

# Comparison of rhinomanometry, symptom score, and inflammatory cell counts in assessing the nasal late-phase reaction to allergen challenge

Elide A. Pastorello, MD,<sup>a</sup> Gian Galeazzo Riario-Sforza, MD,<sup>b</sup>  
Cristoforo Incorvaia, MD,<sup>a</sup> Marco Segala, MD,<sup>a</sup> Mario Fumagalli, MD,<sup>b</sup>  
and Renato Gandini, MD<sup>b</sup> Milan, Italy

*Inspiratory nasal resistance, symptom score, and influx of inflammatory cells into the nose were evaluated before and after a nasal challenge in 15 patients with grass pollen rhinitis and in six nonallergic control subjects, to study the nasal late-phase reaction and its relationship with nasal cytology. All patients had an immediate positive reaction to specific nasal challenge. In seven allergic subjects we observed a late inspiratory nasal resistance increase, but only two had a significant late symptom score. Inflammatory cells increased by 8 hours in all the patients; the higher the cell influx, the higher the symptom score. Close correlations were found between the inspiratory nasal resistance increase, nasal obstruction at hours 8 and 24, and all cell counts. In control challenges there were no significant increases of symptom score or inspiratory nasal resistance either during the challenge or in the next 24 hours. A weakly significant increase was found only for neutrophil counts at 8 hours. These results indicate that an inflammatory reaction constantly occurs after a specific nasal challenge; its extent is closely related to inspiratory nasal resistance and to the intensity of nasal obstruction. Moreover, our data outline the pivotal role played by eosinophils and basophils in the genesis of an allergic late-phase reaction in the nose. (J ALLERGY CLIN IMMUNOL 1994;93:85-92.)*

**Key words:** Rhinomanometry, symptom score, nasal cytology, nose, late-phase reaction, allergen challenge, allergic rhinitis

In most allergic patients a specific nasal provocation test is followed by an immediate reaction and in an undefined percentage of them by a late-phase reaction (LPR).

Unlike the well-known parameters for defining bronchial LPR, the criteria for diagnosing nasal LPR have not been thoroughly investigated, and most authors<sup>1-5</sup> employ different investigation techniques and positivity cutoffs. The lack of reliable assessment criteria makes it impossible to identify properly patients with late nasal reactions, the clinical meaning of which thus remains partially unknown.

## Abbreviations used:

|      |                              |
|------|------------------------------|
| AUR: | Activity units by RAST       |
| INR: | Inspiratory nasal resistance |
| IR:  | Immediate reaction           |
| LPR: | Late-phase reaction          |
| MGG: | May-Grünwald-Giemsa stain    |
| NAR: | Nasal airway resistance      |
| NSS: | Normal saline solution       |
| RMM: | Rhinomanometry               |
| SS:  | Symptom score                |

Assessment by an objective method such as rhinomanometry (RMM) has often been criticized for not giving reliable results. This technique measures nasal obstruction, which is influenced by various factors, including posture and especially the nasal cycle, during which nasal resistance may vary widely with respect to baseline values.<sup>6-8</sup> Moreover, patients vary in their severity of obstruction. Accompanying this they may have as their main symptoms rhinorrhea,

From <sup>a</sup>the First Department of Internal Medicine, University of Milan; <sup>b</sup>the Allergy/Immunology Unit, Ospedale Buzzi; and <sup>c</sup>the Scientific Direction, Ospedale Maggiore.

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Reprint requests: Elide A. Pastorello, MD, Istituto di Clinica Medica Generale, Pad. Granelli, Via Francesco Sforza 35, 20122 Milano, Italy.

