

Contralateral differences among biomarkers determined by a modified nasal lavage technique after unilateral antigen challenge

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The concentration of biomarkers from vessels and inflammatory cells in nasal lavage fluid reflects the degree of hyperresponsiveness in patients with allergic rhinitis. The lavage has usually been performed of both nasal cavities together after prewashings and administration of decongestants. To improve the technique, we introduced a modification involving lavage of the nasal cavities separately without any prewashings or decongestants. We challenged 20 rhinitic subjects sensitive to timothy unilaterally with timothy extract. In nasal lavages performed before, immediately after, and 6 h after the challenge, we determined the concentrations of albumin, histamine, bradykinin, TAME (*N*- α -tosyl-L-arginine methyl ester)-esterase, and leukotriene C₄ (LTC₄). In eight subjects, the procedure was repeated 1 and 2 weeks later. After the challenge, albumin, bradykinin, TAME-esterase, and LTC₄ in the nasal lavage fluid increased on the ipsilateral side but not on the contralateral side. Histamine did not increase after antigen challenge. After 6 h, the biomarkers were not increased. The concentrations of biomarkers did not differ between sides before the challenge and not between visits. Thus, the modified nasal lavage technique is reliable and improved compared to previous methods because it involves reproducible determinations of different biomarkers, and it is simple and easy to perform.

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Patients with allergic rhinitis are characterized by an increased responsiveness to antigen challenge and natural exposure of the nasal mucosa. There are several ways to assess the degree of hyperresponsiveness: the case history, the nasal airway resistance, the amount of nasal secretions, and the concentrations of biomarkers, i.e., mediators from vessels, cells, and nerves in nasal secretions after antigen challenge. Determination of biomarkers in nasal secretions reflects not only the degree of disease but also pathophysiologic mechanisms, and it is an important way to assess nasal reactivity and hyperreactivity of different origin.

Biomarkers have been determined in nasal secretions mainly collected by a lavage technique including pretreatment with decongestants, several prewashings, and washing of both nasal cavities together (28, 34). The use of decongestants involves effects on vessels, and prewashings make the technique un-

suitable for repeated lavages over several hours. By lavage of the nasal cavities separately, local and nerve-mediated reactions can be studied. We believe that there is a need to improve the nasal lavage techniques so far used. In addition, the reproducibility of the nasal lavage technique has to be tested.

In this paper, the purpose was to introduce a modification of the nasal lavage techniques previously used but without using decongestants or prewashings. To study the reproducibility of the modified nasal lavage technique, we determined several biomarkers from separate irrigations of both sides of the nose before and after unilateral antigen challenge in rhinitic subjects sensitive to timothy pollen. The biomarkers determined in the nasal lavages were albumin, histamine, bradykinin, TAME (*N*- α -tosyl-L-arginine methyl ester)-esterase, and leukotriene C₄ (LTC₄), reflecting vascular and cellular reactions.

Material and methods

Subjects and design

Twenty subjects (14 men, six women) with a mean age of 26 years (range 17–39) underwent unilateral nasal antigen challenge. They were selected from the Allergy Division, Department of Otorhinolaryngology, Malmö University Hospital, Sweden. Inclusion criteria were at least 2 years of early summer hay fever, as confirmed by positive skin prick test and radioallergosorbent test (RAST) using timothy extract, and by positive nasal provocation test with timothy pollen grains. Exclusion criteria were nasal polyps; gross nasal anatomic deviations; ongoing symptoms of nasal disease, as revealed by history and inspection of the nasal mucosa; and local or general treatment with antihistamines, corticosteroids, or other antiallergic drugs during the last 4 weeks. Before the antigen challenge, each subject underwent anterior rhinoscopy and nasal lavage. After the nasal lavage, the subject expelled any remaining fluid by sniffing or blowing. Nasal patency was determined by the nasal peak inspiratory flow rate (NPIFR) 10 min after the nasal lavage. Ten minutes after a positive reaction to the antigen (see below), the procedure with rhinoscopy, nasal lavage, and NPIFR was repeated. For assessment of any late-phase reaction, the same procedure was repeated after 6 h.

After the first visit, the subjects were randomized into a double-blind, placebo-controlled study evaluating the effect of a new antiallergic drug given orally. The subjects were tested twice more by the same procedure and after the interval of 1 week. The results from eight of the patients – the placebo group – were used to study the reproducibility of the method. Intake of a placebo tablet cannot be supposed to influence the nasal reactivity justifying this comparison. The results of treatment with the active drug will be published elsewhere. The study was carried out well out of season (January–February 1990). Each subject was included after informed consent, and the study was approved by the local ethics committee.

