

Cytological changes in nasal secretions accompanying delayed nasal response to allergen challenge

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ABSTRACT

Background: Patients with allergic rhinitis when challenged with allergen develop various types of nasal response, such as an immediate nasal response (INR), late nasal response (LNR), dual late nasal response (DLNR), or delayed nasal response (DYNR), probably because of different hypersensitivity mechanisms. This study was designed to investigate the cytological changes in the nasal secretions (NSs) during the DYNR, beginning 24–32 hours, reaching maximum at 32–36 hours, and resolving within 56 hours after the nasal provocation tests (NPTs) with allergens.

Methods: In 45 allergic rhinitis patients developing 45 positive DYNRs ($p < 0.01$), the NPTs and the phosphate-buffered saline (PBS) controls were repeated and supplemented with cytological examination of the NSs and determination of interferon (IFN) γ and IL-4 in nasal lavage fluid (NLF).

Results: The repeated DYNR ($p < 0.001$) was accompanied by significant changes ($p < 0.05$) in the counts of neutrophils, monocytes, lymphocytes, epithelial and goblet cells, and, to a limited extent, of plasma cells and platelets in the NSs and increased concentrations of IFN- γ but not of IL-4, in NLF between 24 and 48 hours after the NPT. No significant cytological changes were found in NSs during the PBS controls ($p > 0.1$).

Conclusion: The DYNR is associated with cytological profiles in the NS different from those observed during the INR or LNR. The significant count changes of neutrophils, monocytes, lymphocytes, epithelial and goblet cells in NSs, and increased IFN- γ but not of IL-4 concentrations in the NLF, suggest a possible involvement of the cell-mediated hypersensitivity in the DYNR. These results also emphasize the diagnostic value of NPTs combined with cytological examination of the NSs in patients with nasal allergy.

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Allergic rhinitis, a very common disorder affecting a large population segment, having also an important impact on the other related organs, such as lower airways, paranasal sinuses, middle ear, and eyes, is a multifactorial disorder due to the complex immunologic mechanism(s).^{1–6} The allergic component involved in allergic rhinitis has classically been attributed to the IgE-mediated hypersensitivity on involvement of mast cells (basophils), eosinophils, and Th2 lymphocytes.^{1–4} However, evidence has already been gathered for possible involvement of other hypersensitivity mechanisms in patients with allergic rhinitis.^{2,4,7–10} Patients with nasal allergy may develop various nasal response (NR) types to nasal provocation with allergen (nasal provocation test [NPT]).^{2,4–7,11–21} The immediate/early NR (INR), late NR (LNR), and dual LNR (DLNR; a combination of an immediate and a late) response types have been already extensively investigated by several authors and also analyzed in our previous studies.^{1–6,11,12,15–21} Additionally, an unusual NR type appearing later than 24 hours after the allergen challenge had been recorded in ~7% of the allergic rhinitis patients at our department.^{4,11} This response, beginning within 26–32 hours, reaching its maximum within 32–36 hours and resolving within 56 hours after the NPTs, has been designated by us as the delayed NR (DYNR). The DYNR differs from the INR, LNR, and DLNR, not only in clinical course, but also in clinical features, association with various diagnostic parameters and pharmacologic modulation, as it has already been reported in our previous articles.^{4–6,11} The clinical importance of the DYNR deals with the fact that this NR type appears later than 24 hours after the allergen exposure, which is the time period when most patients as well as doctors would not expect such late response.

The nasal secretions (NSs) may be considered to be an indicator for the pathological processes in the nasal mucosa and therefore are an important diagnostic parameter of the nasal allergy.^{2–4,12,13,14–34} However, there is a dearth of information concerning the appearance and changes in the counts of individual cell types in NSs accompanying

particular NR types to allergen challenge recorded by rhinomanometry, especially those associated with the DYNR.^{4,11–13,20,21}

The purpose of this study, being a continuation of our earlier work,^{4,11–13,35–38} was (1) to investigate the changes in the counts of particular cell types in the NSs during the DYNR and (2) to evaluate the significance of such changes for the diagnostic approach as well as for the clarification of the immunologic mechanism(s) underlying the clinical DYNR.

MATERIALS AND METHODS

Patients

Forty-five patients with allergic rhinitis, referred to our Department of Allergology and Immunology (Institute of Medical Sciences “De Klokenberg”, Breda, the Netherlands) for diagnostic analysis and developing a DYNR to NPTs with allergens (NPTs) during the routine diagnostic procedure, volunteered to participate in this study. These patients, 17–51 years of age (Table 1), showed a positive disease history to inhalant allergens and insufficient compliance to various H₁-receptor antagonists. None of these patients suffered from infection or systemic disease or had previously been treated with nasal glucocorticosteroid or immunotherapy.