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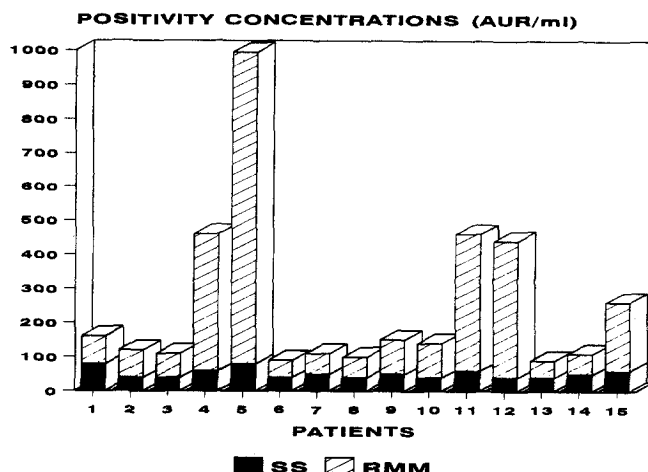


FIG. 1. Allergen threshold concentrations, expressed in AUR per milliliter, eliciting immediate positivity to nasal challenge.

pruritus, or sneezing.<sup>9</sup> Some authors<sup>10, 11</sup> have found that the increase of certain mediators in nasal lavages is related to the LPR, but the measurement of these mediators is still experimental and not available for routine clinical purposes.

Nasal cytology investigated during LPR<sup>10, 12-16</sup> showed an increased influx of inflammatory cells, especially eosinophils, in patients with a positive LPR. In the present study nasal challenges with a specific allergen, an irrelevant antigen and allergen diluent were carried out in grass pollen-allergic patients and in healthy subjects. The changes in RMM, symptom score (SS), and eosinophil, neutrophil, and basophil counts in nasal secretion were recorded hourly up to 8 hours, then again 24 hours after challenge. The resulting data were compared, and correlations were sought in those patients who had a nasal LPR.

## METHODS

### Patients

We enrolled from the allergy clinic of Buzzi Hospital, Milan, Italy, 15 patients, nine females and six males, 17 to 58 years of age (mean age 29.6 years). Inclusion criteria were as follows. Patients had a typical history of seasonal rhinitis. Patients had a positive skin prick test with an extract of *Phleum pratense* (timothy grass) (Dome/Hollister-Stier Laboratories, Slough, U.K.). Positivity was determined by a wheal of at least 3 mm diameter. For inclusion wheal size had to be equal to or larger than that elicited by histamine dihydrochloride, 10 mg/ml, according to the Nordic Guidelines<sup>17</sup> as usually followed in Europe. Prick tests were made by standardized lancets with 1 mm tips (Dome/Hollister-Stier Allergy Pricker) as reported by Malling et al.<sup>18</sup> Participants also had a positive RAST (Phadebas

RAST, Pharmacia, Uppsala, Sweden) of at least class 3 for grass pollen and negative skin tests for a panel of common seasonal allergens such as birch, hazel, ragweed, pellitory, and mugwort. Exclusion criteria were (1) previous episodes of asthma; (2) immunotherapy with grass pollen extracts in the previous 3 years; (3) nasal abnormalities such as septal deviations or polyps; or (4) any other acute or chronic disease, so that none of the patients enrolled had required any medications for 3 months before the start of the study. All patients gave their informed consent, and the study was approved by the ethical committee of Buzzi Hospital.

### Control groups

We selected two control groups as follows: Group 1 consisted of six of the 15 grass-allergic patients (three male, and three female; mean age 27.7 years) who were rechallenged with normal saline solution (NSS) out of the grass pollen season. We tested only six patients in the study group because some subjects had begun immunotherapy and others were not available for a second challenge.

Group 2 comprised six healthy volunteers (three male, three female; mean age 28.4 years) who were challenged with *P. pratense* extract at increasing concentrations. Inclusion criteria were (1) no previous history of allergic diseases and (2) negative skin tests and RAST for a panel of common seasonal allergens such as birch, hazel, ragweed, pellitory, and mugwort. Exclusion criteria were the same as for the allergic patients.

### Grass extract

A lyophilized extract of *P. pratense* was dissolved in NSS at concentrations of 10, 20, 40, 80, 100, 200, 400, 800, 1000 activity units by RAST (AUR) per milliliter. In comparison with the international unit as established in the standardization of allergenic extracts,<sup>19</sup> 1 AUR corresponds to 1.91 IU.

