

A Preliminary Study on Interleukin-8 Detection During Grass or Ragweed Nasal Provocation

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ABSTRACT

Early- (ER) and late-phase reactions (LPR) occur in the nasal mucosa with release of inflammatory mediators and a mixed cellular influx. In a preliminary study, we evaluated ten atopic subjects with confirmed allergic rhinitis (age range 29–45 years) and five nonatopic controls (age range 35–53 years) by nasal provocation with 10 to 1,000 protein nitrogen units (PNU) of grass or ragweed extract. In 80% of all patients, symptom scores for sneezing rose during the ER after one hour and LPR after 3 to 8 hours from a mean baseline of 0.2 to 4.9 (ER) to 3.7 (LPR). Other symptoms for rhinorrhea, nasal congestion, and pruritus rose in 80% to 90% of all patients from a mean of 2.8 to 15.1 (ER) and 2.8 to 3.7 (LPR). Nasal albumin levels rose during the ER from 52 to 102 $\mu\text{g/L}$ but less at LPR to 58 $\mu\text{g/L}$ ($p < 0.05$). Nasal histamine rose after 10 minutes into challenge from 2.8 to 4.2 ng/ml and peaked over LPR from 4.5 to 11.0 ng/ml. Nasal interleukin-1 (IL-1) levels increased from 3 to 6 hours approaching diluent control during the LPR, which paralleled a rise in nasal epithelial cells. Nasal IL-8 levels at 3 and 6 hours during the LPR from 119 to 178 pg/ml corresponded to nasal smear neutrophilia from $\bar{x}$ of 3.5% to 11.6%. Thus, nasal provocation allergic rhinitis is a useful tool to monitor nasal cytokine mediators and new therapeutic agents.

INTRODUCTION

ALLERGIC RHINITIS IS an IgE-mediated inflammatory reaction with both acute and delayed or late-phase reactions (LPR) associated with an inflammatory infiltrate of mononuclear cells, neutrophils, and eosinophils.⁽¹⁾ The spontaneous LPR was first reported in 1873⁽²⁾ and its occurrence after nasal challenge has been reported in several studies.^(3–5)

Cytokines have been implicated in chronic inflammation. Interleukin-1 α (IL-1 α) and IL-1 β both bind to the same cell surface receptor.⁽⁶⁾ IL-1 can augment the capacity of monocytes to produce prostaglandins and IL-8.⁽⁷⁾ IL-1 α can induce a three- to five-fold increase in cytokine expression, including IL-8 in cultured bronchial epithelial cells from normal subjects.⁽⁸⁾

IL-8 from monocytes can stimulate neutrophils, modulate neutrophil adhesion and receptors, and stimulate T lymphocytes to release potent enzymes.^(10–18) IL-8 can also cause primed basophils to release hista-

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mine and leukotrienes as well as amplify the LPR during allergic inflammation for allergic rhinitis.⁽¹⁹⁾ Basophils are responsible for the LPR of histamine and studies from our center demonstrated basophil histamine release from mononuclear cells obtained from patients with allergic rhinitis.⁽²⁰⁾ Human bronchial epithelial cells can be induced to express the IL-8 gene and protein in culture when stimulated with IL-1.⁽²¹⁾ Mast cell and basophil histamine release, which occurs in both in the early phase reaction and LPR can increase vascular permeability in nasal secretions.^(22–24) A previous study has correlated the early inflammatory cell response and histamine release into nasal secretions during ragweed challenge.⁽²⁵⁾

Evidence suggests that the LPR after experimental provocation is a good model to study ongoing allergic disease.⁽²⁶⁾ IL-8 has been shown to produce rapid neutrophilia in mice and rabbits following intraperitoneal injection in vivo.⁽¹⁷⁾ The protective effect of nedocromil sodium on the IL-1-induced production of IL-8 in human bronchial epithelial cells has recently been demonstrated.⁽²⁷⁾ Our study in human subjects is the first to demonstrate IL-8 levels in nasal secretions, which correspond with an increase in neutrophil numbers, during the late phase nasal response during both grass and ragweed challenge. Nasal provocation in allergic rhinitis is a useful tool to monitor nasal cytokine mediators and new anti-inflammatory therapeutic agents.