Antigen challenge

The antigen challenge was performed in the following way. By means of rhinoscopy and a Carlsberg pipette, a purified and biologically standardized extract of timothy pollen (25 µl; Aquagen®, ALK, Hørsholm, Denmark) was applied to the inferior nasal turbinate without touching it. The challenge was performed as an end-point titration, starting with an allergen concentration of 100 SQ/ml and increasing in 10-fold steps until the subject reacted convincingly; i.e., symptom score 2 or 3 each in at

least two of the following symptoms: sneezing, nasal obstruction, and increased nasal secretion. The symptom scores were as follows: 0 – no, 1 – mild, 2 – moderate, 3 – strong. Thus, a total score of at least 4 was regarded as a positive reaction. To avoid priming the nasal mucosa (11), the antigen challenge was performed in the right nasal cavity at the first and third visit, and in the left nasal cavity at the second visit. To reduce any diurnal variation, the challenge was performed at the same time on the day.

Nasal lavage

Each nasal cavity was lavaged separately with 10 ml saline (room temperature) with a syringe (60 ml) to which a nasal olive was adapted. The test subject was sitting and leaning forward at an angle of about 60°, and breathing through the mouth (Fig. 1). This position prevented fluid from reaching the throat. To avoid leakage of fluid, the olive was gently applied to the external part of the nostril. To achieve an equilibrium of biomarkers between the fluid and the nasal mucosa, the lavage fluid was passed slowly into the nasal cavity and back into the syringe five times. If the volume of the nasal cavity was less than 10 ml, some lavage fluid started to drop out on the contralateral side. Then, the insertion of fluid was stopped and the volume was noted. To avoid further spill-over, this lower volume was used at the following passages. After the fifth passage, the regained fluid was measured from each side separately, and, if necessary (in a few subjects), further saline was added to reach a minimum of 5 ml, which was the volume required for the analyses of the biomarkers. The volume of fluid recovered after the nasal lavage was, on average, 7.1 ml ± 0.1 (144 measurements in eight subjects), and did not differ between the challenged and the nonchallenged sides ($P > 0.05$), or

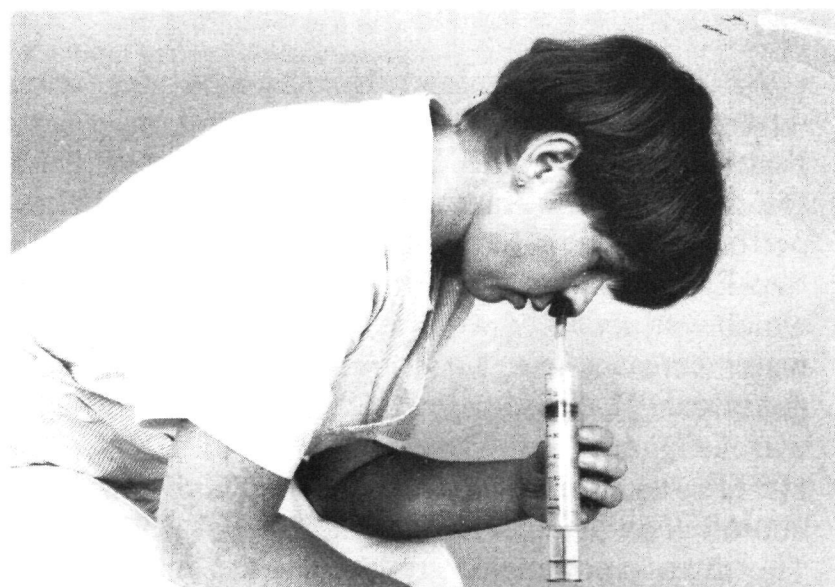


Fig. 1. Test position of subject in modified nasal lavage technique.

between visits ($P > 0.05$). When saline was added, the values for the analyzed biomarkers were adjusted to the dilution. After centrifugation (1500 g for 10 min), the lavage fluids were stored as aliquots at -30°C until analyzed. The lavage fluids were analyzed for albumin, histamine, bradykinin, TAME-esterase, and LTC_4 , respectively.

Biomarker assays

Albumin was determined in the lavage fluid by a double-antibody radioimmunoassay (RIA) (4). The sensitivity was 1 ng/ml. The intra- and interassay coefficients of variation were less than 3%. The concentration of albumin was given as $\mu\text{g/ml}$.

