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Original article

Differential expression of the interleukin 5 receptor α isoforms in blood and tissue eosinophils of nasal polyp patients

Background: Given the key role of interleukin-5 (IL-5) in eosinophil function, we investigated the regulated expression of the membrane-anchored (TM-IL-5Rα) isoform, or a secreted (SOL IL-5Rα) isoform, on both protein and transcript level *in vitro* and *in vivo*.
Methods: A real-time PCR, FACS and ELISA were established to determine IL-5Rα isoform expression in peripheral blood and nasal tissue from control subjects and nasal polyp (NP) patients with or without asthma. Human peripheral blood eosinophils were incubated with IL-5 and were analyzed for SOL-IL-5Rα and TM-IL-5Rα mRNA and protein levels in comparison with CD-69 expression.
Results: SOL-IL-5Rα and TM-IL-5Rα mRNA and protein expression was significantly increased in NP *vs* controls. In polyp tissue, SOL-IL-5Rα expression correlated to disease severity and eosinophils counts, whereas TM-IL-5Rα levels were inversely correlated to eosinophils counts and SOL-IL-5Rα expression. FACS analysis revealed increased CD-69 and decreased TM-IL-5Rα expression in NP tissue eosinophils *vs* blood eosinophils. Incubation of blood eosinophils with IL-5 caused up-regulation of CD-69 and down-regulation of TM-IL-5Rα after 2 and 24 h.
Conclusion: The expression of SOL-IL-5Rα and TM-IL-5Rα differs according to the eosinophil activation state and localization in the body (blood *vs* tissue) and may therefore be involved in the fine-tuning of the eosinophil homeostasis. Exposure of eosinophils to IL-5 reduces their responsiveness to IL-5 by regulated expression of the IL-5Rα isoforms. Since, TM-IL-5Rα is down-regulated and SOL-IL-5Rα (antagonistic) is upregulated in NP tissue, our findings are important to understand the clinical trials with anti-IL-5 in humans.

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Key words: eosinophils; IL-5 receptor alpha; nasal polyps; rhinosinusitis.

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Interleukin-5 (IL-5) plays a central role in an eosinophil's life span: it supports eosinophilopoiesis and eosinophil differentiation, contributes to eosinophil migration, tissue localization and function, and prevents eosinophil apoptosis (1, 2). Given the likely role of eosinophils in chronic inflammatory diseases, many research focused at antagonizing the IL-5 function. It appears from recent studies that, although this can easily be achieved *in vitro* (3, 4), blocking IL-5 function *in vivo* is much more difficult than originally anticipated (5). In nasal polyposis we demonstrated that a single intravenous injection of anti-IL-5 (mepolizumab) is effective and reduces the size of nasal polyps in 50% of the patients, and that nasal IL-5 levels predict the response to anti-IL-5 treatment (6). Flood-Page et al. observed a significant differential effect of IL-5 blockade on eosinophil counts in various body compartments (7). After multiple dosing with mepolizumab, there was a 100% median reduction in eosinophils in blood, 79% in bronchoalveolar lavage, but only a 52% in the bone marrow and a 55% decrease in the bronchial mucosa (7). The exact reason for a different effect of anti-IL-5 in different body compartments is unclear, but recent data indicated varying IL-5 sensitivity and IL-5 receptor expression at different time points in the cell's formation and localization (8).

The interleukin-5 receptor (IL-5R) is a complex consisting of two chains (9). The soluble (SOL) variant of the IL-5Rα has antagonistic properties *in vitro*, whereas the transmembrane (TM) IL-5Rα can bind with the βc-chain leading to a high affinity receptor complex. The βc-chain does not bind IL-5 by itself but provides the major determinants for signaling whereby IL-5 ligation results in internalization and degradation of the βc-chain (10). It

Abbreviations: βc, β-common chain; BAL, bronchoalveolar lavage; ECP, eosinophil cationic protein; GM-CSF, granulocyte macrophage colony stimulating factor; IQR, interquartile range; MMP, matrix metalloproteinases; NP, chronic sinusitis with nasal polyps; SOL-IL-5Rα, soluble interleukin 5 receptor alpha; TM-IL-5Rα, trans-membrane interleukin 5 receptor alpha.

