

Henrik H. Jacobi^a
Lars K. Poulsen^a
Claus M. Reimert^a
Per S. Skov^b
Ann-Kristin Ulfgren^c
Ilona Jones^c
Lena B. Elfman^c
Hans-Jørgen Malling^a
Niels Mygind^a

^a Allergy Unit, National University Hospital, and

^b Reference Laboratory, Copenhagen, Denmark;

^c Pharmacia AB Diagnostics, Uppsala, Sweden

IL-8 and the Activation of Eosinophils and Neutrophils following Nasal Allergen Challenge

Abstract

Background: A growing body of evidence suggests that proinflammatory cytokines play a role in allergic inflammation by attracting and activating inflammatory cells. In this study, we have investigated the relationship between interleukin-8 (IL-8) in nasal lavage fluid and the local activation of eosinophils and neutrophils following nasal allergen challenge of allergic patients. **Methods:** Nasal challenges were performed with grass pollen extract in 14 allergic patients and 5 nonallergic controls. Nasal lavage fluid was collected repeatedly for 10 h, and the levels of eosinophil cationic protein (ECP) and myeloperoxidase (MPO) were used as markers of eosinophil and neutrophil activation, respectively. The levels of these molecules were compared with that of IL-8 in nasal lavage fluid. **Results:** Allergen challenge of allergic patients produced a significant late-phase increase in the levels of ECP and MPO. Furthermore, the level of MPO showed a highly significant correlation with the level of IL-8 in lavage fluid ($r = 0.8$, $p < 0.0001$), whereas there was no significant relationship between the levels of ECP and IL-8. **Conclusion:** Interestingly, our findings suggest that both eosinophils and neutrophils are activated following nasal allergen challenge. In addition, our results are consistent with the hypothesis that IL-8 acts as a chemoattractant/activator of neutrophils during the late phase of the allergic inflammation. In contrast, we were not able to demonstrate any significant relationship between the level of IL-8 in lavage fluid and the activation of eosinophils.

Key Words

Allergic rhinitis
Allergen challenge, nasal
Interleukin-8
Eosinophils
Eosinophil cationic protein
Neutrophils
Myeloperoxidase

Introduction

A growing body of evidence suggests that proinflammatory cytokines play a role in allergic inflammation by attracting and activating inflammatory cells. Interleukin 8 (IL-8) is a potent proinflammatory cytokine belonging to the family of C-X-C chemokines [1]. IL-8 was originally described as a neutrophil chemotactic factor produced by lipopolysaccharide-stimulated human blood mononuclear

leukocytes [2], and a number of studies indicate that this cytokine is an important activator of neutrophils [1, 3–6].

Recently, considerable attention has been focused on the possible effects of IL-8 on eosinophils. Thus, nasal challenge with IL-8 produced a marked eosinophil influx into nasal secretions in a subgroup of allergic patients [7]. In contrast, nasal challenge with IL-8 produced no eosinophil influx in nonallergic controls [7], just as intradermal injection produced no eosinophil skin infiltrate in healthy sub-

KARGER

E-Mail karger@karger.ch
Fax +41 61 306 12 34
www.karger.com

© 1998 S. Karger AG, Basel
1018–2438/98/1161–0053 \$15.00/0

This article is also accessible online at:
<http://BioMedNet.com/karger>

Correspondence to: Dr. Henrik H. Jacobi
Allergiklinikken 7511
Rigshospitalet, Tagensvej 20
DK-2200 Copenhagen N (Denmark)
Tel. +45 35 45 75 11, Fax +45 35 45 75 83

jects [8]. As eosinophil chemotaxis is sensitive to cytokine priming [9, 10], these differences in the eosinophil response to IL-8 might reflect differences between atopics and healthy subjects in the priming of their eosinophils. This hypothesis is supported by the finding that eosinophils isolated from patients with atopic dermatitis [9], or from the blood of asthmatic patients after bronchoprovocation with allergen [11] showed a chemotactic response to IL-8, whereas eosinophils from healthy donors did not. Similar results were obtained when eosinophils isolated from the patients with blood eosinophilia were compared with eosinophils from healthy subjects [10].

