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Interleukin-5 receptor α levels in patients with marked eosinophilia or mastocytosis

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Abstract

Background—Interleukin (IL)-5 plays a central role in the development and maintenance of eosinophilia and eosinophil activation in a wide variety of eosinophilic disorders. Although IL-5, IL-3 and GM-CSF can modulate expression of IL-5 receptor α (IL-5Rα) on eosinophils *in vitro*, little is known about soluble and surface IL-5Rα levels *in vivo*.

Objective—To assess surface and soluble IL-5Rα levels in patients with eosinophilia and/or mastocytosis.

Methods—Surface IL-5Rα expression was assessed by flow cytometry in blood and/or bone marrow from subjects with eosinophilia (n=39), systemic mastocytosis (n=8) and normal volunteers (n=28). Soluble IL-5Rα (sIL-5Rα) was measured in a cohort of 177 untreated subjects and correlated with eosinophilia, eosinophil activation, serum tryptase and cytokine levels.

Results—Whereas IL-5Rα expression on eosinophils inversely correlated with eosinophilia ($r=-0.48$, $p<0.0001$), serum levels of sIL-5Rα increased with eosinophil count ($r=0.56$, $p<0.0001$), serum IL-5 ($r=0.40$, $p<0.0001$) and IL-13 levels ($r=0.29$, $p=0.004$). Of interest, sIL-5Rα was significantly elevated in patients with systemic mastocytosis without eosinophilia. Although sIL-5Rα levels correlated with serum tryptase levels in these patients, eosinophil activation, assessed by CD69 expression on eosinophils and serum eosinophil-derived neurotoxin levels, was increased compared to normal subjects.

Conclusion—These data are consistent with an *in vivo* IL-5Rα regulatory pathway in human eosinophils similar to that described in vitro and involving a balance between surface and soluble

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receptor levels. This may have implications with respect to the use of novel therapeutic agents targeting IL-5 and its receptor in patients with eosinophilia and/or mastocytosis.

Keywords

Hypereosinophilic syndrome; interleukin-5; mast cell; eosinophil; mastocytosis

Introduction

The IL-5 receptor (IL-5R) is a high affinity receptor expressed on eosinophils, basophils and mast cells and occurs as a heterodimer of IL-5Rα with the β subunit common to IL-5, IL-3 and GM-CSF receptors[1]. Data from numerous studies in animals and humans have demonstrated that IL-5 signaling through this receptor is essential for eosinophil development, survival and activation. Consequently, recent attention has focused on the development of therapeutic agents that target the IL-5/IL-5R axis for use in a wide variety of disorders characterized by eosinophilic inflammation. These include humanized monoclonal antibodies to IL-5 itself, already proven to be effective in the treatment of both hypereosinophilic syndrome (HES)[2] and eosinophilic asthma[3,4] , and a monoclonal antibody to IL-5Rα (benralizumab, MEDI-563) that results in enhanced susceptibility of eosinophils to antibody-dependent cell-mediated cytotoxicity[5,6].

A soluble form of IL-5Rα (sIL-5Rα) can be generated by differential splicing of mRNA transcripts[7] or by cleavage of surface receptors[8]. Increased serum levels of sIL-5Rα have been described in subjects with acute exacerbations of chronic obstructive pulmonary disease[9] and in patients with nasal polyposis with asthma[10] and have been suggested to play a role in the down-modulation of the eosinophilic inflammatory response. In fact, recombinant sIL-5Rα has been shown to bind IL-5 with high affinity and to prevent binding of IL-5, but not IL-3 or GM-CSF, to surface IL-5R, attenuating signal transduction, mediator release and survival of eosinophils *in vitro*[11].

Decreased surface expression of IL-5Rα on eosinophils has been reported following *in vitro* incubation with IL-5, IL-3, GM-CSF or IL-9 and is accompanied by a reciprocal increase in sIL-5Rα in the culture supernatants[8,12-14]. A similar phenomenon has been observed *in vivo* in association with eosinophil recruitment to the airways following airway allergen challenge and resulted in a lack of eosinophil responsiveness to exogenous IL-5[15]. Theoretically, such alterations in membrane and soluble IL-5Rα levels could interfere with the efficacy of monoclonal antibodies to bind IL-5Rα on eosinophils, as well as on basophils and mast cells. Despite these concerns, little is known about IL-5Rα regulation in patients with peripheral eosinophilia and/or mastocytosis.