is suggested that eosinophils are able to control their responsiveness to IL-5 by regulating the expression of the IL-5Rα isoforms. However, this regulated expression may change during eosinophil differentiation, maturation and localization. On CD34+ progenitor cells, it has been demonstrated that IL-5 induces a switch from predominantly SOL isoform to TM-IL-5Rα mRNA expression at the splicing level (11). In mature blood eosinophils, IL-5, IL-3 and GM-CSF down-regulate IL-5Rα mRNA in a dose dependent manner at the promoter level (12). Liu et al. showed that IL-5 receptor expression on airway eosinophils is down-modulated *in vivo* after inhaled allergen challenge and that this may be due to cleavage by membrane associated metalloproteinases (13, 14). In addition, Gregory et al. have demonstrated that exposure of blood eosinophils to IL-3, IL-5, or granulocyte-macrophage colony-stimulating factor *in vitro* leads to sustained down-regulation of surface IL-5Rα expression and reduced responsiveness to IL-5 (15). It seems that IL-5R expression is regulated by different mechanisms ranging from alternative splicing of the IL-5Rα isoforms to cleavage by membrane associated metalloproteinases.

We hypothesize that the expression of the IL-5Rα isoforms may be different depending on eosinophil activation state, maturation and localization. Therefore, we studied SOL-IL-5Rα and TM-IL-5Rα expression in blood and in nasal tissue from control subjects and nasal polyp (NP) patients. At the transcript level IL-5Rα isoform expression was determined by a quantitative real-time PCR. On the protein level SOL- IL-5Rα concentrations were measured by ELISA (16), whereas TM-IL-5Rα receptor expression on tissue and blood eosinophils was determined by flow-cytometry. The eosinophil activation was determined by CD69 expression and/or ECP release. Furthermore, we aimed to investigate whether *in vitro* stimulation of peripheral blood eosinophils with IL-5 could regulate the IL-5Rα isoform expression similar to the expression found in tissue eosinophils and its relation to membrane type-1 matrix metalloproteinases.

Material and methods

Patients

Thirty-four subjects with Chronic rhinosinusitis with NP and 16 controls were recruited at the Departments of Otorhinolaryngology at the Ghent University Hospital, Belgium and the Karolinska Hospital in Stockholm, Sweden (Table 1). Peripheral blood and NP samples were collected in 34 NP subjects (mean age 51.4 years, range 22–79 years; 12 females/22 males) during routine endoscopic sinus surgery and immediately processed for ELISA and PCR measurements, whereas flow-cytometry was performed on blood and tissue samples of 11 NP subjects. Nasal polyps were diagnosed based on history, clinical examination, nasal endoscopy and sinus CT-scan. A history of asthma was reported in 17 NP patients, with seven subjects additionally demonstrating a history of aspirin intolerance. Subjects with concurrent asthma were allowed on no more than 1000 mcg/day BDP or the equivalent, whereas nasal glucorticosteroids were only allowed up to one week before surgery. Patients treated with oral corticoids within the last four weeks prior to surgery were excluded.

Table 1. Patient characteristics

	Controls	Nasal polyps
Number (*n*)	16	34
Age (years)	34.5 (18–71)	51.4 (22–79)
Male : female	9 : 7	22 : 12
Allergy (SPT+)	0% (0/16)	44% (15/34)
Asthma	0%	50% (17/34)
Aspirin intolerance	0%	21% (7/34)
Recurrent NP after surgery	0%	32% (11/34)

Blood and nasal tissue (inferior turbinates) from healthy controls were obtained during routine corrective nasal surgery. All controls (mean age 34.5 years, range 18–71 years; seven females/nine males) 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 ethical committees of both universities approved this study and a written informed consent was obtained from all subjects before inclusion in the study.

Preparation of nasal tissue

Removed polyps were transferred to +4°C HEPES (10 mM)-buffered RPMI 1640 medium (Gibco, Paisley, UK). The polyp tissue was either homogenized or prepared to single cell suspension. To obtain tissue homogenates, nasal tissue specimens were weighed, and 1 ml of 0.9% NaCl solution was added for every 0.1 g of tissue and then homogenized with a mechanical homogenizer (B. Braun, Melsungen, Germany) as described previously (17).

For single cell suspensions, the tissue was cut into small pieces, passed through a fine wire mesh by a syringe stamper and rinsed with RPMI. The cell suspension was centrifuged at 200 ***g*** for <1 min at +4°C and remaining tissue elements were sedimented. The cell supernatant was further centrifuged at 300 ***g*** for 12 min at +4°C. The cells were separated, one part was lysed and stored at −80°C until RNA purification. The other part was resuspended in 5 ml PBS and used for FACS analysis.

Preparation of blood and serum

Peripheral blood was collected in tubes containing EDTA (Vacutainer, 5 ml, with 50 µl of 21% EDTA) (Terumo, Leuven, Belgium). Leukocytes were isolated by hemolysing 150 µl portions of blood in 3 ml +4°C isotonic NH_4Cl-EDTA lysing solution (154 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2) for 5 min at +15ºC. Cell suspensions were then centrifuged at 300 ***g*** for 6 min at +4ºC and washed in PBS. The cells were used for RNA preparations and FACS analysis. Serum was collected in tubes with no additives, was allowed to coagulate for 30 min and centrifuged at 1500 ***g*** for 15 min at +4°C.

