

Comparison of the nasal release of IL-4, IL-10, IL-17, CCL13/MCP-4, and CCL26/eotaxin-3 in allergic rhinitis during season and after allergen challenge

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ABSTRACT

Background: Allergic rhinitis is an inflammatory disease characterized by local overproduction of type 2 cytokines and tissue eosinophilia. Recent research suggests the involvement of additional cytokines such as IL-17, chemokine (C-C motif) ligand (CCL) 26/eotaxin-3, and CCL13/monocyte chemoattractant protein-4 (MCP-4) in its pathophysiology. Furthermore, bronchial epithelial cells treated with IL-17 and type 2 cytokines distinctively up-regulated eotaxin-3 gene expression. In this study we investigated the kinetics of IL-4, IL-10, IL-17, eotaxin-3, and MCP-4 in seasonal allergic rhinitis volunteers after nasal allergen challenge (NAC) and their release during natural pollen exposure.

Methods: The nasal lavages of 15 symptomatic allergic and 14 nonallergic subjects were collected during the pollination season. Additionally, six allergic subjects underwent a single unilateral nasal allergen and control challenge out of season, and nasal secretions were collected. Levels of IL-4, IL-10, IL-17, eotaxin-3, and MCP-4 in nasal lavages and secretions were measured using an electrochemiluminescent assay.

Results: After NAC, allergic subjects had a significant immediate response of nasal symptoms as well as a significant increase at 5 hours of IL-4, IL-10, and IL-17 and at 2, 5, and 24 hours significantly raising levels of eotaxin-3. IL-17 and eotaxin-3 concentrations at 5 hours were correlated ($r = 0.94$; $p = 0.005$). During natural pollen exposure, barely detectable levels of IL-17 in allergic subjects were also correlated with eotaxin-3 ($r = 0.62$; $p = 0.01$). Eotaxin-3 and MCP-4 levels were significantly elevated 9- or 3.7-fold, respectively, and IL-10 and, unexpectedly, IL-4 were significantly lower in allergic subjects compared with nonallergic subjects.

Conclusion: Nasal IL-17, MCP-4, and, possibly, eotaxin-3 may aggravate and IL-10 may alleviate nasal mucosal allergy.

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Allergic rhinitis is caused by an IgE-mediated inflammatory reaction to allergen exposure mainly in the nasal mucosa.^{1,2} Symptoms comprise sneezing, nasal obstruction, rhinorrhea, and itching and considerably affect the quality of life. Untreated allergic rhinitis leads to, in many cases, the development of asthma.^{3–5} Along with the burden of the disease and decrease in quality of life, allergic rhinitis and asthma have a significant economic impact.⁶

The expansion of the T helper 2 (Th2) cell subset of CD4⁺ T cells (responsible for the humoral response *via* Th2 cytokines such as IL-4) and the inhibition of the Th1 cell subset (responsible for the cellular immune responses *via* Th1 cytokines such as interferon- γ) is one basic hallmark of allergic immune responses.⁷ The confirmation of the existence of another subset of CD4⁺ T cells, the regulatory T cells (T_{Reg}), which limit allergic responses by suppressing the function of Th2 cells through inhibitory cytokines such as IL-10, complements this Th1/Th2 model.^{1,8,9} Additionally, another newly identified type of CD4⁺ T cells has been named the Th17 cell based on the secretion of IL-17 with possible roles in allergic inflammation.^{10,11} Interestingly, a subset of Th2 cells also secretes IL-17.¹² Besides Th cells, other cells such as eosinophils, basophils, mast cells, epithelial cells, and/or nuocytes have also been described as sources of the described cytokines.^{9,13}

The aim of this study was to characterize the release of IL-4, IL-10, IL-17, eotaxin-3, and monocyte chemoattractant protein-4 (MCP-4) in

allergic rhinitis patients on nasal allergen challenge (NAC) and natural pollen exposure. To date, this type of direct comparison has seldom been performed.¹⁴ The choice of the specific cytokines in our study was based on the following considerations: because of their central roles in allergic inflammation and however insufficient characterization *e.g.*, during natural allergen exposure, we chose the type 2 cytokine IL-4^{9,15} and the regulatory cytokine IL-10,^{16–18} IL-17 and the two C-C chemokines CCL26/eotaxin-3 and CCL13/MCP-4 were chosen because their nasal release has not been investigated in allergic rhinitis thus far, although several reports suggest their contribution to the pathophysiology of allergic rhinitis.^{12,19–24}

MATERIALS AND METHODS

Subjects and Design

The study was approved by the Ethics Committee of the Heinrich-Heine University of Duesseldorf. All participants provided written informed consent. R. Baumann and M. Rabaszowski contributed equally to this work.