Thus, the aim of the present study was to determine whether modulation of membrane and soluble IL-5Rα levels occurs *in vivo* in patients with marked eosinophilia and/or mastocytosis. Not only did surface and soluble IL-5Rα levels increase with eosinophil count and serum IL-5 level, as predicted from *in vitro* studies, but serum levels of sIL-5Rα were significantly increased in patients with systemic mastocytosis (SM) without eosinophilia. Consequently, the relative contributions of eosinophils and mast cells to sIL-5R levels were also explored.

Subjects and Methods

Patient populations

Subjects with eosinophilia or mastocytosis underwent detailed clinical and laboratory evaluation as part of Institutional Review Board (IRB)-approved clinical protocols to study

these disorders (NCT00001406, NCT00044122). Healthy volunteers were recruited under an IRB-approved clinical protocol designed to obtain controls for in vitro research (NCT00090662). All participants gave written informed consent. Subjects with eosinophilia were defined as subjects with a peak peripheral eosinophil count >1500/mm^3 evaluated between January 1991 and November 2009 and were classified into subtypes as follows: chronic eosinophilic leukemia (CEL; presence of the *FIP1L1/PDGFRA* fusion gene), hypereosinophilic syndrome (HES; unexplained eosinophilia >1,500/mm^3 with evidence of signs or symptoms attributable to the eosinophilia), parasitic (PARA; evidence of helminth infection and resolution of eosinophilia with treatment), benign eosinophilia (BE; persistent eosinophilia >1500/mm^3 for more than 5 years without signs or symptoms), secondary eosinophilia (Other EO; eosinophilia >1,500/mm^3 of known cause, such as due to a neoplasm or drug hypersensitivity). Subjects with mastocytosis were defined according to the 2008 WHO classification of myeloproliferative diseases[16] as systemic mastocytosis (SM; eosinophilia <1000/mm^3), systemic mastocytosis with eosinophilia (SM-eo; eosinophilia >1000/mm^3) or cutaneous mastocytosis (CM; urticaria pigmentosa in the absence of bone marrow involvement). Demographic and laboratory characteristics of the different patient populations are presented in Table 1.

Assessment of surface receptor expression by flow cytometry

Surface expression of IL-5Rα and activation markers on peripheral blood eosinophils was assessed using whole blood from all subjects evaluated for eosinophilia or mastocytosis between March and November 2009. A normal volunteer was included as a control with each assay. Whole blood was stained with CDw125-PE (clone A14; BD Biosciences, San Jose, CA), CD25-FITC, CD40-FITC, CD9-FITC, CD69-FITC, and CD16-PE (BD Biosciences, San Diego, CA). Irrelevant, directly conjugated, murine IgG1 was used to ascertain background staining. CD9 was used as a positive control for eosinophils. Samples were analyzed on a FACSCanto II flow cytometer using Cellquest software (BD Biosciences). Eosinophils were separated from granulocytes by their characteristic high side scatter and dim staining for CD16. Percent positive for each surface molecule was ascertained using a FITC conjugated subclass control and setting a marker so that >98% of the control was defined as negative. The normal ranges for surface receptor expression represent the 95% confidence interval for % expression on eosinophils from 40 normal volunteers. Quantibrite PE (BD Biosciences) was used to estimate the antibodies bound per cell (ABC) for CDw125-PE according to the manufacturer's instructions.

Bone marrow aspirates from subjects with SM (n=17) or HES (n=6) were stained using CD45-PerCP (clone 2D1), CD117-APC (clone 104D2), CD34-FITC (clone 8G12), CD49d-PE (clone 9F10), CD16-FITC (clone 3G8), CD9-PerCP-Cy5.5 (M-L13), CD3- FITC (clone SK7), CD19-APC (clone SJ25C1), CDw125-PE (clone A14) (BD Biosciences, San Jose, CA) and CD14-APC (TuK4; Invitrogen, Carlsbad, CA). In each sample, a minimum of 300,000 events was acquired. Median number of events for eosinophils was 6,262 (range 2,692-122,789), mast cells 103 (range 11-549) and lymphocytes 38,635 (range 8,002-66,597). Mast cells were identified as CD117 bright positive, CD34 negative, CD45 positive cells with characteristic forward and side scatter properties. Eosinophils were identified as CD45 positive, CD49d positive, CD16 dim positive, CD9 positive, CD14 negative, CD3 negative, CD19 negative cells cells with characteristic high side scatter properties. FlowJo (Version 8.8.2, Tree Star, Ashland, OR) software was used for data analysis. Flow sorting of bone marrow mast cells and cDNA preparation were performed as previously described[17]. Sort purity routinely exceeded 98%.