Preparation of purified peripheral blood eosinophils

Peripheral blood eosinophils from healthy blood donors (age 18–64 years) were purified by the magnetic cell separation system

MidiMacs (Miltenyi, Biotec, Bergisch Gladbach, Germany) (18). Briefly, blood was layered onto Percoll solution (Pharmacia-Upjohn, Uppsala, Sweden) and centrifuged (30 min, 1000 ***g***, 20°C). The mononuclear cell layer was removed and the remaining cell suspension was hemolysed in isotonic lysing solution (154 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2). Eosinophils and neutrophils were washed in PBS and anti-CD16 magnetic beads were added for 20 min at +4°C. The eosinophils were obtained by negative selection using a separation column in a magnetic field where magnetically labeled cells (CD16+ neutrophils) were trapped and unlabelled cells (eosinophils) were collected.

In vitro incubation of purified eosinophils with recombinant human IL-5

Purified eosinophils (1.0×10^6/ml) were incubated with recombinant human (rh) IL-5 (10 ng/ml) (Immunokontact, Frankfurt, Germany) diluted in HEPES (10 mM)-buffered RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 10% heat-inactivated fetal calf serum (RPMI). As control, eosinophils were incubated in RPMI alone. The cell suspensions were incubated in 24-well plates for 2 h or 24 h at +37°C in 5% CO_2. Supernatants were collected and stored at −30°C until ELISA measurements. The cells were washed twice in PBS (300 ***g*** for 5 min at +4°C) and used for FACS.

Immunofluorescence staining of eosinophils and flow-cytometry

Polyp tissue cells ($0.5–1.0 \times 10^6$), isolated blood leukocytes or purified eosinophils (0.1×10^6) were incubated with non-conjugated mAb to IL-5Rα (10 μg/ml, Clone: α16, non-neutralizing). Secondary immunostaining was performed with fluoroscein isothiocyanate (FITC)-conjugated Rabbit Anti Mouse immunoglobulin, $F(ab')_2$ (50 μg/ml, Code: F313) (DAKO A/S). Polyp tissue cells and blood leukocytes were double stained with phycoerythrin (PE)-conjugated mAb to CD16 (Immunotech) or stained with FITC-conjugated mAb to CD69 (5 μg/ml, Clone: L78) (Becton Dickinson, Meylan-Cedex, France) together with mAb to CD16 (Immunotech). Cells and antibodies were incubated for 30 min at +4°C, and then washed in PBS. Non-specific binding was determined with isotype-matched control antibodies in corresponding concentrations. Cells were finally diluted in 0.5 ml PBS and a minimum of 1000 eosinophils was analyzed in an EPICS XL-MCL (Beckman Coulter Inc., Fullerton, CA, USA) flow-cytometer. Polyp tissue and peripheral blood eosinophils were detected and analyzed as a separate CD16 negative population by using depolarized light (19). The flow-cytometer was calibrated daily with Flow Check and Flow Set (Beckman Coulter).

Measurement of IL-5 and SOL IL-5 Rα protein concentrations

A SOL-IL-5Rα specific sandwich ELISA was developed by combining two monoclonal antibodies (Innogenetics, Gent, Belgium). A detailed description and characterization of this ELISA was previously published (16). Serum, tissue homogenates and eosinophil supernatants were assayed by a research ELISA for SOL-IL-5Rα (Innogenetics) and IL-5 (R&D Systems, Minneapolis, MN, USA).

SOL and TM IL-5 Rα real-time PCR

RNA was isolated from snap frozen blood leukocytes and nasal tissue cells of 16 controls and 34 NP patients using the Rneasy Kit (Qiagen, Hilden, Germany), whereas RNA from eosinophil cell pellets was extracted using the TriReagent method (Sigma, Bornem, Belgium). Total RNA was quantified using RiboGreen Kit (Molecular Probes, Merelbeke, Belgium) and 0.5 μg was reverse transcribed using Superscript II Reverse Transcriptase (Invitrogen, Merelbeke, Belgium), oligo dT and random hexamers (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the manufacture's protocol. cDNA equivalent to 25 ng total RNA 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 forward primer and specific reverse primers for SOL hIL-5Rα and TM hIL-5Rα isoforms as previously published (20). Two plasmids containing the cDNA sequences for the soluble or membrane-anchored encoding transcripts of hIL-5Rα were used to prepare the template for the standards (provided by Prof. Dr Jan Tavernier). MMP-14 RT-PCR was done with Assay-on-Demand Gene Expression products (Applied Biosystems, Foster City, CA, USA). PCR was performed in a 25 μl reaction mixture composed of cDNA (equivalent to 25 ng total RNA), primers and FAM-labeled probes and TaqMan Universal PCR Master mix (Applied Biosystems). 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 (21).