MATERIALS AND METHODS

After institutional board review and approval, ten grass- and ragweed-sensitive adults (age range 29–45 years) along with five nonatopic healthy controls (age range 35–53 years) consented to bilateral grass or ragweed nasal challenge out of season. All patients were history positive for seasonal allergic rhinitis confirmed by prick skin tests, 1:20 w/v of aeroallergens (mixed trees, grasses, ragweed, molds, and dust). Skin tests were graded as follows: 0 = no wheal, erythema; 1+ = erythema only; 2+ = wheal > 3 mm, erythema > 21 mm; 3+ = wheal 3 to 8 mm/erythema; 4+ = wheal > 8 mm with pseudopods. Patients were not on nasal steroids or cromolyn prior to challenge. Volunteer control subjects without allergic rhinitis and prick skin test negative were also used. All subjects gave informed consent.

Nasal challenge technique

Antigen challenge was performed three times at 10-minute intervals with either mixed grass or ragweed pollen extract at a concentration of 10, 100, and 1,000 protein nitrogen units (PNU) (Nelco Laboratories, Deer Park, NY), according to the method of Togias et al.⁽²⁸⁾ and secretions were collected up to 8 hours.

The diluted antigen was insufflated into the nostrils of sensitive and control subjects every 10 minutes for challenges 1 to 3 after 2 prewashes and washes following diluent control and decongestant (oxymethazoline) installation. Nasal smears were obtained at baseline and the LPR at 3 and 6 hours.

Because most of our nasal lavage fluid collected was required for multiple cytokine determinations and markers of permeability, nasal cytospin cytology was unrevealing due to an inadequate sample size. Nasal specimens for cytologic examination were collected with cotton-tipped swabs from the inferior bulb of the inferior turbinate on three occasions. Nasal smears were consistently obtained by the same physician, fixed in methanol, and stained with Wright giemsa and Alcian blue, and examined blindly for cell count and differential. Nasal secretions were obtained by instilling 2.5 ml of normal saline into each nostril at baseline, following two prewashes, diluent, decongestant, and three doses of antigen challenge from 10 minutes up to 1 hour. For LPR studies, nasal lavage was performed at 3, 6, and up to 8 hours. Nasal lavage secretions were collected for measurement of albumin, histamine content, and cytokine levels (IL-1, IL-8) by enzyme-linked immunosorbent assay (ELISA).

Nasal albumin assay

The albumin content of nasal lavage fluids was determined using an ELISA. Samples at 100/μl were run in duplicates in microtiter wells previously overnight coated with rabbit antibody directed against human albumin.⁽²⁹⁾ After 2 hours of incubation at room temperature, wells were aspirated and washed three times

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with phosphate-buffered saline containing Tween 20. Peroxidase-conjugated-antibody at 100 μ l was added to each well and incubated at room temperature for 1 hour.

After incubation wells were aspirated and washed again followed by the addition of 100 μ l of the orthophenylenediamine (OPD) color reagent. After 15 minutes of incubation at room temperature protected from light, the enzymatic reaction was stopped with 1M sulfuric acid and the absorbance was measured at 492 nm on a Titertech spectrophotometer. Values were expressed in micrograms per liter of albumin.