Measurement of serum levels of sIL-5Rα

Soluble IL-5Rα levels in serum were assessed using a chemiluminescence capture ELISA. Briefly, FluoroNunc Maxi-sorp plates were coated with monoclonal anti-IL-5Rα antibody (KM 1257) at 1.0 μg/ml in PBS overnight at 4°C. The plates were blocked for 1 hour with PBS/Tween/20% casein prior to incubation with sera for 2 hours at room temperature. sIL-5Rα was detected by sequential incubation with biotinylated goat anti-human IL-5Rα (R&D) and streptavidin-horseradish peroxidase (GE Healthcare). After washing with 0.2M phosphate buffer, plates were developed with FemtoGlow (Michigan Diagnostics) and read on a luminometer. All assays were performed in duplicate, and values were calculated based on a standard curve. The sensitivity of the assay was 7.8 pg/ml.

Cytokine analysis

Serum cytokine and eotaxin levels were measured by suspension array technology in multiplex using a Milliplex kit for human IL-2, IL-3, IL-5, IL-13, IL-17, GM-CSF and eotaxin (Millipore Corp, St. Charles, MO) according to the manufacturer's instructions[18]. The minimal detectable concentrations were as follows: IL-2 (0.4 pg/ml), IL-3 (9.8 pg/ml), IL-5 (0.1 pg/ml), IL-13 (0.3 pg/ml), IL-17 (0.4 pg/ml), GM-CSF (2.3 pg/ml) and eotaxin (2.1 pg/ml).

Serum EDN assay

Serum levels of EDN were measured by ELISA as described previously[19]. Briefly, Immulon 4 flat bottom plates were coated with monoclonal antibody (6D1.5/A5 anti-EDN) at 2.5 μg/ml in PBS overnight at 4°C. The plates were blocked for 1 hour with PBS/Tween/0.1% bovine serum albumin prior to overnight incubation of sera. Granule proteins were detected by sequential incubation with rabbit polyclonal anti-EDN followed by alkaline phosphatase-labeled goat anti-rabbit IgGFc and alkaline phosphatase substrate (Sigma). All assays were performed in duplicate, and values were calculated based on a standard curve. The limit of detection for the assay was approximately 2 ng/ml.

Quantification of isoforms IL-5Rα mRNA transcripts for soluble and membrane forms

Eosinophils were purified from peripheral blood by negative selection as described previously[19] and frozen in TRIzol (Life Technologies) to be processed at a later date. Total RNA was quantified using a NanoDrop, ND-1000 (NanoDrop Technologies) and 1.0 μg of RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's protocol. Approximately 50 ng of RNA equivalent cDNA template was used per 1X SYBR Green (SaBiosciences) in a final 10 μl reaction volume. Real-time amplification was performed in a 384 well plate using a GeneAmp 7900HT Sequence Detection System (Applied Biosytem). Transmembrane (TM) IL-5Rα and soluble (SOL) IL-5Rα isoform variants were amplified using a common forward primer annealing to exon 10 (5′-GCAGCAGTGAGCTCCATGTG-3′) and isoform-specific reverse primers (TM: 5′-AGGGCTTGTGTTCATCATTTCC-3′, SOL: 5′-TGGATGTTATCTCCTTTATCTTGAGAA-3′), generating PCR products of 89 bp and 95 bp, respectively[20]. Each sample was run in duplicate, and cycle threshold (C_T) values were normalized using β-actin C_T values from corresponding samples. Real-time PCR efficiencies were compared and were equivalent between IL-5Rα transcript variants using a standard curve consisting of 10-fold serial dilutions of plasmids containing their respective target sequences.

Statistical analyses

Both medians and geometric means (GM) were used as measurements for central tendency. The Mann-Whitney-U or Fisher's exact test was used for comparisons between groups and

Spearman correlations were used with approximate confidence intervals based on Fisher's Z transformation. Responses below the level of detection were given the lowest rank. *P* values were corrected for multiple comparisons where stated using the Holm's correction[21]. All analyses were performed using Prism V5.0 (GraphPad Software) or R version 2.11.1.

Results

Serum sIL-5Rα levels are elevated in patients with eosinophilia and/or mastocytosis

Levels of sIL-5Rα were measured in serum from a cohort of patients with untreated eosinophilia >1500/mm^3 (n=74), SM (n=59) or CM (n=6) and in normal controls (n=38) (see Table 1). Serum sIL-5Rα levels were positively correlated with eosinophilia in the group as a whole (r=0.56, p<0.0001, Spearman rank correlation), in subjects with eosinophilia (r=0.52, p<0.0001) and in subjects with SM (r=0.31, p=0.02), but not in normal subjects (r=−0.04, p=0.8) (Figure 1).

