

IL-3, IL-5, granulocyte-macrophage colony-stimulating factor receptor α -subunit, and common β -subunit expression by peripheral leukocytes and blood dendritic cells

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Background: IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors consist of cytokine-specific α -subunits, which associate with a shared signalling common β -subunit (β c) to form a high-affinity complex. The expression of IL-3, IL-5, and GM-CSF is upregulated in atopic inflammation, and these cytokines are thought to contribute to pathology through mechanisms that include eosinophil activation.

Objective: We sought to examine the distribution of receptor expression between cells relevant to allergic inflammation from individual subjects and to compare atopic and non-atopic individuals.

Methods: Peripheral blood was obtained from atopic and nonatopic volunteers. Cytokine-receptor expression was examined by flow cytometry with monoclonal antibodies specific for α -subunits and β c in combination with phenotypic markers for eosinophils, basophils, neutrophils, dendritic cells, monocytes, and T cells.

Results: Using a ligand-independent system, we confirmed the cellular distribution of IL-5R α , IL-3R α , and GM-CSFR α . IL-3R α and GM-CSFR α were detected on high-affinity IgE receptor blood dendritic cells. β c expression was detected on basophils, eosinophils, neutrophils, and, at low levels, on monocytes and dendritic cells. There was intense staining of basophils for IL-3R α relative to IL-5R α , GM-CSFR α , and β c, whereas eosinophil-staining intensity was similar for IL-3R α , IL-5R α , GM-CSFR α , and β c. There were no significant differences between atopic and nonatopic subjects in cytokine-receptor staining.

Conclusion: IL-3R α and GM-CSR α are shown on a newly defined population of Fc ϵ RI-high dendritic cells. The intense staining of basophils for IL-3R α , relative to that of IL-5R α and GM-CSFR α , is in contrast to eosinophils from the same subjects and may explain the higher sensitivity of basophils to IL-3 compared with IL-5 and GM-CSF. We found no evi-

dence for downregulation of receptor expression in atopic compared with nonatopic subjects, suggesting that these receptors remain accessible as potential targets for therapeutic intervention in atopic allergic disease. (*J Allergy Clin Immunol* 1998;101:677-82.)

Key words: IL-3 receptor, IL-5 receptor, GM-CSF receptor, basophils, eosinophils, monocytes, neutrophils, T lymphocytes

IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) share many activities of relevance to atopic allergic disease.¹ These cytokines act in myeloid differentiation,² modulate mature cell function,³⁻¹⁰ prime both eosinophils and basophils for activation, and enhance tissue survival.³⁻⁹

The receptors for IL-3, IL-5, and GM-CSF have cytokine-specific α -subunits that bind to cytokine with low affinity and a common signal-transducing β -subunit that can associate with any of the three specific α -subunits to form a high-affinity complex.¹¹⁻¹³ Different activities of IL-3, IL-5, and GM-CSF on various cell types may be determined by α -subunit distribution because the same signal is presumably delivered on cytokine-receptor interaction.^{11, 12} Support for this concept comes from studies in which transgenic mice expressing IL-5R α on all myeloid cells (in common with IL-3R α) showed identical activity of both IL-3 and IL-5 in hematopoietic cell-differentiation assays.¹⁴

Mutant cytokine molecules with amino acid substitutions act as cytokine antagonists for GM-CSF^{15, 16} and IL-5¹⁷ in vitro through interference with normal receptor assembly and activation. If such an approach is to be used in therapy, it is important to define the cellular distribution of receptors for IL-3, IL-5, and GM-CSF. We therefore used monoclonal antibodies (mAbs) to examine receptor expression by peripheral blood cells from atopic and nonatopic donors, including a recently defined dendritic cell population. This approach had several advantages over previous radioligand-binding studies. First, measurements are ligand independent. In addition, we were able to examine expression of low-affinity receptor subunits, such as IL-3R α and the common β -chain, which does not itself bind cytokine on a cell-by-cell basis, to determine whether there were subgroups of receptor-bearing cells.