The aim of the present study was to investigate the relationship between IL-8 and the local activation of eosinophils and neutrophils following nasal allergen challenge in pollen-allergic patients and nonallergic controls. Nasal lavage fluid was collected before and repeatedly for 10 h after allergen challenge, and the level of IL-8 in lavage fluid was measured. The levels of eosinophil cationic protein (ECP) and myeloperoxidase (MPO) in lavage fluid were used as markers of eosinophil and neutrophil activation in the nose. ECP and MPO are localized to the cytoplasmic granules of eosinophils and neutrophils, respectively, and the proteins are released when these cells are activated and degranulate.

Materials and Methods

Design, Terminology, Ethics

The study was performed more than 2 months after the end of the pollen season and all subjects were free of nasal symptoms at the time of investigation. Nasal challenges were performed with grass pollen extract in grass pollen-sensitive patients and in nonallergic controls. During nasal challenge, sneezing was recorded, and nasal lavage fluid was collected before and repeatedly for 10 h after the challenge. The clinical and biochemical response to challenge, occurring within the 1st hour, is referred to as the early-phase (EP) response. The response, occurring within 1–10 h after challenge, is referred to as the late-phase (LP) response. The protocol was approved by the Ethical Committee of Copenhagen.

Volunteers

Fourteen grass pollen-sensitive patients (7 males, 7 females) volunteered to take part in the study. Their median age was 24 years (range 21–39 years). They all had hay fever symptoms in June to July and a positive skin prick test [12] and specific IgE $\geq$ class 2 to timothy pollen (Pharmacia CAP System). Five nonallergic subjects (2 males, 3 females) of a similar age participated as healthy controls. All volunteers were students or hospital staff members. None suffered from asthma or perennial rhinitis, and rhinoscopy did not reveal any anatomic abnormalities. No medication was taken at the time of investigation and none had received immunotherapy.

Allergen Extract

A standardized timothy grass pollen extract was used (Aquagen SQ®, ALK, Hørsholm, Denmark), supplied as a freeze-dried stock solution. Ampoules, all from the same batch, were stored at 5°C, and fresh solutions of allergen in 0.9% saline were made before challenge testing. Doses of allergen are expressed in standardized quality units (SQ-U) and, according to the manufacturer, 100,000 SQ-U of timothy grass pollen extract equal 30,000 biologic units [12].

Allergen Challenge

One puff of 0.14 ml of the challenge solution was sprayed into each nostril from a metered-dose pump spray. Allergic patients and non-allergic controls were challenged with 0.9% saline twice followed by 140, 1,400, and 14,000 SQ-U of pollen extract (dose per nostril) with 10-min intervals. The nasal challenges were performed between 8 and 10 a.m. Saline and allergen solutions were kept at room temperature for 1 h before provocation.

Recording of Nasal Symptoms

The number of sneezes was counted throughout the study period. Nasal blockage and rhinorrhea could not be recorded in a reliable way due to the nasal lavage procedure.

Nasal Lavage Protocol

The nasal lavage protocol has been described in detail elsewhere [13]. The protocol consisted of 4 prechallenge lavages. Five milliliters of prewarmed physiological saline (30°C) were instilled into each nostril of the volunteer while sitting with his neck extended approximately 30° from the horizontal level. After 10 s the fluid was expelled in a beaker made of polypropylene. To limit mucosal swelling, interfering with subsequent lavages, the first challenge with saline (control challenge) was preceded by spraying 0.1 ml of oxymetazoline hydrochloride 0.5 mg/ml into each nostril. A nasal lavage was performed 10 min after each challenge (saline or allergen) and 20 and 30 min after the last allergen challenge. Hereafter lavages were performed hourly during the time period of 2–10 h after allergen challenge.

Sample Processing

The recovered lavage fluid was immediately put on ice. Mucus lumps were dissolved by pipetting and the fluid was centrifuged for 10 min at 4°C and 1,800 g. The supernatant was separated from the cell pellet by pipetting and stored at –20°C. The supernatant was later assayed for IL-8, ECP and MPO.

