

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

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

Objective: To assess soluble and surface IL-5R α levels in patients with EO and/or mastocytosis.

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

Results: IL-5R α expression on eosinophils inversely correlated with EO ($r = -0.48$; $P < .0001$), whereas serum levels of sIL-5R α increased with the eosinophil count ($r = 0.56$; $P < .0001$) and serum IL-5 ($r = 0.40$; $P < .0001$) and IL-13 ($r = 0.29$; $P = .004$) levels. Of interest, sIL-5R α level was significantly elevated in patients with systemic mastocytosis without EO. Although sIL-5R α 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 with that in normal subjects.

Conclusions: These data are consistent with an *in vivo* IL-5R α regulatory pathway in human eosinophils similar to that described *in vitro* and involving a balance between soluble and surface receptor levels. This may have implications with respect to the use of novel therapeutic agents targeting IL-5 and its receptor in patients with EO and/or mastocytosis. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Hypereosinophilic syndrome, IL-5, mast cell, eosinophil, mastocytosis

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-5R α 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 mAbs to IL-5 itself, already proven to be effective in the treatment of both hypereosinophilic syndrome (HES)² and eosinophilic asthma,^{3,4} and an mAb to IL-5R α (benralizumab, MEDI-563), which results in enhanced susceptibility of eosinophils to antibody-dependent cell-mediated cytotoxicity.^{5,6}

A soluble form of IL-5R α (sIL-5R α) can be generated by differential splicing of mRNA transcripts⁷ or by cleavage of surface receptors.⁸ Increased serum levels of sIL-5R α have been described in subjects with acute exacerbations of chronic obstructive pulmonary disease⁹ and in patients with nasal polypsis with asthma¹⁰ and have been suggested to play a role in the downmodulation of the eosinophilic inflammatory response. In fact, recombinant sIL-5R α 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-5R α 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-5R α 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 sIL-5R α levels could interfere with the efficacy of mAbs to bind IL-5R α on eosinophils, as well as on basophils and mast cells. Despite these concerns, little is known about IL-5R α regulation in patients with peripheral eosinophilia (EO) and/or mastocytosis.

Thus, the aim of the present study was to determine whether modulation of membrane and sIL-5R α levels occurs *in vivo* in patients with marked EO and/or mastocytosis. Not only did surface and sIL-5R α levels increase with eosinophil count and serum IL-5 level, as predicted from *in vitro* studies, but serum levels of

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Abbreviations used

AEC:	Absolute eosinophil count
EDN:	Eosinophil-derived neurotoxin
EO:	Eosinophilia
HES:	Hypereosinophilic syndrome
IL-5R:	IL-5 receptor
sIL-5R:	Soluble IL-5 receptor
SM:	Systemic mastocytosis
SOL:	Soluble
TM:	Transmembrane

sIL-5R α were significantly increased in patients with systemic mastocytosis (SM) without EO. Consequently, the relative contributions of eosinophils and mast cells to sIL-5R levels were also explored.

METHODS**Patient populations**

Subjects with EO or mastocytosis underwent detailed clinical and laboratory evaluation as part of Institutional Review Board–approved clinical protocols to study these disorders (NCT00001406, NCT00044122). Healthy volunteers were recruited under an Institutional Review Board–approved clinical protocol designed to obtain controls for *in vitro* research (NCT00090662). All participants gave written informed consent. Subjects with EO were defined as subjects with a peak peripheral eosinophil count of $>1500/\text{mm}^3$ evaluated between January 1991 and November 2009 and were classified into subtypes as follows: chronic eosinophilic leukemia (presence of the *FIP1L1/PDGFR* fusion gene), HES (unexplained EO $>1500/\text{mm}^3$ with evidence of signs or symptoms attributable to the EO), parasitic (evidence of helminth infection and resolution of EO with treatment), benign EO (persistent EO $>1500/\text{mm}^3$ for more than 5 years without signs or symptoms), and secondary EO (other EO; EO $>1500/\text{mm}^3$ 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 diseases¹⁶ as SM (EO $<1000/\text{mm}^3$), SM with eosinophilia (EO $>1000/\text{mm}^3$), or cutaneous mastocytosis (urticaria pigmentosa in the absence of bone marrow involvement). Demographic and laboratory characteristics of the different patient populations are presented in Table I.

