

Original article

Enhanced soluble interleukin-5 receptor alpha expression in nasal polyposis

Background: Alternative splicing of the interleukin-5 receptor alpha (IL-5R α)-subunit leads to the generation of a signalling, membrane-anchored (TM) isoform, or a secreted [soluble (SOL)], antagonistic variant. Given the key role of IL-5 in eosinophil function, we investigated SOL IL-5R α expression pattern in an eosinophil-associated disease such as nasal polyposis (NP).

Methods: An SOL IL-5R α enzyme-linked immunosorbent assay and quantitative real-time polymerase chain reaction (PCR) were established and applied in serum, nasal secretion and nasal tissue of controls ($n = 12$), and NP patients ($n = 42$) with or without asthma.

Results: Analysis of serum, nasal secretion, and nasal tissue samples revealed that SOL IL-5R α protein concentrations were significantly increased in NP vs control tissue. Within the NP group, there was a significant up-regulation of SOL IL-5R α in patients with systemic airway disease. These findings were confirmed at the mRNA level, using an optimized real-time reverse-transcriptase PCR procedure.

Conclusions: This report demonstrates SOL IL-5R α transcript and protein up-regulation in NP. Soluble IL-5R α differentiates nasal polyps with or without concomitant asthma. As SOL IL-5R α is strongly up-regulated for disease and has antagonistic properties *in vitro*, our studies shed new light on the mechanisms of specific immunomodulatory therapies, such as anti-IL-5.

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Key words: asthma; enzyme-linked immunosorbent assay; eosinophils; interleukin-5 receptor alpha; nasal polyps; polymerase chain reaction.

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Interleukin-5 (IL-5) is a haematopoietic growth factor essential for eosinophil development, activation, and survival. Eosinophil is regarded as an important effector cell in various chronic pathologies including asthma, allergic rhinitis, and nasal polyposis (NP). However, the recent failure to demonstrate clinical efficacy of humanized antibodies directed against IL-5 in human asthma trials has led to a re-evaluation of the role of eosinophils (1). A better understanding of the biology of IL-5 and the regulation of its receptors seems to be mandatory for understanding the effect of anti-IL-5 treatment in eosinophil-associated diseases.

Nasal polyposis is an excellent model for eosinophil-associated diseases, as 80–90% of bilateral nasal polyps are characterized by abundant eosinophilic infiltration. Nasal polyps are swellings of the lamina propria mucosa, a pathology that is frequently associated with asthma and often treated by surgical removal of polyps that block airways, facilitating the use of this tissue for investigation. High amounts of IL-5 can be detected at the mRNA as

well as at the protein level in NP (2, 3). The highest levels of IL-5 protein were observed in polyp homogenates of patients with generalized eosinophilic diseases such as asthma and aspirin sensitivity (2, 4, 5).

The biological signal from IL-5 is mediated through a receptor consisting of a specific IL-5-binding α -chain, and a signal-transducing common β -chain, which is shared with the receptors for IL-3 and granulocyte macrophage-colony stimulating factor (GM-CSF) (6, 7). When the β -chain associates with the specific IL-5-binding α -chain, a high affinity complex is formed (8). In contrast to the pan-haematopoietic β c-chain, the IL-5R α receptor seems to be expressed on a restricted range of cell types in humans, principally eosinophils, basophils, and their precursors. However, recent publications have demonstrated eosinophil-independent airway hyperresponsiveness and IL-5R α gene expression in human bronchus smooth muscle (9, 10).

The IL-5 receptor α -subunit (IL-5R α) can appear in either a membrane-anchored (TM) active form or a soluble (SOL) variant with antagonistic properties *in vitro* (6, 11). The various expression patterns of these forms are regulated at the transcriptional level by alternative splicing (12). The antagonistic properties of SOL IL-5R α have been demonstrated in binding assays, eosinophil differentiation assays (11), and in nasal tissue explants (13). It is suggested that eosinophils are able to control

Abbreviations: SOL IL-5R α , soluble interleukin-5 receptor alpha; TM IL-5R α , membrane-anchored interleukin-5 receptor alpha; NP, nasal polyposis; ECP, eosinophil cationic protein; CV, coefficient of variation; ROC, receiver operating characteristic; IQR, interquartile ranges.

their responsiveness to IL-5 by regulated expression of the IL-5R α isoforms. Surprisingly, mature, circulating eosinophils predominantly express the SOL IL-5R α transcript (own data) (11), which raises the question whether this reflects a role *in vivo*. However, to date, the human SOL IL-5R α protein could not be demonstrated in clinical samples or in supernatants of cultured eosinophils.

The aims of this study were to develop specific enzyme-linked immunosorbent assays (ELISAs) for studying the SOL IL-5R α isoform at the protein level and to set up a polymerase chain reaction (PCR)-based approach to monitor the expression of the SOL IL-5R α mRNA, and to test the diagnostic potential of SOL IL-5R α in eosinophil-associated disease such as NP.

Methods

Patients

We collected serum, nasal secretions, and nasal polyp tissue of 42 nasal polyp patients (13 females/29 males; mean age 42.6 years, range 22–78 years) during routine sinus surgery at the Department of Otorhinolaryngology at the Ghent University Hospital, Belgium. Bilateral polyposis was diagnosed based on history, clinical examination, nasal endoscopy, and sinus CT-scan. A history of asthma was reported in 18 nasal polyp patients, with nine subjects additionally demonstrating a history of aspirin sensitivity. Twenty-one patients were atopic, as indicated by positive cutaneous skin prick test responses to common aeroallergens. Subjects were excluded if they had received any steroids or antibiotics during the month before the surgery.