## Nasal challenge

For patients a 100  $\mu$ l volume of NSS followed by increasing concentrations of allergenic extract, starting with 10 AUR/ml, was nebulized into the challenged nostril with a modified airbrush (Humbrol, Hull, U.K.) at an airflow rate of 7 L/min.<sup>20</sup> The patients held their breath during insufflation to minimize the risk of bronchial allergen exposure. Nasal airway resistance (NAR) and SS were recorded 5 and 20 minutes after each insufflation, every hour for 8 hours after challenge and at 24 hours. The test was stopped only at RMM positivity (defined later), regardless of the SS recorded.

In control group 1 a 100  $\mu$ l volume of NSS was nebulized four times into the challenged nostril with a 20-minute interval between each challenge.

In control group 2 a 100  $\mu$ l volume of NSS followed by increasing concentrations (80, 100, 200, and 400 AUR/ml) of allergenic extract was nebulized into the challenged nostril with a 20-minute interval between each challenge. The final concentration of 400 AUR/ml was chosen because almost all the allergic patients had an immediate reaction (IR) with this or a lower dose. The insufflation and recording techniques were the same as described previously. All challenges were carried out with the subjects maintaining the same posture. Subjects remained indoors during the challenge procedure and during the following 8 hours. All challenges were performed in the same period of the year (from January to February) and at the same time of the day, starting at 8:30 AM in a room with controlled temperature (20° C) and relative humidity (70%).

## Rhinomanometry

RMM was performed with an anterior active rhinomanometer (Amplifon S.p.A., Milan, Italy) connected by a 16-bit analog-digital converter to a PC/AT 80286. The results of RMM were reported to have good reproducibility<sup>21</sup> that was further increased by the use of computerized rhinomanometers.<sup>9</sup> We found that nasal resistance values measured with a computerized RMM varied within 10%. We measured the inspiratory nasal resistance (INR) because inspiratory curves were reported to be less variable from breath to breath than expiratory curves.<sup>3</sup>

NAR was calculated as the average of four consecutive breaths at a fixed reference pressure of 150 Pa, because many patients were too obstructed after the challenge to reach the fixed flow rate of 0.5 L/sec, at which Solomon et al.<sup>22</sup> suggested to calculate NAR. A baseline RMM was performed to choose the nostril with lower INR values for the challenge. The NSS control was then nebulized into the challenged nostril. If the INR increased to more than 100% of the basal value, the patient was dropped from the study.

RMM was considered positive when the INR reached five times the baseline value. This value was chosen to limit interference with normal diurnal variations as much as possible.

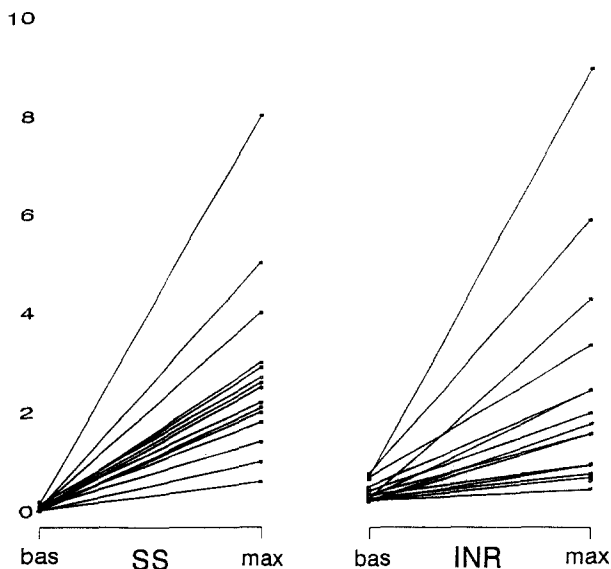


**FIG. 2.** SS and INR late changes. Values reported, expressed respectively as total score and in pascals per cubic centimeter per second are baseline (*bas*) and highest value reached (*max*) 4 hours after challenge.

