDN. Release of EDN was measured 4 h after ex vivo exposure of eosinophils to medium alone, 1 ng/ml IL-5, or 1 ng/ml GM-CSF. Boxes represent medians within 25 and 75 quartiles (n = 7), *, p < 0.05 compared with spontaneous release; †, p < 0.05 compared with IL-5-treated cells. Total EDN release (median with 25 and 75 percentiles) by lysed blood and BAL eosinophils was 3152 (2369, 4734) and 2862 (1941, 3965) ng/l × 10⁶ cells, respectively.

erythroleukemia line (TF-1) have shown that IL-5 induces proteasome-mediated cleavage of the βc cytoplasmic tail, followed by receptor internalization and degradation in the lysosome (32). Second, the receptor could be internalized via lysosomal-mediated mechanisms and be either degraded or recycled to the cell surface. For example, loss of the chemokine receptor CCR3 involves internalization with both degradation and limited recycling of receptors to the cell surface (33, 34). Finally, the ectodomain of mIL-5Rα could be shed from the cell surface through a proteolytic process to give rise to a soluble form of the receptor. A number of integral membrane proteins are known to be enzymatically cleaved from the cell surface, including cytokine receptors (TNF-α, IL-6, TGF-β1), Ig receptors (FcRyIII, FcRyII, FcRe), and adhesion molecules (VCAM-1, CD14, L-selectin) (35). Based on the observations that the sIL-5Rα protein was detected in BAL fluid and that levels increased following Ag challenge in the absence of increased steady state levels of sIL-5Rα mRNA, we speculate that the presence of sIL-5Rα in BAL fluid may result from cleavage of the mIL-5Rα ectodomain from the cell surface and examine this possibility in the accompanying manuscript (40).

The demonstration of sIL-5Rα in human biological fluids is a novel and potentially important finding toward an understanding of eosinophil function in allergic inflammation. The significance and functional activity of this protein remain to be determined, as other soluble receptors have been shown to inhibit or enhance cytokine function (36). It has been suggested that sIL-5Rα may function as an IL-5 antagonist. This is based on the observation that rIL-5Rα binds with high affinity to IL-5 (37), and is a potent in vitro antagonist for IL-5-mediated signal transduction (18) and differentiation of eosinophil progenitor cells (19). In addition, Yasruel et al. (20) have reported that the presence of sIL-5Rα mRNA-positive
References


