

Nasal airway changes assessed by acoustic rhinometry and mediator release during immediate and late reactions to allergen challenge

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Background: We have found that acoustic rhinometry is a reliable means of assessing nasal airway caliber changes during the immediate reaction to nasal allergen challenge of sensitive subjects. Comparison of such changes with symptoms and patterns of mediator release could help in the understanding of mechanisms of immediate and late-phase reactions after allergen challenge and their clinical relevance. **Methods:** Nasal minimal cross-sectional area (MCA) was assessed sequentially for 6 hours after two blinded challenges in random order with pollen antigens and buffer diluent in five sensitive human subjects. Comparisons were made with: (1) symptom scores; (2) olfaction changes; and (3) nasal secretion levels of histamine, tryptase, leukotriene C₄, serum albumin (a marker of vascular permeability), lactoferrin (a marker of local glandular secretion), and inflammatory cells in nasal scrapings.

Results: In four of five subjects there was a significantly greater decrease in MCA after antigen challenge than after diluent challenge, correlating with the degree of subjective nasal congestion. In two of these four subjects there was a prominent second late-phase decrease in MCA at 3 to 5 hours, whereas the MCA was persistently decreased in an additional subject with accompanying subjective congestion. No significant decrease in olfactory acuity occurred. Levels were significantly higher in nasal secretions obtained after antigen challenge than in those obtained after buffer challenge with histamine (9 ± 2.7 ng/ml vs 1.2 ± 0.5 ng/ml; $p = 0.04$); tryptase (95 ± 83 ng/ml vs 3 ± 0.9 ng/ml; $p = 0.02$), leukotriene C₄ (5293 ± 1385 ng/ml vs 578 ± 183 ng/ml; $p = 0.02$), and albumin (123 ± 9 ng/ml vs 19 ± 1.6 ng/ml; $p = 0.005$) but not with lactoferrin (4.6 ± 1.2 ng/ml vs 4.1 ± 28 ng/ml; $p =$ not significant). Granulocyte exudation was seen after antigen challenge but not after buffer diluent challenge. However, there was not a precise correlation between de-

creases in MCA with changes in levels of these mediators in individual subjects.

Conclusions: Acoustic rhinometry can quantitatively assess congestion during immediate and late-phase reactions after nasal challenge without significant correlation to the degree of individual inflammatory events assessed. (*J Allergy Clin Immunol* 1997;100:624-31.)

Key words: Acoustic rhinometry, late phase, allergic reaction, inflammatory responses

Nasal pollen antigen challenge in sensitive human beings is usually followed by prominent transient congestion, among other symptoms, starting within 60 minutes; in some cases, these symptoms recur hours later.^{1,2} Some investigators have postulated that this nasal late-phase reaction (LPR) likely plays an important role in the pathogenesis of chronic allergic rhinitis after environmental exposure to antigen.^{3,4}

However, it has been difficult to quantify nasal congestion in a noninvasive manner, including responses to nasal antigen challenges. Anterior rhinomanometry, the most commonly used approach to date, provides an indirect estimate of nasal airway dimensions as a correlate of measured airway resistance.⁵⁻⁷ However, anterior rhinomanometry is limited by the invasive aspects of the technique and the frequently high coefficients of variation in repetitive measurements.⁷ Anterior rhinomanometry is also limited to areas of laminar airflow with resistance determined only indirectly by measurement of flow-pressure relationships, which are not linear.^{8,9}

Acoustic rhinometry (AR) is a recently described technique that uses the reflection of sound waves transmitted into the anterior nares to estimate geometry of the nasal passages.^{10,11} Acoustic reflection signals from the nose are collected by a microphone, analyzed with an analog to digital conversion by a computer, and processed to determine the minimal cross-sectional area (MCA) and total volume of the nasal cavity.¹² AR is noninvasive, rapid, and simple to perform at frequent intervals; requires minimal cooperation from the subject; and has repeatedly proven to be a reproducible and accurate technique for the quantitative assessment of nasal airway caliber.^{11,12} MCA determined by AR correlates very well with total nasal volume and measurements made by other methods, such as com-

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Abbreviations used

AR:	Acoustic rhinometry
BD:	Buffer diluent
LPR:	Late-phase reaction
LTC ₄ :	Leukotriene C ₄
MCA:	Minimal cross-sectional area
PNU:	Protein nitrogen units

puted tomography and water displacement ($r = 0.90$).¹³ AR has been used to measure changes in nasal patency associated with posture, the nasal cycle, allergic and vasomotor rhinitis, and surgical procedures.¹⁴ We recently reported that AR can be used to reliably assess the early changes in nasal airway geometry after nasal antigen challenge in sensitive subjects.¹⁵

Although inflammatory responses in the nasal mucosa are most likely responsible for the nasal congestion that occurs during early and late allergic reactions to antigen challenge, the precise underlying mechanisms involved are still not well-defined. The availability of the AR method allows us to repeatedly compare the changes in nasal airway caliber with the patterns of local inflammatory responses. This report describes our findings in a group of allergic subjects undergoing controlled nasal challenges in an attempt to help elucidate underlying pathogenic mechanisms in early and late allergic reactions in the nose.