When serum levels of sIL-5Rα were compared among the different patient groups, as expected, sIL-5Rα levels were significantly elevated in patients with a wide variety of eosinophilic disorders as compared to normal subjects (Figure 2). However, serum levels of sIL-5Rα were also increased in patients with SM without eosinophilia (geometric mean [GM] 251 pg/ml, 95% CI 164.9, 381.9) as compared to normals (GM 42.3 pg/ml, 95% CI 22.4, 79.7). In fact, sIL-5Rα was detectable in serum from 70/75 patients with eosinophilia and 47/54 patients with SM without concomitant eosinophilia compared to 17/38 normal controls (P<0.0001, Fisher's exact tests comparing each group to normal). When the ratio of sIL-5Rα to absolute eosinophil count (AEC) was examined comparing the 8 patient groups to the normal group, only the SM group had a significantly higher ratio (sIL-5Rα/AEC) than the normal group (P=0.007 Holm's adjusted for multiple comparisons).

Paired serum samples and eosinophil counts were available from two time points for 22 subjects with eosinophilia, 2 subjects with SM and 2 normal subjects with measurable sIL5Rα levels. As expected, the change in sIL5Rα level was strongly correlated with the change in eosinophil count (r=0.66, p<0.001, Spearman rank correlation).

Elevated sIL-5Rα is associated with increased serum IL-5 levels and eosinophil activation

Since *in vitro* studies had demonstrated an increase in sIL-5Rα levels in response to stimulation with IL-5, GM-CSF and IL-3, serum levels of these, as well as other cytokines important in eosinophilia and eosinophil activation, were measured in 142 of the subjects for whom sufficient serum was available. After correction for multiple comparisons, sIL-5Rα levels were correlated only with serum IL-5 and IL-13 levels (P <0.001 and P=0.004, respectively, Spearman rank correlation; Table 2). Interestingly, serum levels of IL-13 were significantly increased in eosinophilic patients (median 2.18 pg/ml, range 0-557 pg/ml) as compared to normal controls (median 0 pg/ml, range 0-326 pg/ml; P=0.04) and correlated with eosinophil count in the group as a whole (r=0.27, p=0.0012).. Serum levels of sIL-5Rα were also correlated with serum levels of the eosinophil granule protein, eosinophil-derived neurotoxin (EDN) (r=0.55, P<0.0001, Spearman rank correlation) and with serum IgE levels (r=0.26, P=0.006, Spearman rank correlation).

Although subjects with SM and AEC <1000/mm3 had eosinophil counts that were comparable to those in the normal control group, serum levels of EDN were increased in these subjects compared to normal controls (GM 66.15 ng/ml, 95% CI 54.40, 80.43 vs. GM 26.84 ng/ml, 95% CI 17.21, 41.88; p=0.001, Mann-Whitney-U test), consistent with increased eosinophil activation in the patients with SM. As expected, EDN levels in the patients with eosinophilic disorders were significantly elevated (GM 296.3 ng/ml, CI 218.6, 401.6) compared to patients with SM and AEC <1000/mm^3 or to normal controls

(P<0.0001). The surface phenotype was assessed on peripheral blood eosinophils in 9 consecutive subjects with SM without eosinophilia. Expression of the eosinophila ctivation marker, CD69, ranged from 0.9% to 14.8% (normal range ≤2.2%) and was elevated in 6 of the 9 subjects. Surface expression of CD40 and CD25 were within normal limits.

IL-5Rα expression on peripheral blood eosinophils is negatively correlated with eosinophilia

After preliminary, semi-quantitative studies showed a decrease in membrane expression of IL-5Rα on eosinophils from eosinophilic patients as compared to normals (data not shown), surface IL-5Rα expression was quantified on peripheral blood eosinophils from patients with eosinophilic disorders (n=39), KIT D816V-positive systemic mastocytosis (n=8) and normal volunteers (n=28). As shown in Figure 3, an inverse correlation was observed between surface expression of IL-5Rα and absolute eosinophil count ($r = -0.48$, $p<0.0001$, Spearman rank correlation).

To examine the role of mRNA expression in the downregulation of surface IL-5Rα on peripheral eosinophils in vivo, transmembrane (TM) and soluble (SOL) IL-5Rα mRNA was quantified in eosinophils from 21 untreated subjects with varied eosinophilic disorders and in 3 normal controls. The level of TM IL-5Rα mRNA was negatively correlated with eosinophilia (Spearman rank correlation: r=−0.52 (−0.781, −0.096), p=0.016). Although TM IL-5Rα mRNA appeared to increase with increasing surface receptor expression (r=0.56), paired data were available for only 10 subjects, and the correlation was not statistically significant. SOL IL-5Rα mRNA levels were not correlated with eosinophil count (n=21) or soluble receptor levels (n=18).