Overall, 17 nonallergic control subjects and 25 patients with seasonal allergic rhinitis, according to Allergic Rhinitis and its Impact on Asthma and World Health Organization classified as moderate-severe persistent allergic rhinitis, were recruited based on medical history and a skin-prick test²⁵ showing sensitization to either grass (grass mix; Allergopharma Joachim Ganzer KG, Reinbek, Germany) and/or tree pollen (tree mix [birch, European beech, plane, and oak]; Allergopharma). Ten otherwise healthy subjects with seasonal allergic rhinitis underwent NAC out of the pollination season. Control challenges using allergen diluent were performed before or 2 weeks after NAC. During the NAC study, four subjects had to be excluded because of procedural adverse events or noncompliance. Six subjects were included for analysis (age, 19–53 years; mean age, 28.3 years). For the nasal lavage study, we recruited 15 seasonal allergic and 17 nonallergic control subjects during the pollination season. Their clinical symptoms at the time were assessed *via* a questionnaire comprising nasal symptoms (secretion, obstruction, itching, and sneezing),

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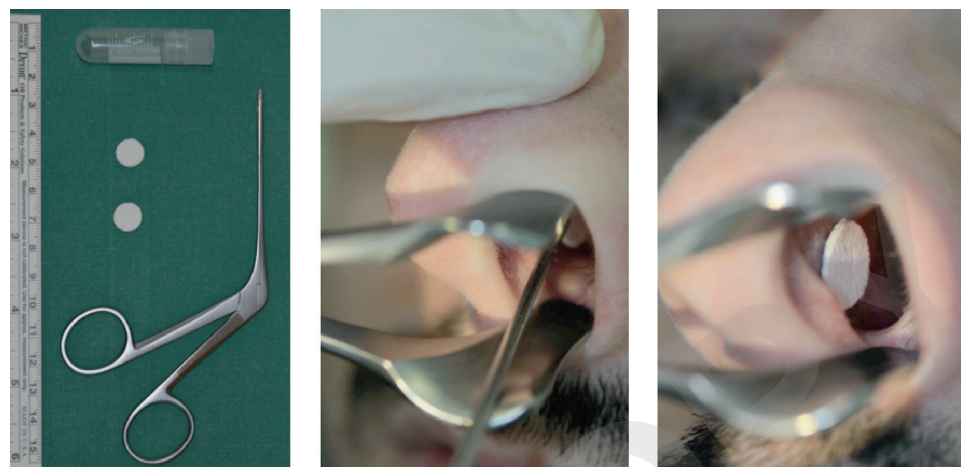
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Figure 1. Nasal allergen challenge (NAC) and collection of nasal secretions with the disk method. (A) Filter paper disks with 10-mm diameter and 1.2-mm thickness (Shandon filter cards; Shandon, Inc., Pittsburgh, PA), cryotube, and duckbill forceps; (B) insertion of the disk into the nasal cavity; (C) disk located on the middle portion of the anterior nasal septum, posterior to the mucocutaneous junction.



overall severity of allergic symptoms, and a differential blood count. Three control subjects were excluded before analysis because of procedural adverse events or elevated eosinophil counts. Fifteen allergic subjects (age range, 22–52 years; mean age, 28.4 years; 53.3% women) and 14 control subjects (age range, 23–50 years; mean age, 29 years; 64.3% women) were included for analysis. Among others, exclusion criteria included any rhinitis or rhinosinusitis other than allergic within 1 month before study, any history of chronic rhinosinusitis, undergoing a specific immunotherapy within the last 3 years, or any surgery 3 months before study participation. All volunteers did not take any medication (except oral contraceptives) for the last 3 months before or during the study with the exception of antiallergic medication until 10 days before nasal lavage.

Nasal Challenge and Sample Collection

Filter paper disks were applied for 1 minute for NAC or 45 seconds for sample collection (Fig. 1). Challenge disks were soaked with 75 μ L of NAC solution (Allergopharma) in a concentration of 50,000 BU/mL (3750 BU/disk) or diluent alone (sterile phenol-buffered saline; Allergopharma). For the sample collection, preweighed disks were stored inside an assigned 2-mL tube (Cryo S PP; Greiner Bio-One, Frickenhausen, Germany). Immediately after collection, disks were placed back inside the corresponding tube and weighed measuring the acquired secretion weight. For elution purposes, 500 μ L of 0.9% sodium chloride solution (B. Braun Melsungen A.G., Melsungen, Germany) were added to each tube. After 3 hours of gentle shaking at 4°C, the disks were removed and the elution was centrifuged at 2800 rpm (Biofuge 15R; Heraeus Instruments, Duesseldorf, Germany). The supernatant was aliquoted into 0.5-mL tubes (LoBind; Eppendorf AG, Hamburg, Germany) and stored at –80°C. Four nasal lavages (5 mL of 0.9% sodium chloride solution) were performed to wash out preexisting mediators before all challenges. Baseline and diluent samples were collected before challenges. The clinical reaction on NAC or diluent challenge was observed and documented after 10 minutes and 2, 5, and 24 hours. The parameters, namely sneezing, nasal secretion, obstruction, and itching, were obtained using a visual analog scale (VAS) and scored as follows: 0 = 0–20 mm, 1 = 21–40 mm, 2 = 41–60 mm, 3 = 61–80 mm, and 4 = 81–100 mm; sneezing: 0 = 0–1 sneezes, 1 = 2 sneezes, 2 = 3 sneezes, 3 = 4 sneezes, 4 = >4 sneezes within 10 minutes. The mean of these scores is shown.