The patients were examined by a routine diagnostic procedure including basic laboratory tests, roentgenogram of paranasal sinuses, nasoscopy, skin tests with inhalant allergens, serum immunoglobulin determination, nasal histamine threshold, control nasal challenge with phosphate-buffered saline (PBS; $n = 45$), and NPTs with inhalant allergens ($n = 83$) chosen with respect to the disease history and skin tests (Table 2).

The routine diagnostic procedure revealed positive skin tests with various inhalant allergens (100%), hyperemic/dermatic nasal mucosa (100%), increased eosinophil, and, especially, neutrophil counts in NSs (93%). In these 45 patients showing positive DYNR during the routine procedure (Fig. 1), the NPTs with the same allergens and the PBS controls were repeated 2–4 weeks later, according to a double-blind, crossover schedule, and supplemented with the collection of the NSs. A 5-day interval was always inserted between the end of the preceding and the beginning of the following test to prevent the carryover effect. The patients were investigated in a period without acute nasal complaints, outside the allergen-relevant season, and during a short hospitalization.

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The author has no conflicts of interest to declare pertaining to this article

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Table 1 Characteristics of the patients

	DYNR (n = 45)	Control Subjects (n = 32)		
		INR (n = 10)	LNR (n = 8)	Healthy (n = 14)
Age (yr)	27 ± 6	31 ± 7	29 ± 4	33 ± 5
Sex (M/F)	21/24	5/5	3/5	5/9
Disease history (yr)	4.2 ± 1.3	3.5 ± 1.0	3.1 ± 0.6	0
Blood leukocyte count ($\times 10^9/L$)*	9.1 ± 1.8	8.3 ± 1.1	7.7 ± 0.9	7.2 ± 1.5
Blood eosinophil count ($\times 10^6/L$)#	289 ± 26	315 ± 21	323 ± 18	261 ± 13
Increased total IgE in the serum§	0	0	0	0
Positive specific IgE in the serum¶	3	6	4	0
Nasal complaints	0			
Obstruction	45	9	8	
Hypersecretion	37	10	7	
Sneezing	0	10	6	
Itching	0	10	3	
Nasoscopy				
Mucosal edema	+ +	0	±	0
Skin-prick tests response				
Immediate	8	7	4	1
Positive skin response				
Immediate type	14	10	4	0
Late type	9	0	4	0
Delayed type	22	0	0	0
Nasal histamine threshold**				
Decreased (<8.0 mg/mL)	17	6	4	1
Normal (>8.0 mg/mL)	28	4	4	13
Nasal provocation tests				
Positive	45	10	8	0
Negative	38	15	11	0
NS				
IFN- γ pg/mL##	<1.0	<1.0	<1.0	<1.0
IL-4 pg/mL§§	<0.6	1.4 ± 0.3	1.5 ± 0.2	<0.6

*Normal value $4.0\text{--}10.0 \times 10^9/L$.#Normal value $<300 \times 10^6/L$.§Normal value <500 kU/L.¶Normal value <0.70 kU/L.

||Positive skin response to the relevant allergen (allergen producing positive nasal and ocular or nasal response only).

**Normal value = >8.0 mg/mL.

##Minimal detection limit, 1.0 pg/mL.

§§Minimal detection limit, 0.6 pg/mL.

DYNR = delayed nasal response; INR = immediate nasal response; LNR = late nasal response; NS = nasal secretion; IFN = interferon.

The long-acting H_1 -receptor antagonists were withdrawn at 4 weeks, and other treatments it was withdrawn 48 hours before each of the NPTs. The study protocol was approved by the local ethical committee (Intuition Review Board-MC De Klokenberg) and written informed consent was obtained from all study participants.

Allergens

Dialyzed and lyophilized allergen extracts (Allergopharma, Reinbek, Germany) diluted in phosphate-buffered saline (PBS) were used for skin tests in concentrations of 100–500 BU/mL and for NPTs in concentrations of 1000–3000 BU/mL (Table 2), as recommended by the manufacturer.

Skin Tests

Skin-prick tests with allergenic extracts in concentrations of 500 BU/mL were evaluated after 20 minutes, followed by intracutaneous tests in dilutions of 1:5 and, if necessary, in our standard concentrations of 100–300 BU/mL (Table 2). The intracutaneous tests were evaluated 20 minutes and 6, 12, 24, 36, 48, 56, 72, and 96 hours after

the intradermal injection. A skin “wheal and flare” (>7.0 mm in diameter) occurring after 20 minutes was qualified as a positive immediate skin response, the skin infiltration appearing between 6 and 12 hours as a late skin response, and the skin induration recorded later than 48 hours as a delayed skin response (Table 1).^{4–6,11–13} PBS was used as a negative control and histamine diphosphate (0.1 and 0.01 mg/mL) as a positive control.