Bone marrow mast cells may contribute to the increased levels of soluble IL-5Rα in patients with SM

In order to confirm that mast cells express IL-5Rα, bone marrow aspirates were obtained from 17 patients with KIT D816V-positive SM and 6 patients with FIP1L1/PDGFRA-negative HES. As expected, bone marrow eosinophilia was significantly increased in HES patients, whereas mast cells were markedly increased in patients with SM (See Table E1 in the Online Repository). IL-5Rα expression was detected on mast cells from all patients tested and was comparable to that seen on bone marrow eosinophils (Figure E1 in the Online Repository). To determine if mast cells are capable of producing transcript for the soluble form of the receptor, bone marrow mast cells were flow sorted from a patient with KIT D816V-positive SM. Soluble transcript was detected by RT-PCR (Figure E2). Serum levels of sIL-5Rα were positively correlated with serum tryptase, a surrogate for total mast cell burden[22], in patients with SM (r=0.62, P<0.0001, Spearman rank correlation; Figure 4a), but not in patients with eosinophilia in the absence of SM (r=0.19, P=0.2, Spearman rank correlation; Figure 4b).

Discussion

IL-5 and its receptor play a central role in eosinophilia and eosinophil activation in eosinophilic disorders and have been exploited successfully as therapeutic targets in hypereosinophilic syndrome[2], Churg-Strauss syndrome[23] and eosinophilic asthma[3,4,6]. In the current study, we examined a large cohort of patients with a broad range of peripheral eosinophil counts and quantified expression of both membrane and soluble IL-5Rα protein *in vivo*. Not only did we observe an increase in soluble receptor levels and a decrease in membrane receptor expression on eosinophils with increasing peripheral blood eosinophilia, but these changes were also correlated with serum levels of IL-5 and IL-13. In contrast to *in vitro* studies, no correlation was observed with serum levels of IL-3 or GM-CSF. Although

eosinophil production of IL-13 cannot be entirely excluded as the reason for the observed correlation with serum IL-13 levels, recent data demonstrating that T cells are the primary source of IL-13 in patients with eosinophilic lung disease[24] suggest that the relationship between IL-13 and sIL-5Rα is more likely due to a generalized skewing of the immune response towards a type 2 phenotype. This is supported by the fact that sIL-5Rα levels were also correlated with serum IgE levels. In contrast to a recent study describing the association of a polymorphism in the promoter for IL-13 with SM[25], serum IL-13 levels were not increased in our patients with SM as compared to normal controls (data not shown).

Of note, levels of sIL-5Rα were significantly increased in subjects with SM without eosinophilia. Although the expression of functional IL-5Rα on mast cells cultured *in vitro* from human cord blood derived CD133+ progenitors has been previously reported[26-28], information on IL-5Rα expression on bone marrow mast cells *ex vivo* is lacking. In the current study, we demonstrate that bone marrow mast cells from patients with either KIT D816V-positive SM or FIP1L1/PDGFRA-negative HES express IL-5Rα at levels comparable to bone marrow eosinophils. Moreover, sIL-5Rα mRNA was detected in the bone marrow mast cells of a patient with KIT D816V-positive SM, suggesting that mast cells are capable of producing the soluble form of the receptor. Serum levels of sIL-5Rα did correlate with serum tryptase levels in patients with SM, consistent with mast cells as a potential source of the increased soluble receptor levels in these patients. However, the increased surface expression of CD69 on eosinophils and elevated serum EDN levels in patients with SM without eosinophilia suggest that activated eosinophils may also play a role. Although eosinophilia has been reported in a subset of patients with SM, the mechanism of eosinophil activation in SM without peripheral eosinophilia has not been studied and may be due to mast cell production of mediators known to activate eosinophils, including IL-33 and IL-5 or direct effects of the KITD816V mutation, which has been shown to be present in eosinophils from patients with SM[29].