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. Differences between the paired data were calculated by using the Wilcoxon test. Spearman rank correlation coefficient (r) was used to assess relationships between parameters.

Results

SOL-IL-5Rα and TM-IL-5Rα protein expression in blood and tissue

We found increased median (IQR) concentrations of SOL-IL-5Rα in serum from 17 NP patients with concomitant asthma (778 pg/ml; 501–1191) compared to 16 controls (285 pg/ml; 226–465; $P = 0.009$) and 17 NP patients without asthma (328 pg/ml; 272–448; $P < 0.001$). Whereas SOL-IL-5Rα could be measured in all serum samples, IL-5 protein concentrations were only measurable in four subjects (all with concomitant asthma). In controls the median percentage of blood eosinophils was 1.2% (0.5–2.5), whereas increased percentages were found in NP (3.6%; 2.5–5.9; $P = 0.02$) and NP with asthma (9.7%; 6.9–19.5; $P < 0.001$) and with a significant difference between the NP groups ($P < 0.001$).

In supernatants prepared from nasal tissue homogenates median (IQR) concentrations of SOL-IL-5Rα were significantly higher in NP (1504 pg/ml; 1037–3757; $P = 0.04$) and NP with concomitant asthma (21069 pg/ml; 13 177–29 784; $P < 0.001$) compared to control nasal tissue (919 pg/ml; 735–1416). Whereas IL-5 was not detectable in control nasal tissue, increased IL-5

concentrations were found in NP tissue of patients without (56 pg/ml; 43–197; $P = 0.03$) and with concomitant asthma (244 pg/ml; 107–367; $P = 0.002$). Within the NP group, there was a significant further increase of SOL-IL-5Rα ($P < 0.001$) and IL-5 ($P = 0.01$) in patients with concomitant asthma compared to patients without asthma. Increased eosinophil percentages were found in NP tissue with (11.3%; 8.1–25.7; $P < 0.001$) and without concomitant asthma (2.4%; 1.2–5.8; $P = 0.006$) compared to control tissue (0.2%; 0.1–0.5). Moreover, in NP tissue, SOL-IL-5Rα significantly correlated with concentrations of IL-5 ($r = 0.500$; $P = 0.02$), and percentage of eosinophils ($r = 0.589$; $P = 0.003$).

When peripheral blood and polyp tissue cells were analyzed by flow-cytometry the proportion of TM-IL-5Rα positive eosinophils was significantly ($P = 0.003$) lower in polyp tissue eosinophils, 6.1% (1.3–10.4) when compared to peripheral blood eosinophils, 72.6% (60.6–86.4) ($n = 11$). The proportion of CD69 positive eosinophils was significantly ($P = 0.018$) up regulated on polyp tissue eosinophils, 63.9% (63.0–78.4), as compared to peripheral blood eosinophils, 2.7% (1.9–3.6) (Fig. 1). No relevant correlations were found for CD69 and TM-IL-5Rα expression on blood and tissue eosinophils.

The percentage of TM-IL-5Rα positive eosinophils ranges in peripheral blood from 70.4% (41.5–86.4) to 72.6% (69.0–82.4), whereas in tissue from 6.9% (1.3–10.4) to 6.1% (2.2–12.7) in NP without asthma ($n = 6$) and with asthma ($n = 5$). The proportion of CD69 positive eosinophils ranges in peripheral blood from 2.2% (1.3–2.74) to 3.0% (2.5–3.7), whereas in tissue from 66.9% (63.0–78.4) to 63.9% (60.3–70.6) in NP without asthma ($n = 6$) and with asthma ($n = 5$) any significance between the groups. There was no significant differences in expression of TM-IL-5Rα or CD69 between NP with or without asthma.

