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Nasal levels of soluble IL-33R ST2 and IL-16 in allergic rhinitis: inverse correlation trends with disease severity

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Summary

Background Serum levels of IL-16, IL-33 and the decoy receptor of IL-33, soluble ST2, are elevated in allergic rhinitis. Recent studies show that IL-16, soluble ST2 or anti-IL-33 reduce type 2 cytokines (such as IL-5) and eosinophilia in murine models of allergic asthma or allergic rhinitis respectively.

Objective In this study, we studied the release of IL-5, IL-16, IL-33 and soluble ST2 in allergic rhinitis patients after nasal allergen challenge and natural pollen exposure.

Methods The nasal lavages of 15 allergic and 14 non-allergic volunteers were collected during the pollen allergy season. In addition, six allergic volunteers underwent unilateral nasal allergen and control challenge out of season and nasal secretions and sera were collected. IL-5, IL-16, IL-33 and soluble ST2 in nasal secretions and sera were measured by electrochemiluminescent assay or ELISA, respectively.

Results Nasal IL-5, IL-16 and soluble ST2 levels were significantly increased in seasonally pollen exposed allergic volunteers compared to control subjects ($P < 0.001$, $P = 0.018$ and $P = 0.002$ respectively), whereas IL-33 remained undetectable. Nasal IL-16 showed a weak inverse correlation trend with nasal symptoms ($r = -0.48$, $P = 0.07$). Nasal soluble ST2 concentrations were inversely correlated with nasal symptoms ($r = -0.61$, $P = 0.02$) and positively correlated with IL-16 ($r = 0.56$, $P = 0.03$). Significant increases of nasal IL-5, IL-16 and ST2 but not IL-33 were observed after nasal allergen challenge. At 24 h after allergen challenge, local ST2 and IL-5 concentrations showed an inverse correlation trend ($r = -0.83$, $P = 0.04$). Serum levels of IL-5, IL-16 and soluble ST2 rose in at least five of six volunteers tested at 5 or 24 h post-challenge.

Conclusions and Clinical Relevance The observed upregulation of soluble ST2 and IL-16 after nasal allergen challenge and during peak pollination season suggests potential regulatory roles of these cytokines in the inflammatory reaction in allergic rhinitis.

Keywords allergic rhinitis, IL-16, IL-33, IL-5, nasal allergen challenge, nasal lavage, nasal secretion, natural pollen exposure, soluble ST2

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Introduction

Allergic rhinitis is the most abundant allergic disease with a prevalence of approximately 20% in Western countries [1, 2] and substantially impairs the quality of life. Allergic rhinitis typically precedes the development of asthma [3, 4]. Other co-morbidities include atopic dermatitis, chronic rhinosinusitis and food allergy.

IL-33 is a newly discovered member of the IL-1 cytokine family including IL-1 α , IL-1 β , and IL-18 [5]. Its receptors are IL-1 Receptor Accessory Protein (IL-1 RAcP) and ST2L [6, 7]. Initially described as an orphan receptor, ST2L binds exclusively to IL-33 and is also called IL-1 receptor-like 1 (IL-1 RL1) or IL-1 R4. It is preferentially expressed by Th2 cells, mast cells, eosinophils, basophils and type 2 innate lymphoid cells (ILC2) [5, 8].

IL-33 binding to its receptors activates NF- κ B and MAP kinase pathways and thus drives the expression of mainly type 2 cytokines [9]. Alternative splicing of the transmembrane form ST2L generates a secreted form. This soluble ST2 functions as a decoy receptor that blocks the ability of IL-33 to signal through transmembrane ST2L [10–13]. Genome-wide association studies identified IL-33 and its receptor ST2L as susceptibility genes for asthma and allergic diseases recently [14]. Elevated IL-33 serum levels were found in atopic patients during anaphylactic shock. Furthermore, IL-33 enhanced IgE-driven anaphylactic shock in a T cell independent, mast cell-dependent manner in murine experimental atopic models [15]. Elevated levels of IL-33 were also found in severe asthma [16, 17], allergic conjunctivitis [18], and in the sera, nasal epithelia and secretions of allergic rhinitis patients [19–21]. In murine models of either asthma or allergic rhinitis, anti-IL-33 treatment showed therapeutic effects [5, 22, 23]. Interestingly, elevated soluble ST2 protein levels could be found in sera of patients with asthma with an acute exacerbation [24] and in the sera of birch pollen atopics [25].