Nasal Provocation Tests

Nasal challenges with allergens were performed using rhinomanometry, described in our previous studies.^{4–6,11–13,39} The nasal obstruction due to the mucosal edema was evaluated by means of nasopharynx–nostril pressure gradient (NPG) parameters, which are the pressure differences (ΔP) between the nasopharyngeal cavity and the outside air, expressed in cmH_2O . NPTs were performed using the following schedule: (1) baseline values recorded at 0, 5, and 10 minutes; (2) PBS control values recorded at 0, 5, and 10 minutes after a 3-minute application of PBS to the nasal mucosa of the nonintubated nasal cavity by means of a saturated wad of cotton wool on a nasal probe inserted under the middle turbinate;

Table 2 Survey of the allergens used for nasal challenge

Allergen	Concentration BU/mL	NR		Control Subjects	
		DYNR (n = 45)	NNR (n = 38)	INR (n = 10)	LNR (n = 8)
<i>Dermatophagoides</i>					
<i>pteronyssinus</i>	100/1000	5 (1/4)	4 (0/4)	2 (1/1)	1 (0/1)
<i>farinae</i>	100/1000	1 (0/1)	2 (0/2)		
Animal danders					
Dog	300/3000	2 (0/2)	1 (0/1)	1 (0/1)	
Cat	200/2000	3 (1/2)	2 (0/2)	1 (1/0)	1 (0/1)
Guinea pig	200/2000	1 (0/1)	1 (0/1)		
Hamster	200/2000	2 (1/1)	2 (0/2)	1 (0/1)	1 (1/0)
Rabbit	300/3000	1 (0/1)			
Horse	100/1000	2 (0/2)	2 (0/2)		
Cow	200/2000	1 (0/1)			1 (0/1)
Sheep	200/2000	1 (0/1)			
Feathers					
Parrot	300/3000	1 (0/1)	1 (0/1)		
Parakeet	300/3000	1 (1/0)			
Canary	300/3000	2 (0/2)	3 (0/3)	1 (0/1)	
Pigeon	200/2000	1 (1/0)	2 (0/2)		1 (0/1)
<i>Aspergillus fumigatus</i>	100/1000	2 (1/1)	1 (0/1)		
Pollen					
Grass mix I	100/1000	4 (1/3)	2 (0/2)	2 (1/1)	1 (0/1)
Grass mix II	100/1000	1 (0/1)	3 (0/3)		
Flower mix	500/5000	1 (0/1)	3 (0/3)		
Tree mix	300/3000	2 (0/2)	1 (0/1)		1 (0/1)
Weed mix	100/1000	1 (0/1)	2 (0/2)		
Timothy	100/1000	2 (0/2)			
Buckhorn plantain	100/1000	1 (0/1)			
Birch	100/1000	3 (1/2)	2 (0/2)	1 (0/1)	1 (1/0)
Poplar	100/1000	1 (0/1)	1 (0/1)	1 (0/1)	
Ragweed short	100/1000	1 (0/1)	2 (0/2)		
Ragweed giant	100/1000	2 (0/2)	1 (0/1)		

Grass pollen mix I, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratensis* and, *Poa pratensis*; *grass pollen mix II*, *Festuca pratensis*, *Holcus lanatus*, *Agrostis alba*, and *Anthoxanthum odoratum*; *flower pollen mix*, *Dahlia variabilis*, *Solidago virgaurea*, *Primula variabilis*, and *Forsythia suspensa*; *tree pollen mix*, *Betula pendula*, *Corylus avellana*, *Juniperus communis*, and *Salix alba*; *weed pollen mix*, *Artemisia vulgaris*, *Plantago lanceolata*, *Rumex acetosa*, and *Taraxacum officinale*.

BU/mL = biological units per mL; DYNR = delayed nasal response; INR = immediate nasal response; LNR = late nasal response; NR = nasal response.

(3) postchallenge values recorded after a 3-minute challenge with allergen, performed in the same manner as the challenge with PBS, at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes, and then every hour up to the 12th hour, and every second hour between the 24th–38th and 48th–56th (60th) hour.^{4,6,11–13}

The NR was considered to be positive when the postchallenge NPG values increased by at least 2.0 cmH₂O (mean $\pm$ SE, 1.2 $\pm$ 0.3) with respect to the baseline values, recorded at least at three consecutive time intervals.^{4,5,8,19–24} The NPG changes recorded within 120 minutes after the allergen challenge were considered to be an INR, those recorded within 4–12 hours were considered to be a LNR, and the changes measured later than 24 hours were considered to be a DYNR.^{4,11–13}

Control Challenge with PBS

The control nasal challenge with PBS was performed following the same schedule as that used for the allergen challenge.