Because IL-3, IL-5, and GM-CSF are upregulated at

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Abbreviations used

β c:	Common β -subunit
CHO:	Chinese hamster ovary
Fc ϵ RI:	High-affinity IgE receptor
FITC:	Fluorescein isothiocyanate
GM-CSFR α :	Granulocyte-macrophage colony-stimulating factor receptor α -subunit
IL-3R α :	IL-3 receptor α -subunit
IL-5R α :	IL-5 receptor α -subunit
mAb:	Monoclonal antibody
SMFI:	Specific mean fluorescence intensity

sites of allergic inflammation and in serum from atopic subjects,¹⁸⁻²¹ and these cytokines downregulate their own receptors *in vitro*.^{22, 23} It is possible that expression of receptors for IL-3, IL-5, and GM-CSF on effector cells might be downregulated in disease, thus reducing the potential for therapeutic intervention. We therefore compared atopic and nonatopic donors.

METHODS

Subjects

Eight normal volunteers and 19 atopic volunteers were examined (Table I). Patients were recruited from the outpatient department of the Royal Brompton Hospital and from volunteers responding to advertisements. Atopy was defined by positive skin prick test results (defined as a wheal reaction >3-mm in diameter compared with control at 15 minutes) or positive RAST results (>0.7 IU/ml) (Phadebas, Pharmacia, Sweden) to one or more of a panel of common aeroallergens. Pregnant women and smokers were excluded. None of the subjects was currently taking oral or inhaled steroids. This study was performed with the approval of the Ethics Committee of the Royal Brompton Hospital, and informed written consent was obtained from all patients and volunteers.

Antibodies

Mouse IgG1 isotype control antibody (DAK-G01) was obtained from Dako (Denmark). The following were used to identify cytokine receptors: anti-IL-3R α mAb (6H6: mouse IgG1),²⁴ anti-IL-5R α mAb (α 14: mouse IgG1), anti-GM-CSFR α mAb (8G6: mouse IgG1),²⁵ and anti- β c mAb (1C1: mouse IgG1).²⁶ For the production of mAb specific for the IL-5R α subunit, BALB/c mice were immunized with Chinese hamster ovary (CHO) cells transfected with human IL-5R α subunit cDNA. The mice were boosted four times before fusing splenocytes with NS-1 cells by standard methods. Hybridoma supernatants were screened on FDCP-1 cells expressing human IL-5 receptors. MAb α 14 was found to stain IL-5R α -expressing FDCP-1 cells but not untransfected FDCP-1 cells. The mAb α 14 also specifically recognized other primary cells or cell lines expressing IL-5R α , such as eosinophils, basophils, and COS transfectants. In immunoprecipitation assays, α 14 specifically recognized a 50 to 55 kD protein from cell lysates of CHO cells expressing the IL-5R α subunit. It failed to detect any specific protein from CHO parent cells. The optimal antibody concentrations for flow cytometric analysis (5.0 μ g/ml for α 14 and 1.0 μ g/ml for 6H6, 8G6, and 1C1) were determined by dose-response experiments on purified eosinophils, and isotype control antibody was used at the same concentrations. TRI-

color conjugated goat anti-mouse IgG1 (gamma) was purchased from Caltag Laboratory (San Francisco, Calif.). Phycoerythrin-conjugated anti-human CD3 mAb (Leu-4, mouse IgG1), PE-conjugated anti-human CD14 mAb (Leu-M3, mouse IgG1), and phycoerythrin-conjugated anti-human CD16 mAb (Leu-11c, mouse IgG1) were obtained from Becton-Dickinson (San Jose, Calif.). Fluorescein isothiocyanate (FITC)-conjugated anti-human CD9 mAb (FMC 56, mouse IgG1) was purchased from Harlan Sera-Lab (Crawley Down, Sussex, England). FITC-conjugated anti-human Fc ϵ RI mAb (22E7, mouse IgG1) was a gift from Dr. Jarima Kochan (Hoffman La Roche Inc, Nutley, N.J.).²⁷