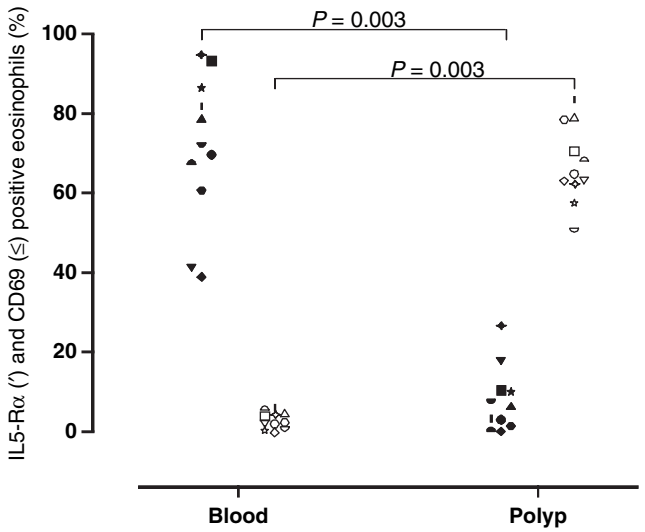

Figure 1. TM-IL-5Rα and CD-69 (eosinophil activation marker) expression was evaluated by FACS analysis in blood and in tissue of 12 NP patients. The results are presented as the proportion of TM-IL-5Rα (filled symbols) and CD-69 (open symbols) positive eosinophils. Statistical analyses were performed using the Wilcoxon test.

SOL-IL-5Rα and TM-IL-5Rα mRNA expression in blood and tissue

Analysis of peripheral blood and nasal tissue samples with quantitative real-time PCR revealed that relative amounts of SOL-IL-5Rα and TM-IL-5Rα isoforms (copies of mRNA/μl), were significantly higher in samples of patients with NP compared to controls. Within the NP group, SOL-IL-5Rα and TM-IL-5Rα mRNA levels were increased in peripheral blood of patients with concomitant asthma compared to patients without asthma (Fig. 2A). In NP tissue of patients with asthma the SOL-IL-5Rα mRNA was significantly increased whereas the TM-IL-5Rα mRNA was significantly decreased when compared to NP patients without asthma (Fig. 2B). In peripheral blood samples of NP patients, relative amounts of SOL-IL-5Rα and TM-IL-5Rα mRNA were significantly correlated to eosinophil percentages (Fig. 2A). In polyp tissue, protein concentrations of IL-5, SOL-IL-5Rα, and eosinophil percentages correlated positively to SOL-IL-5Rα, but inversely to TM-IL-5Rα mRNA expression (Fig. 2B).

Analysis of SOL-IL-5Rα and TM-IL-5Rα protein and mNRA expression in subgroups

Although the presence of asthma seems to be the strongest differentiator in SOL-IL-5Rα and TM-IL-5Rα protein and mNRA expression, other factors play a role. It needs to be mentioned that recurrent nasal polyposis, asthma and aspirin intolerance are strongly linked (not atopy). Within the NP group, atopy based on positive skinprick tests was only associated with increased SOL-IL-5Rα protein expression. Recurrent nasal polyposis and aspirin intolerance were associated with significantly lower TM-IL-5Rα protein and mRNA expression.

SOL-IL-5Rα and TM-IL-5Rα protein expression after *in vitro* stimulation with rh IL-5

Purified eosinophils from seven healthy blood donors were incubated with rh IL-5 for 2 h and 24 h. Flow cytometric analysis showed that rh IL-5 induced a significant ($P = 0.018$ for all) down-regulation in the proportion of TM-IL-5Rα positive eosinophils at 2 h, 37.5% (27.4–61.8) and 24 h, 13.9% (8.8–22.0) as compared to the RPMI controls, 78.3% (68.1–86.0) and 55.0% (46.9–62.3), respectively (Fig. 3A). The SOL-IL-5Rα concentrations in the supernatants were significantly higher after incubation with rh IL-5 compared to RPMI controls at 2 h ($P = 0.002$) and