Collection and Processing of the NSs

The NS specimens have been collected from the nonintubated nasal cavity by having the patient blow onto a polyethylene sheet (a) before the allergen challenge and (b) after the challenge every 2nd hour between the 0 and 12th hour, 24th and 38th hour, and 48th and 56th hour. The NS specimens were divided into three portions and processed

within 10 minutes. The first air-dried specimen series was fixed additionally by polyethylene glycol and stained with Hansen's method. The second air-dried specimen series was stained by the May–Grünwald–Giemsa method. The third series of methanol-fixed specimens was stained with the toluidine blue method.^{4,12,13} After dehydration with methyl alcohol the specimens were mounted in Canada balsam and scanned microscopically in a double-blinded manner.

Two counting techniques were used simultaneously: (a) the counting of absolute numbers of the particular cell types per microscopic field at 250 $\times$ magnification and the mean values per microscopic field were calculated from 25 fields spread over the whole slide; (b) The differential count expressed as a percentage of 100 cells, counted in 25 microscopic fields (magnification, $\times$ 250). Doubtful cells were always reexamined under oil immersion at 1200 $\times$ magnifications (12 $\times$ 100). The particular cell types were distinguished according to the cytological criteria described in our previous articles.^{4,12,13} The statistically significant magnitude of changes in the cell counts in the NSs between two consecutive count degrees (mean $\pm$ SEM) was as follows: eosinophils 4 (4.17 $\pm$ 0.31), neutrophils 5 (4.93 $\pm$ 0.62), basophils 0.6 (0.58 $\pm$ 0.27), mast cells 0.8 (0.75 $\pm$ 0.23), lymphocytes 2 (2.05 $\pm$ 0.24), monocytes 1 (0.83 $\pm$ 0.39), plasma cells 0.2 (0.15 $\pm$ 0.07), epithelial cells 5 (5.11 $\pm$ 0.33), goblet cells 0.6 (0.57 $\pm$ 0.19), and thrombocytes 0.1 (0.08 $\pm$ 0.04).^{4,12,13}

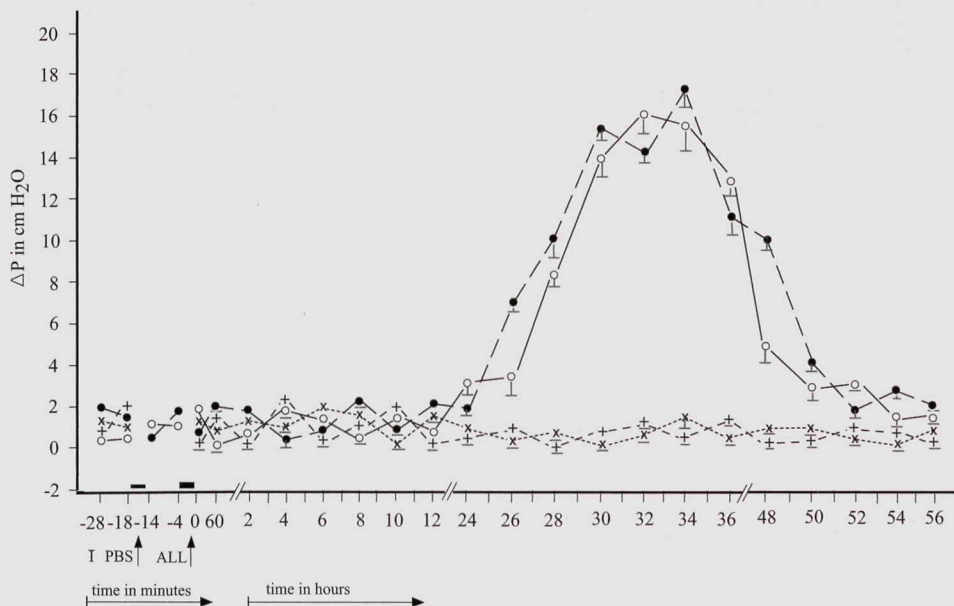


Figure 1. The delayed nasal response (DYNR; $n = 45$) to allergen challenge and PBS control challenge ($n = 45$). The mean rhinomanometric values (NPG) recorded during the initial DYNR (●); repeated DYNR (○); initial PBS (×); and repeated PBS (+). DYNR, delayed nasal response; NPG, nostril pressure gradient; I, initial (baseline) values; PBS, phosphate-buffered saline; ALL, allergen challenge; bars = mean $\pm$ SEM.

The nasal lavage (NL) with 5 mL of PBS was performed by means of a syringe in the nonintubated nasal cavity before and 11 times after the allergen challenge. The recovered NL fluid (NLF) was centrifuged at $1000 \times g$ at 4°C for 5 minutes and stored at -80°C . The cytokines were measured in the supernatants by means of immunoassay ELISA-kits (Bender MedSystem, Vienna, Austria), according to the manufacturer's instructions. The detectable limits were 1.0 pg/mL for interferon (IFN) γ and 0.6 pg/mL for IL-4. The intra-assay coefficients of variations of these assays were $<9\%$ and the interassay coefficients of variations were $<10\%$.