IL-16 is a unique cytokine with no significant sequence homology to other currently known cytokines [5, 26]. This cytokine attracted particular attention due to its ability to suppress HIV replication [5, 27]. IL-16 is a natural ligand of CD4 [28] and CD9 [29]. Pro-IL-16 (the precursor of secreted IL-16) acts – like IL-33 – as a nuclear factor in regulating transcription [30, 31]. IL-16 is produced by epithelial cells as well as immune cells such as eosinophils, mast cells, lymphocytes, macrophages and dendritic cells [5]. IL-16 has been characterized as an immunomodulatory cytokine in allergic diseases [5]. It was both systemically and locally upregulated in a murine allergic rhinitis model with OVA-sensitization [32]. In allergic rhinitis patients, significantly elevated IL-16 levels compared to normal controls were reported in serum, nasal gland cells and infiltrating lymphocytes [33]. Increased secretion of IL-16 was observed in experimentally challenged allergic rhinitis patients [34] and allergic asthma patients [35]. IL-16 inhibited antigen-induced airway hyper-reactivity, eosinophilia and type 2 cytokine production in mice [36]. Furthermore, IL-16 induced preferential migration of human T regulatory cells [37]. Moreover, IL-16 inhibited IL-5 secretion by allergen-stimulated peripheral blood T cells [38], IL-13 production by allergen-stimulated blood mononuclear cells [39], and IgE production by B lymphocytes [40] from atopic subjects.

IL-5 is a Th2 cytokine which plays a central role in allergic asthma and allergic diseases [5]. It is mainly produced by Th2 cells, activated eosinophils, and mast cells and targets besides basophils and mast cells mainly eosinophils enhancing their growth, activation, mobilization, differentiation and survival [5, 41].

Increases in IL-5 in nasal secretions of allergic rhinitis patients after nasal allergen challenge and during season have been described [42, 43].

Thus far, levels of soluble ST2 in nasal secretions of allergic rhinitis patients after nasal allergen challenge or during peak pollination season have, to our knowledge, not been described. Likewise, no studies exist about levels of IL-16 in nasal secretions of allergic rhinitis patients during peak pollination season. In this study, we studied the release of IL-5, IL-16, IL-33 and soluble ST2 in allergic rhinitis patients after nasal allergen challenge and natural pollen exposure.

Methods

Subjects

The study was approved by the Ethics Committee of the Heinrich-Heine University Duesseldorf (approval number: 3530) and all participants provided written informed consent.

Adult subjects were chosen based on a total nasal symptom score questionnaire and a skin prick test showing sensitization for either grass (grass mix, Allergopharma Joachim Ganzer KG, Reinbek, Germany) or tree pollen [tree mix (birch, european beech, plane, oak), Allergopharma]. Initially, thirty-two volunteers were recruited for the nasal lavage study during the peak pollination season including 15 subjects with seasonal allergic rhinitis and 17 non-allergic control subjects. Any anti-allergic medication was stopped 10 days before nasal lavage. Ten otherwise healthy subjects with seasonal allergic rhinitis were enrolled for the nasal allergen challenge study. Nasal allergen challenges were performed out of the pollination season. Due to lack of compliance or procedural adverse events, four patients had to be excluded from the nasal allergen challenge study. In the peak season study, three control individuals had to be excluded due to elevated eosinophil counts in blood differential counts or procedural adverse events. Table 1 shows the age range, mean age and gender proportions of the study participants. Exclusion criteria among others where any surgery 3 months before study participation, any rhinitis or rhinosinusitis other than allergic within 1 month prior to study, specific immunotherapy within the last 3 years and any history of chronic rhinosinusitis.

Table 1. Demographic data of study participants

	Age range	Mean age	% female
15 allergics during peak season	22–52	28.4	53.3
14 non-allergics during peak season	23–50	29.0	64.3
6 allergics out of season (nasal allergen challenge)	19–53	28.3	66.7

Nasal lavage and localized nasal challenge

Nasal lavages were performed with 10 mL of Sodium Chloride Solution (0.9%, B. Braun Melsungen A.G., Melsungen, Germany). After centrifugation at 4°C, the supernatant was aliquoted into 0.5 mL tubes (LoBind; Eppendorf AG, Hamburg, Germany) and stored at -80°C. Nasal provocation and collection of nasal secretions was performed using a modified version of the disc method (Fig. 1) [44]. Out of season challenges were performed after five nasal lavages with 0.9% saline to wash out pre-existing mediators. Baseline nasal secretions were then collected and a diluent challenge was performed to exclude unspecific nasal reactivity (Fig. 1) [45]. For nasal allergen challenge discs from filter paper (10 mm diameter, 1.2 mm thickness; Shandon filter cards; Shandon Inc., Pittsburgh, PA, USA) were soaked with 75 µL containing 3.750 BU of allergen extracts (Allergopharma) and placed on the anterior portion of the nasal septum for 1 min. To collect nasal secretions, pre-weighed filter discs were placed on the same area of the septum at 10 min, 2 h, 5 h and 24 h time-points for 45 s and secretion weights were assessed immediately afterwards. The filter discs were then eluted in 500 µL 0.9% Sodium Chloride Solution (B. Braun Melsungen A.G.) in 2 mL tubes (Cryo S PP, Greiner Bio-One, Frickenhausen, Germany) for approximately 3 h while gently shaking at 4°C. Eluates were centrifuged at 720 g (Biofuge 15R; Heraeus Instruments; Duesseldorf, Germany). Aliquots were stored at -80°C until analysis. Clinical reactions upon nasal allergen challenge were assessed at each time-point using a visual analogue scale symptom score including total nasal symptom score parameters sneeze, nasal secretion, obstruction and nasal itching. Two weeks after or at least 3 days prior to nasal allergen challenge a full protocol diluent control challenge was performed in the study participants.