Assessment of surface receptor expression by flow cytometry

Surface expression of IL-5R α and activation markers on peripheral blood eosinophils was assessed using whole blood from all subjects evaluated for EO 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, Calif), CD25-FITC, CD40-FITC, CD9-FITC, CD69-FITC, and CD16-PE (BD Biosciences). 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 an 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 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) and CD14-APC (TuK4; Invitrogen, Carlsbad, Calif). In each sample, a minimum of 300,000 events were acquired. The median number of events for eosinophils was 6262 (range, 2692–122,789), mast cells 103 (range, 11–549), and lymphocytes 38,635 (range, 8002–66,597). 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 with characteristic high-side-scatter properties. FlowJo (Version 8.8.2, Tree Star, Ashland, Ore) 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-5R α

sIL-5R α levels in serum were assessed using a chemiluminescence capture ELISA. Briefly, FluoroNunc Maxi-sorp plates were coated with monoclonal anti-IL-5R α antibody (KM 1257) at 1.0 $\mu\text{g}/\text{mL}$ 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-5R α was detected by sequential incubation with biotinylated goat anti-human IL-5R α (R&D Systems, Minneapolis, Minn) and streptavidin-horseradish peroxidase (GE Healthcare, Piscataway, NJ). After washing with 0.2 mol/L phosphate buffer, plates were developed with FemtoGlow (Michigan Diagnostics, Royal Oak, Mich) and read on a luminometer. All assays were performed in duplicate, and values were calculated on the basis of 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 eosinophil-derived neurotoxin assay

Serum levels of eosinophil-derived neurotoxin (EDN) were measured by ELISA as described previously.¹⁹ Briefly, Immulon 4 flat-bottom plates were coated with mAb (6D1.5/A5 anti-EDN) at 2.5 $\mu\text{g}/\text{mL}$ in PBS overnight at 4°C. The plates were blocked for 1 hour with PBS/Tween/0.1% BSA prior to overnight incubation of sera. Granule proteins were detected by sequential incubation with rabbit polyclonal anti-EDN followed by alkaline phosphatase-labeled goat antirabbit immunoglobulin G, Fc fragment-specific and alkaline phosphatase substrate (Sigma-Aldrich, St Louis, Mo). All assays were performed in duplicate, and values were calculated on the basis of a standard curve. The limit of detection for the assay was approximately 2 ng/mL.

Quantification of isoforms IL-5R α 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, Carlsbad, Calif) to be processed at a later date. Total RNA was quantified using a NanoDrop, ND-1000 (NanoDrop Technologies, Wilmington, Del), and 1.0 μg of RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, Calif) according to the manufacturer's protocol. Approximately 50 ng of RNA equivalent cDNA template was used per 1X SYBR Green (SA Biosciences, Frederick, Md) in a final 10 μL reaction volume. Real-time amplification was performed in a 384-well plate by using a GeneAmp 7900HT Sequence Detection System

TABLE I. 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)
Age (y), median (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
Eosinophils (/mm ³), median (range)*	124 (30-480)	7067 (3719-23,074)	2950 (471-45,990)	1908 (540-10,496)	2220 (1696-4270)	1818 (340-8437)	1999 (1186-2806)	164 (18-952)	106 (40-876)
Serum tryptase (ng/mL), median (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)
Serum IgE (IU/mL), median (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)

BE, Benign eosinophilia (EO >1500/mm³ without clinical manifestations); CEL, FIP1L1/PDGFR α -positive chronic eosinophilic leukemia; CM, cutaneous mastocytosis; F, female; HES, hypereosinophilic syndrome, including idiopathic and lymphocytic variants; M, male; ND, not done; other EO, secondary eosinophilic disorders (eg, neoplasms, drug hypersensitivity, allergic disease); PARA, helminth infection; SM-eo, systemic mastocytosis with eosinophilia.

*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 α assay. Consequently, the lower range of eosinophils/mm³ is <1500/mm³ in some eosinophilic groups.