METHODS

Subjects and challenge protocol

Five subjects between the ages of 21 and 37 years with a history of seasonal allergic rhinitis and positive intradermal skin test responses to less than or equal to 10 protein nitrogen units (PNU/ml) of grass or ragweed pollen extract (Greer Labs, Lenoir, N.C.) were studied after informed consent was obtained. All subjects were studied outside the pollinating season, were symptom-free, and were not taking any medications. The protocol was approved by the University of Pennsylvania Institutional Review Board Committee on Studies Involving Human Beings. The responses of subjects to blinded intranasal challenges with antigen extract diluted in buffered saline solution on 1 day were randomly compared with their responses to challenge with only the buffered diluent (BD) at the same hour of the day on a separate day, at least 1 week later. One of the two nasal cavities was chosen at random for all spray challenges and measurements on both challenge days.

Each challenge day began with two baseline measurements of nasal patency in the designated nasal cavity at 15-minute intervals. In between these measurements the subject performed the University of Pennsylvania Smell Identification Test (see below). Four sequential challenges were then carried out in the designated nasal cavity with a metered-dose inhaler, which delivered a consistent volume of 0.3 ml per spray. On the BD challenge day, one spray of BD solution was instilled in the nasal cavity on 4 occasions at 10-minute intervals. On the antigen challenge day, one spray of BD was instilled, followed by one spray each of three increasing concentrations of the appropriate pollen antigen extract (10, 100, and 1000 PNU/ml) at 10-minute intervals. Two minutes after each spray, a 7 mm diameter filter paper disk (Millipore) was applied to the

anterior portion of the nasal septal mucosa to collect nasal secretions. The disk was removed after being in place for 5 minutes and placed in a tube containing 0.2 ml of phosphate-buffered saline for study (see below). After disk removal, the subjects graded their rhinorrhea, congestion, and sneezing on scales of 1 to 5 (5 = most severe). AR measurements were then performed. Subjects were then assessed again at 1, 3, 5, and 6 hours after the fourth spray of antigen or BD solutions, with application of the paper disk, followed by symptom reporting and AR as described above at each time period.

Symptomatic responses

Responses were reported by the subjects on a 0 to 5⁺ scale as follows: Congestion was scored as follows: 1⁺ = mild obstruction, barely noticeable; 2⁺ = moderate obstruction, readily noticeable; 3⁺ = considerable obstruction with difficulty breathing through nose; 4⁺ = severe obstruction, almost complete; and 5⁺ = complete obstruction. Sneezing was scored by the number of sneezes within 2 minutes after spray (≥ 5 sneezes graded as 5⁺). Rhinorrhea was scored as follows: 1⁺ = slight sniffing; 2⁺ = intermittent mild watery drainage; 3⁺ = intermittent moderate drainage; 4⁺ = continuous moderate drainage; and 5⁺ = continuous severe drainage.

AR

AR was carried out by using an acoustic rhinometer (Hood Co., Pembroke, Mass.) for data acquisition and processing. In this way a cross-sectional area-distance curve for a distance from the nasal vestibule into the nasal cavity was described graphically. The MCA was defined at the lowest point of the curve. As shown in Fig. 1, the curves resulting from repeated baseline measurements are quite similar (<5% coefficient of variation). Any percentage change in MCA after a challenge was defined as the baseline (prechallenge) value minus the postchallenge value divided by the prechallenge value. A change greater than or equal to 20% in MCA from baseline measurements was considered significant. The changes in MCA observed after antigen challenge were compared with those after BD challenge in the same subject.

Mediator measurements

The containers containing individual filters in 0.2 ml of phosphate-buffered saline were incubated with gentle rocking overnight at 4°C and centrifuged, and the supernates were analyzed for levels of histamine, leukotriene C₄ (LTC₄), serum albumin, lactoferrin, and tryptase. Histamine was sensitive to 0.2 ng/ml according to an ELISA (AMHAC Inc., Westbrook, Me.) as used previously by us.¹⁶ LTC₄ was sensitive to 40 pg/ml according to the CAST-ELISA system (Bühlmann Labs, AG, Allschwil, Switzerland). In initial quality control studies of this assay system, we validated the detection range described above with an independent source of LTC₄ and the lack of cross-reactivity with leukotriene B₄. Serum albumin (as a marker of vascular permeability) was sensitive to 1 µg/ml according to an ELISA as described by Raphael et al.¹⁷ Lactoferrin (as a marker of local glandular secretion) was sensitive to 0.01 µg/ml according to an ELISA as used previously by us.¹⁸ Tryptase was a marker of mast cell (but not basophil) activation according to the B12 monoclonal antibody ELISA sensitive to 2 ng of tryptase per milliliter in nasal fluid eluates.¹⁹

Other measurements

Olfaction. The University of Pennsylvania Smell Identification Test (UPSIT) approach, involving attempted blinded identification of 20 coded odor strips, was performed in the baseline