Preparation of peripheral blood cells

One hundred milliliters of blood was collected from all subjects, and the percentage of eosinophils was assessed in each sample by using Kimura stain. Twenty milliliters of 6% dextran (Fluka Chemie, Dorset, U.K.) in Hank's balanced salt solution (Sigma Aldrich, U.K.) was added, and the blood/dextran mixture was left for 30 minutes at room temperature. Granulocytes and mononuclear cells in the buffy coat layer collected were separated by density gradient centrifugation over the Histopaque (Sigma). Contaminating red blood cells in the granulocyte preparation were lysed with distilled water.

Immunofluorescent staining and flow cytometric analysis

Mononuclear cells and granulocytes (2.5×10^5 cells per tube) were incubated with primary mAbs to cytokine receptor subunits for 15 minutes on ice. Cells were washed twice with PAB (phosphate-buffered solution with 0.1% wt/vol azide and 0.5% wt/vol bovine serum albumin). Then secondary mAb (goat anti-mouse IgG1-TRI-color) was added to the cells and incubated for 15 minutes on ice. After two further washes with PAB, anti-Fc ϵ RI (22E7)-FITC, anti-CD3-PE, and anti-CD14-phycoerythrin were added to the mononuclear cells, and anti-CD9-FITC and anti-CD16-phycoerythrin mAbs were added to the granulocytes. After 15 minutes of incubation, cells were washed with PAB and fixed with 0.5% Formaldehyde-Isoton (Coulter Corporation, Hialeah, Florida) solution. Stained cells were analyzed on a Coulter Epics Elite ESP flow cytometer (Coulter) by using identical gain settings for each sample. Specific mean fluorescence intensity (SMFI) was calculated from the difference in mean fluorescence intensity (MFI) between samples stained with negative control antibody and cytokine receptor mAb.

Statistical analysis

Between patient groups, comparisons were performed with the nonparametric Mann-Whitney U test with the aid of a commercially available statistical package (Minitab for Windows, Minitab Release 9.2; Minitab Inc., State College, Pa.). Probability (*p*) values less than 0.05 were considered significant.

RESULTS

The combination of forward and side scatter characteristics and phenotypic markers allowed clear delineation of different peripheral blood cell types. Two Fc ϵ RI⁺ cell populations were seen in the mononuclear population separated after Ficoll density centrifugation. The lower of these two populations expressed high levels of HLA-DR and corresponded to the cell population previously characterized by Maurer et al. as dendritic

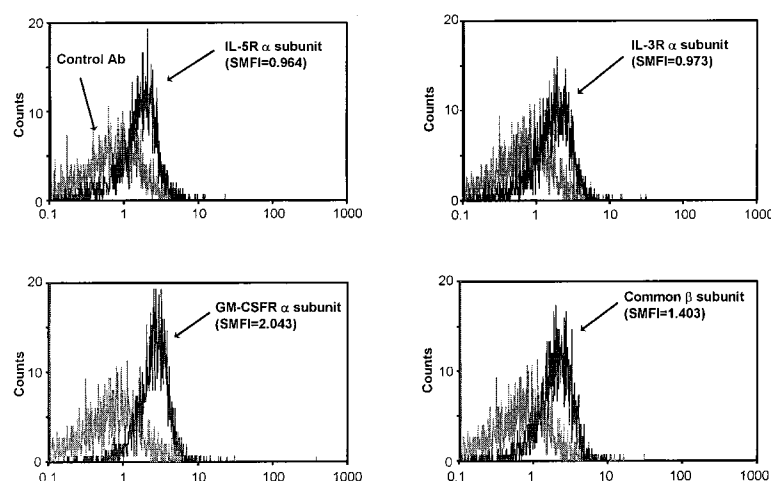


FIG. 1. Examples of cytokine-receptor immunostaining on eosinophils. Cytokine-receptor expression was detected as shift in immunofluorescent staining of cell populations. Expression of IL-3R α , IL-5R α , GM-CSFR α , and β c were detected. AB, Antibody.