Electrochemiluminescent Immunoassay

IL-4, IL-10, IL-17, MCP-4, and eotaxin-3 in nasal secretions were measured using an electrochemiluminescent Multi-Spot assay (Meso Scale Discovery [MSD], Gaithersburg, MD). The IL-17 assay detects IL-17A and, to a lesser extent, the heterodimer IL-17A/F. Standards or

samples were measured in duplicates and zero standards in triplicates. The manufacturer's recommendations were adapted using a 2½ hour initial incubation. The plates were immediately read on the Sector Imager 6000 (MSD). Raw data were analyzed with the Discovery Workbench 3.0 software (MSD). Measurements with a CV value of >20% were repeated.

Statistics

Statistical analyses were performed using Medcalc software 12.1.0 (Kagi, Berkeley, CA). GraphPad Prism (Graph Pad, San Diego, CA) was used to create graphs. Nonparametric statistics were used for the analysis of the data of the NAC study because they were not normally distributed. For the examination of differences between points on either the diluent or the NAC curves, Friedman's analysis of variance was used. If a significant overall difference was found, *post hoc* analysis was performed using the method provided by Conover²⁶ to identify time points showing significantly different levels compared with the diluent time points. The Spearman rank test was used for correlation analyses. If not otherwise stated, all values are displayed as mean $\pm$ SEM. The *t*-test for independent samples or the Mann-Whitney test was used for the comparison of allergic rhinitis and healthy control subjects during natural allergen exposure, depending on the fact whether or not the data were normally distributed. Generally, a two-tailed value of $p \leq 0.05$ was considered significant.

RESULTS

Six volunteers with seasonal allergic rhinitis underwent unilateral NAC out of the pollination season. Immediately after NAC, nasal symptoms increased significantly (diluent [5 minutes before NAC], 0.0 ± 0.0 ; allergen [10 minutes], 2.1 ± 1.1) and remained significantly elevated until 5 hours postchallenge (allergen [5 hours], 0.6 ± 0.7 ; Fig. 2 A). The reactions were considered specific, because control diluent challenges performed on a separate day did not cause any significant nasal symptoms (diluent [10 minutes], 0.0 ± 0.0 ; diluent [5 hours], 0.1 ± 0.3 ; Fig. 2 A).

After NAC, amounts of IL-4, IL-10, IL-17, and eotaxin-3 in nasal secretions changed significantly over the course of time (Fig. 2, B–E). IL-4 amounts peaked in nasal secretions from three of six individuals 5 hours after NAC (allergen [5 hours], 7.5 ± 2.7 pg) and remained undetectable at all other time points (Fig. 2 B). IL-10 was undetectable before the challenge and reached its highest amount 5 hours after the challenge, where it was detectable in four of six individuals (allergen [5 hours], 1.1 ± 0.5 pg). It remained elevated 24 hours after the challenge (allergen [24 hours], 0.4 ± 0.2 pg; Fig. 2 C). After NAC, IL-17 amounts increased at 10 minutes (allergen [10 minutes], 5.4 ± 2.6 pg) with a decline at 2 hours –allergen [2 hours], 2.4 ± 0.9 pg; however,