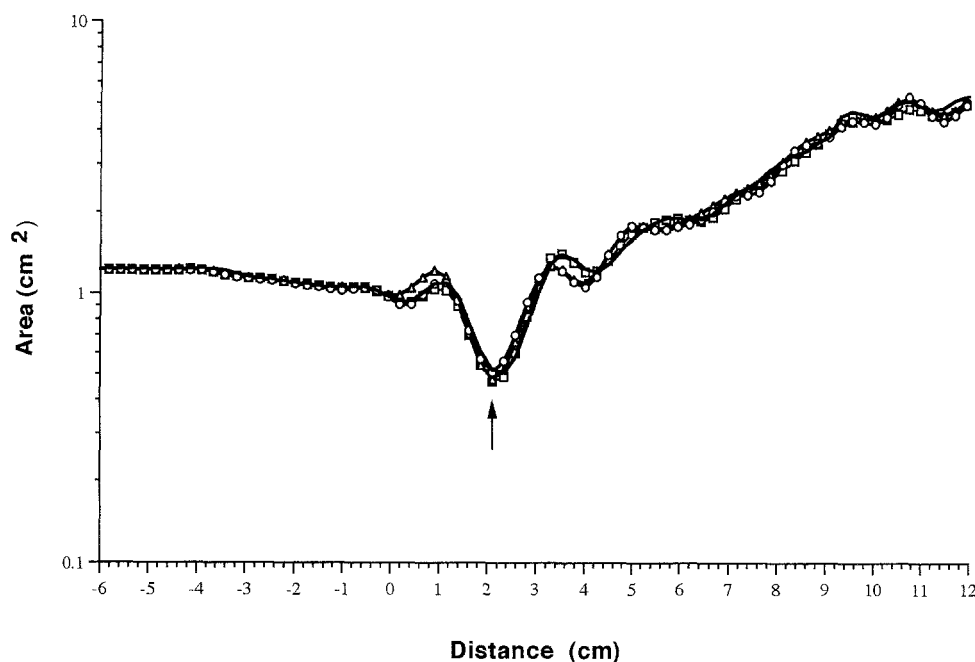


FIG. 1. Representative AR graph of four baseline measurements repeated at 10-minute intervals in the same nares. Ordinate represents cross-sectional area of nasal cavity. In abscissa 0.0 is at the external opening to the nares, with numbers to right representing distances into nares. MCA is usually at the nasal valve location, approximately 2.0 cm from the nasal opening (arrow). In experienced hands there is little variation in curves obtained in repeated measurements as shown. (Data from Hood acoustic rhinometer transferred to Cricket Graph III for graphic display.)

period 20 minutes after the fourth spray of antigen or BD solution and again at the end of the 6-hour observation period. The number of incorrectly identified odors was recorded at each assessment. The validity of such repeated testing in the same individual has been shown previously.²⁰

Nasal cytology. After the olfaction test at the end of the 6-hour observation period, scrapings of the superficial mucosa of the inferior turbinate were obtained with a Rhino probe (Apotex Scientific, Arlington, Texas) and smeared onto coded slides, which were then fixed in 95% ethanol and stained with Wright Giemsa solution as previously described.²¹ The relative frequency of eosinophils and basophilic cells (as a percent of the total inflammatory cells in the smear) was determined.

Statistical analyses

The patterns of relative changes in MCA at the designated time points after antigen challenge were compared with those after BD challenge in the five subjects by analysis of variance. When it was determined that these patterns were significantly different, the relative changes at each time point on the antigen and BD challenge days were compared by paired *t* tests.

In a similar manner the levels of peak mediator release after antigen versus BD challenge were compared in each subject by paired *t* tests when the data were normally distributed and by Wilcoxon signed-rank tests when the data were not normally distributed, with separate analyses for each mediator assessed.

The extent of MCA changes at each time point after the nasal antigen and BD challenges were compared with degrees of symptomatic congestion, olfactory changes, inflammatory cell responses, and mediator levels in nasal secretions obtained at the same time after challenge in each of the five subjects (Spearman correlation analysis).

RESULTS

AR findings

In all subjects there was a prominent decrease in the nasal MCA within 15 minutes after the fourth nasal spray of antigen solution (1000 PNU/ml) (range, 35% to 93% decrease from baseline MCA) (Fig. 2). In four of the five subjects (Fig. 2, A, B, C, and D), these decreased responses were significantly greater than the decrease in MCA within 30 minutes after the similarly timed fourth spray of BD solution on the control challenge day. In the fifth subject (SD) there was also an impressive decrease in MCA after BD challenge (Fig. 2, C).

In one of the five subjects (JM) the marked decrease in MCA occurring shortly after antigen challenge persisted for at least 6 hours (Fig. 2, B). In two other subjects (MK and ES) there was a pattern of return to baseline MCA by 1 hour after the 1000 PNU/ml spray, followed by a second decrease in MCA (maximally 29% and 24%, respectively) at the third to fifth hours (Fig. 2, D and E). MCA either decreased only slightly or frequently increased at these later times after the BD challenge in the same subjects.