Modulation of IL-5Rα expression on eosinophils *in vitro* has been described in response to IL-5, IL-3, GM-CSF and IL-9[8,12-14], although the mechanism of this modulation remains unclear. Although internalization of the common β-chain of the IL-5R has been described in response to IL-5 binding and is essential for signaling[30], the mechanism of IL-5Rα modulation has been less well-studied. Some *in vitro* studies have demonstrated changes in isoform splicing in response to cytokine stimulation[12,13]; whereas others have supported cleavage of surface receptors as the primary mechanism[8]. In a mouse model of schistosomiasis, serum IL-5 levels had no effect on differential splicing of the isoforms in Siglec-F+ bone marrow cells[31]. In contrast, in a study of 34 patients with nasal polyposis (NP), the relative amount of soluble to membrane IL-5Rα mRNA expression was increased in nasal polyp tissue from asthmatic patients with NP as compared to non-asthmatic patients with NP and normal controls[32]. Furthermore, mRNA levels for the soluble and membrane isoforms were correlated (positively and negatively, respectively) with tissue eosinophilia. Serum levels of sIL-5Rα (and peripheral blood eosinophilia) were also increased in the patients with NP and asthma, although no correlation with eosinophilia was observed. In the present study, we found evidence to support modulation of membrane, but not soluble, IL-5Rα levels at the mRNA level in peripheral eosinophils *in vivo*. Whether the discordance between our results and those reported in the nasal polyp study is due to differences between blood and tissue eosinophils, technical issues or other factors remains to be elucidated.

Modulation of IL-5Rα on the cell surface, as well as increased serum levels of the soluble receptor, in subjects with eosinophilia and mastocytosis raise the possibility that agents targeting the IL-5R may be less effective in these patient groups. Although serum levels of soluble receptor exceeded 5 ng/ml in many subjects in the current study, this is unlikely to be sufficient to interfere with agents currently in development. In fact, in a recent study of

the IL-5Rα monoclonal antibody, benralizumab (MEDI-563) therapy in asthma, mean maximum antibody concentrations after a single dose of 0.03 to 3 mg/kg ranged from 1 μg/ml to 82 μg/ml and were dose-dependent[6]. The effect of downmodulation of surface receptor on dose response is less clear and will require additional studies. In summary, surface expression of IL-5Rα on eosinophils is decreased and serum levels of sIL-5Rα are increased in patients with eosinophilia and/or mastocytosis. These data are consistent with an *in vivo* IL-5R regulatory pathway in human eosinophils (and possibly mast cells) similar to that described *in vitro* and involving a balance between surface and soluble receptor levels. The observed increase in serum sIL-5Rα is correlated with serum levels of IL-5 and IL-13, as well as with serum EDN levels. Serum sIL-5Rα levels are also correlated with serum tryptase levels in patients with SM. Whether mast cells are directly responsible for the increase in sIL-5Rα observed in SM patients without eosinophilia and/or act indirectly by stimulating eosinophils remains to be elucidated. Increases in serum levels of soluble IL-5Rα and downmodulation of IL-5Rα on the cell surface in patients with eosinophilic and mast cell disorders may have important implications with respect to the efficacy of novel therapeutic agents targeting IL-5 and its receptor in these patient groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ABC	antibodies bound per cell
AEC	absolute eosinophil count
BE	benign eosinophilia
CEL	chronic eosinophilic leukemia
CM	cutaneous mastocytosis
C_T	cycle threshold
EDN	eosinophil-derived neurotoxin
EO	eosinophilia
GM-CSF	granulocyte macrophage-colony stimulating factor
HES	hypereosinophilic syndrome
IL	interleukin
IL-5R	IL-5 receptor

sIL-5R	soluble IL-5 receptor
PARA	helminth infection
PBS	phosphate-buffered saline
SOL	soluble
SM	systemic mastocytosis
TM	transmembrane
SM-eo	systemic mastocytosis with eosinophilia

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Key Messages

- Surface expression of IL-5Rα on eosinophils is decreased and serum levels of sIL-5Rα are increased in patients with eosinophilia and/or systemic mastocytosis.
- Soluble IL-5Rα levels are positively correlated with eosinophilia, serum levels of IL-5 and IL-13 and markers of eosinophil activation in all subjects and with serum tryptase levels in subjects with systemic mastocytosis.
- These findings are consistent with an *in vivo* IL-5R regulatory pathway in human eosinophils (and possibly mast cells) similar to that described *in vitro* and involving a balance between surface and soluble receptor levels and may have therapeutic implications.

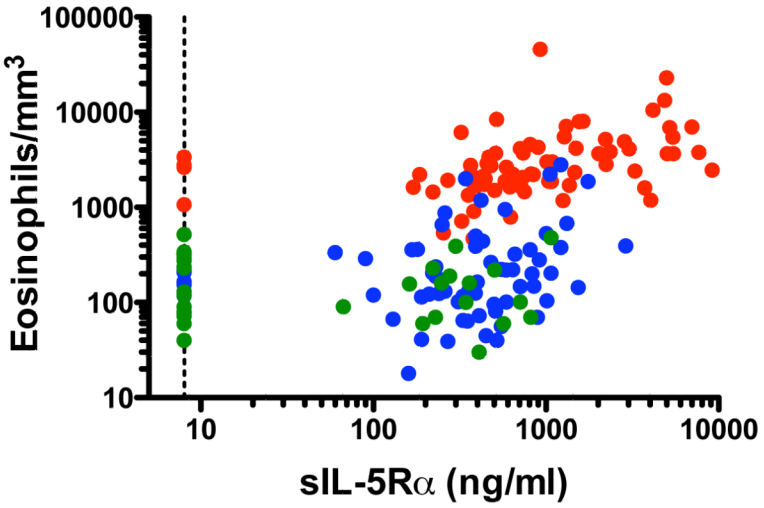

Figure 1.