IL-8 Assay

IL-8 was measured in lavage fluid samples collected immediately after challenge with saline or 14,000 SQ-U of pollen extract and in all samples collected 2–10 h after allergen challenge. A sandwich fluorescent enzyme immunoassay (Pharmacia AB Diagnostics, Uppsala, Sweden) was used. This assay is a modified version of the IL-8 assay described by Peichl et al. [14]. The lavage fluid samples were incubated in microtiter wells precoated with a murine monoclonal antibody against IL-8. After washing, a goat anti-IL-8 antibody conjugated to β -galactosidase was added. The amount of IL-8 was measured fluorometrically after adding 4-methyl-umbelliferyl- β -D-galactoside. Recombinant human IL-8 [15] was used as standard. The measuring range was 10–2,000 pg/ml. Recovery of IL-8 added to the samples was 86–91%, and the test of samples in various dilutions confirmed the absence of interference in nasal lavage fluid.

ECP Assay

ECP was measured in lavage fluid samples collected 2–10 h after allergen challenge. A solid phase sandwich ELISA was used. The assay has been described in detail elsewhere [16]. The measuring range was 15–1,000 pg/ml and before measurement the samples were diluted in PBS containing 0.1% Tween 20, 0.1% CTAB (N-cetyl-N,N,N-trimethyl-ammoniumbromide), 20 mM EDTA, 0.2% HSA, pH 7.4. The serial 2-fold dilution of lavage fluid samples produced dilution curves parallel to the standard curve. Recovery of ECP added to the samples was 90–97%.

MPO Assay

MPO was measured in lavage fluid samples collected 2–10 h after allergen challenge. A double antibody radioimmunoassay (Pharmacia AB Diagnostics, Uppsala, Sweden) was used. The assay has been described in detail elsewhere [17]. MPO present in the samples competed with a fixed amount of ^{125}I -labeled MPO for the binding sites of specific antibodies. Bound and free MPO were separated by the addition of a second antibody immunosorbent followed by centrifugation and decanting. The measuring range of the assay was 8–1,000 ng/ml. Recovery of MPO was 90–105%, and testing samples in various dilutions confirmed the absence of interference in nasal lavage fluid.

Statistical Analysis

Statistical analyses were performed using SigmaStat for Windows, Version 1.0, Jandel Corporation. Nonparametric techniques were used and results are presented as medians and quartiles. The Friedman test was used to test whether there was an overall difference between time points, and Dunn's test was used to identify individual time points that were significantly different from baseline. The correlation between two variables was tested using the Spearman rank order correlation test. Two-tailed *p* values of <0.05 were considered significant. The area under the curve (AUC) was calculated using trapezoid integration.

Results

Symptoms following Allergen Challenge

Allergen challenge of pollen-allergic patients produced symptoms of allergic rhinitis in all patients. EP sneezing was marked with a median number of 15 (quartiles 8–19) sneezes after challenge with the highest dose of allergen. LP sneezing was scattered without a well-defined maximum in time. Allergen challenge of nonallergic controls and challenge with saline (control challenge) did not induce any sneezing.

IL-8 in Lavage Fluid following Allergen Challenge

IL-8 was detectable in most lavage fluid samples obtained both before and after nasal allergen challenge. Ten minutes after nasal challenge with saline (control challenge), the median level of IL-8 in lavage fluid was 30 pg/ml (quartiles <10 –88 pg/ml). Ten minutes after nasal challenge with 14,000 SQ-U of pollen extract, the median value

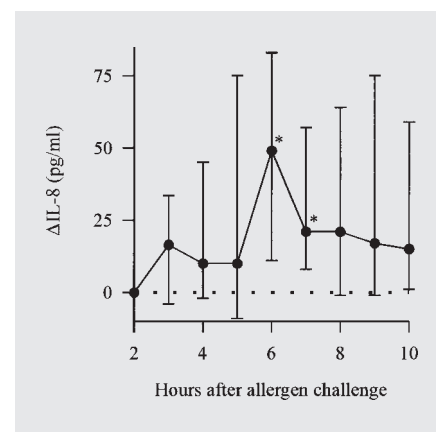


Fig. 1. Change in the level of IL-8 in nasal lavage fluid during the LP response to allergen. Medians and quartiles are shown. For each patient, the value obtained 2 h after allergen challenge was used as baseline and subtracted from the other values. * $p < 0.05$, significant increase in IL-8 during the LP response (paired comparisons between baseline and other time points).

increased to 48 pg/ml (26–169 pg/ml) but this increase was not statistically significant ($p = 0.4$). Two hours after the start of the allergen challenge, the median IL-8 level was 48 pg/ml (19–73 pg/ml), and in the following hours, we observed a small increase in the level of IL-8 reaching statistical significance 6 h after allergen challenge ($p < 0.05$; fig. 1).