TABLE I. Characteristics of patient groups studied

Subjects (no.)	Age range (yrs)	Sex (M/F)	Blood eosinophil (%)‡	Serum IgE (IU/ml)‡	Histamine PC ₂₀ (mg/ml)
NC (8)	21-25	6/2	2.88 $\pm$ 0.41	48.5 $\pm$ 7.8	>16
NAA (5)	18-36	4/1	1.62 $\pm$ 0.42	83.7 $\pm$ 17.3	>16
AA (14)	20-47	4/10	4.87 $\pm$ 0.61*	398.7 $\pm$ 81.4†	3.87 $\pm$ 1.07‡

NC, Normal control subjects; NAA, nonasthmatic atopic subjects; AA, atopic asthmatic subjects.

* $p < 0.05$ versus normal control subjects.

† $p < 0.001$ versus normal control subjects.

‡Mean $\pm$ SEM.

cells.²⁸ We have previously characterized the Fc ϵ RI-high mononuclear cells as basophils.²⁹

Cytokine receptor expression was detected as a shift in the fluorescence of the whole cell populations, without subgroups in all cases. Results are therefore expressed as SMFI relative to isotype control. Examples of cytokine receptor immunostaining on eosinophils are shown in Fig. 1.

IL-5R α was detected on eosinophils and basophils, but not other cell types studied (Fig. 2). IL-3R α was expressed by eosinophils, basophils, dendritic cells, and monocytes (Fig. 2), and GM-CSFR α was expressed by eosinophils, basophils, neutrophils, dendritic cells, and monocytes (Fig. 3). β c was expressed by all cell types studied, except T lymphocytes, although at low levels on monocytes and dendritic cells (Fig. 3).

Comparing relative intensity of cytokine-receptor staining on basophils and eosinophils showed that basophils had intense IL-3R α staining with considerably lower fluorescence intensity for IL-5R α and GM-CSFR α , whereas eosinophil expression of IL-3R α , IL-5R α , and GM-CSFR α was of equal fluorescence intensity (Figs. 2 and 3). Intensity of staining for β c was similar on both eosinophils and basophils and appeared similar to that for IL-5R α . However, β c staining of

monocytes and dendritic cells was minimal, whereas GM-CSFR α expression was an order of magnitude greater than that for IL-3R α . IL-3R α was detected on neutrophils from neither atopic nor nonatopic subjects in this study, but GM-CSFR α and β c staining intensity was roughly equivalent.

No significant differences in immunostaining patterns for cytokine receptors on any of the cell types studied were detected when atopic and nonatopic subjects were compared (Figs. 2 and 3), nor were there significant differences in relative numbers of circulating leukocytes between the two groups.

DISCUSSION

In this study we have confirmed the cellular distribution of receptor α -subunits for IL-3, IL-5, and GM-CSF among mature blood eosinophils, basophils, neutrophils, and monocytes by using a ligand-independent method. We also showed expression of IL-3R α , GM-CSFR α , and, at lower levels, β c by circulating dendritic cells. Relatively high intensity of expression of IL-3R α by basophils was detected, whereas eosinophil immunostaining for IL-3R α , IL-5R α , GM-CSFR α , and β c was of similar intensity. These findings are in accord with the relative in vitro sensitivity of these cells to cytokines.³⁻¹⁰