(Applied Biosystems). Transmembrane (TM) IL-5R α and sIL-5R α isoform variants were amplified using a common forward primer annealing to exon 10 (5'-GCAGCAGTGAGCTCCATGTG-3') and isoform-specific reverse primers (TM: 5'-AGGGCTGTGTTCATCATTCC-3', soluble: 5'-TGG ATGTTATCTCCTTTATCTTGAGAA-3'), generating PCR products of 89 and 95 bp, respectively.²⁰ Each sample was run in duplicate, and cycle threshold values were normalized using β -actin cycle threshold values from corresponding samples. Real-time PCR efficiencies were compared and were equivalent between IL-5R α transcript variants by using a standard curve consisting of 10-fold serial dilutions of plasmids containing their respective target sequences.

Statistical analyses

Both medians and geometric means (GMs) were used as measurements for central tendency. The Mann-Whitney *U* test or Fisher exact test was used for comparisons between groups, and Spearman correlations were used with approximate confidence intervals based on Fisher Z transformation. Responses below the level of detection were given the lowest rank. *P* values were corrected for multiple comparisons where stated using Holm's correction.²¹ All analyses were performed by using Prism V5.0 (GraphPad Software, La Jolla, Calif) or R version 2.11.1.

RESULTS

Serum sIL-5R α levels are elevated in patients with EO and/or mastocytosis

Levels of sIL-5R α were measured in serum from a cohort of patients with untreated EO >1500/mm³ (n = 74), SM (n = 59), or cutaneous mastocytosis (n = 6) and in serum from normal controls (n = 38) (see Table I). Serum sIL-5R α levels were positively correlated with EO in the group as a whole ($r = 0.56$; $P < .0001$; Spearman rank correlation), in subjects with EO ($r = 0.52$; $P < .0001$), and in subjects with SM ($r = 0.31$; $P = .02$), but not in normal subjects ($r = -0.04$; $P = .8$) (Fig 1).

When serum levels of sIL-5R α were compared among the different patient groups, as expected, sIL-5R α levels were significantly elevated in patients with a wide variety of eosinophilic disorders as compared with normal subjects (Fig 2). However, serum levels of sIL-5R α were also increased in patients with SM without EO (GM, 251 pg/mL; 95% CI, 164.9-381.9) as compared with normals (GM, 42.3 pg/mL; 95% CI, 22.4-79.7). In fact, sIL-5R α was detectable in serum from 70 of 75 patients with EO and 47 of 54 patients with SM and without concomitant

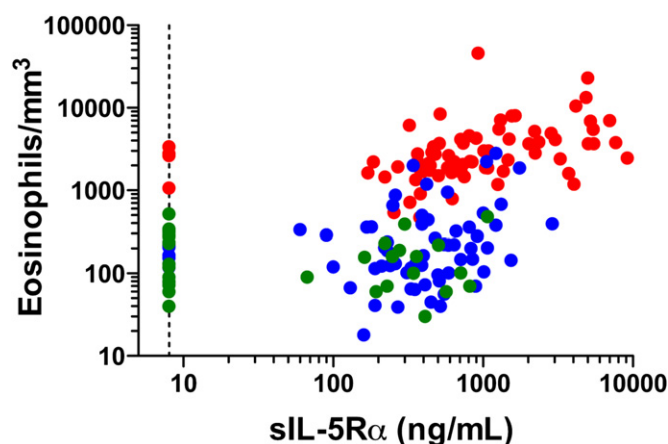


FIG 1. Serum levels of sIL-5R α are positively correlated with peripheral blood EO. Each circle represents an individual data point. Subjects with eosinophilic disease are shown in red, those with mastocytosis in blue, and normals in green. The dotted line represents the limit of detection of the assay. $r = 0.54$; $P < .0001$; Spearman rank correlation.

EO compared with 17 of 38 normal controls ($P < .0001$; Fisher exact tests comparing each group to the normal group). When the ratio of sIL-5R α to absolute eosinophil count (AEC) was examined by comparing the 8 patient groups to the normal group, only the SM group had a significantly higher ratio (sIL-5R α /AEC) than the normal group ($P = .007$; Holm's adjusted for multiple comparisons).