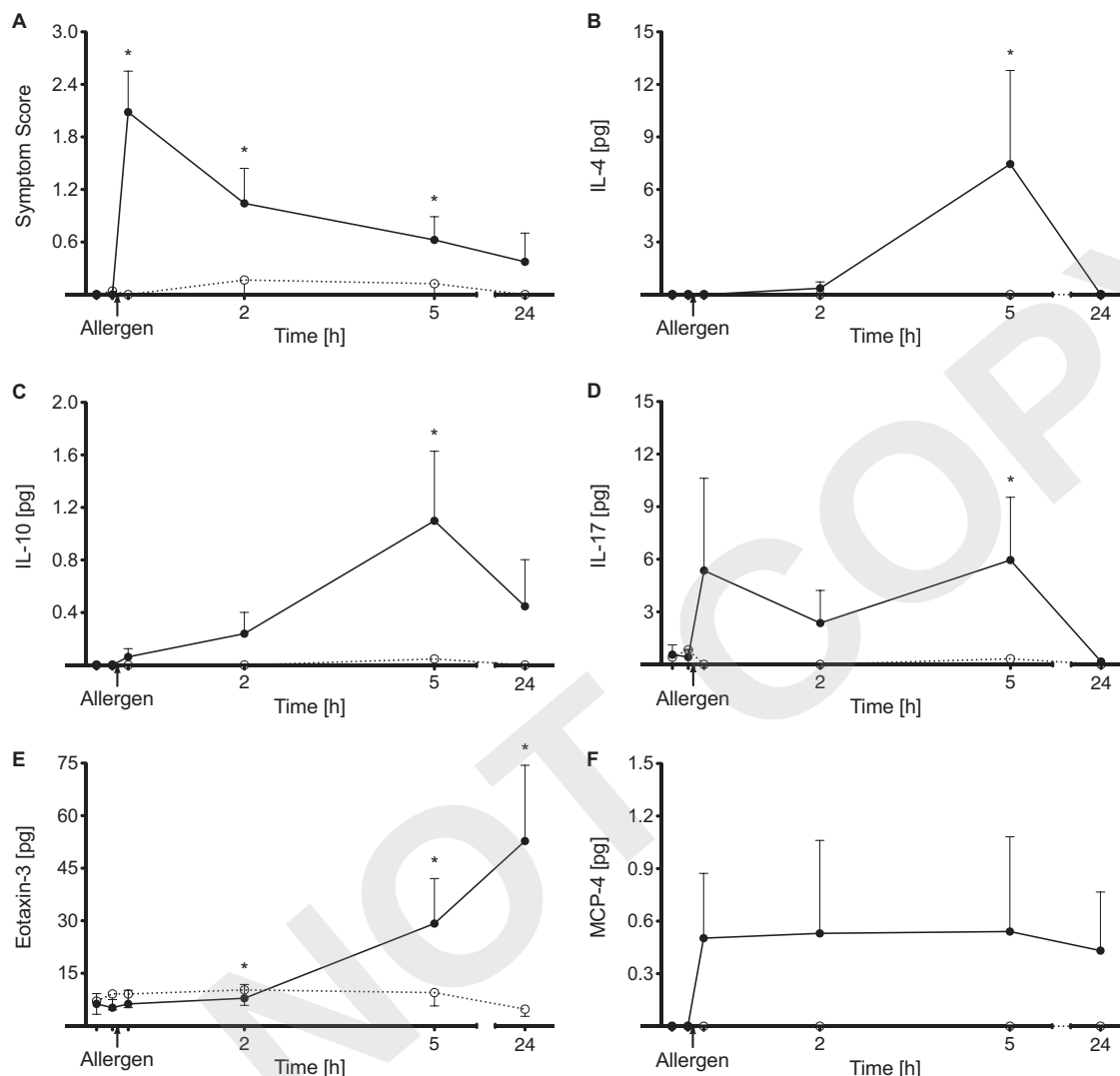


Figure 2. Time course of symptoms, ILs, and chemokines in ipsilateral nasal secretions after unilateral nasal allergen challenge (NAC). (A) The symptom score as well as the contents of (B) IL-4, (C) IL-10, (D) IL-17, (E) eotaxin-3, and (F) MCP-4 in ipsilateral nasal secretions are shown. The mean of the scores ranging from 0 to 4 (0 = no, 1 = mild, 2 = moderate, 3 = moderate/severe, and 4 = severe symptoms) for sneezing, nasal secretion (visual analog scale [VAS]), obstruction (VAS), and itching (VAS) was calculated. Nasal symptoms were recorded and nasal secretions were collected before and at all time points after the allergen challenge. The dashed line represents values obtained after the control challenge on a separate day using the same protocol. Values presented are means $\pm$ SEM ($n = 6$; $*p < 0.05$).

this was observed only in two of the six individuals. Five hours after the challenge, IL-17 was detectable in five of the six individuals (allergen [5 hours], 6.0 ± 1.8 pg), subsequently decreasing to barely detectable levels at 24 hours (Fig. 2 D). Eotaxin-3 amounts remained detectable in similar amounts before (allergen [0 hours], 6.3 ± 1.5 pg; diluent [0 hours], 7.1 ± 1.9 pg) until 2 hours after the challenge with either allergen or diluent. Eotaxin-3 amounts were significantly increased at 5h (allergen [5 hours], 29.2 ± 6.4 pg) and continued to rise significantly until 24 hours in all volunteer's nasal secretions after NAC (allergen [24 hours], 52.8 ± 10.8 pg; Fig. 2 E). Very small amounts of MCP-4 were detectable after NAC within two of six subjects (diluent [5 minutes before NAC], 0.0 ± 0.0 ; allergen [10 minutes], 0.5 ± 0.2 pg; Fig. 2 F). No statistically significant changes for any of the tested parameters were observed after the control challenge with the diluent alone.