Allergen challenge of nonallergic subjects did not induce any EP or LP increase in the level of nasal lavage IL-8 (data not shown).

Activation of Eosinophils and Neutrophils following Allergen Challenge

Allergen challenge of allergic patients produced a significant LP increase in the level of ECP. Two hours after allergen challenge, the median value of ECP was 15 ng/ml (quartiles 2–30 ng/ml), and we observed a steady increase in the level of this mediator reaching statistical significance 6 h after allergen challenge ($p < 0.01$; fig. 2).

Nine patients also showed an LP increase in the level of MPO reaching a maximum 9–10 h after allergen challenge, whereas the remaining patients showed variable levels without a well-defined maximum in time. Two hours after allergen challenge, the median value for all 14 patients was 50 ng/ml (quartiles 24–191 ng/ml), and in the following hours, we observed a small increase in the level of MPO reaching statistical significance 9 h after allergen challenge ($p < 0.05$, data from all 14 patients included in the statistical analysis; fig. 2).

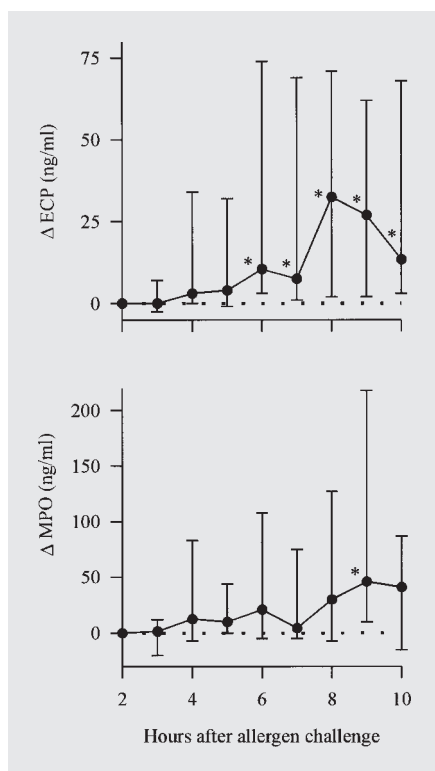


Fig. 2. Change in the levels of ECP/MPO in nasal lavage fluid during the LP response to allergen. Medians and quartiles are shown. For each patient, the value obtained 2 h after allergen challenge was used as baseline and subtracted from the other values. * $p < 0.05$, significant increase in ECP/MPO during the LP response (paired comparisons between baseline and other time points).

Allergen challenge of nonallergic subjects did not induce any LP increase in the levels of ECP or MPO (data not shown).

Activation of Eosinophils and Neutrophils versus IL-8 in Lavage Fluid

There was a marked interindividual variation in the overall levels of ECP and MPO (as determined by the area under the curve during the time period 2–10 h after allergen challenge). When the overall level of MPO was compared with the overall level of IL-8 we found a highly significant correlation between these two molecules ($r = 0.8$, $p < 0.0001$; fig. 3). In contrast, there was no significant relationship between the overall levels of ECP and IL-8 ($r = 0.5$, $p = 0.1$; fig. 4). We also compared the level of IL-8 in individual lavage fluid samples with the level of ECP in samples obtained at later time points. Like the comparison between overall levels, the latter comparison also failed to demonstrate any

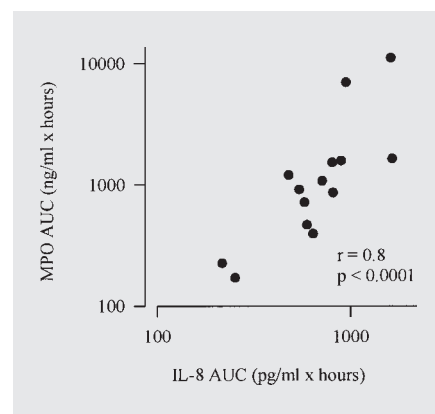


Fig. 3. Relationship between the overall levels of IL-8 and MPO during the LP response to a nasal allergen challenge. The values shown are calculated as the AUC (trapezoid integration) for all nasal lavage fluid samples collected 2–10 h after allergen challenge.