Control Group ($n = 32$)

There were 18 allergic rhinitis patients developing 10 INRs and 8 LNRs to NPTs and 14 healthy adults who volunteered to participate as controls. In the rhinitis patients the NPTs with particular allergens were repeated and in the healthy subjects the NPTs with PBS were repeated and supplemented with cytological examination of NSs up to 10 hours after the challenge.

Statistical Analysis

The NRs and the PBS controls were evaluated by Wilcoxon matched-pair signed-rank test, comparing the postchallenge NPG values with the mean prechallenge (baseline) NPG values. The mean NPG values were compared with corresponding mean PBS values at each of the time points and analyzed by Mann-Whitney U test. The postchallenge cell counts were compared with the baseline counts and analyzed by Wilcoxon matched-pair signed-rank test. The mean postchallenge cell counts during the repeated NPTs were compared with the corresponding counts during the PBS controls and statistically evaluated by the Mann-Whitney U test. A value of $p < 0.05$ was always considered to be statistically significant.

RESULTS

Nasal Responses

The 45 patients developed 45 DYNRs and 38 negative NRs to nasal allergen challenge (Tables 1 and 2). None of these patients demonstrated other NR types. The initial as well as the repeated DYNRs (Fig. 1) were statistically significant in comparing both the postchallenge with the baseline NPG values ($p < 0.01$ and $p <$

0.001 , respectively) and with the PBS control values ($p < 0.01$ and $p < 0.001$, respectively; Fig. 1). No significant differences were found in the appearance of DYNRs with respect to the particular allergens ($p > 0.2$) or between the initial and the repeated DYNRs ($p > 0.1$; Fig. 1). The 38 negative NRs were statistically not significant ($p > 0.05$). The 45 PBS control challenges were all negative ($p > 0.1$). The DYNR was accompanied by nasal complaints in 100%, governed by pronounced nasal obstruction (Table 3), increased blood leukocyte counts in 82%, decreased nasal histamine threshold in 46%, significant changes in IFN- γ in NSs in 96%, and by other diagnostic parameters to various degrees (Table 1). The DYNR was associated with positive intracutaneous skin tests in 100%, distributed as follows: immediate skin response in 31%, late skin response in 20%, and delayed skin response in 49% (Table 1).

Cytological Changes in NS during the DYNR

Before the allergen challenge only sporadic cells were found in the NSs (Table 4). The repeated DYNRs were associated with significant changes in the counts ($p < 0.05$) of neutrophils, lymphocytes, monocytes, epithelial cells, goblet cells, and thrombocytes (Table 4; Fig. 2).

The cellular changes, an increase followed by a decrease in the cell counts, appeared between 24 and 48 hours after the challenge. The pattern of the cellular changes ran almost parallel to the clinical course of the DYNR (Fig. 2). In cells appearing in the NSs during the DYNR the following changes were observed: (1) neutrophils—degranulation or disappearance of their cytoplasmic granules, intracellular vacuolization, diminished stain uptake, and irregular cellular membrane in 96%; (2) lymphocytes—cytoplasmic vacuolization, diminished uptake of the stain, and cellular membrane fissures in 91%; (3) monocytes—intracellular vacuolization and diminished cytoplasmic homogeneity; (4) epithelial cells—cellular deformation, sporadic cell disruption, and diminished stain uptake in 100%; (5) goblet cells—wrinkled cellular membrane and absence of secretion in 100%; (6) platelets—cellular deformation and little cytoplasmic substance. The eosinophils, basophils, plasma cells, and sporadic mast cells did not display any significant changes. The DYAR was accompanied by increased concentration of IFN- γ , but not of IL-4, in NLF between 24 and 36 hours after the challenge (Table 4).

Table 3 Survey of the nasal symptoms accompanying the positive DYNR

Patients (n = 45)	Before the Challenge	After the Challenge (hr)						
		1	12	24	36	48	56	72
Nasal complaints								
Obstruction	±	±	±	++*	++++**	++++**	++**	±
Hypersecretion	0	±	0	+	++*	+±*	±	0
Sneezing	0	0	0	0	+	±	0	0
Itching	0	0	0	0	0	0	0	0
Nasoscopy								
Mucosal edema	±	+	+±	+++*	++++**	++++**	++*	±
Small solitary mucosal hemorrhages (on the middle and inferior turbinate)	0	0	0	±	++*	+	0	0

Statistical significance: * $p < 0.05$; ** $p < 0.01$.

Evaluation criteria: 0 = absent; ± = very mild (present to a slight degree or intermittent); + = mild; ++ = moderate; +++ = pronounced (moderately severe); ++++ = severe.

DYNR = delayed nasal response.

Cytological Changes in NSs during the PBS

Controls

The particular cell types appeared in NSs during the PBS controls irregularly without any significant changes in their counts (Table 4).