Histamine was determined by fluorimetry (37). Nasal lavage fluid, 0.8 ml, was mixed with 8% (20% of 10 mg/ml) HClO_4 , 0.2 ml, for protein removal at the end of the trial before storage at -30°C . After thawing and centrifugation, the supernatant was assayed. The sensitivity was 1 ng/ml. The intra- and interassay coefficients were less than 7%. The concentration of histamine was given as ng/ml.

Bradykinin was determined by an RIA (32). Nasal lavage fluid, 0.8 ml, was mixed with 0.2 M EDTA, 0.2 ml. The sensitivity was 20 pg/ml. The intra- and interassay coefficients of variation were less than 2%. The concentration of bradykinin was given as pg/ml.

TAME-esterase activity was determined by a radiochemical assay based on the liberation of ^3H -labeled methanol from the substrate ^3H -TAME (18). Nasal lavage fluid, 40 μl , and 0.2 M Tris-buffer, pH 8.0, 10 μl , were put into a 1.5-ml Eppendorf microtube. ^3H -TAME, 10 μl ($\sim 1.5 \times 10^{-5}$ cpm), was then added with mixing, and the tube was placed in a counting vial containing 10 ml Econofluor, and 50 μl stop solution. After 1 h, the reaction was terminated. The sensitivity was 500 cpm/ml. The intra- and interassay coefficients were less than 2%. The concentration of TAME-esterase was given as cpm/ml.

LTC_4 was determined by an RIA (9) using dextran-coated charcoal as a separation agent. Before the RIA, the LTC_4 was extracted from the washes with methanol and eluted by high-performance liquid chromatography (HPLC) with Sep-Pak cartridges (Millipore, Bedford, MA, USA), which was activated with methanol and washed with water between use. Leukotrienes were eluted with methanol (9). Cross-reactivity with LTD_4 and LTE_4 was 64% and $<0.1\%$ for LTB_4 and 5- and 15-HETEs, as compared with cross-reactivity with the homologous ligand. The sensitivity was 150 pg/ml. The intra- and interassay coefficients of variation were less than 9%. The concentration of LTC_4 was given as ng/ml.

Nasal peak inspiratory flow rate (NPIFR)

To confirm a positive nasal challenge and to test any congestion in the late phase, we determined the nasal inspiratory flow rate. The NPIFR was performed in both nasal cavities together with a Youlten peak nasal inspiratory flow meter (39, 40). The subject inspired maximally through a nasal adapter mask attached to a peak-flow meter. The highest value of three consecutive recordings was used in calculations. We chose NPIFR for determining nasal patency because it is reproducible, fast, and convenient in repetitive recordings and has been shown to correlate well with rhinomanometry (39).

Statistical analysis

Data were reported as mean $\pm$ standard error of the mean (SEM). Differences of the allergen concentration causing a positive reaction and of NPIFR among the eight subjects (the placebo group) between visits were tested by Friedman two-way ANOVA by ranks; with a calculated power of 94.6% ($1-\beta$, $\beta = 0.054$), the number of subjects was regarded as sufficient. Mean values for different measurements of biomarkers were compared by two-way ANOVA. Differences between individual pairs of means were tested by Fisher's procedure for least significant difference. The reproducibility of data between visits and the agreement of data between sides were tested by assessment of the coefficient of repeatability (8), which is twice the analytic (experimental) error. The analytic error was calculated in a two-step procedure: firstly, the two-way ANOVA was used; secondly, since no difference was found between visits, one-way ANOVA was used to compare the analytic error. For the method to be regarded as valid, 95% of the values – corresponding to two standard deviations – had to be within the coefficient of repeatability. Correlations between measurements were calculated for both the 20 subjects (first visit only) and the eight subjects, respectively, by Spearman's rank correlation coefficient, r_s . P -values <0.05 were considered to be significant.

Results

All the subjects reacted with nasal congestion and increased nasal secretion at various doses of antigen, but the dose required for a positive reaction did not change ($P > 0.05$) at the second and third visits (Fig. 2) ($n = 8$). No late-phase reaction was detected in any of the patients ($n = 20$).

The NPIFR (both nasal cavities together) decreased ($P < 0.01$) in all subjects ($n = 20$), on average, by $40 \pm 9\%$, after antigen challenge, but after 6 h, it did not differ from baseline values (Fig. 3).