## Symptom score

The following symptoms were recorded: rhinorrhea; nasal obstruction; itching of the nose, ear, and throat; sneezing; and conjunctivitis. The baseline SS had to be 0 (no symptoms) for a subject to be included. The scoring was defined according to Bousquet et al.,<sup>23</sup> who established that the total SS for a positive immediate challenge has to be 5 or more. This score has been validated by a close correlation obtained with prostaglandin D<sub>2</sub> release, which is the mediator most involved in immediate nasal reactivity.<sup>24</sup> However, such a correlation has not yet been demonstrated for nasal late reactions.

## Cell counts

We followed the technique previously described by Pelikan and Pelikan-Filipek.<sup>16</sup> Briefly, three series of specimens were collected by having the patient blow his or her nose into a polyethylene sheet before allergen challenge (basal time) and 5 minutes, 8 hours, and 24 hours after a positive challenge. In control groups the specimens were collected before the challenge (basal time) and 5 minutes, 8 hours, and 24 hours after the end of the scheduled insufflations. Each series was stained with three different methods: a modified May-Grünwald-Giemsa (MGG) stain,<sup>25</sup> toluidine blue,<sup>26</sup> and Hansel's stain.<sup>27</sup> The results were assessed by the count obtained with MGG; slides stained with toluidine blue and Hansel's stain were used only as controls.

Specimens were examined microscopically by a technician not directly involved in the study and not aware of the clinical data. Two different counting techniques were used: (1) a count of the absolute number of each

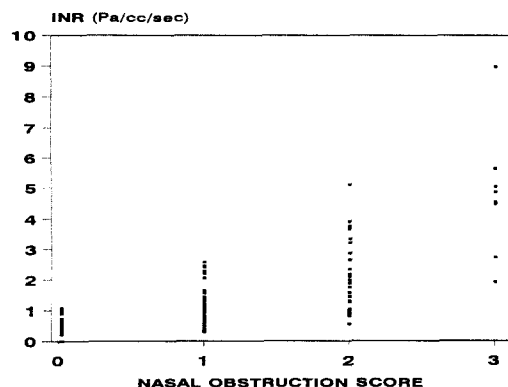


**FIG. 3.** Correlation between INR and nasal obstruction score recorded 1 to 24 hours after nasal challenge ( $r = 0.488$ ;  $p < 0.05$ ).

cell type at  $\times 250$  in 25 microscopic fields spread over the whole slide and (2) a differential count, used as a control. The number of each cell type was expressed as a percentage of 100 leukocytes counted in 25 microscopic fields spread over the whole slide. The reproducibility of the cell-counting technique was satisfactory; our results from control specimens did not significantly differ from counts with MGG-stained slides. These data were in agreement with those reported by Pelikan.<sup>28</sup>

### Statistical analysis

Correlations between parametric data such as cell counts and INR values were analyzed by Pearson's correlation test, and correlations between nonparametric data (such as SS values) and parametric data were analyzed by the Spearman's rank correlation test. Cell counts were compared with basal values by Wilcoxon's rank test, and cell counts in late and non-late groups were matched for each sampling time by the Mann-Whitney  $U$  test. Threshold doses and cumulative doses administered in nasal challenges were correlated by Student's  $t$  test. Statistical analysis was carried out with the Statistical Package for Social Sciences program (SPSS/PC+, SPSS Inc., Chicago, Ill.).

## RESULTS

### Immediate reaction

The results are depicted in Fig. 1. All patients had an immediate positive reaction as judged by a SS of 5 or more and an increase of INR at least 5 times the baseline value.

Twelve of the 15 patients reached symptomatic positivity at an allergen concentration lower than that causing a positive RMM value. In the remaining three patients the two parameters were positive at the same concentration. No significant correlation was found between the SS and the INR. In the control groups no one had symptoms or significant INR increases during challenge.