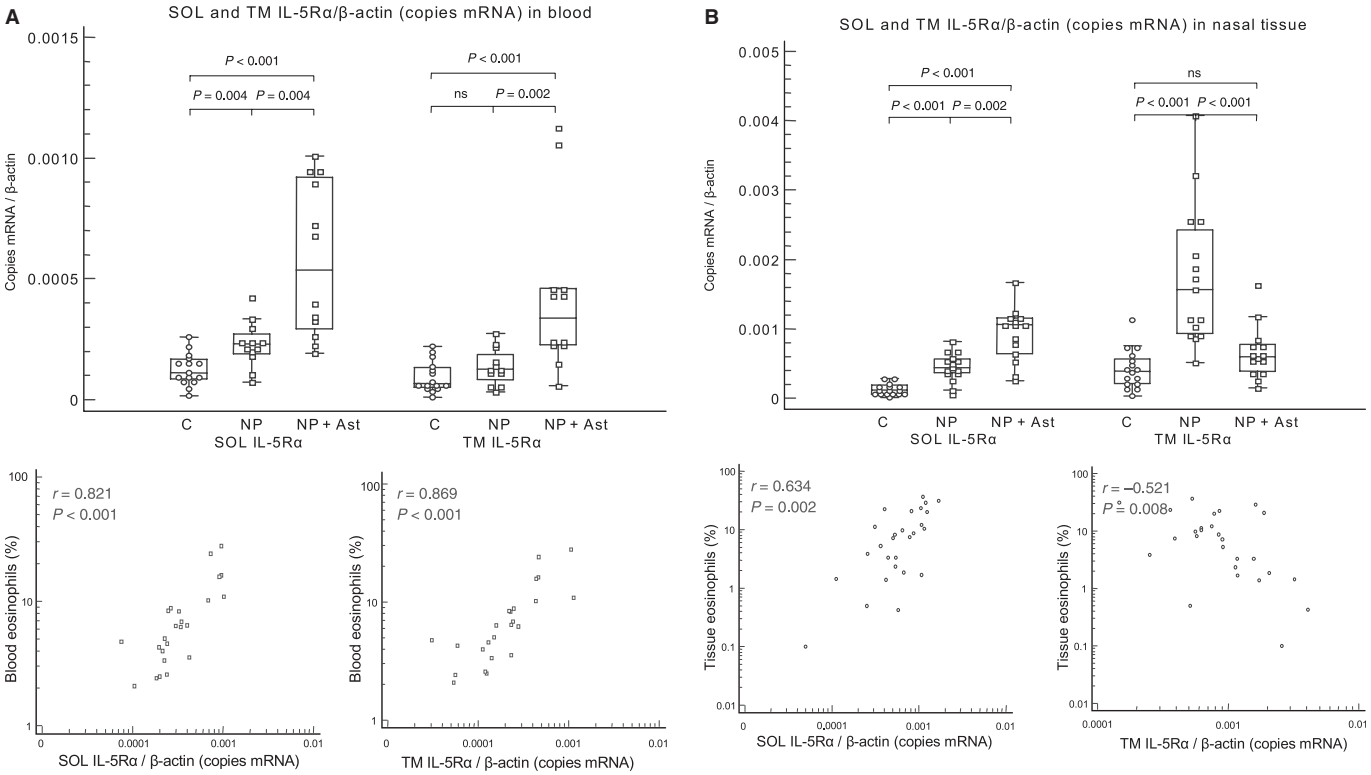

Figure 2. Relative amounts of SOL-IL-5Rα and TM-IL-5Rα/β-actin (copies mRNA/μl) were determined by a isoform specific real-time PCR in peripheral blood A; and in nasal tissue B; of 16 controls (C), 17 nasal polyps (NP) and 17 nasal polyp patients with concomitant asthma (NP + Asth). The Box-and-whisker 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 (ns = not significant). Spearman rank correlation coefficient (*r*) was used to assess the relationships between the parameters.

24 h ($P = 0.03$) (Fig. 3B). SOL-IL-5Rα and TM-IL-5Rα protein expression correlated negatively at 24 h ($r = -0.612$; $P = 0.03$).

SOL-IL-5Rα and TM-IL-5Rα mRNA expression after *in vitro* stimulation with rh IL-5

Stimulation of blood eosinophils with rh IL-5 resulted in a significant decrease in TM-IL-5Rα mRNA expression after 2 and 24 h compared to baseline, which is at both time points significantly lower when compared to the RPMI controls (Fig. 3C). SOL-IL-5Rα mRNA expression decreased significantly after 2 and 24 h, without difference between rh IL-5 incubation and RPMI controls (Fig. 3D). There was no difference in relative MMP-14 mRNA expression (β-actin corrected) between different time points or between stimulation with rh IL-5 and RPMI controls. MMP-14 expression was positively correlated with SOL-IL-5Rα (protein and mRNA) ($r = 0.354$; $P > 0.05$ and $r = 0.786$; $P = 0.005$) and TM-IL-5Rα mRNA expression($r = 0.638$; $P = 0.02$), whereas a negative correlation was shown with TM-IL-5Rα surface expression ($r = -0.750$; $P = 0.007$).