Clinical symptoms

Four of the five subjects described prominent nasal congestion (≥ 3 of 5 on a subjective scale) reaching maximum levels within 15 minutes after the antigen challenge (1000 PNU/ml) spray (mean score, 3.2 ± 0.6 for all five subjects) (Table I). The degree of subjective

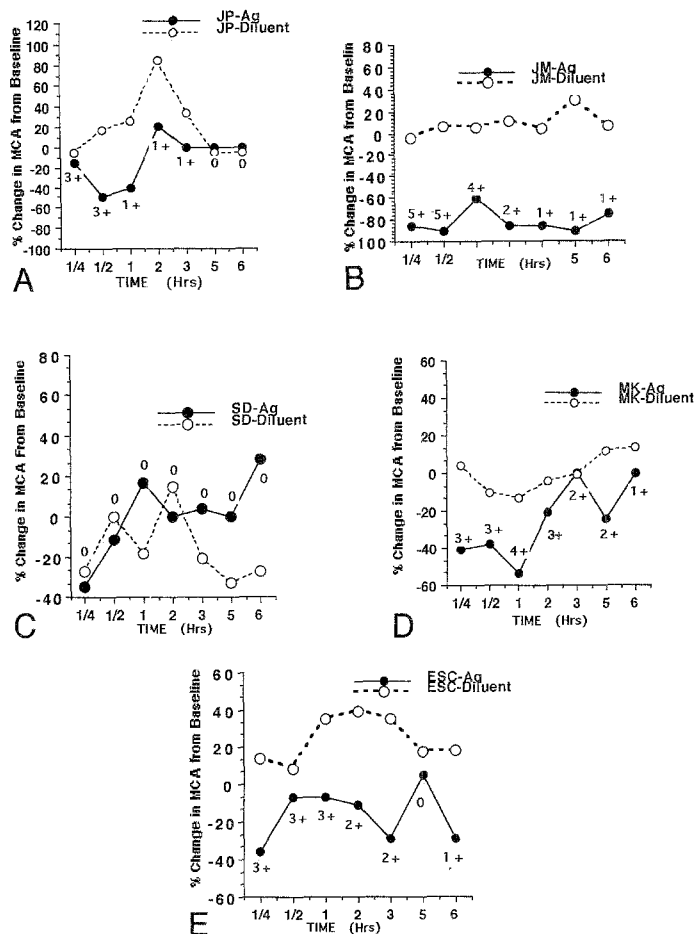


FIG. 2. Relative changes in MCA in five subjects challenged with pollen antigens and BD on 2 different days in a random, blinded protocol. Degrees of subjective congestion (0 to 5+ scale) are listed next to percent change of MCA at each time point after antigen challenge. Greater decreases in MCA were seen after antigen challenge than after diluent challenge in four subjects (A, B, D, and E).

congestion for each time point after antigen challenge in each subject is shown next to the MCA change at that time in Fig. 2. The one subject without subjective congestion (SD) was the same person in whom there was no significant difference in the MCA changes after antigen versus BD challenge (Fig. 2, C). Congestion was accompanied by prominent rhinorrhea (3.8 ± 0.5 of a maximum score of 5) and modest sneezing (1.8 ± 0.9 of a maximum score of 5). After the fourth spray of diluent on the BD challenge day, there was no nasal congestion (0 ± 0 ; $p = 0.008$ vs postchallenge congestion) and minimal rhinorrhea (0.8 ± 0.2 ; $p = 0.005$). There was no sneezing (0 ± 0) after the fourth spray of diluent, but the differences between this and the 1.8 ± 0.9 sneezes after antigen spray were not significant ($p = 0.1$).

Impressive nasal congestion (≥ 2 of 5 on a subjective scale) persisted for at least 2 hours in the three subjects in whom a persistent or recurrent decrease in MCA was noted at the same time (86%, 30%, and 29% decrease in MCA, respectively). No nasal congestion was reported after 30 minutes in the two subjects in whom no impressive decrease in MCA occurred after the first 30 to 60

minutes. No congestion was reported after the comparative diluent spray on the BD challenge day. Congestion responses were significantly greater at the second and third hours after antigen challenge than after BD challenge.

Rhinorrhea persisted for at least 3 hours after the antigen challenge, with no rhinorrhea seen in any subject at 3 or more hours after BD challenge (Table I). The difference between these later responses after antigen and BD challenge was not significant. There was little if any sneezing 1 or more hours after the antigen challenge, which was not significantly different from the complete absence of sneezing after the BD challenge.

For the group as a whole, there was a modest decrease in olfactory acuity shortly after the spray of antigen (1000 PNU/ml) (4 ± 0.7 incorrectly identified odors of 20 tested). This was greater than that seen in the subjects after the fourth spray of BD solution (2.6 ± 0.5 incorrect odors of 20), but not significantly so. The olfactory acuity 6 hours after the antigen challenge was quite similar to that seen 6 hours after BD challenge (2.2 ± 0.7 vs 2.8 ± 0.8 incorrectly identified odors of 20).