Nasal histamine assay

The histamine content of nasal lavage fluids was performed using a microbead method courtesy of H. Nolte.⁽³⁰⁾

Briefly, 25 μ l aliquots of nasal lavage fluid was pipetted into a 96-well microtiter plate coated with a glass-fiber matrix that selectively binds histamine.⁽³¹⁾ After a 1-hour incubation at 37°C, the plate was automatically rinsed twice with immunowasher with deionizer water. Then, interfering substances from the nasal lavage fluid were additionally dissolved by adding a solution of biotrinon and renafix⁽³²⁾ into the wells for 30 minutes at 37°C, followed by another rinse. Subsequently, histamine bound to glass fibers was resolved by adding a mixture of 75 μ l of OPT reagent (10 mg of OPT dissolved in 2 ml of methanol, alkalinized by 18 ml of 0.05 N NaOH) to each well. After 10 minutes, the histamine-OPT reaction was stopped by adding 75 μ l 0.6% HClO₄. Histamine fluorescence values were measured and determined with a 122 fluorometer and an autosampler (Gilson Co., Washington, OH), which performed 96 determinations in 5 minutes. Immediately, the absolute histamine values were computed with MS-DOS-compatible personal computers (Microsoft, Inc., Redmond, WA). Values were expressed as nanograms milliliter of histamine.

Cytokine determinations

IL-1 and IL-8 levels were determined using a standard ELISA (R + D Systems, Minneapolis, MN). Briefly, samples were analyzed in duplicate at a concentration of 200 μ l in each well in microtiter plate coated with murine monoclonal antibodies against IL-1 and IL-8. After 2 hours of incubation at room temperature, wells were aspirated and washed three times with 400 μ l of phosphate buffer. A polyclonal antibody (200 μ l) against either IL-1 or IL-8 conjugated to horseradish peroxidase was added to each well and incubated for 2 hours at room temperature. After incubation wells were again aspirated, washed three times with 400 μ l of phosphate buffer, washed again, and 200 μ l of the substrate solution was added for an additional 20-minute incubation at room temperature. The enzymatic reaction was stopped with 2N sulfuric acid and optical density was determined within 30 minutes on a spectrophotometer with a filter of 450 nm. The minimal detectable level of IL-1 and IL-8 by this assay is 0, 3 and 4, and 7 pg/ml, respectively.

Statistical analysis

Statistical analysis was employed using the paired Student *t*-test and Wilcoxon-signed rank test.

RESULTS

Grass and ragweed challenges induced symptoms in all ten subjects with allergic rhinitis. Sneezing scores peaked during the early response at 30 minutes and during the LPR at 6 hours after nasal provocation (Fig. 1). Symptom scores for rhinorrhea, nasal congestion, and nasal pruritus also peaked at 30 minutes, remained elevated at 3 and 6 hours, and declined by 8 hours (Fig. 2). Because our sample size was small, these values did not approach statistical significance. Interestingly, nasal histamine and IL-8 levels were highest during the 6 hour LPR. Control nonallergic subjects had no symptoms. There were no adverse reactions in any challenged subject.

Nasal albumin levels rose during the early response over 10 to 30 minutes and declined by 3 to 8 hours, whereas nasal histamine levels declined at 10 minutes ($p < 0.05$) and peaked at 6 hours during the LPR, slightly exceeding diluent levels (Fig. 3). The rise in albumin from diluent during the early-phase reaction (ER) indicates increased vascular permeability prior to the histamine rise.

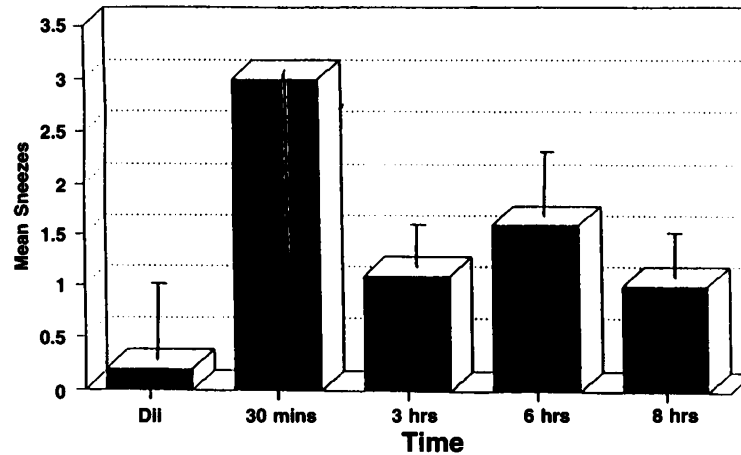


FIG. 1. Mean sneezing scores in patients with allergic rhinitis over 8 hours during the early and late phase after nasal provocation.