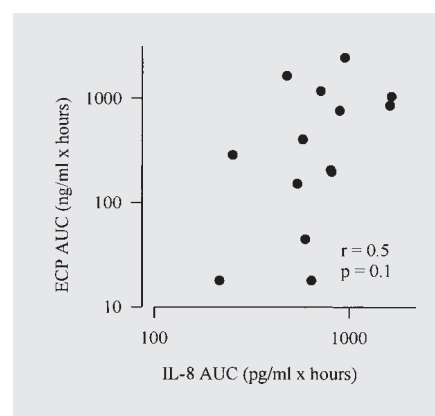


Fig. 4. Relationship between the overall levels of IL-8 and ECP during the LP response to a nasal allergen challenge. The values shown are calculated as the AUC (trapezoid integration) for all nasal lavage fluid samples collected 2–10 h after allergen challenge.

significant correlation between the levels of IL-8 and ECP in nasal lavage fluid (data not shown).

Discussion

Our findings confirm previous reports that IL-8 can be detected in nasal lavage fluid [18–23]. However, the IL-8 levels measured in the present study are lower than reported in previous nasal lavage studies. This could reflect the use of different nasal lavage techniques, differences in the processing of the lavage fluid samples or the use of different

IL-8 assays. IL-8 was present in lavage fluid from both allergic patients and nonallergic controls, and allergen challenge of allergic patients produced a small but statistically significant LP increase in the level of IL-8, reaching a maximum after 6 h. In contrast, no EP increase was observed after allergen challenge of allergic patients. Similar results have been reported by Bachert and Ganzer [23] using the same nasal lavage technique but a different protocol and by Sim et al. [24] using a matrix method to collect nasal secretions.

The source of IL-8 in nasal lavage fluid is not known. The cytokine can be produced by a variety of cells including nasal epithelial cells [25], nasal fibroblasts [26], endothelial cells [27], lymphocytes [28], monocytes [2], macrophages [29], mast cells [30], neutrophils [31] and eosinophils [32]. Immunohistochemical staining of nasal mucosa biopsies indicates that IL-8 is predominantly localized to the nasal epithelium in both healthy subjects and allergic patients [33]. This finding suggests that at least some of the IL-8 recovered in nasal lavage fluid is derived from nasal epithelial cells. However, it is possible that other cell types also secrete IL-8 without accumulating the cytokine in the cytoplasm in a sufficiently high concentration to be detected.

In this study, the levels of ECP and MPO in lavage fluid were used as indices of eosinophil and neutrophil activation in the nose. ECP is specific for eosinophils (only negligible amounts are found in other cell types [34]) and MPO is predominantly localized to neutrophils (small amounts of MPO are also found in monocytes [35]). The proteins are stored in the cytoplasmic granules and are released during degranulation of the cells. As degranulation is an important aspect of cell activation, we believe that ECP and MPO in nasal lavage fluid are valid markers of eosinophil and neutrophil activation in the nose, although other aspects of cell activation, such as altered expression of surface molecules or increased oxidative metabolism, cannot be detected using this approach.

Allergen challenge of allergic patients produced a significant LP increase in the levels of both ECP and MPO (although the increase in MPO was less pronounced than the increase in ECP). This finding suggests that both eosinophils and neutrophils are activated following nasal allergen challenge. In contrast, allergen challenge of nonallergic controls produced no such response. These results are in agreement with other studies showing that nasal allergen challenge in allergic patients (but not in healthy controls) is followed by a marked LP influx of both eosinophils and neutrophils [23, 36].