### Late reaction

**Inspiratory nasal resistance.** Seven of 15 patients showed an increase greater than five times the basal values 4 hours after the challenge. All patients with a late INR increase had symptoms, even though very slight.

**Symptom score.** Fourteen of 15 patients had symptoms of varying severity beginning 4 hours after the challenge, but only two reached an SS of 5 or higher. Fig. 2 shows INR and SS late values for all patients.

The total SS during the first 8 hours and at 24 hours after challenge were significantly correlated with INR values at the same times (Spearman  $r = 0.602$ ,  $p < 0.001$ ).

With regard to the single symptoms, nasal blockage was correlated with INR (Spearman  $r = 0.488$ ,  $p < 0.05$ ) (Fig. 3), but no correlations were obtained with the other symptoms. None of control subjects had symptoms or significant INR increases either during the first 8 hours or at 24 hours after challenge.

### Cell counts

We compared basal counts for each cell type with those obtained at the IR, and at 8 and 24 hours (Fig. 4, A). Eosinophils showed the most consistent increase, rising from a mean basal value of 1 cell/field to 24 cells/field at 8 hours; eosinophil values were also significantly higher than baseline values at IR and at 24 hours. The basophil counts at IR, 8, and 24 hours were higher than baseline values, but the mean number of cells per microscopic field was low. Mean neutrophil counts were significantly higher than baseline with IR and at 8 hours only. In control subjects (groups 1 and 2), only neutrophil counts showed a weakly significant increase over the baseline at 8 hours after challenge ( $p < 0.05$ ); no significant variations in eosinophils and basophils were found in either control group at any sampling time (Fig. 4, B).

We assessed the correlations at IR, 8 hours, and 24 hours for nasal symptoms and for INR (calculated as a percentage of baseline value) with counts of eosinophils, neutrophils, and basophils.

Only nasal obstruction was significantly correlated with cell counts. All cell counts were correlated with obstruction at 8 hours (neutrophils and basophils,  $p < 0.01$ ; eosinophils,  $p < 0.001$ ), but only neutrophils and eosinophils were correlated with obstruction at 24 hours ( $p < 0.05$ ). INR was not correlated with any cell count at IR. At 8 hours INR was correlated with eosinophils ( $p < 0.001$ ) and basophils ( $p < 0.01$ ) but not with