Control serum, nasal secretions, and nasal tissue (inferior turbinates) were obtained from 12 subjects during routine septal surgery. All control subjects (five females/seven males; mean age 33.6 years, range 18–71 years) were skin prick test negative, in generally good health, and none had a history of nasal or sinus disease, allergic disease (asthma, rhinitis or dermatitis), upper respiratory tract infection in the previous month, use of any intranasal medications, decongestants, antihistamines or oral steroids. The ethics committee of the Ghent University Hospital approved this study and a written informed consent was obtained from all subjects.

IL-5R α ELISA

Production of monoclonal antibodies. For the production of monoclonal antibodies (mAbs) against SOL IL-5R α , Balb/C mice were immunized with 30 μ g Baculovirus-secreted human IL-5R α in complete Freund's adjuvant/incomplete Freund's adjuvant (CFA/IFA). Monoclonal antibodies were generated by fusing spleen cells of these immunized mice with SP2/0 myeloma cells. Stable hybridomas were obtained by screening on SOL IL-5R α coated plates. Eleven hybridomas that produced specific antibodies against SOL IL-5R α were selected, classified into four complementation groups, grown under serum-free conditions. The supernatants were harvested and purified on Protein A Sepharose 4 Fast Flow (Pharmacia, Uppsala, Sweden).

Characterization of the mAbs. A checker board experiment was set up whereby each mAb was cross-paired with the other mAbs in an ELISA format. Briefly, microtitre plates were coated with SOL IL-5R α . For this, sub-saturating concentrations for each biotinyl-

ated antibody were determined first. Then competition between biotinylated and unlabelled antibodies for binding to coated SOL IL-5R α was performed.

FDC-P1-C17 originated by transfection of the FDC-P1 cell line to stably express the TM IL-5R α at high levels. Cells were incubated with mAbs at 2 μ g/ml, stained with Alexa488 conjugated anti-mouse Ig (Molecular Probes, Leiden, the Netherlands) and analysed by Fluorescence Activated Cell Sorter (FACS). Nontransfected FDC-P1 cells were used as a negative control.

Development of an ELISA for SOL IL-5R α . Very careful assessment of antibody selection resulted in the development of several different ELISAs. Based on background, sensitivity, and reproducibility in human samples, a sandwich ELISA for SOL IL-5R α detection was selected with combinations of mAbs from groups 2 (15B8) and 4 (1G3) (Innogenetics, Ghent, Belgium).

Microtitre plates (Maxisorb, Nunc, Denmark) were coated overnight with 1G3 at 2 μ g/ml. The plates were washed once with washing buffer (PBS + 0.02% Tween20), blocked for 30 min with blocking buffer (PBS + 0.1% casein), and incubated for 2 h at room temperature on a shaker with the samples or standards diluted in blocking buffer. A cellular extract of COS cells transfected with IL-5R α was used as standard. The concentration of the extract was determined with purified Baculo IL-5R α as standard. After washing, the plates were incubated for 1 h with the biotinylated mAb 15B8 at 0.5 μ g/ml in blocking buffer. The plates were washed and incubated for 30 min with HRP-conjugated streptavidin (Jackson, West Grove, PA) at 100 ng/ml. After washing, the plates were incubated with Tetramethylbenzidine (TMB; Roche, Brussels, Belgium) in phosphate-citrate buffer at pH 4.3 for 30 min. The reaction was stopped by acidification and the plates were read at 450–620 nm.

In vitro bioassay for hIL-5. The Ba/F3-hIL-5R-Luc cells originated by transfection of the Ba/F3 cell line to stably express the IL-5R α . Additionally, luciferase cDNA, driven by a constitutive CMV promoter, was co-transfected and stably integrated into the cell line. Chemiluminescent luciferase detection correlated with proliferation that was dependent on the hIL-5 concentration.

To evaluate the neutralizing capacity of the mAbs the cells were incubated with a low concentration of hIL-5 alone or together with a varying concentration of the mAbs. After 48 h of incubation, the cells were assayed for luciferase by chemiluminescence measurement.

Characterization of the IL-5R α ELISA. The reproducibility of the SOL IL-5R α ELISA was assessed in five different serum samples by serial testing (20 times). The recovery in the ELISA was determined by spiking 400 pg/ml of recombinant IL-5R α in seven different serum samples. To exclude possible interference of IL-5 in ELISA for SOL IL-5R α the standard curve (in blocking buffer; PBS + 0.1% casein) was run both with and without the addition of 250 pg/ml rhIL-5 (R & D Systems, Minneapolis, MN). A similar experiment was carried out on five different serum samples. All serum samples were diluted 1/5 in blocking buffer (PBS + 0.1% casein) and a two-fold dilution series ranging from 500 to 7.8 pg/ml SOL IL-5R α was added. Further, blocking experiments were carried out on three serum and three tissue samples by preincubation with 2 μ g/ml capture antibody (1G3) for 2 h at 4°C.