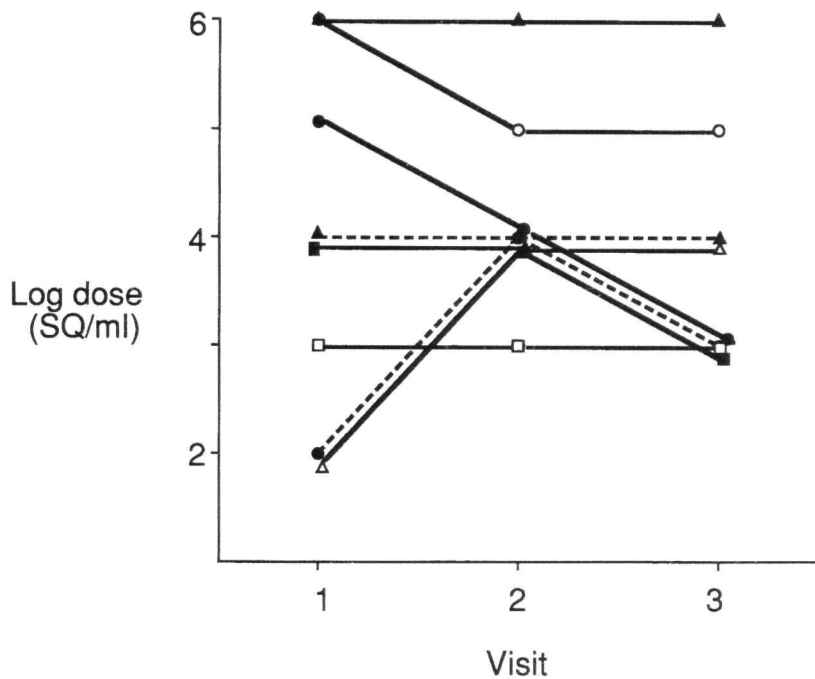


Fig. 2. Concentration (log dose) of timothy extract (SQ/ml) causing positive nasal provocation in subjects with allergic rhinitis ($n = 8$) at three visits at 1-week intervals. Individuals are presented with different symbols.

The concentration of *albumin* in nasal lavage fluid increased three-fold ipsilaterally ($P < 0.01$) and did not change contralaterally from baseline after unilateral antigen challenge ($n = 20$) (Fig. 4). The concentration of albumin differed between sides after ($P < 0.05$), but not before, the antigen challenge. After challenge, the concentration of albumin on the challenged side was, on average, $1531\% \pm 345$ (mean $\pm$ SEM) of the nonchallenged side. Six hours after antigen challenge, the concentration of albumin had returned to baseline values.

The concentration of *histamine* in nasal lavage fluid did not change ipsilaterally, but it decreased contralaterally by, on average, $35.9\% \pm 5.1$ ($P < 0.05$) from baseline after antigen challenge ($n = 20$) (Fig. 4). The concentration of histamine did not differ between sides before or after the antigen challenge. Six hours after antigen challenge, the concentration of histamine was not different from baseline values.

The concentration of *bradykinin* increased three-fold ($P < 0.01$) from baseline ipsilaterally but did not