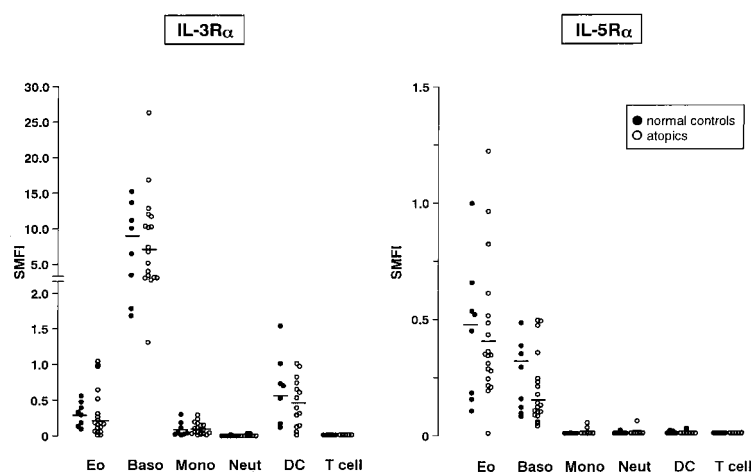


FIG. 2. Expression of receptor subunits on different cell types in blood. IL-5R α was detected on eosinophils and basophils, but not on other cell types studied. IL-3R α was detected on eosinophils, basophils, dendritic cells, and monocytes. No significant differences in immunostaining patterns for cytokine receptors on any cell types studied were detected when atopic and nonatopic subjects were compared. *Eo*, Eosinophils; *Baso*, basophils; *Mono*, monocytes; *Neut*, neutrophils; *DC*, dendritic cells; *T cell*, T lymphocytes.

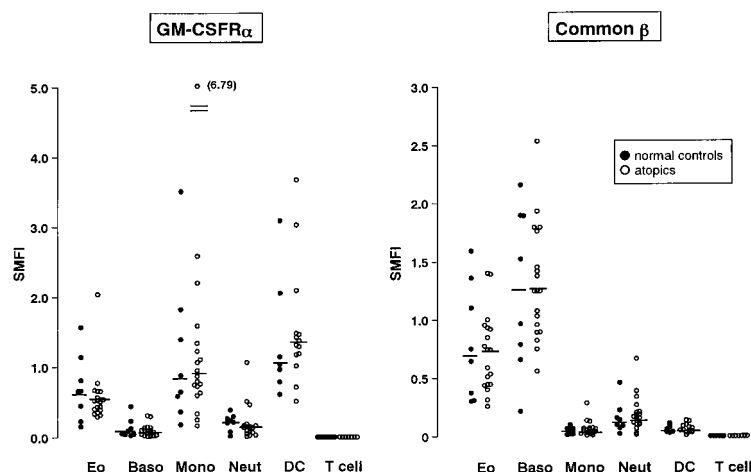


FIG. 3. GM-CSFR α expression by eosinophils, basophils, neutrophils, dendritic cells, and monocytes. β c was expressed by all cell types studied except T lymphocytes, which showed no expression of any receptor types studied. No significant differences in immunostaining patterns for cytokine receptors on any cell types studied were detected when atopic and nonatopic subjects were compared. *Eo*, Eosinophils; *Baso*, basophils; *Mono*, monocytes; *Neut*, neutrophils; *DC*, dendritic cells; *T cell*, T lymphocytes.

No evidence of receptor downregulation was detected in atopic individuals when compared with nonatopic control subjects.

The population of dendritic cells studied here were recently described by Maurer et al.²⁸ GM-CSFR α and β c expression by Langerhans' cells in the skin and spleen dendritic cells has been described,^{30,31} and IL-3 and GM-CSF favor in vitro maturation of dendritic cell function. The derivation and eventual recirculation pattern of this cell population remains to be established, but because expression of Fc ϵ RI may be important in allergen uptake for processing²⁸ and IL-3 and GM-CSF could conceivably act to upregulate the accessory cell

activity of this population at sites of allergic inflammation,³² it will be important to define their role in allergic disease.