Paired serum samples and eosinophil counts were available from 2 time-points for 22 subjects with EO, 2 subjects with SM, and 2 normal subjects with measurable sIL5R α levels. As expected, the change in the sIL5R α level was strongly correlated with the change in the eosinophil count ($r = 0.66$; $P < .001$; Spearman rank correlation).

Elevated sIL-5R α levels are associated with increased serum IL-5 levels and eosinophil activation

Since *in vitro* studies had demonstrated an increase in sIL-5R α levels in response to stimulation with IL-5, GM-CSF, and

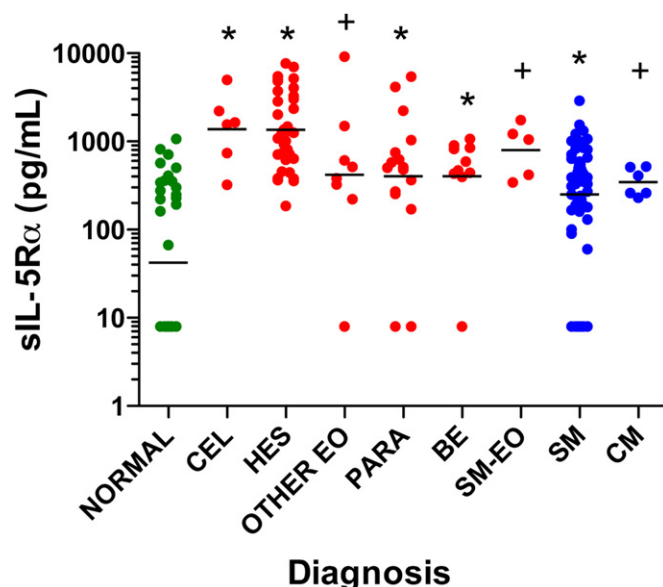


FIG 2. Serum IL-5R α levels are increased in patients with EO and mastocytosis without EO. Each symbol represents an individual data point. Subjects with EO are indicated in red, with mastocytosis in blue, and normal controls in green. Group GMs are indicated by horizontal lines. * $P < .001$ or † $P < .05$ compared with normal group (Holm's adjusted P value using the Mann-Whitney U test).

IL-3, serum levels of these, as well as of other cytokines important in EO and eosinophil activation, were measured in 142 of the subjects for whom sufficient serum was available. After correction for multiple comparisons, sIL-5R α levels were correlated only with serum IL-5 and IL-13 levels ($P < .001$ and $P = .004$, respectively; Spearman rank correlation; Table II). Interestingly, serum levels of IL-13 were significantly increased in patients with EO (median, 2.18 pg/mL; range, 0-557 pg/mL) as compared with normal controls (median, 0 pg/mL; range, 0-326 pg/mL; $P = .04$) and correlated with eosinophil count in the group as a whole ($r = 0.27$; $P = .0012$). Serum levels of sIL-5R α were also correlated with serum levels of the eosinophil granule protein EDN ($r = 0.55$; $P < .0001$; Spearman rank correlation) and with serum IgE levels ($r = 0.26$; $P = .006$; Spearman rank correlation).

Although subjects with SM and AEC $<1000/\text{mm}^3$ had eosinophil counts that were comparable with those in the normal control group, serum levels of EDN were increased in these subjects compared with 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; $P = .001$; 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; 95% CI, 218.6-401.6) compared with patients with SM and AEC $<1000/\text{mm}^3$ or with normal controls ($P < .0001$). The surface phenotype was assessed on peripheral blood eosinophils in 9 consecutive subjects with SM and without EO. Expression of the eosinophila activation marker CD69 ranged from 0.9% to 14.8% (normal range, $\leq 2.2\%$) and was elevated in 6 of the 9 subjects. Surface expression of CD40 and CD25 was within normal limits.

TABLE II. Serum levels of IL-5R α 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	<.0001
IL-13	0.29	.004
IL-17	-0.03	NS
Eotaxin	0.05	NS
GM-CSF	0.08	NS
EDN	0.55	<.0001
IgE	0.26	.038

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

†Holm's adjusted for multiple comparisons.