Serum

To determine systemic effects of the nasal allergen challenge, 5 mL blood was collected [SST II; Becton, Dickinson and Company (BD), Heidelberg, Germany] at baseline, 5 h and 24 h post-challenge and stored in aliquots at -80°C.

IL-5 and IL-16 electrochemiluminescent immunoassay

IL-5 and IL-16 levels in nasal secretion samples were analysed using an electrochemiluminescent immunoassay [Meso Scale Discovery (MSD), Gaithersburg, MD, USA]. Due to limited availability of three control samples IL-16 levels were only measured in eleven instead of 14 control samples during peak season; however, IL-16 could be measured in all samples from allergic volunteers during peak season and was determined in all samples of the nasal allergen challenge study. Standards or samples were measured in duplicates and zero standards in triplicates. If coefficient of variation (CV) values were > 20%, the measurement was repeated. The manufacturer's recommendations were adapted using a 2½ h initial incubation. The plates were immediately read on the Sector Imager 6000 (MSD). Raw data were analysed using the Discovery Workbench 3.0 software (MSD). According to the manufacturer's recommendations, the lower limit of detection (LLOD) was defined as the calculated concentration of the signal that is 2.5 standard deviations over the mean of the zero calibrator. All serum IL-5 and IL-16 values in the nasal allergen challenge study were above the respective serum LLODs of 1.65 pg/mL and 37.7 pg/mL. LLODs of nasal IL-5 and IL-16 were ≤ 0.135 pg/mL and ≤ 11.75 pg/mL respectively. The nasal cytokine concentrations during season presented here are considerably diluted values because the nasal lavage technique does not allow the recalculation of the original local cytokine concentrations. Values below the LLOD in the nasal lavage study

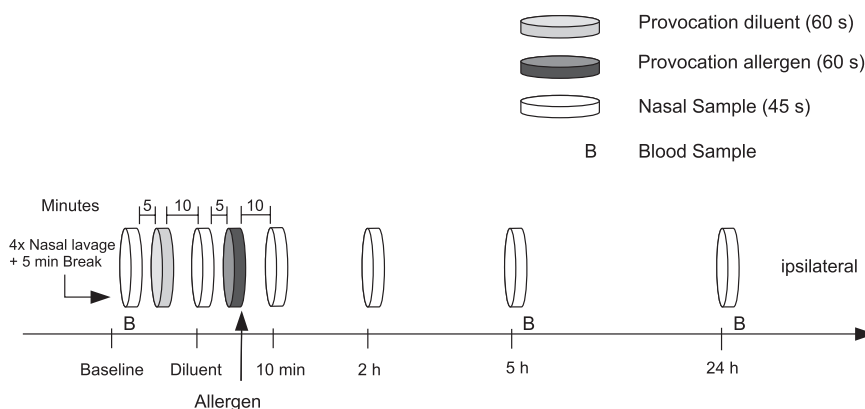


Fig. 1. Protocol of nasal allergen challenge using the disc method. A control challenge using diluent instead of allergen was performed on a separate day using the same protocol.

had CVs < 21% and were depicted graphically in relation to the LLOD. The nasal cytokine contents of the nasal allergen challenge study are the total amounts recovered by the individual collection discs as described before [44]. These values were calculated by multiplying the measured concentration by the volume of the collected secretion plus the volume of the eluate (0.5 mL) [44]. If one measured duplicate value was above and the other duplicate value below the LLOD (with a CV value $\leq$ 20%) in the nasal allergen challenge study, the value was defined as the LLOD. If both duplicate values were below the LLOD in the nasal allergen challenge study, the values were defined as zero values to avoid inappropriately high values due to the described recalculation of original local cytokine contents.

IL-33 and soluble ST2 enzyme-linked immunosorbent assay

Commercially available ultra sensitive pre-coated enzyme-linked immunosorbent assay (ELISA) kits were used to measure soluble ST2 (Quantikine® ELISA ST2 Human ST2/IL-1 R4 Immunoassay, R&D Systems Europe, Abingdon, UK) and IL-33 (LEGEND MAX™ Human IL-33 ELISA Kit, BioLegend, Inc., San Diego, CA, USA) levels in nasal secretions and serum. Sensitivities of nasal and serum soluble ST2 were $\leq$ 5.9 pg/mL and those of IL-33 $\leq$ 6.1 pg/mL respectively. The ELISA protocols were carried out according to manufacturer's recommendations. The ELISA plates were read using an ELISA Reader (DTX 880 Multimode Detector, Beckmann Coulter GmbH, Krefeld, Germany). If CV values were > 20%, the measurement was repeated. As described for the cytokine levels measured via electrochemiluminescent immunoassay, the cytokine concentrations during peak pollination season are highly diluted due to the usage of the nasal lavage technique, and the cytokine contents of the nasal allergen challenge study represent the total amounts recovered by the individual collection discs [44]. The soluble ST2 levels were above the respective detection limits. Nasal IL-33 levels during the pollination season could not be determined due to insufficient sensitivity of the ELISA. For the nasal allergen challenge study, nasal and serum IL-33 were mostly within detection range, and subthreshold IL-33 levels were evaluated as described for IL-5.