TABLE I. Symptoms after nasal challenges

	Congestion			Rhinorrhea			Sneezes		
	Antigen	BD	p Value	Antigen	BD	p Value	Antigen	BD	p Value
15 min	3.2 ± 0.6	0 ± 0	0.008	3.8 ± 0.5	0.8 ± 0.2	0.005	1.8 ± 0.9	0 ± 0	0.1
1 hr	1.8 ± 0.7	0 ± 0	0.07	1.8 ± 0.7	0.2 ± 0.2	0.08	0 ± 0	0 ± 0	—
2 hr	1.6 ± 0.5	0 ± 0	0.04	1.6 ± 0.4	0 ± 0	0.05	0 ± 0	0 ± 0	—
3 hr	1.2 ± 0.4	0 ± 0	0.04	1.6 ± 0.7	0 ± 0	0.08	0.4 ± 0.2	0 ± 0	0.4
5 hr	0.6 ± 0.4	0 ± 0	0.21	1.0 ± 0.8	0 ± 0	0.27	0.2 ± 0.2	0 ± 0	0.5
6 hr	0.8 ± 0.2	0 ± 0	0.15	1.0 ± 0.8	0 ± 0	0.20	0.2 ± 0.1	0 ± 0	0.4

TABLE II. Mediator levels in nasal secretions after nasal challenge

Collection timing	Histamine		Tryptase		LTC ₄		Albumin		Lactoferrin	
	Antigen	BD	Antigen	BD	Antigen	BD	Antigen	BD	Antigen	BD
Baseline	2.2 ± 2.7	3.0 ± 2.4	QNS	QNS	0.2 ± 0.1	0.5 ± 0.2	20 ± 4	31 ± 16	13 ± 5	8 ± 2
Antigen/Diluent†	9.0 ± 2.7‡	2.1 ± 0.5	95 ± 83	3 ± 0.9	5.5 ± 1.3‡	0.6 ± 0.2	123 ± 91§	19 ± 2	12 ± 4	10 ± 3
1 hr	4.1 ± 2.4	1.3 ± 0.6	19 ± 16	2 ± 1.1	0.9 ± 0.3	0.6 ± 0.2	71 ± 20	35 ± 26	5 ± 2	8 ± 4
3 hr	3.3 ± 1.6	1.3 ± 0.6	4 ± 2.1	2 ± 1.2	0.8 ± 0.2	1.3 ± 0.5	86 ± 22	30 ± 20	7 ± 1	5 ± 2
5 hr	6.3 ± 2.5	0.8 ± 0.6	QNS	QNS	1.1 ± 0.3	1.5 ± 1.4	87 ± 18	20 ± 3	7 ± 1	5 ± 2
6 hr	5.4 ± 2.6	4.1 ± 2.7	QNS	QNS	1.4 ± 0.4	0.6 ± 0.1	62 ± 10	24 ± 8	6 ± 1	5 ± 1

QNS, Insufficient chamber fluids to perform this analysis.

*Mean ± SEM/ml in five subjects with histamine (ng/ml); tryptase (ng/ml); LTC₄ (ng/ml); albumin (μg/ml); lactoferrin (μg/ml).

†Ten minutes after 1000 PNU/ml spray on Antigen challenge day; BD spray on BD challenge day; subsequent periods after these sprays.

‡Significantly greater than on BD challenge day ($p = 0.02$).§Significantly greater than on BD challenge day ($p = 0.005$).

Mediator levels in nasal secretions

Histamine levels increased rapidly from baseline (2.2 ± 1.0 to 9.0 ± 2.7 ng/ml) within 15 minutes after spray challenge with antigen (1000 PNU/ml), which was significantly different from the histamine levels in secretions obtained after the comparable BD challenge (2.1 ± 0.5 , $p = 0.03$) (Table II). The mean histamine levels in secretions obtained at 30 minutes, 1 hour, and 3 hours after spray of antigen (1000 PNU/ml) were always higher than those after the comparable BD challenge, but not significantly so. At 5 hours after these challenges, the histamine levels in secretions obtained on the antigen challenge day were close to being significantly greater than those seen on the BD challenge day (6.3 ± 2.5 vs 0.8 ± 0.6 ; $p = 0.07$). This reflected a second increase in histamine levels after the third hour on the antigen challenge day in three subjects. At 6 hours, the difference in histamine levels in secretions on antigen challenge days versus BD challenge days was not significant (5.4 ± 2.0 vs 4.1 ± 2.7).

Tryptase levels were significantly higher in secretions obtained within 15 minutes after the 1000 PNU/ml antigen spray than those seen in secretions obtained after comparable BD challenge (95 ± 83 vs 3 ± 0.9 ; $p = 0.02$) (Table II). Tryptase levels were also higher in nasal secretions obtained 1 hour after antigen challenge than after BD challenge, but not significantly so (19 ± 16 vs 2 ± 1 ; $p = 0.36$). Mean tryptase levels were similar in secretions obtained 3 hours after antigen challenge versus BD challenge (4 ± 2.1 vs 3 ± 0.9 ; $p = 0.71$). There were insufficient

amounts of nasal secretions available from specimens obtained at later observation time points to allow meaningful comparisons of tryptase levels.