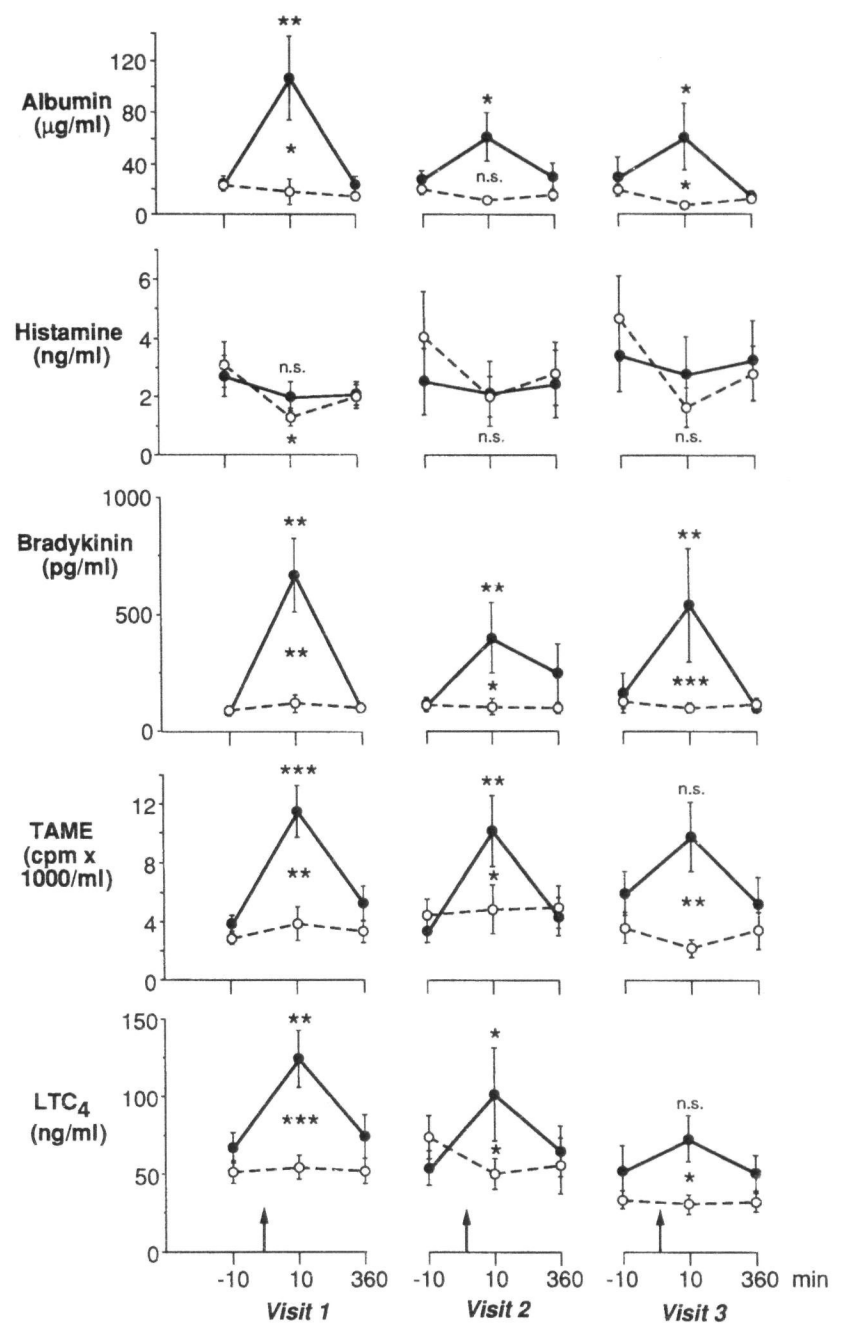


Fig. 4. Concentration of albumin, histamine, bradykinin, TAME-esterase, and LTC_4 in nasal lavage fluid from subjects with allergic rhinitis ($n = 20$) before (-10 min), immediately after ($+10$ min), and in late phase ($+360$ min) after unilateral antigen challenge (arrow). Procedure was repeated identically after 1 (visit 2) and 2 weeks (visit 3) in eight subjects. Concentrations of biomarkers are indicated on challenged side (closed circles, full line) and on nonchallenged side (open circles, interrupted lines). Differences between sides are indicated by asterisks between sides; differences before and after challenge are indicated above (or below) post-challenge value. Values are means $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = $P > 0.05$.

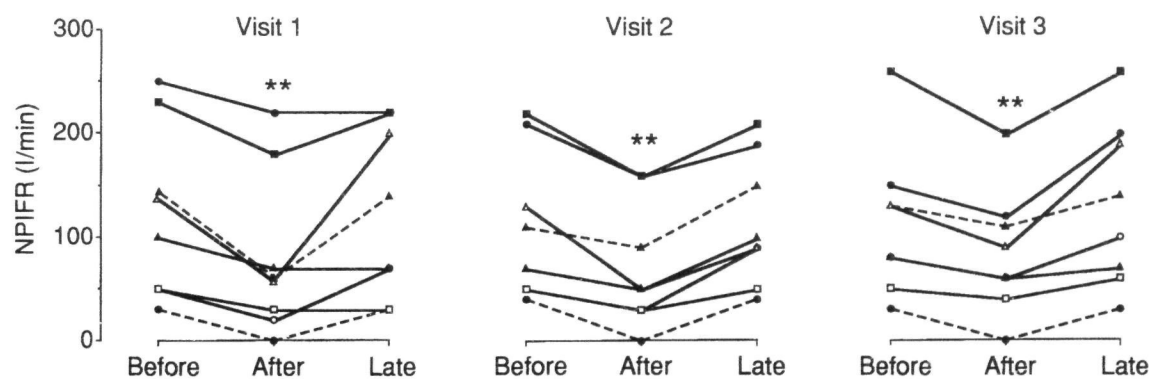


Fig. 3. Nasal peak inspiratory flow rate (NPIFR) (l/min) in allergic rhinitic patients ($n = 8$) before, immediately after, and 6 h after (late) antigen challenge. Individual values are indicated at visits 1, 2, and 3. At all visits, there was significant decrease in NPIFR after antigen challenge. ** $P < 0.01$.