Nasal IL-1 levels increased from 3 to 6 hours, approaching diluent levels at 5.5 pg/ml (data not shown) during the LPR, which paralleled nasal epithelial cell cytology collected at baseline prior to the day of challenge. For this reason, high baseline levels are depicted and the diluent control level of 5.5 pg/ml on the day of provocation should be used for comparison of the 3- and 6-hour IL-1 levels in Figure 4.

Nasal fluid IL-8 levels declined from diluent at 218 pg/ml to 30 minutes and 3 hours, but rose again during the LPR at 6 hours (Fig. 5). The high baseline IL-8 levels could reflect mild nasal irritation in one or two subjects. This elevation did not reach statistical significance. IL-8 levels at 8 hours were still elevated close to those obtained at 6 hours. All nasal IL-8 levels were lower in nonatopic controls (Fig. 5). Comparing baseline levels with levels at 3 or 6 hours, the percentage of neutrophils detected in 11 of 15 (73%) patients corresponded with IL-8 levels (Fig. 6). The increased neutrophilia at 6 hours was significant ($p < 0.05$). As noted above, baseline nasal cytology levels were obtained prior to the day of challenge, reflecting higher levels compared to the diluent control of 219 pg/ml (not shown).

Results of other investigators reported a correlation of neutrophil accumulation with mediator levels and increased vascular permeability.⁽²⁸⁾ Similarly, we observed a neutrophil increase at 3 to 6 hours.

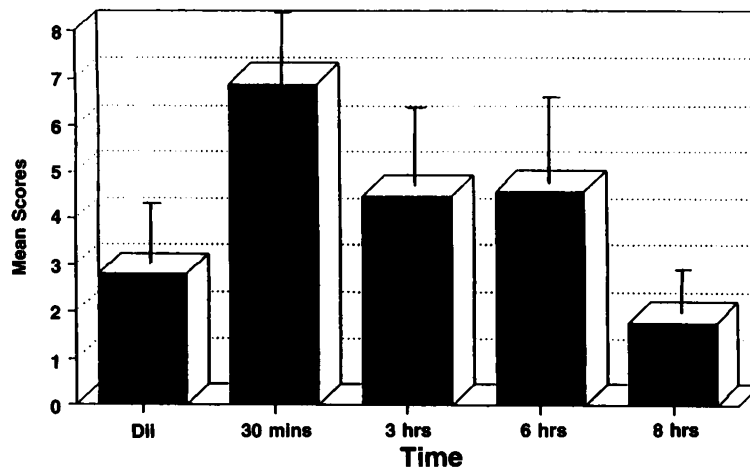
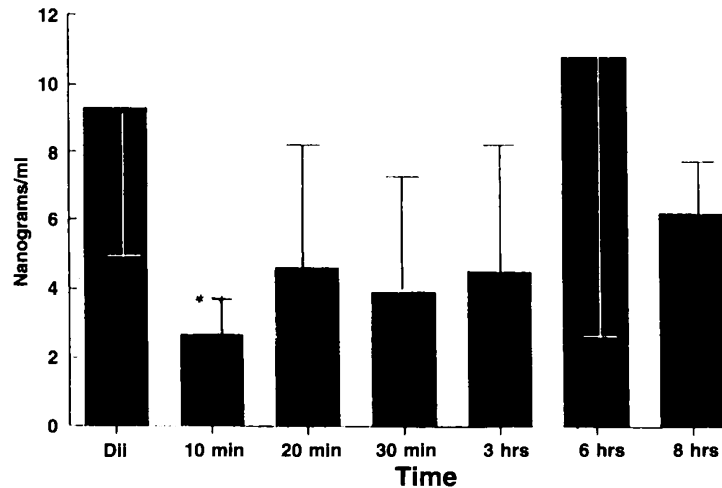


FIG. 2. Mean symptom scores for rhinorrhea, congestion, and pruritus in patients with allergic rhinitis over 8 hours during the early and late phase after nasal provocation.