Measurement of IL-5 R α protein concentrations. All freshly obtained samples were immediately processed, separated, and stored in aliquots at –80°C until analysis. Blood samples were allowed to clot at room temperature for 20–30 min, centrifuged at 1500 g for 10 min at 4°C. Nasal secretions were obtained by placing sinus packs (IVALON® 4000 plus, Surgical products M-Pact,

Eudora, USA) in both nasal cavities for exactly 5 min. The quantity of secretions collected was determined by comparing the weight of the sinus pack before and after insertion. In order to mobilize the nasal secretions out of the sinus pack, 3 ml of 0.9% NaCl solution was added to the tube, which was stored at 4°C for 2 h. The sinus pack was then placed into the shaft of a syringe (placed into another tube) and centrifuged at 1500 *g* for 10 min at 4°C to recover all fluid. Nasal tissue specimens were weighed, and 1 ml of 0.9% NaCl solution was added for every 0.1 g of tissue. The tissue was then homogenized with a mechanical homogenizer (B. Braun Melsungen, Melsungen, Germany) at 1500 *g* for 5 min on ice as described previously (4, 5). After homogenization, the suspensions were centrifuged at 1500 *g* for 10 min at 4°C, and the supernatants separated and stored at -80°C until analysis.

All samples were assayed by a research ELISA for SOL IL-5R α (described above, Innogenetics) and IL-5 (R & D Systems). For comparison to an established marker, eosinophil cationic protein (ECP) (3) was measured by the Uni-CAP system (Pharmacia & Upjohn, Uppsala, Sweden).

IL-5R α real time PCR

As a standard, we used PCR products generated from plasmids containing the cDNA sequences for SOL hIL-5R α . The cDNA sequence of the SOL isoform was amplified using a forward primer complementary to a common sequence of hIL-5R α (nts 1033–1056) and a SOL hIL-5R α specific reverse primer (nts 1279–1298). All primers were designed using the Primer Express 2.0 Software (Applied Biosystems, Foster City, CA) and purchased from Invitrogen, Merelbeke, Belgium. The PCR resulted in a unique band that was purified by using the PCR Gel extraction QIAquick (Qiagen, Hilden, Germany) spin columns. The purified fragments were quantified using PicoGreen reagent (Molecular Probes). Equimolar 10-fold dilutions of the PCR products were used to generate standard curves.

Measurement of SOL IL-5 R α mRNA in blood and nasal tissue. The RNA was isolated from snap frozen blood and nasal tissue of seven controls and 20 NP patients using the Rneasy Kit (Qiagen). Total RNA was quantified using RiboGreen Kit (Molecular Probes) and 0.5 μ g was reverse transcribed using Superscript II Reverse Transcriptase (Invitrogen) and random hexamers (Amersham Pharmacia Biotech, Buckinghamshire, UK). Complementary DNA equivalent to 25 ng was used to perform the real time PCR. The real time amplifications were performed using the 1X SYBR Green I Mastermix (Qiagen) and a set of primers including a common and a specific primer for SOL hIL-5 R α . All the PCRs were performed in a 5700 SDS thermal cycler (Applied Biosystems). Each sample was tested in duplicate. The quantity of each amplicon was calculated from the values of each standard curve and normalized by the quantities obtained for β -actin transcripts (14).

Statistical analysis

Data are expressed as median and interquartile range (IQR). When comparisons were made between groups, significant between-group variability was first established using Kruskal–Wallis test. The Mann–Whitney *U*-test was then used for between-group (unpaired) comparison. Spearman rank correlation coefficient (*r*) was used to assess the relationships between the parameters. The intra- and inter-assay precision was expressed as coefficient of variation (%CV). The ability of a test to discriminate diseased cases from normal cases was evaluated using receiver operating characteristic (ROC) curve analysis. The cut-off values corresponding with the highest accuracy (minimal false negative and false positive results)

were selected and the subsequent sensitivity and specificity were used. Further, comparison of ROC curves was performed to test the statistical significance difference between the areas under two ROC curves. *P*-values <0.05 were considered statistically significant.

Results

IL-5R α ELISA

Careful assessment of antibody selection from different complementation groups resulted in several useful ELISAs. Based on background, sensitivity, and reproducibility in human samples, one sandwich ELISA consisting of two mAbs (1G3/15B8biot) for detection of SOL IL-5R α was selected.

These antibodies were further characterized by FACS analysis and using an IL-5-specific bioassay. As shown in Fig. 1A, strong staining of cells expressing the IL-5R α is