Statistics

Statistical analyses were performed using Medcalc software 12.1.0 (Kagi, Berkeley, CA, USA). Graphs were generated with GraphPad Prism (Graph Pad, San Diego, CA, USA). For nasal allergen challenge data nonparametric statistic tests were employed. To examine differences

between points on either the diluent or the allergen challenge curves, Friedman analysis of variance (ANOVA) was used. If a significant overall difference was found, *post hoc* analysis was performed using the method given by Conover [46] to identify time-points showing significantly different levels compared to the baseline and diluent time-points. Correlations were evaluated using Spearman rank test and Kendall's tau test. If not otherwise stated all values are displayed as mean $\pm$ SEM. To analyse the data during natural allergen exposure *P*-values were calculated using the *t*-test for independent samples. Generally, a two-tailed *P*-value of $P \leq 0.05$ was considered significant.

Results

Nasal soluble ST2 and IL-16 release in peak season and their relation to disease severity

During the peak pollination season, the nasal lavages of 15 allergic and 14 non-allergic volunteers were collected and cytokine levels were evaluated. Nasal lavages were performed with 10 mL of Sodium Chloride Solution; therefore, the cytokine concentrations determined and shown are those in highly diluted nasal secretions. Nasal lavage levels of IL-5 were significantly elevated in subjects with allergic rhinitis (mean: 0.362 pg/mL) compared with non-allergic individuals, whose levels were around and (also with CVs < 20) mostly slightly below the LLOD of 0.078 pg/mL ($P < 0.001$; Fig. 2a). Nasal lavage levels of IL-16 were significantly elevated in allergic subjects (mean: 13.6 pg/mL) compared with non-allergic controls (mean: 8.6 pg/mL; $P = 0.002$; Fig. 2b). Levels of soluble ST2 in the nasal lavages of allergic volunteers (mean: 25.7 pg/mL) were also significantly elevated compared to controls (mean: 12 pg/mL; $P = 0.002$; Fig. 2c). IL-33 was undetectable in the nasal lavages of allergic or control donors during the pollination season. Nasal levels of soluble ST2, IL-5 and IL-16 during the pollination season were tested for correlations to each other or to the symptoms. Nasal IL-16 correlated with soluble ST2 concentrations ($n = 15$; Spearman rank test: $r = 0.56$, $P = 0.03$; Fig. 2d) and showed a weak inverse correlation trend with nasal symptoms in peak season ($r = -0.48$, $P = 0.07$; Fig. 2e). Concentrations of soluble ST2 were negatively correlated with the nasal symptoms in peak season ($r = -0.61$, $P = 0.02$; Kendall's tau test: $\tau = -0.53$, $P = 0.005$; Fig. 2f).

Nasal allergen challenge study: increased levels of nasal soluble ST2 and IL-16 post-challenge

To complement our investigation of allergic rhinitis regarding the more chronic state of nasal allergic