LTC₄ levels in secretions obtained within 15 minutes after 1000 PNU/ml antigen challenge were significantly greater than those in secretions obtained after the comparable (fourth) BD challenge ($5459 \pm 12,978$ vs 579 ± 180 ; $p = 0.02$) (Table II). However, there was no significant difference between LTC₄ levels in secretions obtained after antigen challenges versus BD challenges at 1 hour (895 ± 256 vs 582 ± 181 ; $p = 0.09$), 3 hours (802 ± 240 vs 1310 ± 486 ; $p = 0.48$), 5 hours (1104 ± 339 vs 1499 ± 1423 ; $p = 0.55$), and 6 hours (1372 ± 382 vs 556 ± 72 ; $p = 0.16$).

Albumin levels in secretions obtained within 15 minutes after 1000 PNU/ml antigen challenge were much greater than those in secretions obtained after comparable BD challenge (123 ± 19 vs 20 ± 1.6 ; $p = 0.005$) (Table II). Mean albumin levels in secretions obtained during the next 2 hours were similar after antigen challenge and BD challenge. However, starting at the third hour after challenge, levels were higher on the antigen challenge day than on the BD challenge day (third hour = 86 ± 22.0 vs 30 ± 20.0 ; $p = 0.06$; fifth hour = 87 ± 18.0 vs 20 ± 2.7 ; $p = 0.02$; and sixth hour = 62 ± 9.7 vs 24 ± 8.1 ; $p = 0.09$).

Lactoferrin levels in nasal secretions obtained at any time on the antigen challenge day were not significantly increased when compared with the levels obtained on the analogous BD challenge days (Table II).

TABLE III. Cytology in nasal mucosal scrapings

Subject	Postantigen challenge		Postbuffer challenge	
	Basophilic+	Eosinophils	Basophilic+	Eosinophils
JP	2*	5	0	0
JM	5	8	0	0
SD	2	0	0	0
MK	0	6	0	0
ES	0	15	0	0

+, Includes basophils and mast cells.

*Percent of total inflammatory cells.

Nasal cytology

In four of the five subjects (JP, JM, MK, and ES) a prominent accumulation of granulocytes was seen in nasal scrapings obtained at the end of the sixth hour after antigen challenge (Table III). The majority of these cells were neutrophils with a small minority of eosinophils and basophilic cells, possibly basophils. There was only a sparse granulocyte accumulation in nasal scrapings 6 hours after the BD diluent spray in four of these subjects, with a more prominent accumulation seen in the scrapings from the remaining subject (SD). No eosinophils or basophilic cells were present in these specimens.

Correlations

Association of changes in MCA and symptoms. Within 15 minutes after the 1000 PNU/ml antigen challenge, greater relative decreases in MCA were seen in the subjects with more subjective congestion ($r = 0.9$, $p = 0.005$). There was no significant association between subjective congestion and the relative decreases in MCA 1 hour and 3 hours after antigen challenge ($r = 0.2$, $p = 0.79$; $r = 0.1$, $p = 0.86$, respectively). However, in the sixth hour after challenge there was a significant association between subjective congestion and decreases in MCA ($r = 0.9$, $p = 0.01$).

Within 15 minutes after the 1000 PNU/ml antigen challenge, there was also a significant association between the degree of rhinorrhea and the relative decrease in MCA ($r = 0.9$, $p = 0.02$). There was no such significant association at 1 hour ($r = 0.2$, $p = 0.74$), at 3 hours ($r = 0.05$, $p = 0.97$), and at 6 hours ($r = 0.3$, $p = 0.58$). The number of sneezes within 15 minutes after antigen challenge was associated with changes in MCA, but not significantly so ($r = 0.8$, $p = 0.09$). There was insufficient sneezing at later time points to determine any association. Decreases in olfactory acuity shortly after the antigen spray occurred more prominently (but not significantly) in the subjects with greater maximal decreases in MCA ($r = 0.8$, $p = 0.1$) and subjective congestion ($r = 0.7$, $p = 0.2$).

There was a modest (nonsignificant) correlation between the relative decrease in MCA and the histamine levels in secretions obtained within 15 minutes after the 1000 PNU/ml spray ($r = 0.67$, $p = 0.22$). There was no correlation at all between the relative decrease in MCA

and levels of either LTC₄ ($r = 0.1$, $p = 0.9$) or albumin ($r = 0.1$, $p = 0.9$).

There was no correlation between the decreases in MCA and the relative frequencies of eosinophils or basophilic cells in the nasal mucosal scrapings. There was also no correlation between this relative frequency of basophilic cells and histamine levels in secretions obtained just before these scrapings. However, we could not determine the absolute number of basophilic cells because it was not feasible to reliably calculate the total inflammatory cell exudate in such scrapings. It is of note that the highest percentage of basophilic cells was seen in scrapings from one subject (JM) in whom a pronounced decrease in MCA occurred throughout the 6-hour period after the antigen challenge.