The small LP increase in MPO levels, observed using the nasal lavage technique, is not necessarily detected using

other techniques to sample nasal secretions. Recently, Wang et al. [37] used a microsuction technique to measure the concentration of MPO in nasal secretions. Using this technique, the authors were not able to demonstrate any LP increase in MPO levels following nasal allergen challenge of allergic patients. One possible explanation could be that the microsuction technique (unlike the nasal lavage technique) measures the absolute concentration of mediators in nasal secretions, and that MPO levels are reduced (diluted) by the allergen-induced rhinorrhea.

In the present study, MPO levels correlated significantly with the level of IL-8 in nasal lavage fluid. This finding is consistent with the hypothesis that IL-8 acts as a chemoattractant/activator of neutrophils during the LP of the allergic inflammation. However, we realize that a nasal lavage study cannot provide us with any definite proof of such a hypothesis. Thus, a positive correlation between the level of IL-8 and the activation of neutrophils does not necessarily reflect a causal relationship between these two variables, and it cannot be excluded that other mechanisms are responsible for the observed covariation between the level of IL-8 and the activation of neutrophils.

In contrast to the above finding, no correlation was found between the level of IL-8 in nasal lavage fluid and the LP activation of eosinophils. The reason(s) for this is not known. In a previous study, nasal challenge with IL-8 produced influx of eosinophils into nasal secretions in a small subgroup of allergic patients (3 out of 8 patients) [7]. This finding does not imply, however, that IL-8 actually acts as a major eosinophil chemoattractant during the allergic inflammatory response. Several other cytokines and inflammatory mediators (e.g. IL-5 [38], IL-3 [39], GM-CSF [39], eotaxin [40], RANTES [41], MIP-1 α [41], LTB₄ [39], PAF [39] and C5a [39]) are capable of attracting and/or activating eosinophils, and it is likely that the influx and local activation of eosinophils following allergen challenge is controlled by multiple factors (and some of them may be more important in this respect than the concentration of IL-8 in the nasal mucosa). Furthermore, the study was performed outside the pollen season and it is possible that the outcome would have been different if the study had been performed in the pollen season (due to priming of the eosinophils).

To our knowledge, this is the first report of the relationship between IL-8 and the levels of MPO and ECP in nasal lavage fluid following an experimental nasal allergen challenge. Recently, Kuna et al. [18] used a different lavage technique to study the levels of chemokines and mediators in nasal lavage fluid collected during natural pollen exposure. In the study of Kuna et al., the activation of neutrophils was not investigated, whereas the activation of eosinophils

was assessed using ECP levels in lavage fluid samples. As in our study, the investigators observed no correlation between the levels of IL-8 and ECP when the patients were exposed to allergen. In contrast, the investigators observed a significant correlation between the levels of IL-8 and ECP outside the pollen season, when the patients were asymptomatic. The reason for this finding is not known.

In conclusion, our findings suggest that both eosinophils and neutrophils are activated following nasal allergen challenge. Furthermore, our results are consistent with the hypothesis that IL-8 acts as a chemoattractant/activator of

neutrophils during the LP of the allergic inflammation. In contrast, we were not able to demonstrate any significant relationship between the level of IL-8 in nasal lavage fluid and the activation of eosinophils during the allergic inflammatory response.

Acknowledgements

This study was in part supported by Pharmacia AB Diagnostics, Uppsala, Sweden and the Danish Biotechnology Programme.