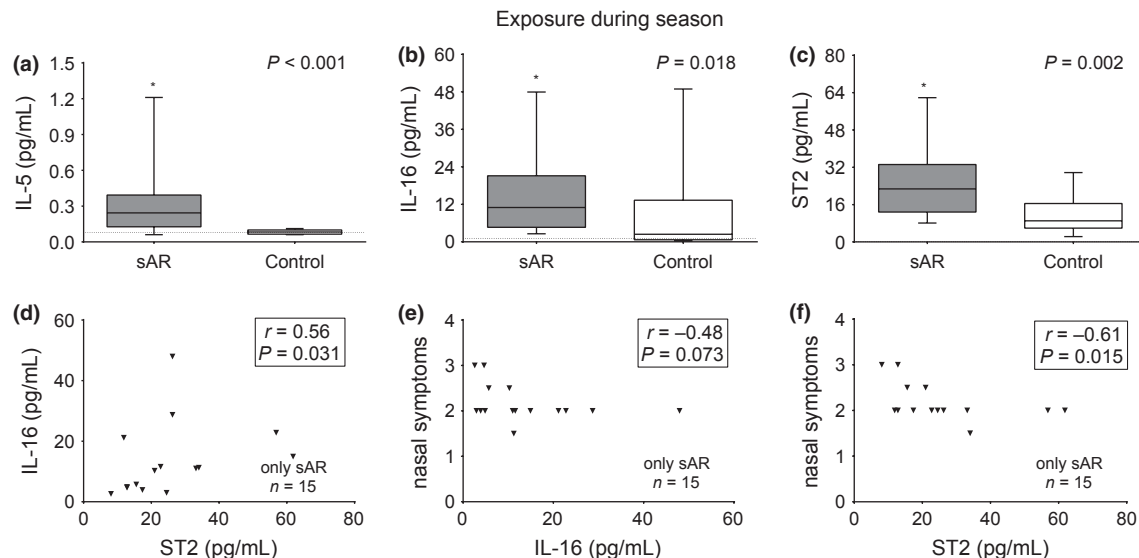


Fig. 2. Cytokine concentrations in nasal lavages of allergic rhinitis and control subjects after natural pollen exposure. (a–c) Nasal IL-5 (a), IL-16 (b) and soluble ST2 (c) concentrations in nasal lavages after natural pollen exposure during peak season in 15 seasonal allergic rhinitis subjects (sAR) compared to non-allergic controls. Nasal lavage concentrations of soluble ST2 were within detection range. In contrast, some values of IL-5 and IL-16 were below the lower limit of detection (LLOD; defined as described in Methods) depicted as a dotted horizontal line: 0.078 pg/mL (IL-5) and 1.11 pg/mL (IL-16), however, CV values were ≤ 21 . (d) Correlation between the nasal soluble ST2 and the IL-16 concentrations of 15 allergic rhinitis volunteers during natural pollen exposure. (e) Weak inverse correlation trend of IL-16 concentrations with the nasal symptoms of 15 allergic rhinitis volunteers during natural pollen exposure. (f) Inverse correlation of the nasal soluble ST2 concentrations with the nasal symptoms of 15 allergic rhinitis volunteers during natural pollen exposure. Nasal symptoms were assessed by questionnaire and comprised secretion, obstruction, sneezing and itching. The correlation coefficient and the P -value were calculated using the Spearman rank test.

inflammation during the pollination season [47], we additionally analysed the early and late-phase responses after a single nasal allergen challenge. Therefore, six volunteers with seasonal allergic rhinitis underwent a unilateral nasal allergen challenge out of the pollination season. Following the nasal allergen challenge using the disc method (Fig. 1), nasal symptoms increased significantly (Figs 3a,b). Post-allergen itching score was significantly elevated almost immediately [allergen (10 min): 43.2 ± 10.8] and gradually abated during the following 24 h (Fig. 3a). In contrast, the control challenge with the diluent alone did not cause any statistically significant changes [diluent (10 min): 2.6 ± 1.5] (Fig. 3a). A similar time course was observed for the obstruction score (data not shown) and the nasal secretion score [diluent (10 min): 3.5 ± 2.3 ; allergen: 49.1 ± 9.5], which remained significantly elevated until the 5 h measuring time-point (allergen: 31 ± 10.2 ; Fig. 3b). Likewise, nasal secretion weights significantly increased after nasal allergen challenge [allergen (before challenge): 17.4 ± 5.8 mg; allergen (10 min): 64.7 ± 11.3 mg] and slowly decreased [allergen (24 h): 37.8 ± 9.4] (Fig. 3c). No significant changes of secretion weights were measured after the control challenge (Fig. 3c).

Before challenge, relatively low levels of IL-5 were measured in nasal secretions (diluent: 0.4 ± 0.3 pg; Fig. 3d). IL-5 levels started to increase significantly 2 h post-challenge, peaked at 5 h (70.5 ± 32.8 pg) and

decreased at 24 h, though still remained significantly elevated (30 ± 12.9 pg; Fig. 3d). Nasal IL-5 levels after the control challenge remained at baseline level (Fig. 3d). IL-16 levels increased significantly at 2, 5 and 24 h after the challenge compared to the baseline with a maximum at 5 h [allergen (0 h): 50 ± 12 pg; allergen (2 h): 204 ± 64 pg; allergen (5 h): 466 ± 146 pg; allergen (24 h): 290 ± 66 pg] (Fig. 3e). After the control challenge, a relatively moderate but significant increase in IL-16 could be observed at 5 h [diluent (0 h): 59 ± 20 pg; diluent (5 h): 178 ± 40 pg] but not at the other time-points (Fig. 3e). The levels of IL-33 in the nasal secretions remained undetectable or at low levels at all time-points after allergen as well as control challenge. The highest IL-33 levels were measured at the 5 h time-point after allergen challenge (8.7 ± 5.5 pg) and 10 min after control challenge (12.4 ± 6.4 pg), however changed without any statistical significance (Fig. 3f). Soluble ST2 was detected at relatively low levels prior to nasal allergen challenge in nasal secretions. Ten minutes after nasal challenge soluble ST2 levels significantly increased (212 ± 59 pg) and decreased to lower levels close to control levels at the 2 h time-point (45 ± 22 pg) but remained significantly elevated in comparison to the baseline (Fig. 3g). During the late-phase response nasal soluble ST2 levels showed a second peak at 5 h (125 ± 48 pg) and reached their maximum at 24 h (225 ± 65 pg; Fig. 3g). No statistically