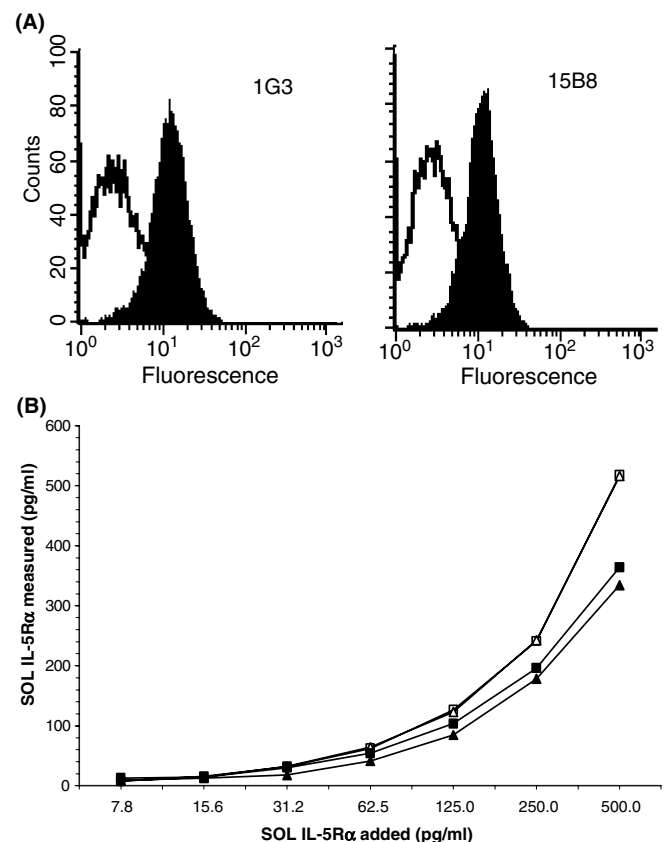


Figure 1. Characterization of monoclonal antibodies (mAb) and SOL IL-5R α ELISA. (A) FACS analysis using mAb 1G3 in the upper and 15B8 in the lower histogram. Open and filled curves represent control FDC-P1 and hIL-5R α -expressing FDC-P1-C17 cells, respectively. (B) To exclude interference of IL-5 with the SOL IL-5R α ELISA, standard curves (7.8–500 pg/ml SOL IL-5R α) were run in blocking buffer (PBS + 0.1% casein) in the absence (□) or presence of 250 pg/ml rhIL-5 (△). A similar experiment was carried out in serum that was diluted 1/5 in blocking buffer in the absence (■) or presence of 250 pg/ml rhIL-5 (▲).

Table 1. The SOL IL-5R α expression in nasal tissue and serum median and IQRs of SOL IL-5R α , IL-5, ECP concentrations and eosinophil percentages are compared in nasal tissue, nasal secretions and serum of controls and nasal polyp patients with or without asthma

	Controls (n = 12) median ($\pm$ IQR)	Versus (Mann–Whitney)	NP (n = 24) Median ($\pm$ IQR)	Versus (Mann–Whitney)	NP & Asthma (n = 18) median ($\pm$ IQR)	Versus (Mann–Whitney)
Nasal tissue						
SOL IL-5 R α (pg/ml)	885 (738–1453)	$P = 0.004$	2496 (1244–7287)	$P < 0.001$	26096 (17640–34287)	$P < 0.001$
IL-5 (pg/ml)	All BDL	$P = 0.002$	100 (43–301)	$P = 0.04$	230 (112–420)	$P < 0.001$
Ratio Sol IL-5 R α /IL-5	/	/	21 (11–41)	$P = 0.002$	95 (32–284)	/
ECP (μ g/l)	220 (94–306)	$P < 0.001$	3268 (856–7036)	$P < 0.001$	15737 (11331–28544)	$P < 0.001$
Eosinophils (%)	0.3 (0–0.7)	$P = 0.001$	5.3 (1.2–21.72)	NS	20.7 (8.7–24.6)	$P < 0.001$
Nasal Secretion						
SOL IL-5 R α (pg/ml)	519 (301–595)	$P = 0.07$	667 (430–1135)	$P < 0.001$	7568 (3389–12869)	$P < 0.001$
IL-5 (pg/ml)	All BDL	/	15/24 BDL	NS	118 72 (30–172)	$P = 0.006$
ECP (μ g/l)	139 (45–300)	$P = 0.03$	516 (200–853)	$P = 0.02$	1064 (389–2243)	$P < 0.001$
Serum						
SOL IL-5 R α (pg/ml)	286 (237–426)	NS	369 (282–562)	$P = 0.01$	759 (475–1030)	$P = 0.005$
ECP (μ g/l)	12 (11–16)	NS	25 (18–29)	NS	43 (23–65)	$P = 0.008$
Eosinophils (%)	0.9 (0.4–1.8)	$P = 0.002$	4.8 (2.6–7.1)	$P = 0.007$	9.4 (6.8–16.9)	$P < 0.001$

BDL, below detection level; NS, not significant; NP, nasal polyps; IQR, interquartile range; SOL IL-5 R α , soluble interleukin-5 receptor alpha; ECP, eosinophil cationic protein.

observed for both antibodies. In an *in vitro* bioassay on Ba/F3-hIL-5R-Luc cells, 15B8 was shown to neutralize IL-5 activity, whereas all other antibodies (including 1G3) were non-neutralizing. Because 15B8 is a neutralizing antibody, the potential noncapture of an IL-5 occupied SOL receptor could bias the results of the ELISA. However, competition experiments between IL-5 and 15B8, analysed by FACS, revealed that at equimolar concentrations 15B8 was able to chase IL-5 from its receptor, even when IL-5 was added 1 h before 15B8. This indicates that the possibility to underestimate the level of receptor, in samples with IL-5 present, is low or negligible, particularly when 15B8 is used as secondary antibody. Furthermore, standard curves in buffer and in human serum were not affected by addition of IL-5, excluding cross interference by endogenous IL-5 (Fig. 1B).

The established ELISA reached a sensitivity of 8 pg/ml for detection of SOL IL-5R α . The intra-assay precision was assessed in five different serum samples by serial testing (20 times) in one run. The CV ranged from 3.1 to 7.0%. The inter-assay precision was determined in five different serum samples by determinations in 20 separate runs, resulting in a %CV of <10% and therefore acceptable. The average recovery – after addition of 400 pg/ml of recombinant SOL IL-5R α in human serum – was 95% with a range between 83 and 103%. Addition of 2 μ g/ml capture antibody (1G3) significantly blocked (86–97%) SOL IL-5R α detection in human serum samples.