Discussion

Our results demonstrate differential expression of IL-5Rα isoforms in blood and tissue eosinophils. In blood, SOL-IL-5Rα and TM-IL-5Rα mRNA and protein expression is up-regulated in NP *vs* controls and correlated to eosinophil percentages, whereas in polyp tissue TM-IL-5Rα levels showed an inverse relation to eosinophilia and SOL-IL-5Rα expression. Blood eosinophils express high levels of surface-anchored TM-IL-5Rα and are not activated as judged by a low CD-69 expression. In contrast, tissue eosinophils are activated and demonstrate a low level of surface receptors to IL-5. Recently, it was demonstrated that following airway antigen challenge of atopic subjects, BAL fluid eosinophils showed a markedly reduced TM-IL-5Rα and βc-chain mRNA and did not release EDN when exposed *ex vivo* to IL-5 compared with circulating eosinophils (14). Julius et al. (22) demonstrated that TM-IL-5Rα on blood and BAL eosinophils was decreased after segmental allergen provocation in 12 atopic asthmatics, whereas CD-69 (activation marker) increased. Interestingly, the net decrease in IL-5Rα

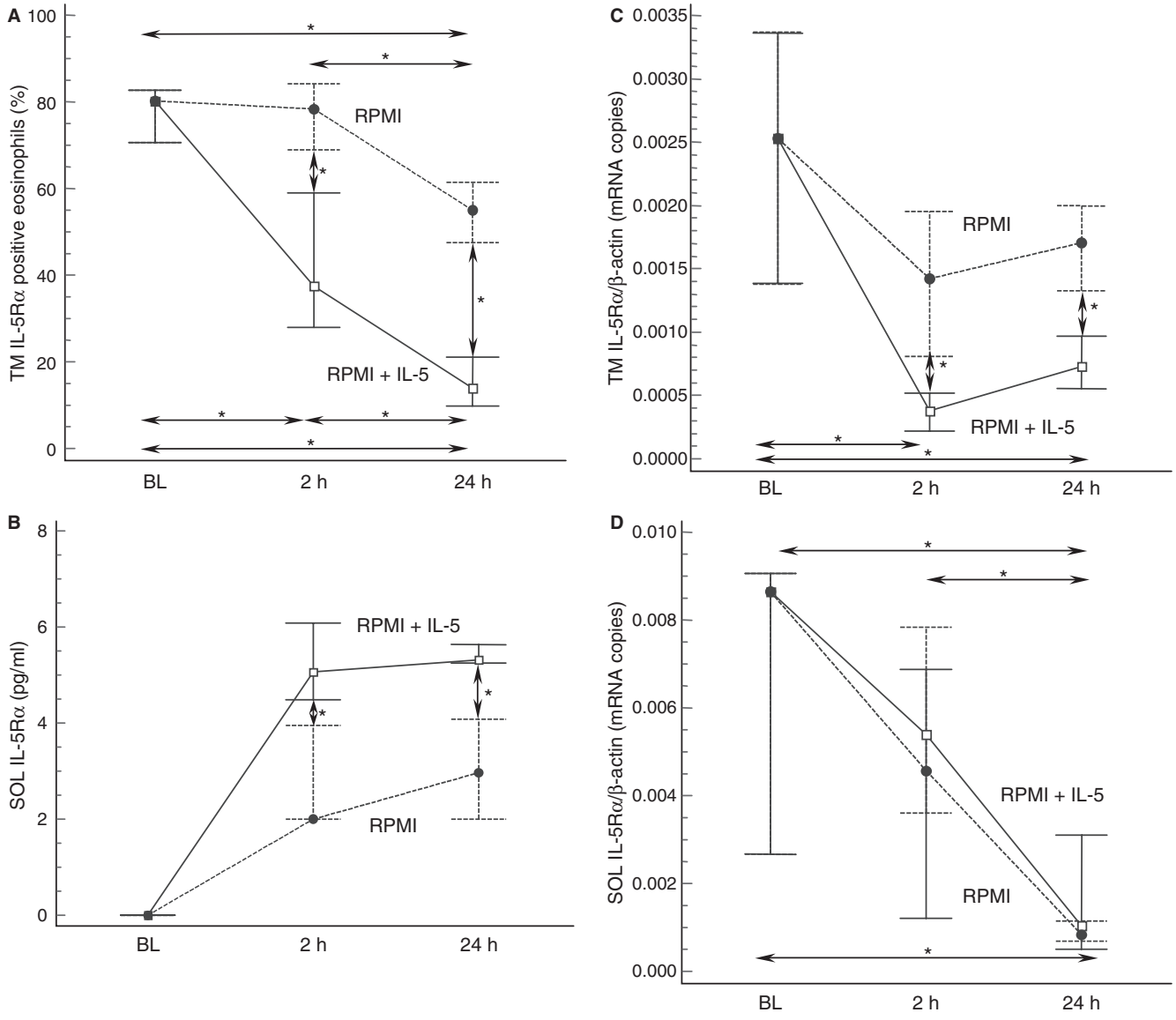

Figure 3. Purified eosinophils from seven healthy blood donors were incubated with 10 ng/ml rh IL-5 for 2 h and 24 h (RPMI + IL-5; full line). As control, eosinophils were incubated in RPMI alone (RPMI; dotted line). TM-IL-5Rα positive eosinophils (%) determined by FACS analysis (A); SOL-IL-5Rα (pg/ml) concentrations in supernatants determined by ELISA (B); TM-IL-5Rα/β-actin (C) and SOL-IL-5Rα/β-actin (copies mRNA) expression determined by real-time PCR (D). The lines represent the median and the lower to upper quartile (*, $P < 0.05$).