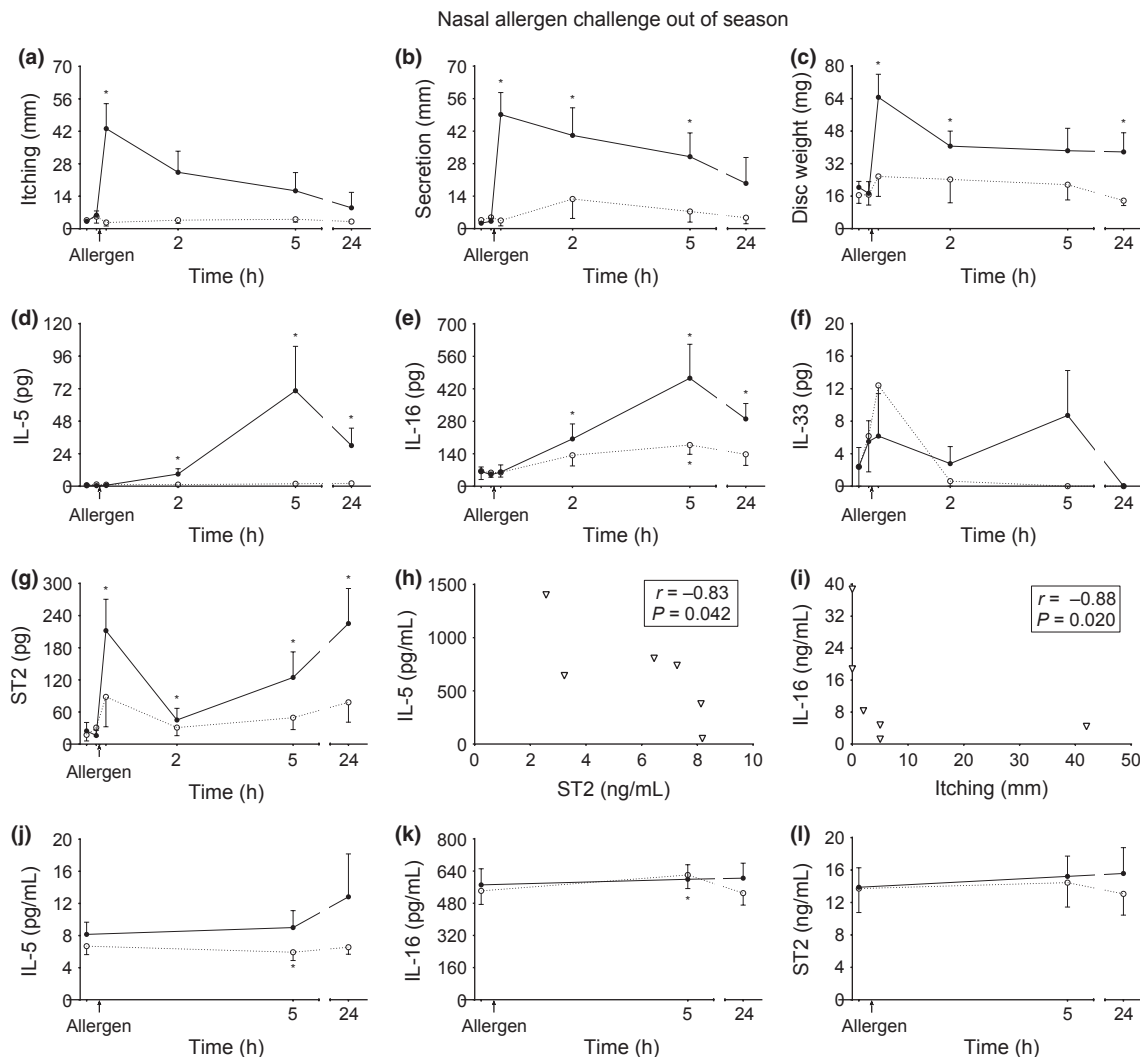


Fig. 3. Nasal and systemic cytokine levels after nasal allergen challenge out of season using the disc method. (a–g) Time course of nasal itching [visual analogue scale (VAS)] (a), nasal secretion (VAS) (b), nasal secretion weights (c), nasal ipsilateral IL-5 contents (d), IL-16 contents (e), IL-33 contents (f) and soluble ST2 contents (g) after unilateral nasal allergen challenge. Nasal secretions were collected using filter discs, and nasal symptoms or nasal secretion weights were recorded at all time-points following allergen challenge. The cytokine contents represent the recalculated (undiluted) total amounts recovered by the individual collection discs. (h, i) Inverse correlation trends between nasal soluble ST2 concentrations and nasal IL-5 concentrations (h) as well as between nasal IL-16 concentrations and itching (i) 24 h after nasal allergen challenge ($n = 6$). The correlation coefficient and the P -value were calculated using the Spearman rank test. (j–l) IL-5 (j), IL-16 (k) and soluble ST2 (l) concentrations in serum before and 5 and 24 h following nasal allergen challenge. (a–g, j–l) The dashed time course lines represent values obtained after control challenge. Values presented are means $\pm$ SEM ($n = 6$; $*P < 0.05$).

significant changes in soluble ST2 could be measured after control challenge (Fig. 3g).