To investigate nasal cytokine levels after natural allergen exposure, we evaluated allergic symptoms and concentrations of IL-4, IL-10, IL-17, eotaxin-3, and MCP-4 in the nasal lavages of 15 allergic volun-

teers with seasonal allergic rhinitis and 14 nonallergic control subjects during the pollination season. Allergic symptoms were distinctively elevated in allergic subjects when compared with nonallergic control subjects ($p < 0.0001$; Fig. 3 A). Nasal lavage levels of IL-10 were significantly lower in subjects with allergic rhinitis (mean, 0.259 pg/mL) compared with nonallergic individuals (mean, 0.272 pg/mL; $p = 0.016$; Fig. 3 C). Nasal IL-4 levels were significantly lower in allergic subjects (mean, 0.172 pg/mL) compared with control subjects (mean, 0.340 pg/mL; $p < 0.0001$; Fig. 3 B). No significant differences were detected for IL-17 (Fig. 3 D). Eotaxin-3 levels were significantly elevated (ninefold) in the nasal lavages of allergic volunteers (mean, 380 pg/mL) compared with controls (mean, 42 pg/mL; $p = 0.0003$; Fig. 3 E). MCP-4 levels were also significantly elevated (3.7-fold) in the nasal lavages of allergic subjects (mean, 22.5 pg/mL) compared with control donors (mean, 6.1 pg/mL) during the pollination season ($p = 0.0001$; Fig. 3 F).

Nasal lavage levels of cytokines after NAC and during the pollination season were tested for correlations to each other or to the symp-

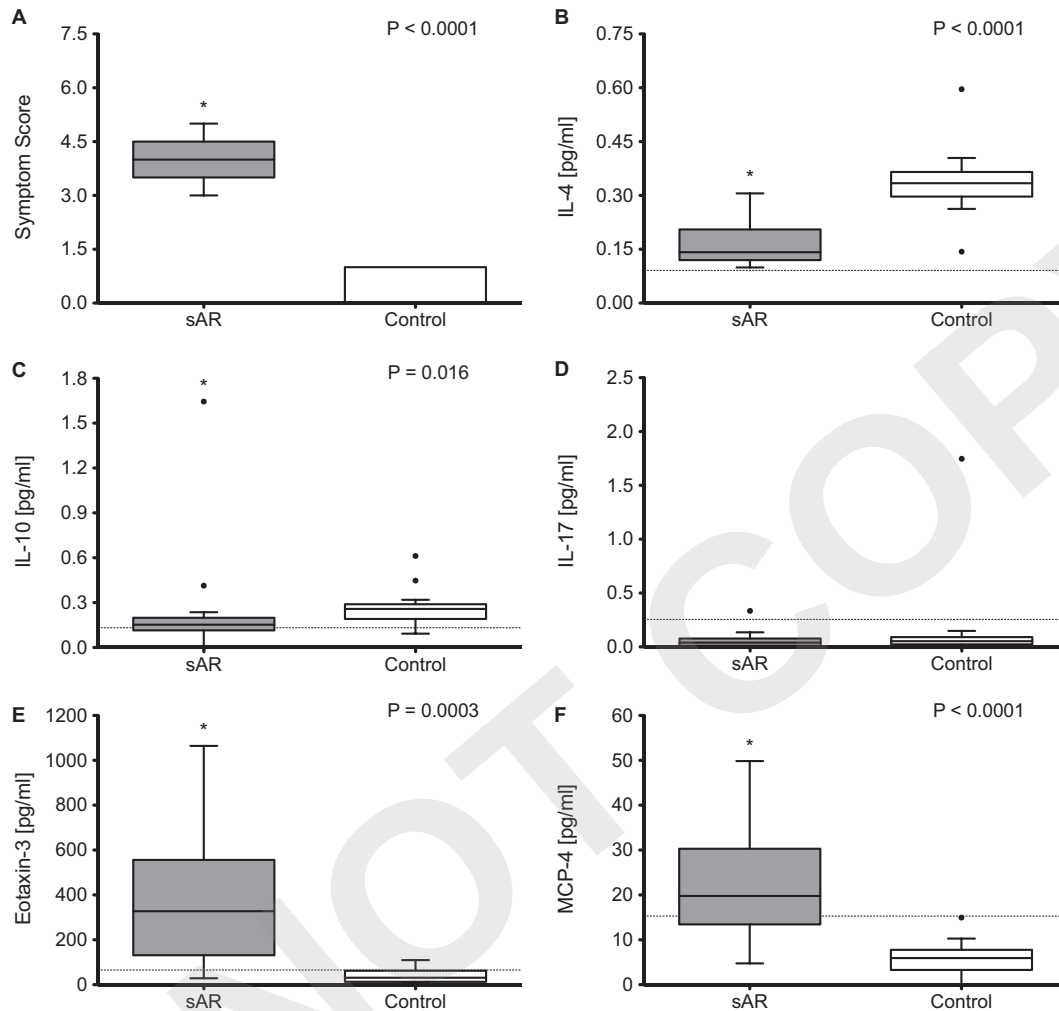


Figure 3. Symptom score and IL and chemokine concentrations in nasal lavages after natural pollen exposure. The (A) symptom score of as well as the concentrations of (B) IL-4, (C) IL-10, (D) IL-17, (E) eotaxin-3, and (F) MCP-4 in 15 seasonal allergic rhinitis subjects (sAR) compared with 14 nonallergic controls are shown. The dotted horizontal line indicates the lower limit of detection of the protein detection assay (* $p < 0.05$).