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* $p < 0.05$

FIG. 3. Mean nasal histamine levels in nasal fluid in allergic rhinitis patients over 8 hours during the early and late phase after nasal provocation.

DISCUSSION

This preliminary investigation has focused on detection of nasal IL-8 during grass and ragweed nasal provocation over the ER and LPR (Fig. 5). The rise in symptom scores during the peak IL-8 nasal LPR is of interest. Nasal IL-8 levels also corresponded to a rise in neutrophil numbers (Fig. 6) at 3 and 6 hours during the LPR.

IL-8 is a polypeptide, macrophage-derived neutrophil chemotactic factor that can contribute to ongoing inflammation. It has recently been cloned and isolated from pulmonary epithelial cells.^(33,34) Monocytes also produce IL-8 in response to various stimuli including IL-1, which is required for the production of epithelial-cell-derived IL-8.⁽¹⁶⁾ IL-8 is an early-acting skin reactive neutrophilic factor, and IL-1 has been demonstrated to possess delayed skin reactivity upon intradermal injection, which presumably is mediated by local release of IL-8.⁽³⁵⁾ Of interest, the addition of IL-1 α to cultured mesothelial cells has resulted in significant quantities of neutrophil chemotactic activity.⁽³⁶⁾ Epidermal IL-1 α functional activity and IL-8 immunoreactivity have been found to be increased in patients with cutaneous T-cell lymphoma (9). Thus, similar to

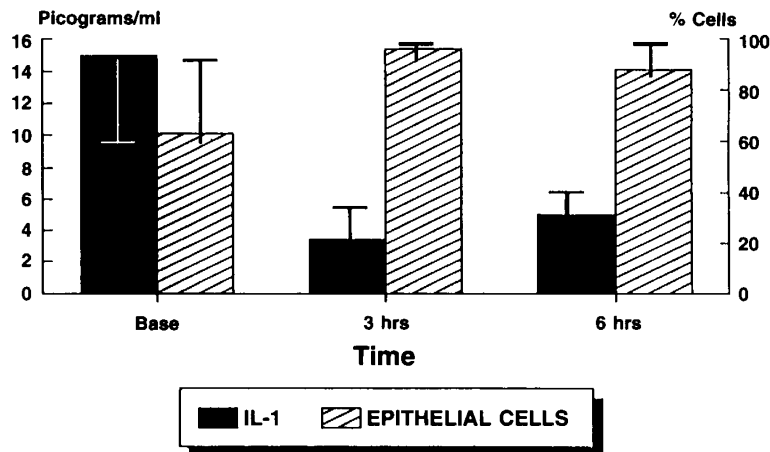


FIG. 4. Mean nasal IL-1 α levels and nasal epithelial cell cytology obtained at baseline prior to the day of challenge and at 3 and 6 hours following challenge. Diluent control IL-1 α level = 5.5 pg/ml prior to challenge.