Table 1. Assessment of agreement between biomarkers from two sides and reproducibility of biomarkers between visits

Biomarker	Test of agreement between sides before challenge at first visit (<i>n</i> =20; 40 values)					Test of reproducibility between visits on control side before challenge (<i>n</i> =8; 24 values)				
	2-way ANOVA		1-way ANOVA	Coefficient of reliability		2-way ANOVA		1-way ANOVA	Coefficient of reliability	
	F	p	Analytic error	No. of values > 95%		F	p	Analytic error	No. of values > 95%	
Albumin	0.041	0.842	20.6	41.2	1	0.048	0.954	12.2	24.4	1
Histamine	0.623	0.440	1.55	3.10	1	0.521	0.605	1.50	2.90	2
Bradykinin	0.024	0.879	68.9	137.8	1	0.976	0.401	49.6	99.2	2
TAME-esterase	0.007	0.933	1049	2099	2	1.215	0.328	2297	4594	1
LTC ₄	2.635	0.124	29.1	58.2	2	3.424	0.070	34.0	68.0	1

change contralaterally after the antigen challenge (*n* = 20) (Fig. 4). The concentration of bradykinin differed between sides after (*P* < 0.01), but not before, antigen challenge. After the challenge, the concentration of bradykinin on the challenged side was, on average, 637% ± 180 of the nonchallenged side. Six hours later, the concentration of bradykinin had returned to baseline values.

The concentration of TAME-esterase increased threefold (*P* < 0.001) ipsilaterally, but it did not change contralaterally after antigen challenge (*n* = 20) (Fig. 4). The concentration of TAME-esterase differed between sides after (*P* < 0.01), but not before, antigen challenge. After the challenge, the concentration of TAME-esterase on the challenged side was, on average, 587% ± 148 of the nonchallenged side. Six hours after antigen challenge, the concentration of TAME-esterase had returned to baseline values.

The concentration of LTC₄ increased twofold (*P* < 0.01) ipsilaterally, but it did not change contralaterally after antigen challenge (*n* = 20) (Fig. 4). The concentration of LTC₄ differed between sides after (*P* < 0.001), but not before, antigen challenge. After the challenge, the concentration of LTC₄ on the challenged side was, on average, 219% ± 25 of the nonchallenged side. Six hours after antigen challenge, the concentration of LTC₄ had returned to baseline values.

The NPIFR did not differ between visits before or after challenge. When the coefficients of repeatability were calculated, only one out of 24 values was outside the 95% limits (data not shown).

The concentrations of albumin, histamine, bradykinin, and TAME-esterase did not differ between sides before challenge (*n* = 20), or between visits before or after antigen challenge (*n* = 8). The test of agreement between biomarkers analyzed from the two sides assessed before challenge at the first visit (*n* = 20) and the test of reproducibility of the analyses of biomarkers assessed by calculating the value of the biomarker from one side (control) in the placebo

group (*n* = 8) at the three visits are shown in Table 1. Only a few values were outside the 95% limits of the coefficient of repeatability.

The correlation coefficients between the biomarkers are shown in Table 2. Albumin, bradykinin, and TAME-esterase correlated well, whereas histamine and LTC₄ did not. The correlations of the biomarkers at different time points were similar.

Discussion

We found that the modified nasal lavage technique without pretreatment with decongestants and pre-washings was highly reproducible, as the concentrations of albumin, histamine, bradykinin, TAME-esterase, and LTC₄ were consistent between sides before challenge and between visits on the challenged and nonchallenged sides.

Repeated antigen challenges within short intervals may result in local priming of the nasal mucosa; i.e.,

Table 2. Spearman rank correlation coefficients (*r_s*) between biomarkers at different visits

Biomarker	Visit (V), <i>n</i>	Histamine	Bradykinin	TAME-esterase	LTC ₄
Albumin	V1 (<i>n</i> =20)	0.163	0.728**	0.838***	0.880***
	V1 (<i>n</i> =8)	0.443	0.976**	0.952**	0.943*
	V2 (<i>n</i> =8)	0.072	0.738*	0.643	0.690
	V3 (<i>n</i> =8)	0.378	0.929**	0.857*	0.714*
Histamine	V1 (<i>n</i> =20)		0.047	0.106	0.031
	V1 (<i>n</i> =8)		0.419	0.347	0.116
	V2 (<i>n</i> =8)		0.311	0.168	0.127
	V3 (<i>n</i> =8)		0.090	0.468	0.090
Bradykinin	V1 (<i>n</i> =20)			0.877***	0.759**
	V1 (<i>n</i> =8)			0.976**	0.943*
	V2 (<i>n</i> =8)			0.905**	0.357
	V3 (<i>n</i> =8)			0.762*	0.810*
TAME-esterase	V1 (<i>n</i> =20)				0.759**
	V1 (<i>n</i> =8)				1.000*
	V2 (<i>n</i> =8)				0.405
	V3 (<i>n</i> =8)				0.643