toms. Eotaxin-3 and IL-17 concentrations were correlated at 5 hours after NAC ($n = 6$; Spearman rank test, $r = 0.94$; $p = 0.005$; Fig. 4 A). Of note, the concentrations of these two cytokines also correlated during the pollination season ($n = 15$; $r = 0.62$; $p = 0.01$; Fig. 4 B). In addition, MCP-4 concentrations correlated with eotaxin-3 ($n = 15$; $r = 0.81$; $p = 0.0003$; Fig. 4 C) and with IL-17 ($n = 15$; $r = 0.69$; $p = 0.005$; Fig. 4 D) during the pollination season. Neither the cytokine concentrations nor the symptom scores during the pollination season correlated with the estimated length of allergen exposure experienced by the “in-season” subjects at the time of measurement (data not shown).

DISCUSSION

To the best of our knowledge, the current study shows for the first time in patients with allergic rhinitis (i) the release of IL-17 and eotaxin-3 in nasal secretions mainly during the late-phase response after NAC and of eotaxin-3 and MCP-4 in nasal secretions during natural allergen exposure, (ii) a correlation of nasal IL-17 and eotaxin-3 protein levels in nasal secretions during natural pollen exposure and at 5 hours after NAC, and (iii) significantly decreased levels of the regulatory cytokine IL-10 in allergic subjects compared with nonallergic subjects during natural allergen exposure.

The proinflammatory cytokine IL-17, also known as IL-17A, binds as a homodimer or as a heterodimer with IL-17F to its receptor,

IL-17RA.⁹ It is responsible for the deterioration of allergic asthma and allergic rhinitis.⁹ It promotes differentiation and increase of numbers of IgE-secreting cells.⁹ IL-17 acts as a potent mediator in delayed-type reactions by increasing the production of chemokines such as eotaxin-3 in various tissues to recruit immune cells to the site of inflammation.^{9,12,28} In comparison with wild-type mice, IL-17-deficient mice showed a significant decrease in allergic symptoms, serum IgE levels, and eosinophil infiltration into the nasal mucosa after intranasal challenge¹⁹ as well as decreased airway hypersensitivity responses after allergen inhalation.²⁹ Patients with severe allergic rhinitis could be identified by elevated serum IL-17 levels.^{20,30} Classic Th2 memory/effector cells revealed the potential to produce IL-17 after stimulation with proinflammatory cytokines.¹² The number of IL-17/Th2 cells was significantly increased in the blood of patients with atopic asthma.¹² Furthermore, asthmatic subjects showed positive correlations between the severity of their illness and the IL-17 protein levels.⁹ We found significantly elevated nasal IL-17 levels in allergic rhinitis subjects compared with controls after NAC, although not during natural pollen exposure. Future research is needed to elucidate if the moderate pollination season in 2010 might be one reason for this. The IL-17 levels in nasal lavages were barely detectable because of the high dilution of the nasal secretions. Because these nasal lavage IL-17 levels were distinctively below the lower limit of detection, we