SOL IL-5R α protein expression is increased in NP. Analysis of SOL IL-5R α , IL-5, and ECP in supernatants prepared from tissue homogenates demonstrated that the concentrations of these mediators were significantly higher in NP tissue, compared with normal control tissue

(Table 1). Within the NP group, there was a significant increase in SOL IL-5R α in patients with concomitant asthma compared to patients without asthma. SOL IL-5R α /IL-5 ratios were calculated in all NP samples and three to 1200 times higher SOL IL-5R α levels were present compared with IL-5 concentrations. Furthermore, within the NP group SOL IL-5R α /IL-5 ratios were significantly higher in NP tissue with concomitant asthma (Fig. 2A). Increased eosinophil percentages were found in NP tissue with and without concomitant asthma compared with control tissue (Table 1). Assessment for any correlations between SOL IL-5R α and eosinophilic markers demonstrated that in NP tissue, SOL IL-5R α significantly correlated with concentrations of IL-5 ($r = 0.405$; $P = 0.006$), ECP ($r = 0.843$; $P < 0.001$), and percentage of eosinophils ($r = 0.536$; $P < 0.001$).

With nasal secretions we aimed to have a less invasive and easy to use technique in comparison with nasal biopsy to monitor SOL IL-5R α concentrations. Within the nasal polyp group SOL IL-5R α and ECP concentrations were higher in secretions of NP patients with asthma than those without asthma (Table 1). Using 2341 pg/ml SOL IL-5R α as best cut-off value, the sensitivity was 83% and specificity was 100% for the differentiation of NP patients with or without asthma, whereas with a best cut-off value for ECP of 1546 μ g/l, a sensitivity of 44% and specificity of 95% could be reached (Fig. 3A). Comparison of areas under the ROC curves for SOL IL5R α and ECP were significantly different in favour of SOL IL-5R α ($P = 0.023$) (Fig. 3B). A significant correlation between SOL IL-5R α concentrations in nasal secretions and nasal tissue confirms that sampling of nasal secretions might be a valuable alternative for biopsies for SOL IL-5R α monitoring (Fig. 3C).

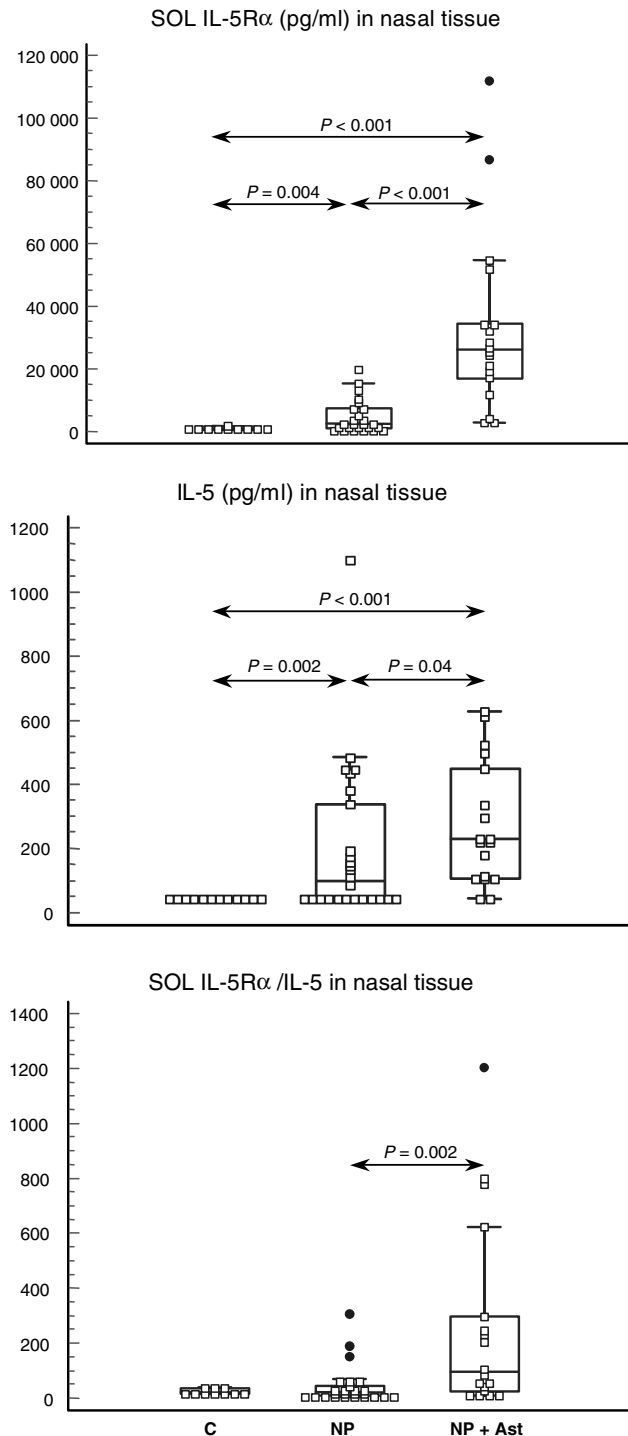


Figure 2. The SOL IL-5R α protein expression in nasal polyp (NP) tissue. Concentrations of SOL IL-5 R α , IL-5, and SOL IL-5 R α /IL-5 in nasal tissue homogenates of 15 controls (C), 24 NP and 18 NP patients with concomitant asthma (NP + Asth). The Box-and-whisker plot represents the median, the lower to upper quartile, and the minimum to the maximum value, excluding 'far out' values. Statistical analyses were performed using the Mann-Whitney *U*-test and *P*-values < 0.05 were considered statistically significant (NS = not significant).