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

the same response can be evoked with a lower dose of antigen at a repeated challenge (11). Priming can also be defined as increasing concentrations of biomarkers in nasal lavage fluid after repeated antigen challenge with the same amount of antigen. A second antigen challenge 24 h after the first challenge was followed by an increased concentration of TAME-esterase (1), but a second antigen challenge 48 h after the first challenge did not change the response in terms of amount of secretion, nasal airway resistance, and amount of histamine (25). In this study, we avoided priming by performing the antigen challenges with 1-week intervals and on alternate sides. The individual dose of antigen that caused a positive reaction did not increase from visit to visit, nor did the concentrations of biomarkers.

The use of a decongestant has been justified because allergen-induced congestion has been said to hamper subsequent lavaging (28). We believe for several reasons that decongestants should not be used. With the modified nasal lavage, we suggest that decongestants are unnecessary, because we had no difficulty in performing the lavage after the antigen challenge, and we found a high degree of reproducibility of biomarkers in nasal lavage fluid. Although they decrease nasal blood flow (5), decongestants do not cause any change in the concentrations of albumin and TAME-esterase in nasal lavage fluid (27, 28), but unpredictable effects on other mediators cannot be excluded. Actually, the concentrations of histamine in nasal lavage fluid increased after the administration of decongestants in allergic subjects in one study (12) but not in another (28). Furthermore, the use of decongestants before antigen challenge hampers the interpretation of nasal airway resistance. Actually, in some studies, decongestants have been omitted without any reported difficulties in performing lavage (10, 16).

The modified nasal lavage technique allows the interpretation of the involvement of neurogenic mechanisms because the nasal cavities are lavaged separately after unilateral antigen challenge. However, in this study, the antigen challenge did not cause any increase of biomarkers contralaterally, suggesting that central neurogenic pathways are not involved in the release of the biomarkers determined. Thus, it seems less likely that nerve reflexes activating ganglia or the central nervous system are involved in the leakage of plasma in the nasal mucosa after antigen challenge, as the concentration of albumin in the nasal lavage fluid increased ipsilaterally but not contralaterally. Likewise, nasal challenge with capsaicin, which activates sensory nerves, did not cause any increase of the concentration of albumin in nasal lavage (ipsi- and contralaterally) (3, 33). However, these findings do not exclude the existence of local reflexes.

The modified lavage technique differs from previously used techniques also in the position of the patient's head and the time allowed for contact with the nasal mucosa. The nasal lavage has been performed with the head bent backward during closure of the pharynx by the soft palate (28), or with the head in an upright position without bending (13). In the latter study, a small, flexible rubber catheter connected to a vacuum was inserted 4 cm into the nose. By bending the head forward in the modified lavage method, we prevented fluid from reaching the pharynx. Unlike the lavage technique in which the patient's head is bent backward, the modified technique is comfortable for the patient and causes no coughing or irritation of the throat. As we aimed to lavage the whole nasal cavity, a fluid volume of 10 ml was chosen. This volume fills the nasal cavity without reaching the epipharynx and risking bacterial contamination and mediator release (2, 12). By using an olive applied to the outer part of the nostril, we avoided contact with the nasal mucosa. Because we were able to repeat the lavage five times with the same fluid, we regard this technique as adequate to achieve equilibration of biomarkers between the nasal mucosa and the lavage fluid. Equilibration may also be achieved by the "nasal pool" device (16). The nasal pool device, which is no longer available commercially, is equipped with a fixed nozzle that cannot be adapted to the individual – in contrast to the present technique.

In general, lavage methods provide relative concentrations of biomarkers in nasal fluid. Thus, any increase of nasal fluid secretion will imply a dilution of the biomarkers measured and a concentration of them if lavage fluid is absorbed. To overcome the problem of dilution and concentration, adding a tracer to the lavage fluid has been tried, but this method has not been commonly used (6, 21, 22). We have tried to use lithium as a tracer in the lavage fluid, but the sensitivity of the analysis method for lithium was not sufficient for the large volume of the lavage fluid. Alternatively, to obtain absolute concentrations of biomarkers, one can collect nasal fluid secretion by sucking (17), blowing (35), or using filter papers (29), when a more restricted area is covered. If an inert tracer could be found, the value of the nasal lavage technique might be further improved.