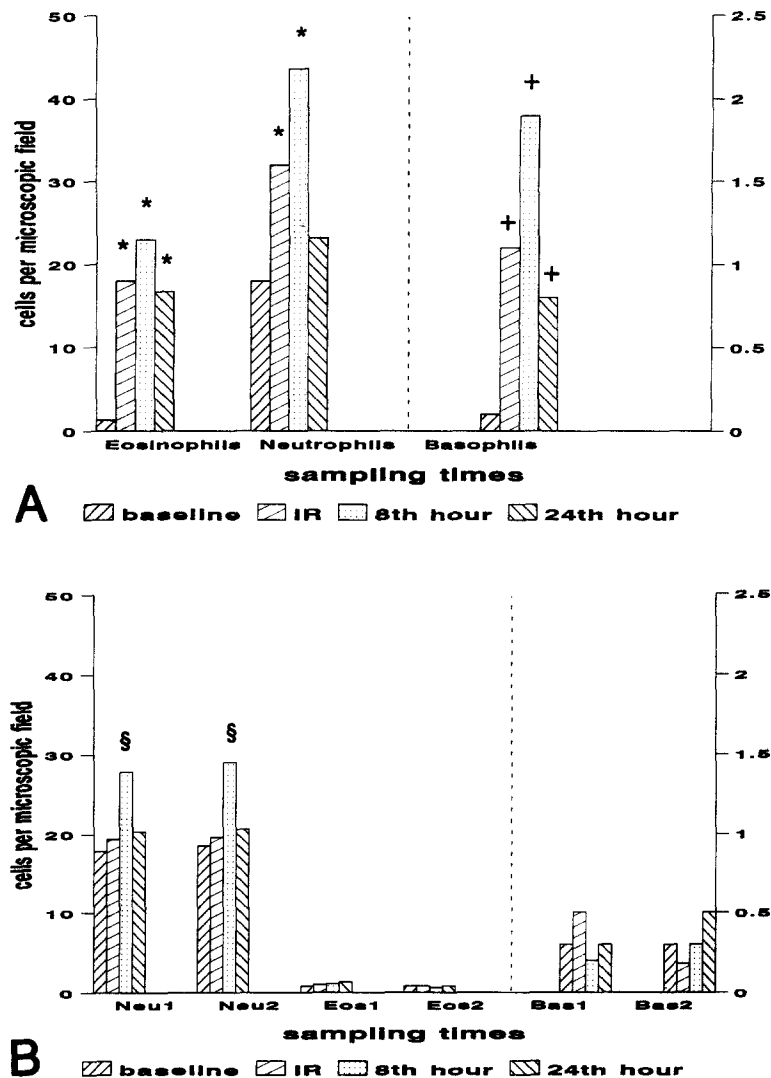


**FIG. 4.** Comparison of baseline cell counts and values recorded at challenge positivity (IR) and 8 and 24 hours after challenge for each kind of cell in the patient population (A) and in control groups 1 and 2 (B). All counts are expressed as mean number of cells per microscopic field at  $\times 250$ . \*  $p < 0.001$ ; †  $p < 0.01$ ; §  $p < 0.05$ . Bas, Basophils; Eos, eosinophils; Neu, neutrophils.

neutrophils, whereas at 24 hours it was correlated only with eosinophils ( $p < 0.001$ ). No correlations between cell counts and INR or SS were found in either control group 1 or 2.

We next considered that patients with an INR increase of five times or more over the baseline value were more likely to have a late reaction. We then divided the population into two groups, late reactors and non-late reactors, and compared cell counts for these two groups (Fig. 5). All cell counts were higher in the late group than in the non-late group. The eosinophil counts of the late group were significantly different from the non-late group counts at IR ( $p < 0.01$ ), 8 hours ( $p < 0.001$ ), and 24 hours ( $p < 0.01$ ). The neutrophil counts of the late group were different

from those of the non-late group values at IR and at 8 hours but not at 24 hours, and basophils were higher in the late group only at 8 hours.

#### IR threshold and cumulative doses

Table I lists the IR threshold RMM doses (AUR per milliliter) and cumulative doses (AUR) for each patient. No differences were found between the late and non-late groups (Student's  $t$  test,  $p = 0.17$ ).

#### DISCUSSION

The significance of late reactions to allergen challenge is currently being evaluated. Although we know only incompletely the factors determining a bronchial LPR in patients with allergic