In serum we found increased SOL IL-5R α concentrations in NP patients with concomitant asthma compared with controls and NP patients without asthma (Table 1). Whereas SOL IL-5R α could be measured in all serum samples, IL-5 protein concentrations were only measurable in four subjects (all with concomitant asthma). The SOL IL-5R α concentrations correlated significantly ($r = 0.595$; $P = 0.005$) between serum and nasal tissue samples.

SOL IL-5R α mRNA real-time PCR

Analysis of peripheral blood and nasal tissue samples revealed that relative amounts of SOL IL-5R α isoform (copies of mRNA/ μ l), were significantly higher in samples of NP patients compared with controls. Within the NP group, there was a significant increase in SOL IL-5R α mRNA in NP tissue from patients with concomitant asthma compared to patients without asthma. A similar tendency was observed for SOL IL-5R α in peripheral blood (Fig. 4). There was a significant correlation between SOL IL-5R α in peripheral blood and nasal tissue ($r = 0.668$; $P = 0.006$). Assessment for any correlations between SOL IL-5R α mRNA isoform expression and eosinophilic markers demonstrated that relative amounts of SOL IL-5R α mRNA were significantly correlated with protein concentrations of IL-5 ($r = 0.619$; $P = 0.002$), SOL IL-5R α ($r = 0.659$; $P = 0.001$), ECP ($r = 0.617$; $P = 0.003$), and the percentage of eosinophils ($r = 0.779$; $P < 0.001$) in nasal tissue. In peripheral blood samples, relative amounts of SOL IL-5R α mRNA were correlated to ECP ($r = 0.604$; $P = 0.02$) and percentage of eosinophils ($r = 0.761$; $P = 0.001$), but not to SOL IL-5R α serum concentrations.

Discussion

This paper demonstrates human SOL IL-5R α protein in clinical samples such as serum, nasal secretion, and tissue homogenates. So far, only expression of the murine SOL IL-5R α protein could be demonstrated in sera and ascitic fluids of mice bearing chronic B-cell leukaemia (15). We have established a sensitive, specific, and reliable ELISA that enables determination of SOL IL-5R α concentrations in various human body fluids such as serum, nasal secretions, urine, and peritoneal fluid (not all data shown). In human serum, the SOL IL-5R α protein is detectable in all samples with a median concentration of 210 pg/ml in healthy controls. Notably, we found that the SOL IL-5R α was up-regulated in samples from patients with NP. These findings were confirmed at the mRNA level, using an optimized real-time reverse-transcriptase PCR procedure.

Nasal polyposis is an excellent model for eosinophil-associated diseases and is often associated with asthma and/or aspirin sensitivity. Interleukin-5 can be detected