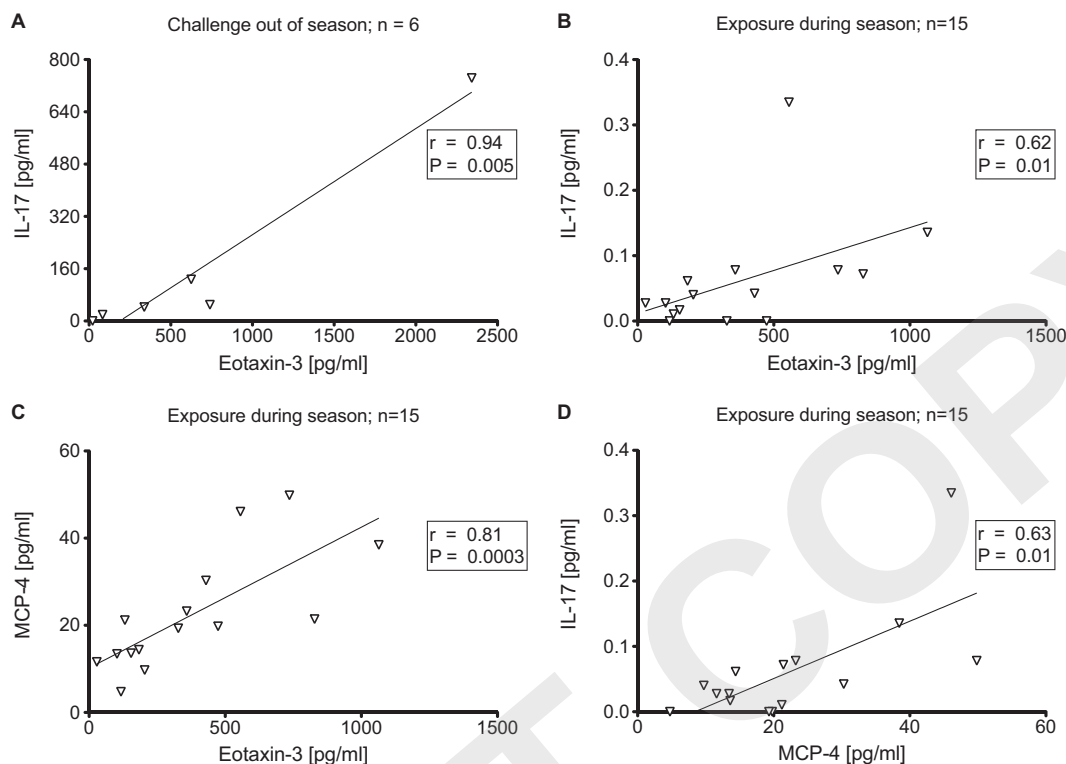


Figure 4. Correlations of cytokine concentrations in nasal secretions in allergic rhinitis. Correlations in nasal secretions (A) after the nasal allergen challenge (NAC; $n = 6$) or (B–D) in nasal lavages during natural pollen exposure ($n = 15$) are shown. (B and D) Nasal lavage values of IL-17 during natural pollen exposure were below the lower limit of detection; however, CV values were <10 . (A and B) IL-17 correlated with eotaxin-3 during natural exposure and after allergen challenge. The correlation coefficient and the p value were calculated using the Spearman rank test.

recommend being careful with their interpretation. However, we report these nasal lavage IL-17 values because (i) they were measured in duplicate with CVs <10 , (ii) they correlated with nasal eotaxin-3 as well as MCP-4 levels in allergic subjects, and (iii) we measured a similar correlation between IL-17 and eotaxin-3 with IL-17 values above the lower limit of detection after NAC.

Eotaxin-3 as a functional ligand for CCR3 acts at high concentrations chemotactic for eosinophils and basophils.³¹ Like IL-17, eotaxin-3 still has not been investigated after NAC; however, elevated nasal eotaxin levels have been described post-NAC.³² Eotaxin-3 mRNA was up-regulated >100 -fold in bronchial biopsy specimens of asthmatic subjects at 24 hours after the allergen challenge.³³ Furthermore, eotaxin-3 mRNA was distinctively up-regulated in epithelial cells treated with IL-17 and Th2 cytokines.¹² In patients with allergic rhinitis, besides IL-17, eotaxin-3 gene polymorphisms have been described.^{21,22} We observed elevated eotaxin-3 protein levels in allergic subjects, 9-fold after compared with nonallergic controls during the pollination season as well as 6-fold at 5 hours and 10-fold at 24 hours after NAC compared with the baseline. In both settings, eotaxin-3 and IL-17 concentrations were significantly correlated. Future research will have to elucidate if eotaxin-3 promotes the ongoing allergic inflammation of upper and lower airways in the later stages (24 hours) after allergen challenge.³³ Alternatively, its increasing levels until 24 hours after allergen challenge, its antagonistic properties for CCR1, CCR2, and CCR5,^{34,35} as well as its weak agonistic properties for CCR3³¹ may indicate a contribution to the modulation or even regulation of allergic inflammation.

In our study, levels of IL-17 and eotaxin-3 both correlated with MCP-4 during the pollination season. The elevated MCP-4 levels during season confirm a gene expression study describing fivefold elevated MCP-4 mRNA levels in allergic rhinitis nasal mucosa compared with control samples.²⁴ After NAC, we did not find a signifi-

cant increase of MCP-4, thus suggesting that it might contribute to chronic inflammation. In allergic asthma, a disease-promoting function of MCP-4 has been suggested.²³

IL-4 is a pleiotropic Th2 cytokine responsible for the class switching from IgM to IgE and the differentiation to a Th2 phenotype of T cells.⁹ The unexpected significantly lower levels of IL-4 in allergic subjects compared with nonallergic controls in our study may be explained by its binding to the more abundantly expressed IL-4R on nasal cells in allergic subjects,^{36,37} or in nonallergic subjects possibly higher (by antibodies usually indistinguishable) protein levels of the human splice variant IL-4delta2, which acts as a natural receptor antagonist of full-length IL-4 protein.³⁸ The increased likelihood of expression of IL-4delta2 but not IL-4 mRNA was described in asthmatic subjects,³⁹ which additionally might decrease the further expression of IL-4. Although there were no significant differences, Chaves and colleagues described still different ranges of nasal IL-4 in allergic subjects (0–0.3) and healthy controls (0.05–0.8) during the pollination season.¹⁸ After a single nasal allergen (but not control) challenge, we found increased nasal IL-4 levels in those three allergic subjects with the highest symptom scores. Hence, different and complementary aspects of allergic rhinitis may be represented by the more chronic state of allergic inflammation during the pollination season¹⁴ and the early and late-phase responses after a single NAC out of season. In contrast to IL-13 polymorphisms, IL-4 polymorphisms did not show an associated risk with allergic rhinitis.⁴⁰ Paradoxical results about IL-4 have been described in allergic and other diseases.^{41–43}