Control Patients

The INRs were associated with a significant increase in the count of mast cells, eosinophils, neutrophils, and goblet cells and a decrease in the count of basophils in the NSs. Most of the mast cells and eosinophils showed degranulation of their cytoplasmic granules. The LNRs were accompanied by a significant increase in the count of eosinophils, basophils, neutrophils, epithelial, and goblet cells in the NSs. Most of the basophils and eosinophils displayed degranulation of their cytoplasmic granules.

DISCUSSION

The particular types of the NR to allergen challenge, INR, LNR, and dual DLNR (a combination of an immediate and a late response) have already been studied from various clinical, immunologic, and pharmacologic points of view.^{1-7,9-21}

The INR, appearing within 10 minutes and resolving within 120 minutes after the allergen challenge, is clinically characterized by nasal obstruction due to the edema of the nasal mucosa, sneezing, itching, and nasal hypersecretion. The INR is caused by the immediate hypersensitivity mechanism on involvement of the IgE antibody, mucosal mast cells, eosinophils, and Th₂ cells. The INR is associated with immediate skin response, increased specific IgE antibody in the serum as well as in the NSs, and appearance of a number of classic mediators and factors in the NSs, such as histamine, tryptase, eosinophil cationic protein (ECP), leukotriene C₄ (LTC₄), IL-4, and granulocyte macrophage colony-stimulating factor^{2,4,12,18,40,55}. The LNR, appearing at 3-4 hours and resolving within 10-12 hours after the allergen challenge, is clinically characterized predominantly by nasal obstruction and hypersecretion. Although the exact hypersensitivity mechanisms underlying the LNR is not yet fully clarified, either a modification of the immediate hypersensitivity or a late (immunocomplex) hypersensitivity mechanism is presumed to be involved in this NR type, on participation of the mast cells, eosinophils, neutrophils, Th₁ and Th₂ cells, and epithelial cells^{2,4,13,14,19,45,55}. The LNR is associated with late skin response; sometimes with increased concentrations of IgE antibodies in the serum; sometimes with increased specific IgE and/or total IgG and IgG subclasses in the NSs; and appearance of a number of mediators and factors such as histamine, ECP, eosinophil-derived neurotoxin, eosinophil peroxidase, LTC₄, LTE₄,

prostaglandin D₂ (PGD₂), IL-4, and IL-5 in the NSs. The DLNR, being a combination or simultaneous appearance of an INR and an LNR, displays a two-phase course and clinical features characteristic for both the response types. Although the immunologic mechanisms underlying the DLNR remain unclear, involvement of some modifications of the immediate (IgE-mediated) hypersensitivity, on participation of mast cells, basophils, eosinophils, neutrophils, and epithelial cells, is presumed. However, the results concerning the appearance and concentration changes of various mediators, cytokines, and other factors in the NSs accompanying the DLNR exhibit relatively high variation.^{1-4,9-14,35, 36,40-48}

In contrast, although the first evidence for the existence of the DYNR to allergen challenge (DYNR) was reported by us some years ago,¹¹ little data are available on this clinical phenomenon.⁴⁻⁶

Allergic reaction in the nasal mucosa is a dynamic process in which various types of cells residing in the mucosal membrane as well as the cells circulating in the local blood and lymphatic vessels participate in various stages of this process.^{3,4,9,10,12-22} This reaction is also an exfoliative process leading to the release and migration of various cell types, usually after their active involvement, into the NSs.^{4,7,12-20,22,23,25-34}

The NSs may therefore be considered not only as a means of conditioning the nasal mucosa, but also as a medium supplemental in the drainage and removal of the cells being expelled or migrating from the nasal mucosa after their active participation in the allergic reaction.^{4,7,12-20}

The numbers and condition of the eliminated/migrating cells in the NSs can also indicate the qualitative as well as the quantitative aspects of the present or just transient allergic reaction in the nasal mucosa.^{4,12,13} However, the current or already passing involvement of particular cell types in the allergic reaction located in the nasal mucosa can only be evaluated by comparing their counts and conditions before and repeatedly after a certain and well-defined event, which is a nasal challenge with a certain allergen.^{4,7,12-21,24} The cytologic examination of the NSs is an easy, noninvasive, and valuable technique for evaluation of the changes in the cells appearing in the NSs during a particular NR type to allergen challenge^{4,12-19,22-34}. However, this method is limited only to NSs and does not indicate fully the cellular events in the nasal mucosa, which can only be derived from a biopsy.^{4,9,10,20,21,49,50}