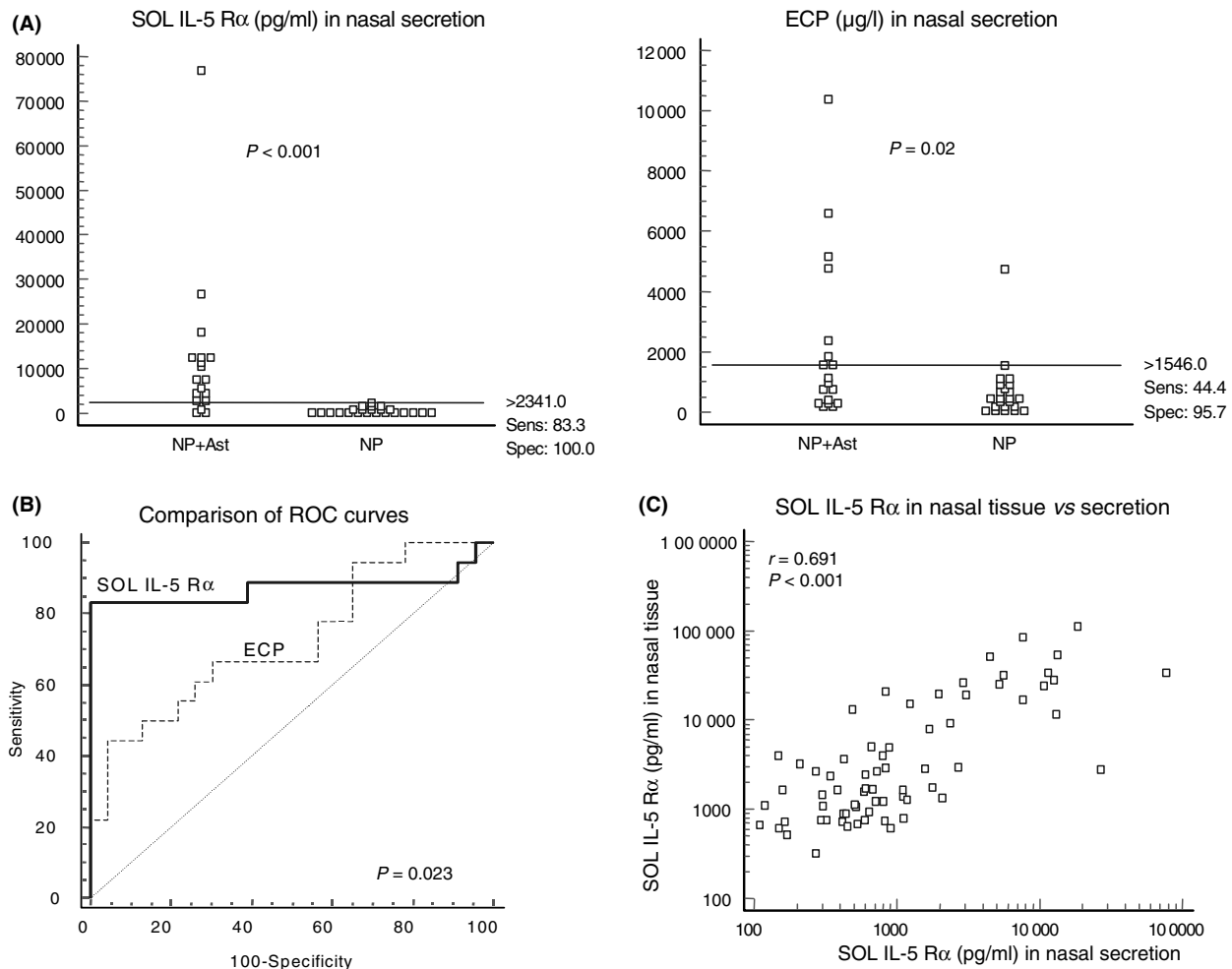


Figure 3. The SOL IL-5R α protein expression in nasal secretions. (A) Concentrations of SOL IL-5 R α and ECP in nasal secretions of 24 and 18 nasal polyp (NP) patients with concomitant asthma (NP + Asth). The mentioned cut-off values are selected corresponding with the highest accuracy (minimal false negative and false positive results). (B) Comparison of ROC curves of SOL IL-5R α and ECP in the differentiation between NP with or without concomitant asthma. The statistically significant difference between the areas under the ROC curves was tested and P -values < 0.05 were considered statistically significant. (C) Correlation between SOL IL-5R α concentrations in nasal secretions and nasal tissue confirms that sampling of nasal secretions might be a valuable alternative for biopsies monitoring SOL IL-5R α .

at the mRNA as well as at the protein level in high amounts by immunohistochemistry and by *in situ* hybridization, and could be co-localized to eosinophils (3). Highest levels of IL-5 protein were observed in polyp homogenates of patients with generalized eosinophilic diseases such as asthma and aspirin sensitivity (2, 4, 5). Our data now reveal that SOL IL-5R α mRNA and protein are up-regulated in NP and are correlated with eosinophil counts, ECP, and IL-5 concentrations. In addition, SOL IL-5R α levels in nasal tissue or blood of NP patients are up-regulated and related to the extension of disease, more specifically with the involvement of local (NP) or systemic airway disease (NP with concomitant asthma). With the collection of nasal secretion we aimed to have a less invasive and easy to use technique in

comparison with the nasal biopsy to determine SOL IL-5 R α concentrations. In nasal secretion, SOL IL-5 R α concentrations were well detectable and there was a strong correlation to the SOL IL-5 R α concentrations in nasal tissue confirming that collection of nasal secretion is a valuable alternative for biopsies. Furthermore, ROC curve analysis demonstrates that SOL IL-5 R α in nasal secretion seems to be a superior marker than an established marker such as ECP for the differentiation of NP patients with or without asthma. Taken together, our results indicate that the measurement of SOL IL-5R α in serum and nasal secretion can be used for objectively monitoring severity of NP, and may offer an attractive alternative for currently used clinical scoring parameters.

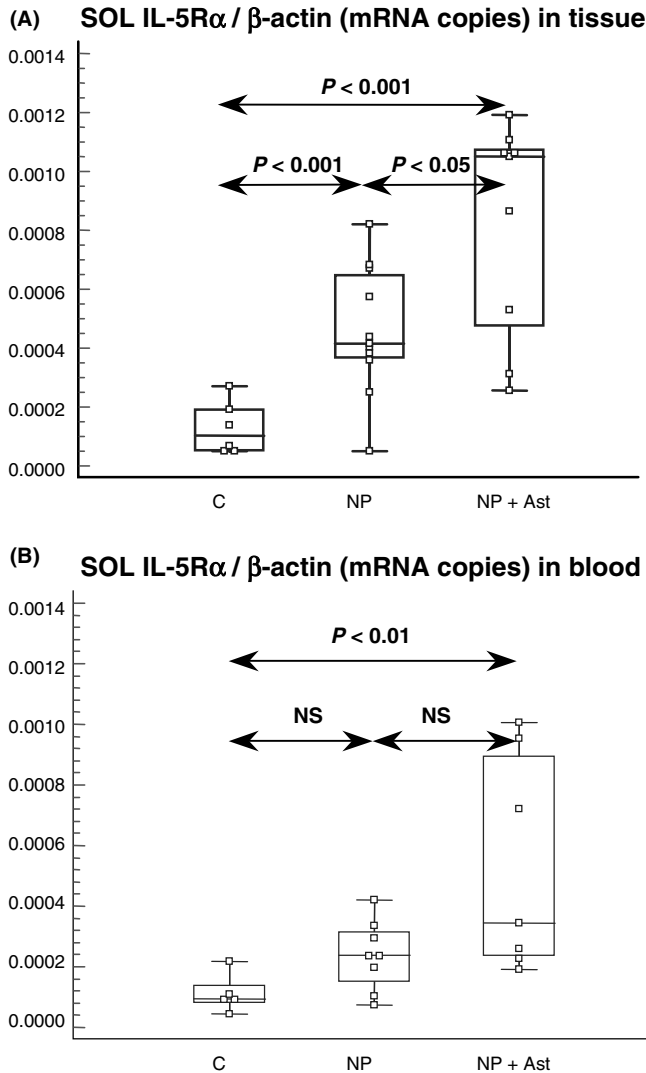


Figure 4. The SOL IL-5R α mRNA expression (real-time PCR). Relative amounts of SOL hIL-5R α / β -actin (copies mRNA/ μ l) in nasal tissue (A) and in peripheral blood (B) of seven controls C, 10 nasal polyps (NP) and nine NP patients with concomitant asthma (NP + Asth). Statistical analyses performed using the Mann-Whitney *U*-test (NS = not significant).