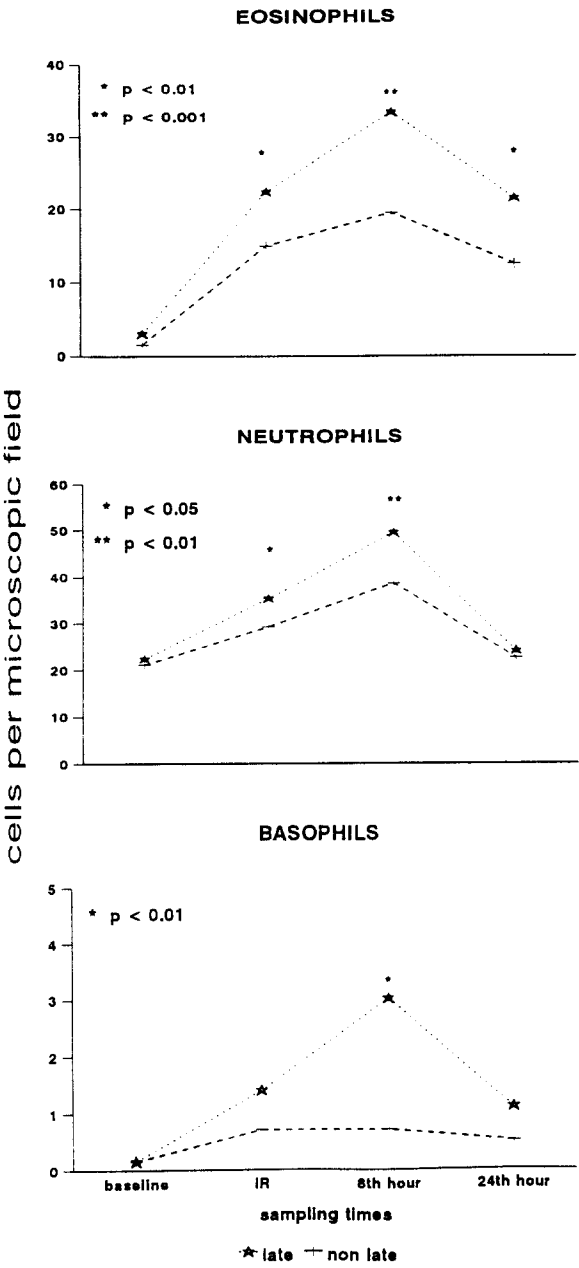


**FIG. 5.** Comparison of eosinophil, neutrophil, and basophil counts, expressed as mean cell number per microscopic field at  $\times 250$ , in late and non-late patients. All values for late patients (except baseline) are significantly greater than those for non-late patients.

asthma, a relationship with more severe clinical symptoms and bronchial hyperreactivity has been reported.<sup>29</sup> Bronchial LPR can nevertheless be easily assessed by an objective parameter such as forced expiratory volume at 1 second.

Nasal late responses to allergen challenge however, are more difficult to establish. The immediate reaction can be clearly assessed by symptoms

**TABLE I.** Threshold and cumulative allergen doses administered in nasal challenges

| Patient No. | Threshold dose (AUR/ml) | Cumulative dose (AUR) |
|-------------|-------------------------|-----------------------|
| 1*          | 80                      | 60                    |
| 2*          | 80                      | 60                    |
| 3           | 40                      | 28                    |
| 4*          | 400                     | 340                   |
| 5*          | 1000                    | 1060                  |
| 6*          | 20                      | 12                    |
| 7           | 20                      | 12                    |
| 8           | 20                      | 12                    |
| 9           | 100                     | 100                   |
| 10*         | 100                     | 100                   |
| 11*         | 400                     | 340                   |
| 12          | 400                     | 340                   |
| 13          | 40                      | 28                    |
| 14          | 20                      | 12                    |
| 15          | 200                     | 180                   |

\*LPR.

such as sneezing, itching, rhinorrhea, and nasal blockage, and the intensity of symptoms is correlated to the release of precise amounts of mast cell mediators.<sup>24</sup> The reappearance of nasal symptoms, particularly obstruction and to a lesser extent itching, within 3 to 12 hours after challenge may be consistent with a nasal LPR.<sup>10, 30</sup> The subjective evaluation of nasal LPR by SS can be supplemented by objective measurement of NAR.<sup>31</sup> Anterior RMM is reported to be reliable for this measurement,<sup>9</sup> but the increase in nasal resistance caused by the nasal cycle may interfere with the RMM values recorded during nasal challenge. Because there are no generally accepted criteria for defining an increase in INR as definitively positive, we chose an increase of five times baseline to minimize such an interference.

In the present study almost all patients challenged with specific allergen had nasal symptoms from 4 hours after the challenge, but considering the same threshold of SS positivity as for an IR, only two patients could be judged as having had a LPR. It is likely that this criterion for SS positivity—a score of 5—was too strict. In any case it was not possible to relate it to any biologic event. No standardized criteria for detecting nasal LPR are available, however, and no studies demonstrate, as for IR,<sup>24</sup> a relationship between a certain SS at late assessment and the release of a given mediator.