References

- Oppenheim JJ, Zacharie COC, Mukaida N, Matsushima K: Properties of the novel pro-inflammatory supergene 'intercrine' cytokine family. *Annu Rev Immunol* 1991;9:617-648.
- Yoshimura T, Matsushima K, Oppenheim JJ, Leonard EJ: Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human blood mononuclear leukocytes: Partial characterization and separation from interleukin 1 (IL-1). *J Immunol* 1987;139:788-793.
- Peveri P, Walz A, Dewald B, Baggiolini M: A novel neutrophil activating factor produced by human mononuclear phagocytes. *J Exp Med* 1988;167:1547-1559.
- Djeu JY, Matsushima K, Oppenheim JJ, Shiot-suki K, Blanchard DK: Functional activation of human neutrophils by recombinant monocyte-derived neutrophil chemotactic factor/IL-8. *J Immunol* 1990;144:2205-2210.
- Smith RJ, Sam LM, Leach KL, Justen JM: Postreceptor events associated with human neutrophil activation by interleukin-8. *J Leukoc Biol* 1992;52:17-26.
- Kurdowska A, Miller EJ, Cohen AB: An anti-interleukin 8 monoclonal antibody that interferes with the binding of interleukin 8 to cellular receptors and the activation of human blood neutrophils. *Hybridoma* 1995;14:225-233.
- Douglass JA, Dhami D, Gurr CE, Bulpitt M, Shute JK, Howarth PH, Lindley IJ, Church MK, Holgate ST: Influence of interleukin-8 challenge in the nasal mucosa in atopic and non-atopic subjects. *Am J Respir Crit Care Med* 1994;150:1108-1113.
- Leonard EJ, Yoshimura T, Tanaka S, Raffeld M: Neutrophil recruitment by intradermally injected neutrophil attractant/activation protein-1. *J Invest Dermatol* 1991;96:690-694.
- Bruijnzeel PL, Kuijper PH, Rihs S, Betz S, Warringa RA, Koenderman L: Eosinophil migration in atopic dermatitis. I. Increased migratory responses to N-formyl-methionyl-leucyl-phenylalanine, neutrophil-activating factor, platelet-activating factor, and platelet factor 4. *J Invest Dermatol* 1993;100:137-142.
- Sehmi R, Cromwell O, Wardlaw AJ, Moqbel R, Kay AB: Interleukin-8 is a chemo-attractant for eosinophils purified from subjects with a blood eosinophilia but not from normal healthy subjects. *Clin Exp Allergy* 1993;23:1027-1036.
- Warringa RA, Mengelers HJ, Raaijmakers JA, Bruijnzeel PL, Koenderman L: Upregulation of formyl-peptide and interleukin-8-induced eosinophil chemotaxis in patients with allergic asthma. *J Allergy Clin Immunol* 1993;91:1198-1205.
- Dreborg S, Frew A: EAACI Subcommittee on Allergen Standardization and Skin Test. Position paper: Allergen standardization and skin test. *Allergy* 1993;48(suppl 14):48-82.
- Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NEJ, Meyers DA, Norman PS, Lichtenstein LM: Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
- Peichl P, Ceska M, Broell H, Effenberger F, Lindley IJD: Human neutrophil activating peptide/interleukin 8 acts as an autoantigen in rheumatoid arthritis. *Ann Rheum Dis* 1992;51:19-22.
- Lindley I, Aschauer H, Seifert JM, Lam C, Brownsky W, Kownatzki E, Peveri P, Dewald B, Tschanner V: Synthesis and expression in *Escherichia coli* of the gene encoding monocyte-derived neutrophil-activating factor: Biological equivalence between natural and recombinant neutrophil-activating factor. *Proc Natl Acad Sci USA* 1988;85:9199-9203.
- Reimert CM, Venge P, Kharazmi A, Bendtzen K: Detection of eosinophil cationic protein (ECP) by an enzyme-linked immunosorbent assay. *J Immunol Methods* 1991;138:285-290.
- Olofsson T, Olsson I, Venge P, Elgefors B: Serum myeloperoxidase and lactoferrin in neutropenia. *Scand J Haematol* 1977;18:73-80.
- Kuna P, Lazarovich M, Kaplan A: Chemokines in seasonal allergic rhinitis. *J Allergy Clin Immunol* 1996;97:104-112.
- Noah TL, Henderson FW, Henry MM, Peden DB, Devlin RB: Nasal lavage cytokines in normal, allergic, and asthmatic school-age children. *Am J Respir Crit Care Med* 1995;152:1290-1296.
- Peden DB, Setzer RW, Devlin RB: Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the nasal airways of perennially allergic asthmatics. *Am J Respir Crit Care Med* 1995;151:1336-1345.
- Wang JH, Devalia JL, Duddle JM, Hamilton SA, Davies RJ: Effect of six-hour exposure to nitrogen dioxide on early-phase nasal response to allergen challenge in patients with a history of seasonal allergic rhinitis. *J Allergy Clin Immunol* 1995;96:669-676.
- McBride DE, Koenig JQ, Lucht DL, Williams PV, Henderson WRJ: Inflammatory effects of ozone in the upper airways of subjects with asthma. *Am J Respir Crit Care Med* 1994;149:1192-1197.
- Bachert C, Ganzer U: Die Rolle der proinflammatorischen Zytokine bei der Rekrutierung von Entzündungszellen an der Nase. *Laryngol Rhinol Otol* 1993;72:585-589.
- Sim TC, Reece LM, Hilsmeier KA, Grant JA, Alam R: Secretion of chemokines and other cytokines in allergen-induced nasal responses: Inhibition by topical steroid treatment. *Am J Respir Crit Care Med* 1995;152:927-933.
- Becker S, Koren HS, Henke DC: Interleukin-8 expression in normal nasal epithelium and its modulation by infection with respiratory syncytial virus and cytokines tumor necrosis factor, interleukin-1, and interleukin-6. *Am J Respir Cell Mol Biol* 1993;8:20-27.
- Xing Z, Jordana M, Braciak T, Ohtoshi T, Gauldie J: Lipopolysaccharide induces expression of granulocyte/macrophage colony-stimulating factor, interleukin-8, and interleukin-6 in human nasal, but not lung, fibroblasts: Evidence for heterogeneity within the respiratory tract. *Am J Respir Cell Mol Biol* 1993;9:255-263.