Serum levels of sIL-5Rα are positively correlated with peripheral blood eosinophilia. Each circle represents an individual data point. Subjects with eosinophilic disease are shown in red, those with mastocytosis in blue and normals in green. The dotted line represents the limit of detection of the assay. $P<0.0001$, $r = 0.54$, Spearman rank correlation

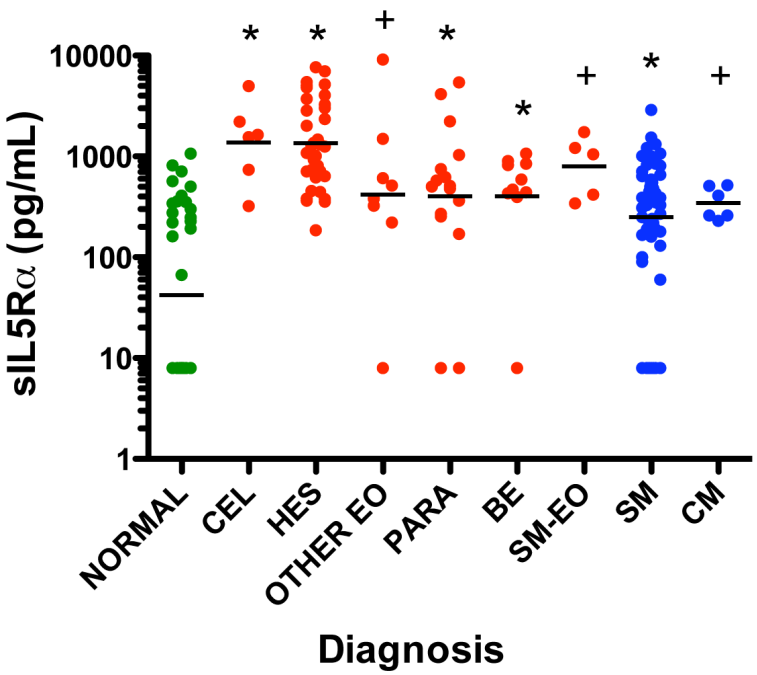

Figure 2.

Serum IL-5Rα levels are increased in patients with eosinophilia and mastocytosis without eosinophilia. Each symbol represents an individual data point. Subjects with eosinophilia are indicated in red, with mastocytosis in blue and normal controls in green. Group geometric means are indicated by horizontal lines. * $p<0.001$ or + $p<0.05$ compared to normal group (Holm's adjusted p-value using the Mann-Whitney-U test)

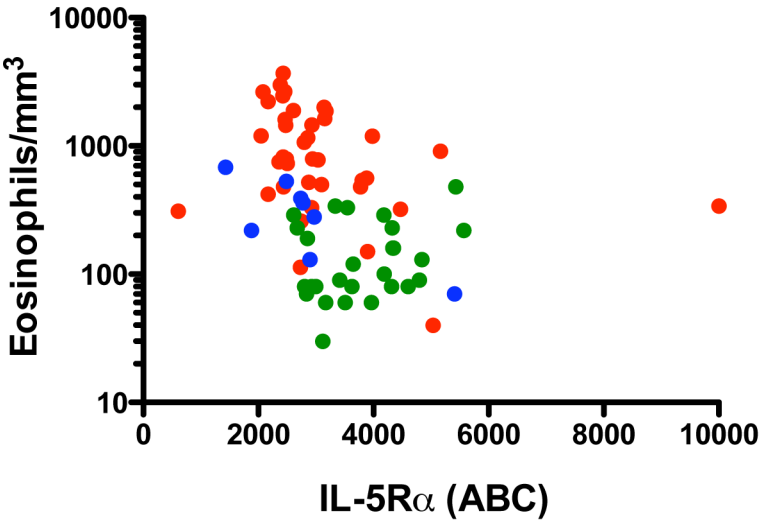

Figure 3.