With regard to the RMM values, 10 of 15 patients (66%) had significant increases of INR

and seven (47%) reached the threshold value of five times baseline. Nasal LPRs have been reported in 3% to 75% of allergen-challenged allergic patients.<sup>3, 4, 10, 11</sup> The frequency of 47% that we considered to be the more objective result appears consistent with studies dealing with pollen-sensitive patients.<sup>3, 10</sup> At present there is no evidence that the nasal LPR is dose dependent, and this suggests that in the nose, in contrast to the skin,<sup>32</sup> the LPR is not affected by the dose of the challenge allergen.

The reliability of the parameters for detection of a nasal LPR can be assessed well on the basis of their correlations with underlying immunologic events such as the release of mediators or the influx of inflammatory cells into the nose after a nasal provocation test. An influx of various kinds of cells into the nose has been reported after challenge. Bascom et al.<sup>12, 14</sup> were the first to report an increase of neutrophils, eosinophils, and mononuclear cells and a significant increase in major basic protein and eosinophil-derived neurotoxin in nasal washings during a nasal LPR. In 104 patients with a nasal LPR Pelikan and Pelikan-Filipek<sup>16</sup> found an increase of neutrophils in 84%, eosinophils in 58%, and basophils only in 8%. Other studies<sup>13, 33</sup> found an increase of Alcian blue-stained cells, especially basophils, and of metachromatic cells in nasal LPR. Iliopoulos et al.<sup>10</sup> reported a significant increase of neutrophils and eosinophils in LPR nasal lavages. Thus data on the influx of basophils are still uncertain, whereas it seems clear that eosinophils and neutrophils increase during an LPR.

In this study we observed that all the patients challenged with specific allergen had significant increases of eosinophils, neutrophils, and basophils in the nasal secretions both in immediate and in late reaction. The most striking rise was in eosinophils; they showed a 17-fold increase compared with only a 2.5-fold increase of neutrophils and a threefold increase of basophils.

Within this population significant differences were observed between patients with and those without a fivefold increase in INR; cell counts were significantly higher in the former. Moreover, highly significant correlations were obtained between cell counts and the symptom of nasal obstruction at 8 hours after challenge; these correlations became weaker and for basophils disappeared at 24 hours. The correlations between cell counts and INR were highly significant for eosinophils both at 8 and 24 hours and for basophils only at 8 hours; no correlation was found between

neutrophils and INR. It is clear that an influx of inflammatory cells was common to all the patients tested by nasal challenge; however, the larger increase was the hallmark of a more marked clinical manifestation. In the control groups challenged with allergen diluent and with an irrelevant antigen, in which no significant changes of INR and SS occurred, only neutrophils showed a weakly significant increase in nasal secretions 8 hours after challenge. These values were still twice as low as those in the patient group at the same sampling time.

On the basis of these results, among the different symptoms occurring after nasal challenge, we can confirm the major importance of nasal obstruction in the assessment of the nasal LPR. In fact, both this clinical symptom and its measurement by RMM (which were associated) were significantly correlated with the influx of inflammatory cells. Even though basophils and neutrophils also increased after challenge, the eosinophil showed the most significant and most persistent rise as well as the most significant correlations with clinical findings. Therefore this cell is likely to play a crucial role in the alterations causing the nasal LPR, similar to what has been reported in late asthmatic reactions.<sup>30, 34</sup> In asthma the behavior of eosinophils appears to be regulated even at a molecular level, as demonstrated by the recent observations of Robinson et al.<sup>35</sup> that T lymphocytes in bronchoalveolar lavages of asthmatic subjects expressed messenger RNA for interleukin-5, which is an eosinophil differentiation and growth factor.

Conversely, the role of neutrophils in the nasal reaction to allergens has still to be clarified. Our data showed no increase of neutrophils immediately after challenge in the two control groups, although there was a significant increase 8 hours after challenge. Hence this cell seems to play a nonspecific role, as demonstrated by its influx into the nose after any kind of stimulus.

In conclusion, an influx of eosinophils and increased airway resistance appear to be the central features of the late nasal allergic reaction, as previously described in the bronchial model.

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