We demonstrate abundant SOL IL-5R α expression in NP with concomitant asthma often dramatically exceeding the IL-5 concentrations. Indeed, in nasal tissue SOL IL-5R α levels up to 1200 times higher than IL-5 concentrations can be found. In severe cases, a regulatory system may inhibit eosinophilic inflammation by the synthesis of SOL IL-5R α . It has been shown that differential splicing of the IL-5R α gene can generate different mRNA isoforms: a signalling competent membrane-anchored TM IL-5R α isoform, and at least one secreted variant (SOL IL-5R α). The expression of the latter depends on the utilization of a specific exon, leading to the loss of the membrane anchor. When expressed in heterologous cells,

this SOL IL-5R α protein shows receptor antagonist activity *in vitro* (6, 11, 16, 17). However, the precise *in vivo* role of the SOL IL-5R α protein requires more detailed study. Secreted receptor variants are a hallmark of the receptor family to which the IL-5R α belongs. This mere evolutionary conservation underscores their functional importance, but the precise physiological role may differ from case to case. Models in which the antagonistic effects prevail include surface receptor down-modulation and ligand capture. However, secreted receptors may bind their ligands in circulation, protecting them from proteolytic inactivation and prolonging their serum half-life or facilitating ligand-mediated signalling. The SOL IL-5R α protein has antagonistic properties *in vitro* as demonstrated in eosinophil differentiation assays as well as in proliferation assays using IL-5 responsive cell lines (11, 18). It captures IL-5 in solution, but this IL-5/IL-5R α complex is unable to associate and activate membrane-bound β c chains (6). Recently, the antagonistic effect of recombinant SOL IL-5R α has been demonstrated on nasal tissue explants. Culturing tissue with recombinant SOL IL-5R α almost completely attenuated the ragweed-induced decrease in eosinophil precursors and increase in major basic protein (MBP)-immunoreactive cell numbers (13). This observation suggests that endogenous production of SOL receptor may regulate IL-5 function *in vivo*. However, the observed correlation between SOL IL-5R α levels and disease severity questions the antagonistic role of SOL IL-5R α *in vivo*.

A number of protein-based IL-5 antagonists have been reported, including SOL IL-5R α that sequesters the ligand in solution (18), single point mutants of IL-5 that occupy the receptor but fail to initiate receptor activation (7, 19), and neutralizing antibodies directed against IL-5 (20). Treatment of eosinophil-infiltrated polyp tissue with neutralizing anti-IL-5 mAb resulted in eosinophil apoptosis and decreased tissue eosinophilia *in vitro* (21). In a monkey model of *Ascaris*-allergic asthma, Egan and coworkers suppressed inflammatory cell migration and airway hyperreactivity for up to 3 months by treating the monkeys with a single dose of the SCH55700 (Reslizumab) neutralizing humanized mAb against hIL-5 (22). Similar anti-IL-5 treatment in humans, however, failed to demonstrate clinical efficacy in two independent studies. A single-dose phase I clinical trial was conducted with SCH55700 in patients with severe persistent asthma and demonstrated a significant decrease in peripheral blood eosinophils (lasting up to 90 days) and a trend towards improvement in lung function at the higher doses (30 days after dosing) (23). SB240563 (Mepolizumab) was tested in mild asthmatics and caused a significant reduction in peripheral blood eosinophils and in postchallenge sputum eosinophils but did not alter FEV1 values upon allergen challenge (1).

Our findings suggest that high amounts of SOL IL-5R α may not be capable of controlling eosinophilia

in vivo. In line with this, antagonizing IL-5 activity in patients with humanized anti-IL-5 mABs was largely unsuccessful. Completely blocking IL-5 activity *in vivo* therefore appears to be much more difficult than *in vitro*. Perhaps, the local tissue microenvironment, and/or the existence of IL-5-dependent autocrine activation mechanisms (3, 21) may limit *in vivo* antagonism (2). A reassessment of the *in vivo* role of the SOL IL-5R α may therefore help to understand recent findings in anti-IL5 treatment. Accurate measurement of SOL IL-5R α levels may distinguish responders from nonresponders, or help in the titration of anti-IL-5 doses. Assessment of endogenous SOL IL-5R α levels might then be considered as a tool for management of anti-IL-5 therapy. With reliable methods now in place to monitor SOL IL-5R α protein and mRNA expression, detailed studies of the mechanisms controlling IL-5 activity under normal conditions and in eosinophil-associated diseases can now be performed.

Conclusion

This report demonstrates the presence of the SOL IL-5R α transcript and protein in various human samples including serum, nasal secretion, and nasal tissue homogenates. SOL IL-5R α is up-regulated in NP and differentiates nasal polyps with or without concomitant asthma. As SOL IL-5R α is strongly up-regulated for disease and has antagonistic properties *in vitro*, our studies shed new light on the mechanisms of specific immuno-modulatory therapies, such as anti-IL-5.

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