IL-10 is a T_{Reg} cell cytokine that inhibits the expression of many proinflammatory cytokines,^{8,9} reduces IgE switching in the presence of IL-4,⁴⁴ inhibits eosinophil survival,⁴⁵ promotes T-cell tolerance to allergens,⁴⁶ and mediates allergen tolerance in allergen-specific immunotherapy and after exposure to high doses of allergen.^{9,47} In contrast with its inhibitory effects on T cells, IL-10 promotes survival,

proliferation, and differentiation of human B cells and increases the production of IgG4.^{9,44} A polymorphism of the IL-10 receptor IL-10RA was found to be significantly associated with allergic rhinitis.⁴⁸ In our study, we found elevated IL-10 protein levels at 5 hours after NAC. Our finding of significantly lower IL-10 levels in the nasal lavages of allergic rhinitis patients compared with nonallergic controls may result in exaggerated and more prolonged inflammatory responses in nasal airways and suggests a local regulatory role of IL-10 in allergic rhinitis. These lower nasal levels contradict several earlier studies describing higher^{16,17,49,50} or comparable IL-10 levels¹⁸ in allergic subjects compared with controls during the pollination season. However, nasal IL-10 levels in allergic rhinitis subjects before immunotherapy were significantly lower than after immunotherapy.¹⁶ Inconsistent results about IL-10 levels have also been described in bronchoalveolar lavage fluid from asthmatic patients compared with healthy controls.⁴⁸

The variability among the subjects regarding the length of pollen exposure before the study did not have a significant effect on the overall range of cytokine levels in our study. This is, from our point of view, not surprising because there is a known high variability in symptoms and the degree of inflammation even between patients that were exposed to identical amounts of allergen.

Wang and colleagues reported an important link between Th2 cells and IL-17 in asthmatic subjects and the up-regulation of eotaxin-3 mRNA in bronchial epithelial cells treated with IL-17 and Th2 cytokines.¹² Quan and colleagues described decreased nasal allergic symptoms in IL-17-deficient mice after intranasal challenge.¹⁹ Our findings of elevated IL-17 and eotaxin-3 protein levels after NAC and the correlation of nasal IL-17 and eotaxin-3 protein levels after NAC and during natural pollen exposure support the hypothesis that besides classically known Th2 or T_{Reg} cytokines, other cytokines may also contribute to or regulate nasal mucosal allergy. The decreased nasal levels of IL-10 in allergic subjects compared with nonallergic subjects during natural allergen exposure underline its potential regulatory role in allergic rhinitis.

CONCLUSION

An advanced understanding of the mechanisms leading to symptoms in allergic rhinitis may help to explain the limited therapeutic value of anti-IL-4, anti-IL-5, and anti-IL-13 strategies⁷ and may also aid with the identification of new therapeutic targets. We found significantly elevated nasal IL-4, IL-10, IL-17, and eotaxin-3 levels during the late phase after NAC in allergic rhinitis patients. Moreover, during natural allergen exposure, eotaxin-3 and MCP-4 concentrations were higher and IL-4 and IL-10 were lower in allergic rhinitis patients compared with nonallergic controls. The exact role of IL-4 in the pathophysiology of allergic rhinitis remains unclear due to lower nasal IL-4 levels in allergic subjects compared with controls during season and elevated IL-4 levels after a single NAC out of season. Lower nasal IL-10 levels in allergic subjects compared with controls may result in exaggerated inflammatory responses in nasal airways and suggest a local regulatory role for IL-10 in allergic rhinitis. Elevated IL-17 levels during the late phase after NAC may contribute to the deterioration of human allergic rhinitis, complementing very recent results obtained in a mouse model where IL-17 deficiency decreased nasal allergic symptoms after intranasal challenge.¹⁹ The contributions of eotaxin-3 are still not clear: it might promote or regulate allergic inflammation.^{31,34,35} Our observation of a correlation between nasal IL-17 and eotaxin-3 in the late phase after NAC as well as during natural pollen exposure adds to the previously reported up-regulation of eotaxin-3 in bronchial epithelial cells treated with IL-17 and Th2 cytokines.¹² In the future, functional assays to explore the local roles particularly of IL-10 and IL-17 in the pathophysiology of allergic rhinitis should be considered. Clearly, additional research is needed to clarify whether IL-10, anti-IL-4, and/or anti-IL-17 strategies might contribute to new allergic rhinitis therapies.

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