IL-5R α expression on peripheral blood eosinophils is negatively correlated with EO

After preliminary, semiquantitative studies showed a decrease in membrane expression of IL-5R α on eosinophils from patients with eosinophilic disorders as compared with normal controls (data not shown), surface IL-5R α expression was quantified on peripheral blood eosinophils from patients with eosinophilic disorders ($n = 39$), *KIT* D816V-positive SM ($n = 8$), and normal volunteers ($n = 28$). As shown in Fig 3, an inverse correlation was observed between surface expression of IL-5R α and AEC ($r = -0.48$; $P < .0001$; Spearman rank correlation).

To examine the role of mRNA expression in the down-regulation of surface IL-5R α on peripheral eosinophils *in vivo*, TM and sIL-5R α mRNA was quantified in eosinophils from 21 untreated subjects with varied eosinophilic disorders and in 3 normal controls. The level of TM IL-5R α mRNA was negatively correlated with EO (Spearman rank correlation: $r = -0.52$; $P = .016$). Although TM IL-5R α 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-5R α mRNA levels were not correlated with eosinophil count ($n = 21$) or soluble (SOL) receptor levels ($n = 18$).

Bone marrow mast cells may contribute to the increased levels of sIL-5R α in patients with SM

To confirm that mast cells express IL-5R α , bone marrow aspirates were obtained from 17 patients with *KIT* D816V-positive SM and 6 patients with *FIP1L1/PDGFR*A-negative HES. As expected, bone marrow EO was significantly increased in patients with HES, whereas mast cells were markedly increased in patients with SM (see Table E1 in this article's Online Repository at www.jacionline.org). IL-5R α expression was detected on mast cells from all patients tested and was comparable to that seen on bone marrow eosinophils (see Fig E1 in this article's Online Repository at www.jacionline.org). To determine whether mast cells are capable of producing transcript for the SOL form of the receptor, bone marrow mast cells were flow sorted from a patient with *KIT* D816V-positive SM. The SOL transcript was detected by RT-PCR (see Fig E2 in this article's Online Repository at www.jacionline.org). Serum levels of sIL-5R α were positively correlated with serum tryptase, a surrogate for

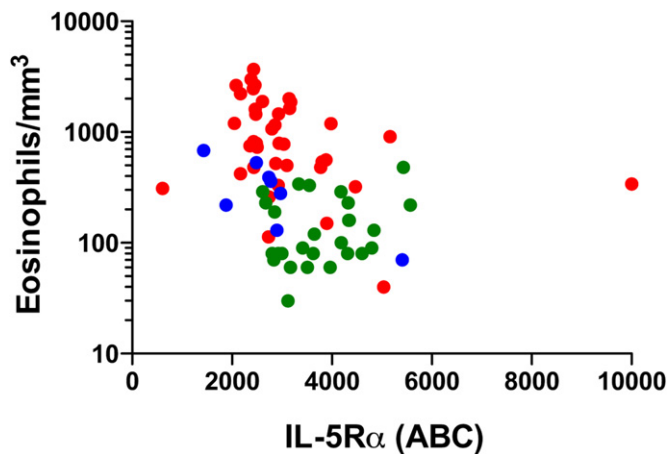


FIG 3. Surface expression of IL-5R α on eosinophils is inversely correlated with peripheral blood EO. Each circle represents an individual data point. Subjects with eosinophilic disease are shown in red, those with mastocytosis in blue, and normal controls in green. ABC, Antibodies bound per cell. $r = -0.48$; $P < .0001$; Spearman rank correlation.

total mast cell burden,²² in patients with SM ($r = 0.62$; $P < .0001$; Spearman rank correlation; Fig 4, A), but not in patients with EO in the absence of SM ($r = 0.19$; $P = .2$; Spearman rank correlation; Fig 4, B).

DISCUSSION

IL-5 and its receptor play a central role in EO and eosinophil activation in eosinophilic disorders and have been exploited successfully as therapeutic targets in HES,² Churg-Strauss syndrome,²³ and eosinophilic asthma.^{3,4,6} In the present study, we examined a large cohort of patients with a broad range of peripheral eosinophil counts and quantified expression of both membrane and sIL-5R α protein *in vivo*. Not only did we observe an increase in SOL receptor levels and a decrease in membrane receptor expression on eosinophils with increasing peripheral blood EO, 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 disease²⁴ suggest that the relationship between IL-13 and sIL-5R α is more likely due to a generalized skewing of the immune response toward a type 2 phenotype. This is supported by the fact that sIL-5R α 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 with normal controls (data not shown).