Nasal levels of soluble ST2, IL-5 and IL-16 after nasal allergen challenge were correlated with each other or to symptom scores. Due to the low number of study participants in the nasal allergen challenge study, Kendall's tau test was performed besides the Spearman rank test and we speak of correlation trends instead of correlations also in the case of significant P -values. IL-16 concentrations showed a negative correlation trend to the itching score at 24 h (Spearman rank test: $r = -0.88$, $P = 0.02$; Kendall's tau test: $\tau = -0.78$, $P = 0.015$;

Fig. 3i) but not at the earlier time-points tested. Concentrations of soluble ST2 showed a negative correlation trend to those of IL-5 at 24 h ($r = -0.83$, $P = 0.04$; $\tau = -0.73$, $P = 0.02$; Fig. 3h) but not at the earlier time-points tested. Using Kendall's tau test for testing the correlation trends of ST2 with the symptom scores, the only association trend found was between ST2 contents at 24 h and the itching score at 24 h ($\tau = 0.93$, $P = 0.02$). In contrast, IL-5 contents at 5 h showed correlation trends with various symptom scores, the most significant being the obstruction score at 5 h ($\tau = 1$, $P = 0.009$).

Systemic changes to serum IL-5, IL-16 and soluble ST2 levels after nasal allergen challenge

After nasal allergen challenge, IL-5, IL-16 and soluble ST2 serum levels rose moderately 5 and 24 h after nasal allergen compared to baseline (Figs 3j–l). In all analysed subjects, IL-5 levels declined weakly 5 h after control challenge (Fig. 3j). In five of six subjects, IL-5 sera levels rose 24 h after nasal allergen challenge (12.8 ± 5.3 pg/mL) compared to the baseline (8.1 ± 1.5 pg/mL; Fig. 3j). In all analysed subjects, IL-16 concentrations increased moderately 5 h after nasal control challenge however decreased at 24 h after control challenge, in contrast to increased mean values of serum IL-16 concentrations post-challenge at 5 and 24 h (Fig. 3k). Soluble ST2 levels rose slightly in the sera of all six subjects 5 h after nasal allergen challenge compared to the baseline (Fig. 3l). No systemic changes to IL-33 were seen after the single nasal allergen challenge (data not shown).

Discussion

Our study demonstrates to the best of our knowledge for the first time in patients with allergic rhinitis (i) the release of soluble ST2 and IL-16 into nasal secretions during natural allergen exposure; (ii) soluble ST2 release into nasal secretions (with maximal levels at 10 min and 24 h) after nasal allergen challenge; and (iii) detectable increases of IL-5 and soluble ST2 serum levels after a single nasal allergen challenge.

A role for IL-33/ST2L signalling in allergic rhinitis was indicated first by the characterization of a high expression of IL-33 and its receptor ST2L in the nasal epithelium of patients with allergic rhinitis [20], elevated serum levels of IL-33 in allergic rhinitis [19] and soluble ST2 in sera of birch atopics working in animal facilities [25]. Eosinophils and basophils accumulate in the nasal mucosa of patients with allergic rhinitis after nasal allergen challenge [44] and both cell types are direct leucocyte targets of IL-33 [48–50]. Furthermore, corticosteroid treatment reduced IL-33-mediated inflammatory responses via NF- κ B in cultured human nasal epithelial cells derived from allergic rhinitis patients [20]. Our finding of reciprocally correlated soluble ST2 and symptom scores in allergic rhinitis volunteers during the pollination season suggests that soluble ST2 might be a natural mediator attenuating the allergic inflammation and perhaps supporting its therapeutic potential. The negative correlation trend between soluble ST2 and IL-5 during the resolution of the nasal inflammation after a single nasal allergen challenge is a further hint in this direction; however, we recommend being careful with the interpretation of these particular nasal allergen challenge data for the following reasons: (i) The numbers of participants are very low ($n = 6$); (ii) The described negative correlation trend was

neither found during season nor in the early nor the start of the late-phase of allergic reactions after nasal allergen challenge; and (iii) We did not determine local eosinophil numbers and/or activation states in this study. Of note, eosinophilic infiltration in the nasal cavity was significantly decreased in a murine allergic rhinitis model after administration of anti-IL-33 [23]. Furthermore, a possible therapeutic effect of anti-ST2 antibodies and/or soluble ST2-Fc including the reduction in IL-5 levels and eosinophilia was reported in a mouse model of Th2-driven eosinophilic viral bronchiolitis [51] and in murine models of allergic asthma [52–54].