It is of note that β c expression by monocytes and dendritic cells was detected at very low mean fluorescence intensity. Cross-competition studies suggest that β c expression is limiting for binding of IL-3, IL-5, and GM-CSF to eosinophils and basophils,^{22,33} and the same may apply for monocytes and dendritic cells. Monocyte and monocytic cell-line responsiveness to IL-3 and GM-CSF was increased by upregulation of expression of β c by IFN- γ and tumor necrosis factor- α ,^{34,35} and it will be of interest to determine whether activation by addi-

tional cytokines is required for IL-3 and GM-CSF responsiveness of circulating monocytes and dendritic cells. Although antibody titration was performed to optimize immunostaining in this study, we cannot necessarily equate staining intensity with function. Indeed, IL-5 activity on human B-cell IgM synthesis was shown despite the inability to detect surface staining for IL-5R α (though mRNA was detectable),³⁶ and the concentration of IL-5 required to support eosinophil survival in vitro was 50- to 100-fold lower than that needed for 50% receptor occupation.³⁷ Thus partial occupancy of very low numbers of IL-5 receptors may be sufficient to transduce signals. Nonetheless, the relative immunostaining and cellular distribution of receptors for IL-3, IL-5, and GM-CSF shown here is in broad agreement with functional studies of these cytokines.

A recent study described a subpopulation of peripheral blood cells expressing high levels of IL-3R α .³⁷ We show here that basophil-staining intensity with antibody to IL-3R α is markedly higher than that for IL-5R α and GM-CSFR α on the same cells, and at least 10-fold higher than that detected on eosinophils. Thus we suggest that the high IL-3R α -expressing population of Macardle et al.²⁵ is basophils. This finding is compatible with the in vitro sensitivity of basophils to IL-3, which acts in priming and activation of IL-4 and IL-13 production, histamine release, and leukotriene synthesis by these cells.^{8,9} IL-3 inhibits the binding of GM-CSF and IL-5 to basophils.²² In contrast, cross-competition experiments show that GM-CSF and IL-5 compete for IL-3 binding to eosinophils,³³ presumably because of an excess of α -subunits for the different cytokines relative to β c expression.¹² Thus it is possible that the different relative expression of receptors for IL-3, IL-5, and GM-CSF determines the differential sensitivity of these key two effector cells in allergic inflammation, with basophils predominantly responsive to IL-3 and eosinophils to GM-CSF and IL-5. Expression of all three of these cytokines has been shown to be upregulated at sites of allergic inflammation.¹⁸⁻²¹ Whether such factors can lead to differential activation of eosinophils or basophils remains to be determined. In addition, the very high expression of IL-3R α by basophils compared with other cell types raises the possibility of using anti-IL-3R α antibodies as a marker for, and in the isolation of, basophils.

We did not find any significant differences in intensity of immunostaining or cellular distribution of cytokine receptors on peripheral blood cells from the atopic individuals studied when compared with nonatopic control subjects, despite previous reports showing detectable circulating GM-CSF and IL-5 in atopic asthmatic subjects¹⁸ and the demonstrated effects of these cytokines on receptor expression by eosinophils and basophils in vitro.^{22, 23, 33} Similarly, although GM-CSF induces IL-3R α expression by neutrophils in vitro,³⁸ we did not detect IL-3R α expression on neutrophils from atopic subjects. The atopic subjects studied here were five rhinitic subjects and 14 mild asthmatic subjects, who,

despite airway hyperreactivity, did not have increased eosinophil numbers in peripheral blood when compared with nonatopic subjects. Thus we cannot exclude the possibility that differences in receptor expression may be detected at the site of inflammation or in atopic subjects with more severe disease or acute exacerbations. Nonetheless, the data in this study do not show receptor downregulation in atopic subjects, suggesting that receptors for IL-3, IL-5, and GM-CSF may remain accessible as therapeutic targets.

In conclusion, we have investigated the cellular distribution of IL-3R α , IL-5R α , GM-CSFR α , and β c by peripheral blood cells and report that IL-5R α detection was limited to eosinophils and basophils among the cell types studied. Basophils showed high staining intensity for IL-3R α , which may account for their relatively high biologic sensitivity to IL-3. Receptor expression for IL-3, IL-5, and GM-CSF was not significantly downregulated in atopic subjects, suggesting that these receptors remain accessible to potential therapeutic intervention in atopic allergic disease.

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