Of note, levels of sIL-5R α were significantly increased in subjects with SM without EO. Although the expression of functional IL-5R α on mast cells cultured *in vitro* from human cord blood–derived CD133⁺ progenitors has been previously reported,²⁶⁻²⁸ information on IL-5R α 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/PDGFR α -negative HES express IL-5R α

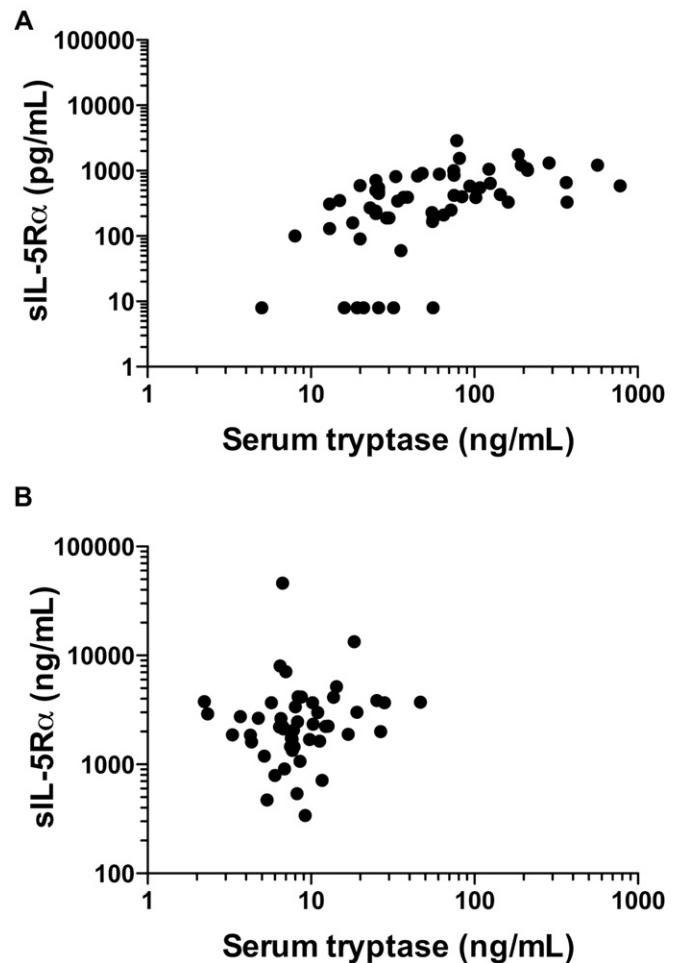


FIG 4. Serum levels of sIL-5R α are correlated with serum tryptase levels in patients with SM, but not HES without SM. Serum tryptase and sIL-5R α levels are shown for subjects with SM ($r = 0.62$; $P < .0001$; Spearman rank correlation, panel A) and EO without SM ($r = 0.19$; $P = .2$; panel B). Each circle represents an individual data point.

at levels comparable to bone marrow eosinophils. Moreover, sIL-5R α 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 SOL form of the receptor. Serum levels of sIL-5R α did correlate with serum tryptase levels in patients with SM, consistent with mast cells as a potential source of the increased SOL receptor levels in these patients. However, the increased surface expression of CD69 on eosinophils and elevated serum EDN levels in patients with SM without EO suggest that activated eosinophils may also play a role. Although EO has been reported in a subset of patients with SM, the mechanism of eosinophil activation in SM without peripheral EO 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 *KIT* D816V mutation, which has been shown to be present in eosinophils from patients with SM.²⁹