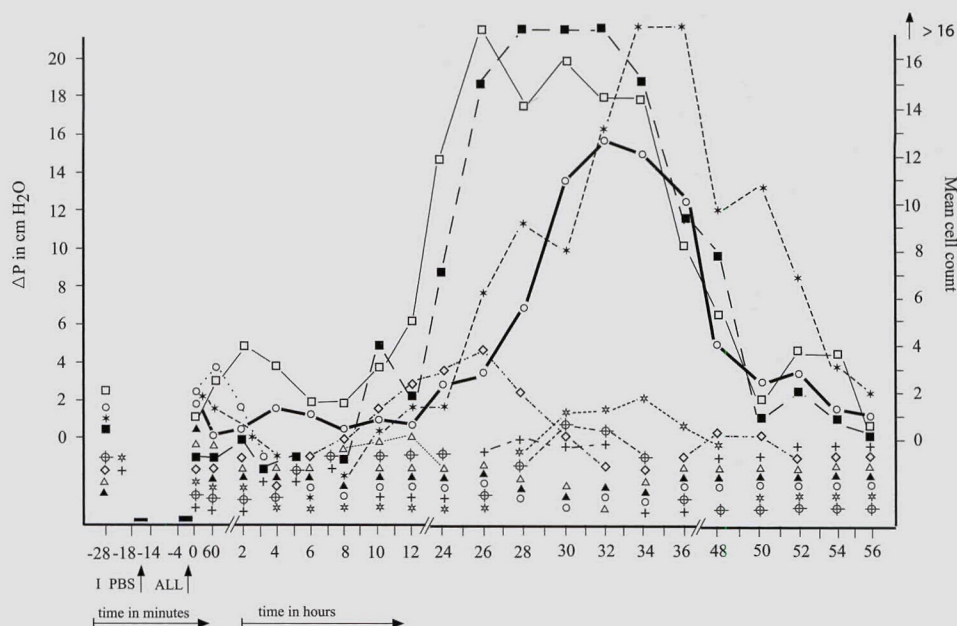
In most studies concerning the cytological examination in patients with allergic rhinitis, the cells released into the nasal cavity have been collected by means of NLF with PBS or other solutions.^{24,26,33,51} In our study the NS specimens were collected by blowing the nose onto a polyethylene sheet. In accordance with our previous studies^{4,12,13,31,32} as well as other investigators' findings,⁵² the NS collection by the

Table 4 Mean numbers of particular cell types in NSs during the positive DYNR and PBS control challenge ($n = 45$)

	Before the Challenge	After the Challenge (hr)																			
		0	1	2	4	6	8	10	12	24	26	28	30	32	34	36	48	50	52	54	56
Eosinophils																					
	DYNR	1.5	2	3	1	1	0	0	1	0	0	0	0	0	0	0	0	0	2	0	1
Neutrophils																					
	DYNR	2	1	3	4	3	1	1	3	5	12*	21*	14*	10*	12*	15*	9*	5	2	4	4
Mast cells																					
	DYNR	0.1	0.4	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0
Basophils																					
	DYNR	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0.2	0
Lymphocytes																					
	DYNR	0.5	0	0	0	0	0	0.1	0.4	0.6*	0	0	0	0	0	0	0	0	0	0	0
Monocytes																					
	DYNR	0	0	0.2	0	0	0	0.5	1*	2*	3*	4*	2*	0.4	0	0	0	0.2	0.1	0	0
Epithelial cells																					
	DYNR	0.8	1.5	1	0.5	0	0	0	1	2	2	6*	9*	8*	13*	27*	21*	10*	11*	7*	3
Goblet cells																					
	DYNR	0	0	0	0	0	0	0	0	0	0	0	0	0.6*	1.5*	1.8*	0.8*	0	0	0	0
Plasma cells																					
	DYNR	0	0	0	0	0	0	0	0	0	0	0	0	0.2*	0.2*	0	0	0	0	0	0
Thrombocytes																					
	DYNR	0	0	0	0	0	0	0	0	0	0	0	0.1*	0.2*	0.1*	0.1*	0	0	0	0	0
IFN- γ (pg/mL) +																					
	DYNR	<1.0	—	<1.0	1.0	—	1.5	—	1.8	—	3.9*	5.7*	5.8*	—	4.9*	3.5*	2.4	—	—	—	<1.0
IL-4 (pg/mL) +																					
	DYNR	<0.6	—	<0.6	<0.6	—	<0.6	—	<0.6	—	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6
Significance with respect to the baseline (before the challenge). *p < 0.05; + minimal detection limit, 1.0 pg/mL; + minimal detection limit, 0.6 pg/mL.																					
DYNR = delayed nasal response; PBS = phosphate-buffered saline; IFN = interferon; NSs = nasal secretions.																					

Significance with respect to the baseline (before the challenge): * $p < 0.05$; + minimal detection limit, 1.0 pg/mL; ++ minimal detection limit, 0.6 pg/mL.
DYNR = delayed nasal response; PBS = phosphate-buffered saline; IFN = interferon; NSs = nasal secretions.