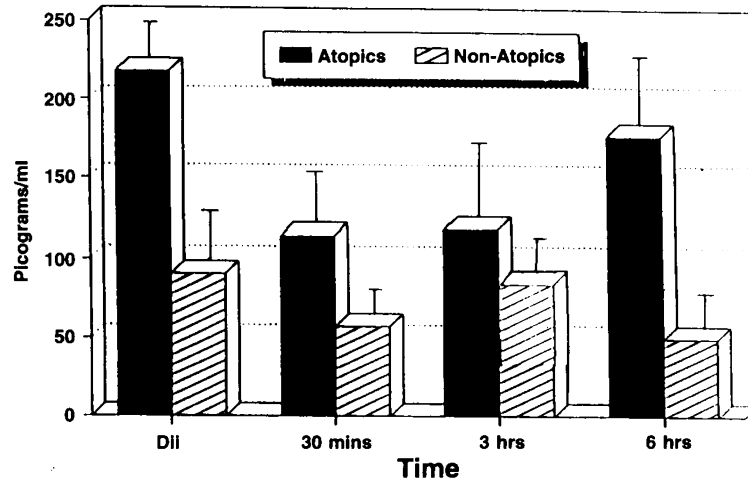


FIG. 5. Mean nasal IL-8 levels during the early and late phase over 6 hours in allergic rhinitis patients and nonatopic controls during nasal provocation.

IL-1, by indirectly leading to chemotactic-inducing IL-8 in the skin and bronchi, we have observed an increase in nasal IL-8 levels by 6 hours (Fig. 5) as well as 8 hours (not shown) after challenge in our patients with allergic rhinitis.

Synthesis and release of granulocyte macrophage colony-stimulating factor (GM-CSF), IL-6, and IL-8 have been reported in the bronchial epithelium of patients with symptomatic asthma.⁽⁹⁾ Several of our patients also had a corresponding history of asthma and we were able to detect a rise in nasal epithelial cells from baseline to 6 hours. GM-CSF can induce the expression of adhesion molecules on epithelial cells and can prime granulocytes to release increased levels of inflammatory mediators.^(37,38) IL-8 immunoreactivity has been demonstrated in nasal biopsy epithelium.⁽³⁹⁾ Since IL-8 is chemotactic for both neutrophils and T cells,^(12,40-42) the local nasal effect of IL-8 can lead to the persistence of nasal inflammation similar to the mechanism in the lung. Therefore, these immunopathologic changes can amplify the nasal mucosal response and ultimately might contribute to pulmonary inflammation. Recently, IL-8 has been demonstrated to induce neutrophil migration through both vascular endothelium and pulmonary epithelium, further contributing to lung inflammation.⁽⁴³⁾

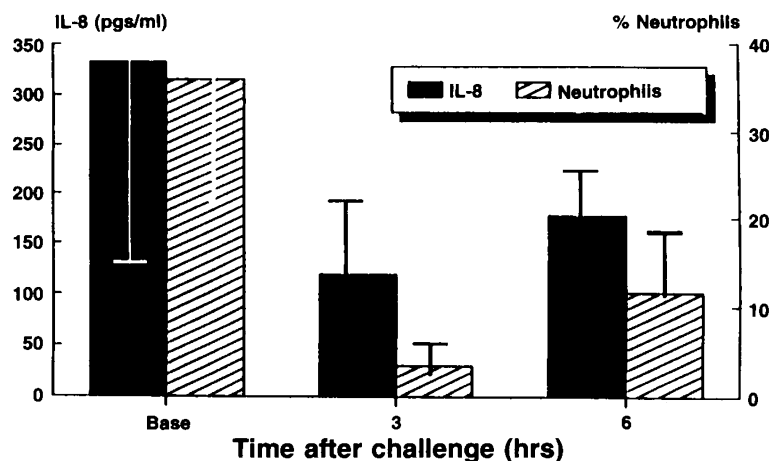


FIG. 6. Mean nasal IL-8 levels and percentage of neutrophils over 3 and 6 hours in allergic rhinitis patients obtained at baseline prior to the day of challenge and at 3 and 6 hours following nasal provocation. Diluent control IL-8 level = 219 pg/ml.

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The results of our study on detection of nasal IL-8 levels especially during the LPR in allergic rhinitis are important. Corticosteroids are known to decrease immunoreactive IL-8 in bronchial epithelial cells.⁽⁹⁾ Cetirizine has been shown to inhibit neutrophil migration during LPR⁽⁴⁴⁾ and perhaps other H1 antagonists could affect IL-8 levels and neutrophil numbers. Thus, nasal provocation in patients with allergic rhinitis is a useful tool to monitor nasal IL-8 levels and new therapeutic agents.

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