Modulation of IL-5R α 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-5R α 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 SOL to membrane IL-5R α mRNA expression was increased in nasal polyp tissue from asthmatic patients with NP as compared with nonasthmatic patients with NP and normal controls.³² Furthermore, mRNA levels for the SOL and membrane isoforms were correlated (positively and negatively, respectively) with tissue EO. Serum levels of sIL-5R α (and peripheral blood EO) were also increased in the patients with NP and asthma, although no correlation with EO was observed. In the present study, we found evidence to support modulation of membrane, but not sIL-5R α 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-5R α on the cell surface, as well as increased serum levels of the SOL receptor in subjects with EO and mastocytosis raise the possibility that agents targeting the IL-5R may be less effective in these patient groups. Although serum levels of the SOL 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-5R α mAb, benralizumab (MEDI-563) therapy in asthma, mean maximum antibody concentrations after a single dose of 0.03 to 3 mg/kg ranged from 1 to 82 μ g/mL and were dose dependent.⁶ The effect of downmodulation of the surface receptor on dose-response is less clear and will require additional studies. In summary, surface expression of IL-5R α on eosinophils is decreased and serum levels of sIL-5R α are increased in patients with EO 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 SOL receptor levels. The observed increase in serum level of sIL-5R α is correlated with serum levels of IL-5 and IL-13, as well as with serum EDN levels. Serum sIL-5R α levels are also correlated with serum tryptase levels in patients with SM. Whether mast cells are directly responsible for the increase in sIL-5R α levels observed in patients with SM without EO and/or act indirectly by stimulating eosinophils remains to be elucidated. Increases in serum levels of sIL-5R α and downmodulation of IL-5R α 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.

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.

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Key messages

- Surface expression of IL-5R α on eosinophils is decreased and serum levels of sIL-5R α are increased in patients with eosinophilia and/or systemic mastocytosis.
- Soluble IL-5R α 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.

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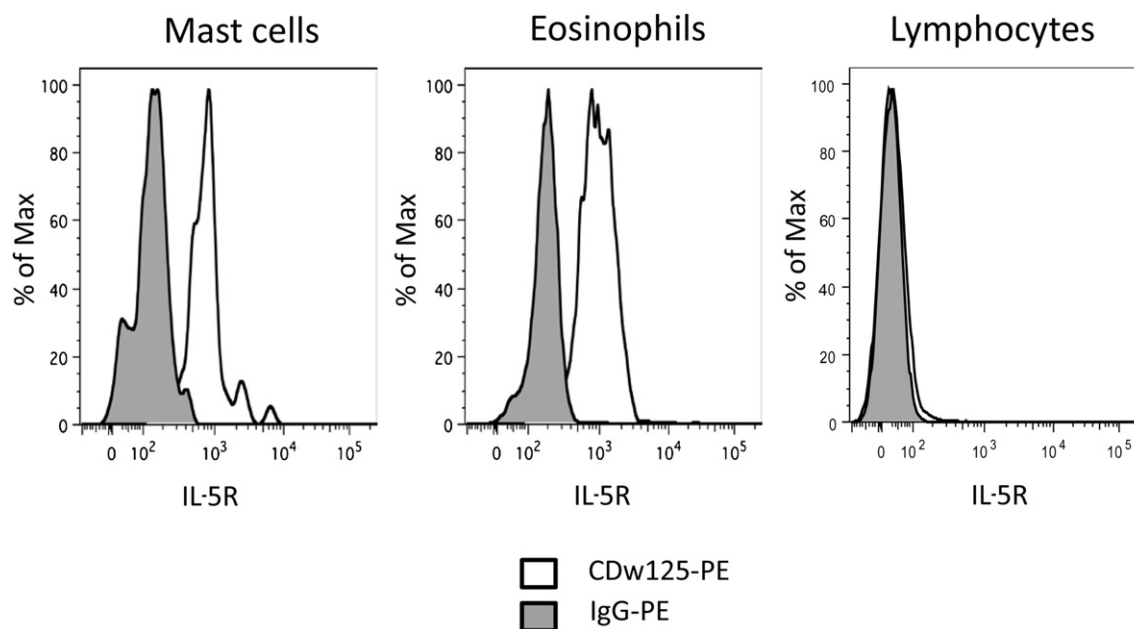


FIG E1. Bone marrow mast cells express IL-5R α on their surface. A representative histogram depicts surface expression of IL-5R α on bone marrow mast cells (*left panel*) and eosinophils (*middle panel*) from a patient with *KITD816V*-positive SM. IL-5R α surface expression was not detected on the patient's bone marrow lymphocytes (*right panel*). The area under the curve for the isotype control (IgG-PE) is indicated in *gray* and for IL-5R α (CDw125-PE) in *white*.