- 27 Schröder JM, Christophers E: Secretion of novel and homologous neutrophil-activating peptides by LPS-stimulated human endothelial cells. *J Immunol* 1989;142:244–251.
- 28 Gregory H, Young J, Schröder JM, Mrowietz U, Christophers E: Structure determination of a human lymphocyte derived neutrophil activating peptide (LYNAP). *Biochem Biophys Res Commun* 1988;151:883–890.
- 29 Sylvester I, Rankin JA, Yoshimura T, Tanaka S, Leonard EJ: Secretion of neutrophil attractant/activation protein by lipopolysaccharide-stimulated lung macrophages determined by both enzyme-linked immunosorbent assay and N-terminal sequence analysis. *Am Rev Respir Dis* 1990;141:683–688.
- 30 Möller A, Lippert U, Lessmann D, Kolde G, Hamann K, Welker P, Schadendorf D, Rosenbach T, Luger T, Czarnetzki BM: Human mast cells produce IL-8. *J Immunol* 1993;151:3261–3266.
- 31 Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, Baggiolini M: Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. *J Exp Med* 1991;173:771–774.
- 32 Braun RK, Franchini M, Erard F, Rihs S, De Vries IJ, Blaser K, Hansel TT, Walker C: Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* 1993;23:956–960.
- 33 Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, Howarth PH: Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993;151:3853–3865.
- 34 Abu-Ghazaleh RI, Dunnette SL, Loegering DA, Checkel JL, Kita H, Thomas LL, Gleich GJ: Eosinophil granule proteins in peripheral blood granulocytes. *J Leukoc Biol* 1992;52:611–618.
- 35 Bos A, Wever R, Roos D: Characterization and quantification of the peroxidase in human monocytes. *Biochim Biophys Acta* 1978;525:37–44.
- 36 Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM: The influx of inflammatory cells into nasal washings during the late response to antigen challenge. Effect of systemic steroid pretreatment. *Am Rev Respir Dis* 1988;138:406–412.
- 37 Wang D, Smits J, Derde M-P, Clement P: Concentrations of myeloperoxidase in nasal secretions of atopic patients after nasal allergen challenge and during natural allergen exposure. *Int Arch Allergy Immunol* 1996;110:85–90.
- 38 Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A: Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701–705.
- 39 Warringa RA, Koenderman L, Kok PT, Kreukniet J, Bruijnzeel PL: Modulation and induction of eosinophil chemotaxis by granulocyte-macrophage colony-stimulating factor and interleukin-3. *Blood* 1991;77:2694–2700.
- 40 Garcia-Zepeda EA, Rothenberg ME, Ownbey RT, Celestin J, Leder P, Luster AD: Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat Med* 1996;2:449–456.
- 41 Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA: RANTES and macrophage inflammatory protein 1 alpha induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 1992;176:1489–1495.

Copyright: S. Karger AG, Basel 1998. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.