Figure 2. The mean counts of particulate cell types in NSs accompanying the DYNR ($n = 45$). $\circ$, eosinophils; $\square$, neutrophils; $\blacktriangle$, mast cells; $\triangle$, basophils; $\blacksquare$, lymphocytes; $\diamond$, monocytes; $\star$, epithelial cells; $\star$, goblet cells; $\oplus$, plasma cells; $+$, thrombocytes; DYNR, delayed nasal response; NSs, nasal secretions; I, initial (baseline) values; PBS, phosphate-buffered saline; ALL, allergen challenge.



"blowing technique" would appear to be a suitable and perhaps even more reliable method than the NLF, because it collects the naturally produced NSs in a physiological manner. Moreover, this technique does not require any additional maneuvers, such as instillation of a solution into the nasal cavity leading to an additional dilution of the cell amounts.^{21,24,31,33} However, both of these semiquantitative techniques also have some disadvantages. The main disadvantage of the "blowing method" is the irregular volume of the collected NSs, and the important disadvantage of the NL is lower volume of the recovered lavage fluid than that initially instilled into the nasal cavity, resulting in the loss of some cells.^{4,21} The NLF is more suitable for collection of liquid factors, such as mediators or cytokines.^{4,7,19,21}

Various grading systems for evaluation of the cytological changes in NSs/NLF have been suggested,^{7,14-21,22-34,49} the most frequently applied system was proposed by Meltzer *et al.*²⁵ However, these systems use the same criteria for all cell types independent of their physiological appearance ratio. They are satisfactory for use in practice but not for research purposes. Therefore, we have developed a more accurate grading system (Pelikan's grading scale), derived from the statistically significant differences calculated for each of the cell types separately,^{4,12,13} which has also been applied in the present study.

In patients with nasal allergy, the influx of cells into the nasal cavity has most commonly been investigated by means of single cell counts performed in the NLF, revealing increased eosinophils, mast cells, basophils, epithelial cells, and sometimes neutrophils.^{15,19,22,23-27,29,30,32,34,53,54} Unfortunately, the articles presenting the repeated counts of individual cell types in the NSs, which means in a dynamic course, during a particular NR type to allergen challenge, are uncommon.^{4,7,12-14,17,18,34,51} Moreover, little data are available to illustrate the cytological changes in NSs during the DYNR.⁴ The cytological changes in NSs accompanying the DYNR, *viz.*, of neutrophils, monocytes, lymphocytes, thrombocytes, epithelial, and goblet cells, together with concentration changes of IFN- γ but not IL-4, differ substantially from the cytological profiles associated with INR and LNR. The INR has been reported to be associated with appearance of mast cells (sometimes basophils), eosinophils, and epithelial cells in NS/NLF.^{7,14,17-19,32,33,51} whereas the LNR was accompanied by count changes of basophils, eosinophils, neutrophils, and epithelial cells.^{7,14,17-19,51} Results of our previous studies of INR and LNR^{4,12,13} as well as cellular profiles found in the NSs of the control patients with

INR and LNR in this study are consistent with the other investigators' findings.

According to the aforementioned outcomes, our previous results,^{4,11-13} and other investigators' findings,^{7,9,10,14,18,19,22,34,50} immediate hypersensitivity may play the pivotal role in the INR,^{1-4,7,18,19,22,34} and in some modification(s) probably also in the LNR.^{2,4,7,9,10,14,18,19,34,50}

Although the exact mechanism(s) underlying the DYNR is not definitely clarified, a role of the cell-mediated hypersensitivity mechanism in that NR type could probably be assumed regarding the following facts and indicators: (1) the clinical course and features (appearance of DYNR later than 24 hours after the allergen exposure)^{4-6,11,39}; (2) the absence of allergen-specific IgE antibody in NSs and peripheral blood^{4-6,11,45,46}; (3) almost full absence of mast cells and eosinophils, whereas significant changes of lymphocytes, neutrophils, and monocytes in NSs during the DYNR^{4,37,38,45}; (4) histological findings in the nasal mucosa biopsy specimens during the DYAR showing reversible damage of the nasal mucosa and indicating a genuine inflammation process^{4,35,45}; (5) our preliminary, not yet fully published results, of increased myeloperoxidase, LTB $_4$, PGF $_{2\alpha}$, IFN- γ , IL-2, and transforming growth factor β , in NSs during the DYNR, whereas mediators and cytokines typical for immediate hypersensitivity mechanism(s) on involvement of mast cells, eosinophils, and Th $_2$ cells, such as histamine, tryptase, ECP, LTC $_4$, PGD $_2$, IL-4, IL-5, IL-12p40, and granulocyte macrophage colony-stimulating factor were absent^{4,40,45,55}; (6) our previous pharmacologic studies showed significant protection of DYNR by glucocorticosteroids but not by cromolyn, whereas the INR was significantly protected by cromolyn but not by glucocorticosteroids, and the LNR was protected by both the drugs.^{4,36,38,45-48} Nevertheless, additional studies will be necessary to clarify satisfactorily the DYNR and the underlying immunologic mechanism(s).

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