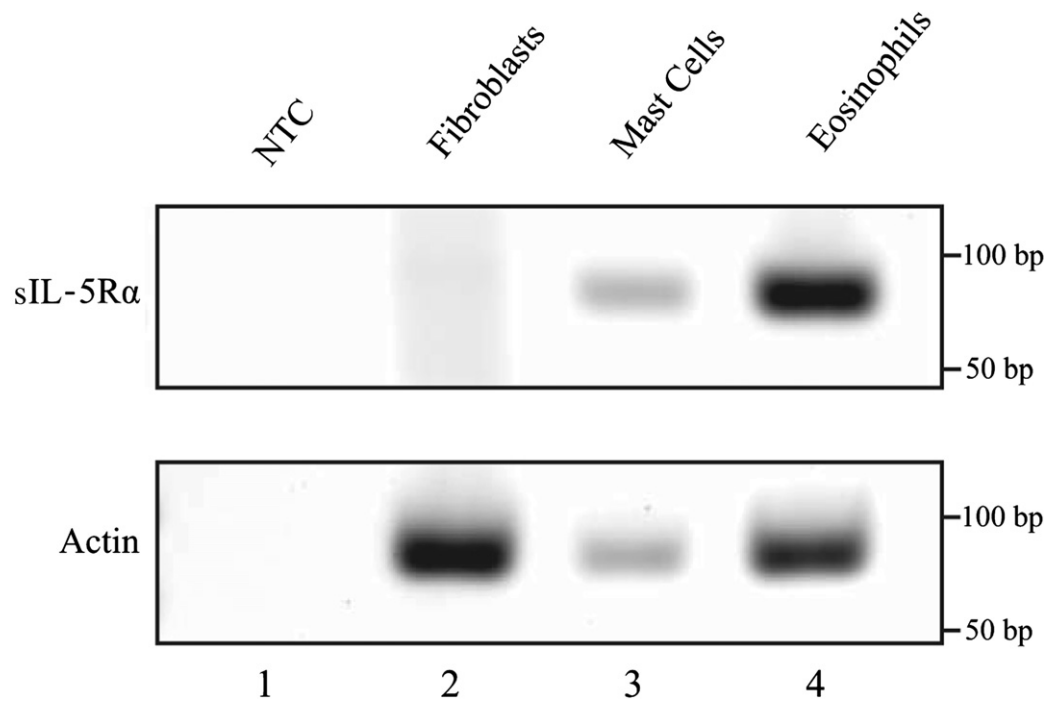


FIG E2. Human bone marrow mast cells express mRNA for sIL5R α . Total RNA was isolated from human fibroblasts (ATCC cell line CCL-202), flow-sorted human bone marrow mast cells from a patient with SM, and purified human peripheral blood eosinophils reverse-transcribed and amplified using primers specific for sIL5R α and β -actin as described in the Methods section. The PCR products were run on a 2% agarose gel and visualized with ethidium bromide. *NTC*, No template control.

TABLE E1. Bone marrow features of subjects with SM or HES

Feature	D816V-positive SM without peripheral EO (n = 17)	FIP1L1/PDGFR α -negative HES (n = 6)	<i>P</i> value*
Cellularity (%), median (range)	55 (30-82)	58 (35-75)	.852
Eosinophils (%), median (range)	5 (1-12)	13 (2-27)	.044
Presence of mast cell aggregates	17/17	1/6†	<.001
Mast cell aggregates (%), median (range)	13 (3-55)	NA	NA
Spindle-shaped mast cells >25%	17/17	1/6	<.001
Presence of CD2 ⁺ mast cells	16/16	0/6	<.001
Presence of CD25 ⁺ mast cells	16/16	1/6	<.001

*Holm's adjusted for multiple comparisons.

†One small mast cell aggregate was seen.