We believe that only one or two of the measurements of albumin, bradykinin, and TAME-esterase are necessary to monitor the response to antigen challenge, because the concentrations of these biomarkers in the nasal lavages correlated significantly. The signal-to-noise ratio was good for albumin, bradykinin, and TAME-esterase but poorer for LTC₄, which increased least. Increased concentration of TAME-esterase after antigen challenge mainly reflects increased plasma leakage and activation of

mast cells (4, 28). Bradykinin is a plasma protein with strong inflammatory properties, and it is activated by mast-cell degranulation (31). LTC₄ also reflects the activation of mast cells. In this study, LTC₄ increased in nasal lavage fluid immediately after the antigen challenge as in some previous observations (15, 36), but in contrast to another observation (7). However, we did not find any increase of LTC₄ in the late phase, in contrast to a previous study (38). Furthermore, unlike the results in our study, Georgitis et al. (15) found an association of the antigen dose and the concentration of LTC₄. These differences might be due to differences in the selection of patients, the lavage techniques, and the biomarker assays. Moreover, the antigen challenge might be important because several authors (e.g., Georgitis et al. (15)) have performed the challenge to a maximum dose, whereas we stopped at the dose of a convincingly positive reaction.

Histamine does not seem to be a suitable biomarker to monitor the reactions after antigen challenge. We did not find any increase of histamine after challenge, in agreement with some previous reports (22, 23, 26), but not with another (28). These discrepancies might be due to different techniques for the nasal lavage. Naclerio et al. (28) have shown that an increase of histamine is detected only if the nose is washed several times before the antigen challenge. The prewashings imply that a rather high baseline value of histamine is reduced, allowing a rather small but significant increase of histamine to be detected. However, although the nasal cavities were prewashed and decongested in the same manner as in the study of Naclerio et al. (28), several authors (20, 38) have failed to demonstrate any increase of histamine. On the contrary, a drop of histamine was found in this and in other studies (20, 30). The fact that the concentration of histamine did not increase in the present study conflicts with the fact that the concentrations of albumin, bradykinin, and TAME-esterase increased although no prewashings were performed. To monitor a response to antigen challenge, we do not find it necessary to perform any prewashings unless histamine is to be measured. In our study, we cannot rule out the possibility that some histamine was released, but in an amount too small to be detected in relation to the baseline concentration. A regional increase in the release of mediators might well be masked by the large volume of lavage fluid. With (30) or without prewashings (19), tryptase might be a more reliable marker for monitoring mast cell degranulation than histamine.

The nasal challenge caused nasal congestion in most subjects immediately after the antigen challenge, as confirmed by the NPIFR, which decreased in all subjects. Because the NPIFR was not decreased 6 h after the challenge, in agreement with

Lozewicz et al. (24), we found no support for a late-phase reaction in the present study. We cannot exclude the possibility that a minor increase of NPIFR occurred on the challenged side, an effect masked by the measurement of both nasal sides together. A missing late phase in the present study is further supported by the fact that no subjects reported any symptoms and the fact that the concentrations of the biomarkers were not elevated ipsilaterally 6 h after antigen challenge. In contrast, a second peak of the concentrations of histamine and TAME-esterase has been demonstrated 3–10 h after antigen challenge (24, 28). We chose to perform the late lavage 6 h after antigen challenge because this is the most likely time point at which late reactions occur (14). We cannot rule out the possibility that a late-phase reaction might have occurred later than at 6 h when we lavaged the subjects. Moreover, a late-phase reaction does not occur in all subjects. The concept of a late-phase reaction is controversial and is reported in 0–63% of cases after antigen challenge (14). It seems that the late-phase reaction depends on the strength of the allergen challenge. The strength of the challenge in our study might have been too weak to cause a late-phase reaction.

In summary, we have found that modified nasal lavage is an improved and simplified technique to determine biomarkers in the human nasal mucosa. It is reliable because it implies a high degree of reproducibility in terms of concentrations of biomarkers. Lavaging the nasal cavities separately offers a new means to evaluate the involvement of neurogenic pathways in the mediation of nasal symptoms and to study the mode of action of different drugs; e.g., direct and indirect effects of topical treatment. The modified nasal lavage technique offers a more accurate evaluation of the pathophysiology of the response of the nasal mucosa and of clinical trials in allergic and nonallergic rhinitis.

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