Surface expression of IL-5Rα on eosinophils is inversely correlated with peripheral blood eosinophilia. Each circle represents an individual data point. Subjects with eosinophilic disease are shown in red, those with mastocytosis in blue and normals in green. ABC = antibodies bound per cell. $P<0.0001$, $r = -0.48$, Spearman rank correlation

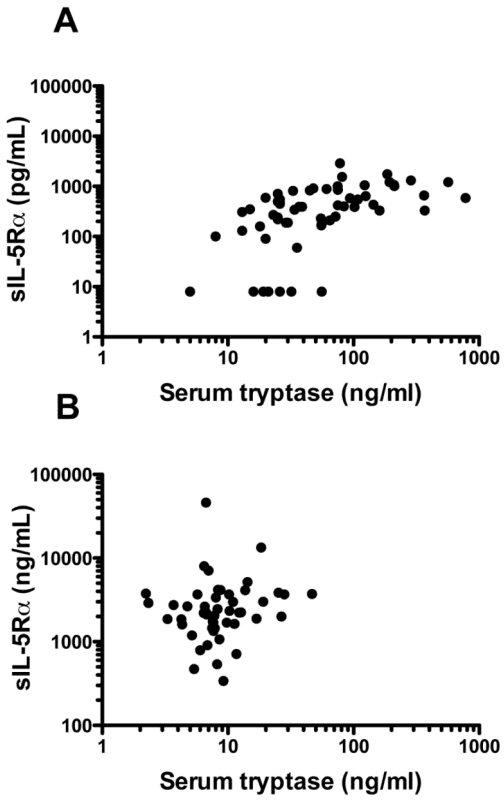

Figure 4.

Serum levels of sIL-5Rα are correlated with serum tryptase levels in patients with SM, but not HES without SM. Serum tryptase and sIL-5Rα levels are shown for subjects with SM ($r=0.62$, $P<0.0001$, Spearman rank correlation, panel A) and eosinophilia without SM ($r=0.19$, $P=0.2$, panel B). Each circle represents an individual data point.

Table 1

Demographic and laboratory characteristics of study population.

Diagnosis*	Normal (n=38)	CEL (n=6)	HES (n=34)	PARA (n=16)	BE (n=10)	Other EO (n=8)	SM-eo (n=5)	SM (n=54)	CM (n=6)
Median Age (range)	44 (22-79)	35 (17-78)	41 (1-82)	27 (11-80)	53 (28-74)	52 (32-73)	59 (54-74)	49 (21-69)	31 (20-54)
Gender (M/F)	11/31	6/0	19/15	9/7	7/3	5/3	3/2	20/34	0/6
Median eos/mm^3 (range)**	124 (30-480)	7067 (3719-23074)	2950 (471-45990)	1908 (540-10496)	2220 (1696-4270)	1818 (340-8437)	1999 (1186-2806)	164 (18-952)	106 (40-876)
Median serum tryptase ng/mL (range)	ND	14.3 (6.46-46.7)	11.2 (2.23-28.1)	7.85 (3.7-16.9)	7.18 (3.31-12.7)	8.78 (4.26-11.7)	123 (34-567)	42 (5-780)	6 (4-13)
Median serum IgE IU/mL (range)	ND	14 (4-22)	243 (4-13263)	898 (15-6293)	60 (4-628)	273 (2-1422)	4 (4-29)	7 (0-1370)	41 (6-173)

*CEL = FIP1L1/PDGFRA-positive chronic eosinophilic leukemia, HES = hypereosinophilic syndrome, including idiopathic and lymphocytic variants, PARA = helminth infection, BE = benign eosinophilia (eosinophilia >1500/mm^3 without clinical manifestations), Other EO = secondary eosinophilic disorders (e.g. neoplasms, drug hypersensitivity, allergic disease)

**Although subjects were classified on the basis of peak recorded eosinophil counts, laboratory results listed in the table are from the date of the sIL-5R assay. Consequently, the lower range of eosinophils/mm^3 is <1500/mm^3 in some eosinophilic groups. ND = not done

Table 2

Serum levels of IL-5Ra are correlated with serum levels of IL-5, IL-13, IgE and EDN, but not with serum levels of eotaxin, IL-2, IL-3, GM-CSF or IL-17

Analyte*	Spearman R	P value**
IL-2	−0.004	NS
IL-3	−0.06	NS
IL-5	0.40	**<0.0001**
IL-13	0.29	**0.004**
IL-17	−0.03	NS
eotaxin	0.05	NS
GM-CSF	0.08	NS
EDN	0.55	**<0.0001**
IgE	0.26	**0.038**

* n=142 for all cytokines, n=129 for IgE and n=151 for EDN

** Holm's adjusted for multiple comparisons