DISCUSSION

The findings reported here confirm and extend those previously reported by our group.¹⁵ As documented by the noninvasive AR procedure, intranasal antigen challenge is followed within 15 to 30 minutes by a marked decrease in the MCA of the nasal cavity. This decrease in MCA occurred after the control BD challenge in only one member of the current study group. We continued to find a significant correlation between the extent of decrease in MCA and the degree of subjective nasal congestion within 15 minutes in individual subjects, thus emphasizing the clinical relevance of the quantitative AR measurements. Of interest, decreases in MCA also correlated with the extent of rhinorrhea in the same period.

In addition, we now found in longer observations after intranasal challenge that three of the five subjects manifested either a persistent or recurrent decrease in MCA for at least 6 hours. These relative decreases in MCA correlated significantly with the degree of subjective congestion at the sixth hour but not at the second, third, or fifth hours after antigen challenge. There was no correlation between decreases in MCA and the modest expression of rhinorrhea or sneezing at these later time points after antigen challenge. Correlations among MCA changes and symptoms after the control BD challenge could not be ascertained because these subjects reported no symptoms after such challenges in this blinded, randomized protocol.

Another new finding reported here is the comparison

of MCA changes and mediator levels in secretions obtained at the same observation periods after nasal challenge. Significantly higher levels of histamine, tryptase, LTC₄, and albumin (but not lactoferrin) were present in secretions obtained within 15 minutes after antigen challenge than after BD challenge. Levels of histamine, LTC₄, and albumin continued to be higher in secretions obtained hourly after antigen challenge than in comparable measurements after BD challenge, although not significantly so. Tryptase levels were not different in secretions obtained later than 1 hour after the fourth spray of antigen versus BD. This suggests that histamine (along with tryptase) was released initially from mast cells. There was subsequent release of histamine at later times after antigen challenge, which likely came from basophils because no accompanying tryptase release was observed. This temporal pattern of histamine release is similar to that reported previously by others.²² The increased levels of albumin but not lactoferrin in the nasal secretions obtained after antigen challenge provide indirect evidence that the protein content of such secretions reflect vascular leak of plasma proteins rather than secretory gland activity, as noted by others.¹⁷ The pattern of increased LTC₄ levels seen in secretions obtained after allergen challenge in this study is also similar to that reported by others.²² However, there was not an impressive correlation between decreases in MCA and increases in levels of any of these mediators in secretions obtained at the same time after antigen challenge.

The prominent granulocyte accumulation seen in nasal scrapings, obtained from three of our five subjects 6 hours after antigen challenges, consisted almost entirely of neutrophils with only a small minority of eosinophils. This pattern is similar to that observed by others after a single nasal antigen challenge, with more pronounced eosinophil accumulation noted after repeated daily antigen challenges.²⁴ The basophilic cells present in low frequency in these scrapings were likely basophils, on the basis of evidence described by others.^{20, 25} There was not an impressive correlation between the relative frequency of such cells and the levels of histamine in secretions obtained shortly before the nasal scrapings were performed. However, we do not know the absolute numbers of basophils because total granulocyte enumeration is likely not accurate in such scraping preparations.

These findings can be evaluated in light of additional findings from previous studies. There have been occasional reports of AR being used to assess immediate and late-phase responses to nasal allergen challenge.^{8, 26} As noted above, there has been no prior systematic comparison of the changes in MCA, as assessed by AR, with levels of mediator in nasal secretions and inflammatory cell exudation in allergic responses to nasal challenge. However, comparisons of such inflammatory responses to rhinomanometric changes during the immediate response and after LPR to nasal allergen challenge have been assessed. Pastorello et al.²⁷ found a late change in inspiratory resistance in 7 of 15 subjects with positive

immediate reactions to nasal allergen challenge; however, only two of these subjects had symptomatic LPR. Late increases in inspiratory resistance correlated with inflammatory cell counts in nasal secretions. Wang and Clement⁴ found late increased inspiratory resistance in 80% of those with immediate reactions, correlating with the eosinophil exudation at that time.

In conclusion, it appears that AR is a promising approach to rapidly, reliably, and repeatedly assess the changes in airway caliber during both immediate and later responses after nasal allergen challenge. AR is relatively noninvasive, thereby reducing chances of alteration of the natural architecture of the nasal passages. With AR we have found either persistent or recurrent decreases in the caliber of the nasal airways in three of five sensitive subjects more than 1 hour after nasal allergen challenge. This frequency of later responses is in the range reported previously with other approaches.²⁶ The clinical relevance of the decreases in MCA observed by us is suggested by the correlation of the relative decrease in MCA with the degree of subjective congestion reported by the subjects at the same time. The modest decrease in olfaction observed after allergen challenge, not correlated with MCA changes, suggests that more pronounced and/or prolonged nasal airway obstruction is required for induction of more prominent anosmia. Variations in the cerebral perception of olfactory stimuli among subjects may also be a factor. The lack of correlation we found between the extent of late changes in MCA and individual inflammatory responses in the nose suggests that the pathogenesis of airway caliber changes in nasal LPR is likely complex and multifactorial.