During natural pollen exposure, nasal soluble ST2 concentrations correlated with those of IL-16, another putative inhibitor in allergic airway disease, strengthening the hypothesis that these two cytokines indeed have inhibitory functions in allergic rhinitis. The presumed different sources and mechanisms of action of these two potential inhibitors in allergic airway disease are reflected by the different kinetics of these two cytokines after nasal allergen challenge. IL-16 was reported to show a therapeutic effect in a mouse model of allergic asthma [36]. In human *ex vivo* allergy studies, IL-16 induced preferential migration of human T regulatory cells [37] and inhibited the release of Th2 cytokines [38, 39].

We determined a biphasic release of nasal soluble ST2 in the nasal allergen challenge model with peak levels at 10 min and 24 h after nasal allergen challenge. Although levels of soluble ST2 at 2 h post-challenge were decreased, they were still significantly elevated compared to the baseline. In a mouse asthma model, a biphasic release of serum soluble ST2 has also been described [52]. Three hours post-challenge (as the first time-point post-challenge investigated) showed a distinct increase in soluble ST2 compared to the baseline [52]. Comparable to our study, this first peak in soluble ST2 levels occurred earlier than the peak in IL-5 levels, and it was suggested to be of mast cell origin [52]. In addition, in a murine lung injury model after LPS challenge, soluble ST2 protein levels remained longer at high levels than the levels of pro-inflammatory cytokines [55]. Furthermore, the biphasic nasal release of soluble ST2 resembles the biphasic release of histamine after nasal allergen challenge previously described by our group [44, 56] with peaks of histamine release 5 min and 5 h after nasal challenge. The immediate increase in histamine was assigned to a direct activation of mast cells after cross-linking of IgE by allergen, and the increase in histamine in the late-phase was attributed to the activation of basophils which enter the nasal mucosa [56]. It is tempting to speculate that soluble ST2 is derived from similar cellular sources. Constitutive expression of high soluble ST2 mRNA levels in mast cells [57] has been described. Moreover, in basophils, soluble ST2 protein is expressed weakly

constitutively and highly at 4 h and particularly 18 h and 24 h after activation, and is released after basophil degranulation [48]. Like basophils, Th2 cells enter the nasal mucosa after nasal allergen challenge [56, 58]. Activated, but not resting human Th2 cells (in contrast to Th1 and T regulatory cells) also produce soluble ST2 [59]. ILC2 cells might also represent another cellular source of soluble ST2 (and IL-5) [8].

We were unable to detect IL-33 in nasal lavages of symptomatic allergics during the peak pollination season. This might be due to high dilution of nasal secretions or a lack of sensitivity in the available assay system. However, Kamekura et al. were also unable to detect IL-33 protein release from human nasal epithelial cells though they reported an upregulation of IL-33 mRNA in IFN- γ and toll-like receptor 9 ligand treated human nasal epithelial cells [20]. Recently, Asaka et al. used aspirated nasal secretion samples of allergic rhinitis patients during peak season and found very low but significantly elevated IL-33 concentrations in allergics during peak pollen season that correlated with the total nasal symptom score [21]. This finding complements results of soluble ST2 levels in nasal secretions being negatively correlated with the total nasal symptom score.

After nasal allergen challenge, we were able to detect elevated nasal IL-33 in the late-phase in two of the six volunteers examined. Occasionally, we observed increased levels of nasal IL-33 after nasal contact with the collection or dilution discs without allergen. This may be explained by the recent observation that IL-33 is secreted by living cells in response to mechanical stimulation [60]. An immediate release seems plausible because IL-33 exists preformed in nasal epithelial cells [20].

Our group published previously nasal IL-1 β in 1000-fold lower quantities than its antagonist IL-1Ra [44]. Similarly, we found high soluble ST2 and relatively low or no IL-33 levels. We speculate that the relatively high ratio of receptor to its ligand might reflect a general regulatory mechanism.

In asthmatics (with early and late responses to allergen inhalation) an association between allergen inhalation and increased IL-5 protein levels in serum and bone marrow was observed at 12 and 24 h after allergen inhalation. Both airway and blood eosinophilia were suggested to be sustained by an IL-5-responsive, eosinophil-differentiative process within the bone marrow [61]. In our study patients with allergic rhinitis revealed an increase in serum IL-5 and soluble ST2 upon a single localized nasal allergen challenge.

Future studies with larger cohorts, optimized IL-33 protein detection, inclusion of other cytokines and functional assays to further investigate the role of the IL-16 and IL-33/ST2L signalling in allergic rhinitis should be performed. In addition, the inclusion of systemic and local eosinophil numbers and activation states, as well as their relation to the corresponding levels of soluble ST2 and IL-16, should be considered. As suggested in murine models [5, 22, 23, 36, 52–54], it remains a promising hypothesis that IL-16 and/or soluble ST2 or other IL-33 inhibitors may have therapeutic potential in allergic airway disease.

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Conflicts of interest

Adam Chaker reports to have given lectures sponsored by GSK and received grant support or consultant arrangements with Allergopharma, ALK-Abello, Stallergenes, Mundipharma, Zeller AG, Novartis Pharma AG. All other authors have no conflict of interest to declare.

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