expression occurred in the presence of significantly elevated IL-5 concentrations in BAL fluid (22). Similarly, the decreased expression of TM-IL-5Rα on tissue eosinophils from nasal polyps might prevent eosinophil activation by IL-5. This insights are especially important in order to understand the clinical trials with anti-IL-5 in humans. We recently showed that nasal IL-5 levels determined the response to anti-IL-5 treatment in patients with nasal polyposis (6). Only in those patients with highly increased nasal IL-5 levels a significant decrease in polyp volume was obtained up to four weeks after one single injection with anti-IL-5. Probably only highly increased local IL-5 levels can overcome the low TM-IL-5Rα expression on tissue eosinophils. Nasal polyposis is frequently associated with asthma and particularly those patients have the highest levels of IL-5 and eosinophil counts in the NP tissues (17, 23). In line with this we now demonstrate that the SOL-IL-5Rα expression is increased whereas the TM-IL-5Rα mRNA expression is decreased in NP tissue of patients with asthma compared to those without asthma. Furthermore, TM-IL-5Rα mRNA is inversely correlated to protein levels of IL-5, SOL-IL-5Rα and the proportion of eosinophils. This suggests TM-IL-5Rα mRNA tissue expression is down-regulated independently of eosinophil counts and this further supports the finding that

tissue eosinophils have a low IL-5Rα surface expression. Hence, in tissue with the highest levels of IL-5 and eosinophils, a regulatory mechanism must operate to control the ongoing eosinophilic inflammation by up-regulation of SOL-IL-5Rα and down-regulation of TM-IL-5Rα. Taken together, our results suggest that the expression of the IL-5Rα isoforms differs according to the eosinophil activation state, maturation and localization in the body.

Hellman et al. demonstrated that surface TM-IL-5Rα was strongly down-regulated by recombinant IL-5, intermediately with both IL-5 and GM-CSF, and weakly with only GM-CSF, suggesting that GM-CSF binding partially inhibits the surface IL-5 receptor to be down-modulated (24). In the present study we show that freshly isolated mature human blood eosinophils express more SOL-IL-5Rα than TM-IL-5Rα mRNA expression. *In vitro* exposure of mature human blood eosinophils with rh IL-5 for 2 h and 24 h induces an extensive down-regulation of IL-5Rα TM protein and mRNA, whereas SOL-IL-5Rα was only significantly up-regulated at the protein level but not on the transcript level. The discrepancy between the mRNA and the protein level point to the fact that SOL-IL-5Rα expression is mainly dependent of proteolytic cleavage of the receptor rather than on regulation the transcript level. Liu et al. (13) demonstrated that the down-modulation of TM-IL-5Rα from the cell surface and the increased release of SOL-IL-5Rα into culture supernatant fluid were partially inhibited by the MMP-specific inhibitor BB-94 (13). These data suggest that exposure of peripheral blood eosinophils to IL-5 results in a rapid, sustained loss of TM-IL-5Rα, that is, at least in part, dependent on MMP activity. In line with this we found a significant inverse correlation between MMP-14 and IL-5Rα surface expression, which suggests a role in proteolytic cleavage of the receptor. Taken all observations into account we suggest that an IL-5 driven inflammation generates an eosinophil tissue phenotype that is characterized by a low TM but high SOL-IL-5Rα expression and that this process is partially the result of proteolytic receptor modulation and down-regulation of TM-IL-5Rα gene transcription.

Conclusions

This report demonstrates differential expression of SOL-IL-5Rα and TM-IL-5Rα in blood and tissue eosinophils. Interestingly, in polyp tissue SOL-IL-5Rα expression is increased and correlated to disease severity and eosinophil percentages, whereas TM-IL-5Rα levels decreased and were inversely correlated to eosinophils and SOL-IL-5Rα expression. *In vitro* exposure of blood eosinophils to IL-5 reduces the expression of TM-IL-5Rα, but induces SOL-IL-5Rα protein release. SOL-IL-5Rα protein has antagonistic properties *in vitro*, however endogenous concentrations might be insufficient to block IL-5 activity. The expression of the IL-5Rα isoforms differs according to the eosinophil activation state, maturation and localization in the body and may therefore be involved in the fine-tuning of the eosinophil homeostasis.

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