REFERENCES

1. Dvoracek JE, Yunginger JW, Kern EB, Hyatt RE, Gleich GJ. Induction of nasal late phase reactions by insufflation of ragweed pollen extract. *J Allergy Clin Immunol* 1984;73:363-8.
2. Naclerio RM, Proud D, Togias AG, Adkinson NF Jr, Meyers DA, Kagey-Sobotka A, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
3. Iliopoulos O, Proud D, Adkinson NF Jr, Norman PS, Kagey-Sobotka A, Lichtenstein LM, et al. Relationship between the early, late, and rechallenge reactions to nasal challenge with antigen. *J Allergy Clin Immunol* 1990;86:851-61.
4. Wang D, Clement P. Assessment of early- and late-phase nasal obstruction in atopic patients after nasal allergen challenge. *Clin Otolaryngol* 1995;20:368-73.
5. Gleeson MJ, Youlton LFJ, Shelton DM, Siodlak MZ, Eiser NM, Wengraf CL. Assessment of nasal airway patency: a comparison of four methods. *Clin Otolaryngol* 1986;11:99-107.
6. Cole P. Stability of nasal airflow resistance. *Clin Otolaryngol* 1989;14:177-82.
7. Sandham A. Rhinomanometric method error in the assessment of nasal respiratory resistance. *Rhinology* 1988;26:191-202.
8. Lai VWS, Corey JP. The objective assessment of nasal patency. *ENT Journal* 1993;72:397-400.
9. Jones AS, Willatt DJ, Durham LM. Nasal airflow: resistance and sensation. *J Laryngol Otol* 1989;103:909-11.
10. Jackson AC, Butler JP, Millet EJ, Hoppin FG Jr, Dawson SV. Airway geometry by analysis of acoustic pulse response measurements. *J Appl Physiol* 1977;43:523-36.
11. Hilberg O, Jackson AC, Swift DL, Pederson OF. Acoustic rhinom-

- etry: evaluation of the geometry of the nasal cavity by acoustic reflections. *J Appl Physiol* 1989;66:295-303.
12. Grymer LF, Hilberg O, Pedersen OF, Rasmussen TR. Acoustic rhinometry: values from subjects with subjective normal patency. *Rhinology* 1991;29:35-47.
 13. Mayhew TM, O'Flynn P. Validation of acoustic rhinometry by using Cavalier principle to estimate nasal cavity volume in cadavers. *Clin Otolaryngol* 1993;18:220-5.
 14. Fouke JM, Jackson AC. Acoustic rhinometry: effects of decongestants and posture on nasal patency. *J Lab Clin Med* 1992;19:371-6.
 15. Lane AP, Zweiman B, Lanza DC, Swift D, Doty R, Dhong H-J, et al. Acoustic rhinometry in the study of the acute nasal allergic response. *Ann Otol Rhinol Laryngol* 1996;105:811-7.
 16. Zweiman B, Kucich U, Shalit M, von Allmen C, Moskovitz AR, Weinbaum G, et al. Release of lactoferrin and elastase in human allergic skin reactions. *J Immunol* 1990;144:3953-60.
 17. Raphael GD, Baraniuk JN, Kaliner MA. The pathophysiology of rhinitis. V. Sources of protein in allergen-induced nasal secretions. *J Allergy Clin Immunol* 1991;88:33-42.
 18. Zweiman B, Kaplan AP, Joseph K, Moskovitz AR. Cytokine levels and inflammatory responses in developing allergic late phase reactions in the skin. *J Allergy Clin Immunol* 1997 (in press).
 19. Schwartz LB, Bradford TR, Rouse C, Irani A-M, Rasp G, der Zwan JK, et al. Development of a new, more sensitive immunoassay for human tryptase. Use in systemic anaphylaxis. *J Clin Immunol* 1994;14:190-204.
 20. Doty RL, Frye R. Influence of nasal obstruction on smell function. *Otolaryngol Clin North Am* 1989;22:397-411.
 21. Meltzer EO, Jalowayski AA. Nasal cytology in clinical practice. *Am J Rhinol* 1988;2:47-54.
 22. Naclerio RM, Hubbard W, Lichtenstein LM, Kagey-Sobotka A, Proud D. Origin of late phase histamine release. *J Allergy Clin Immunol* 1996;98:721-33.
 23. Georgitis JW, Stone BD, Gottschlich G. Nasal inflammatory mediator release in ragweed allergic rhinitis: correlation with cellular influx into nasal secretions. *Int Arch Allergy Appl Immunol* 1991;96:231-7.
 24. Godthelp T, Holm AF, Fokkens WJ, Doornenbal P, Mulder G, Hoefsmits EC, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. *J Allergy Clin Immunol* 1996;97:800-11.
 25. Iliopoulos O, Baroody F, Naclerio RM, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. *J Immunol* 1992;148:2223-8.
 26. Rasp G. Acoustic rhinometry: measuring the early and late phase of allergic immediate reaction in allergic rhinitis. *Laryngorhinootologie* 1993;72:125-30.
 27. Pastorello EA, Riario-Sforza GG, Incorvaia C, Segala M, Fumagalli M, Gandiini R. Comparison of rhinometry, symptoms score and inflammatory cell counts in assessing the nasal late phase reaction to allergen challenge. *J Allergy Clin Immunol* 1994;93:85-92.

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