

Local IgE production and positive nasal provocation test in patients with persistent nonallergic rhinitis

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Background: Allergic rhinitis is an IgE-mediated inflammatory disease of the nasal mucosa, which is usually diagnosed by typical symptoms, positive skin tests, and/or serum specific IgE antibodies to allergens. Despite suggestive symptoms of allergic rhinitis, some patients have a negative diagnostic test for atopy.

Objective: To evaluate in the nose the inflammatory response, specific IgE to *Dermatophagoides pteronyssinus* (DP), and the response to a nasal allergen provocation test with DP (NAPT-DP), in patients with persistent nonallergic rhinitis (PNAR) compared with patients with persistent allergic rhinitis (PAR) and healthy controls.

Methods: Fifty patients with PNAR, 30 with PAR to DP, and 30 healthy controls were studied by determining the nasal leukocyte-lymphocyte phenotype by flow cytometry (CD16, CD8, CD4, CD33, CD3, and CD45), nasal eosinophil cationic protein (ECP), albumin, total and specific IgE to DP, and NAPT-DP.

Results: The PNAR patients showed a similar leukocyte-lymphocyte phenotype in nasal lavage to the PAR patients and was different to the healthy controls. Within the PNAR group, 54% showed a positive NAPT-DP, with 22% of these having nasal specific IgE to DP.

Conclusion: These data support the hypothesis that in persistent nonallergic rhinitis some patients may have local inflammation, nasal IgE production, and a positive response to a nasal allergen provocation test despite no evidence of systemic atopy. Further research is needed to evaluate the influence of other perennial allergens and/or immunologic mechanisms.

Clinical implications: The local production of IgE antibodies without systemic detection is a condition that should be considered in patients with PNAR. (*J Allergy Clin Immunol* 2007;119:899-905.)

Key words: Flow cytometry, lymphocyte subtypes, nasal allergen provocation test, nasal lavage, nasal specific IgE, persistent allergic rhinitis, persistent nonallergic rhinitis

Abbreviations used

CG:	Control group
DP:	<i>Dermatophagoides pteronyssinus</i>
ECP:	Eosinophil cationic protein
FHA:	Family history of atopy
IAR:	Intermittent allergic rhinitis
IgE-DP:	Specific IgE to DP
IR:	Idiopathic rhinitis
NAPT:	Nasal allergen provocation test
PAR:	Persistent allergic rhinitis
PNAR:	Persistent nonallergic rhinitis
SPT:	Skin prick test
TSS:	Total symptom score
VAS:	Visual analog scale

Persistent rhinitis is a highly prevalent disease of the nasal mucosa, which affects up to 20% of the general population.¹ It can be induced by different mechanisms, and several etiological agents can be involved.² Allergic rhinitis is common, affecting 50% of the patients with persistent rhinitis³ and 1-40% of the general population.¹⁻⁶ This condition is an IgE-mediated inflammatory disease of the nasal mucosa, characterized by obstruction, rhinorrhea, sneezing, itching, and/or postnasal drip; ocular symptoms are also frequent. The diagnosis is based on clinical manifestations and a positive skin prick test (SPT) and/or serum specific IgE antibodies to airborne allergens.^{1,7-10} In contrast, idiopathic rhinitis (IR) is another form of persistent rhinitis with an unknown pathogenesis that is diagnosed by exclusion after using conventional diagnostic methods, including those mentioned above.^{1,7,8}

Idiopathic rhinitis is difficult to define and may be induced by different mechanisms.⁸ Patients with IR are considered nonallergic because they have no evidence of atopy (negative skin prick test, absence of specific IgE to aeroallergens, and normal IgE levels in serum) despite many of them having the clinical ARIA criteria¹ of persistent allergic rhinitis (PAR). Symptoms are present more than 4 days a week and for more than 4 weeks a year. This group is also designated as having persistent nonallergic rhinitis (PNAR).¹¹ Evidence of local IgE synthesis exists in the nasal mucosa in rhinitis patients,¹²⁻¹⁶ and the concept that some IR might be a form of localized allergy in the absence of atopy has been proposed.^{15,17} The aim of

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Disclosure of potential conflict of interest: All of the authors have declared that they have no conflict of interest.

Received for publication October 5, 2006; revised January 3, 2007; accepted for publication January 9, 2007.

Available online March 10, 2007.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2007.01.006

this work was to study and characterize the nasal inflammatory response, the presence of nasal specific IgE, and the response to a nasal allergen provocation test (NAPT) in patients with PNAR. To test this process, after a classic allergologic evaluation, the following determinations were made in patients with PAR, PNAR, and a control group of healthy nonatopic subjects (CG): leukocyte-lymphocyte phenotype by flow cytometry with monoclonal antibodies (CD16, CD8, CD4, CD33, CD3, and CD45) in nasal fluid, nasal levels of eosinophil cationic protein (ECP) and albumin, nasal specific IgE to *Dermatophagoides pteronyssinus* (DP), and NAPT-DP.

The results obtained in this study showed a nasal mucosal inflammation with a similar leukocyte-lymphocyte phenotype compared with persistent allergic rhinitis patients and the presence of nasal specific IgE and positive NAPT-DP in patients with PNAR that substantiate the hypothesis of a new form of localized nasal allergy in the absence of atopy.

METHODS

Study subjects

The study included a total of 110 subjects observed in our clinic divided into 3 groups: 50 subjects with PNAR, 30 with PAR, and 30 healthy nonatopic subjects as a CG, recruited consecutively over 9 months. The study was approved by the ethical committee of our institution.

Subjects had to fulfill the following requirements:

1. General inclusion criteria for the PNAR and PAR groups: subjects aged 18-70 years, with no evidence of other immunologic disease, chronic rhinosinusitis and/or nasal polyposis by CT scanning, vasomotor rhinitis (clear rhinorrhea and response to ipratropium bromide), or respiratory infection during the previous 4 weeks (purulent sputum or rhinorrhea, fever, or abnormal laboratory test for white blood cells); no treatment with systemic or nasal corticosteroids during the previous month or systemic antihistamines or nasal vasoconstrictors during the previous 2 weeks. No patient was pregnant or breast feeding.
2. Specific inclusion criteria for the PNAR group: a history of persistent rhinitis for at least 2 years, negative skin prick test and serum specific IgE to perennial aeroallergens, negative intradermal skin test to DP, and fulfilling the exclusion criteria for idiopathic rhinitis outlined in the ARIA¹ and Rijswijk reviews.⁹
3. Specific inclusion criteria for the PAR group: history of persistent rhinitis for at least 2 years, positive skin prick test (>5-mm wheal) and/or positive serum specific IgE to DP. All PAR subjects had to have a positive response to NAPT-DP and no evidence of treatment with immunotherapy during the previous 10 years.
4. Inclusion criteria for the CG: age 18-70 years, healthy, no allergic or nasal diseases, no pregnancy or lactation, negative skin prick test, negative serum specific IgE to aeroallergens, and negative NAPT-DP.

Symptom score

The study was carried out while the patients were symptomatic. Patients who fulfilled the inclusion criteria were enrolled in the study. After inclusion all patients recorded the nasal symptoms of obstruction, rhinorrhea, itching, and sneezing during the week before the

nasal lavage. Each symptom was scored using the following scale: 0 = no symptoms, 1 = mild (symptom was present but was of short duration and not annoying or troublesome), 2 = moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep), or 3 = severe (symptom was troublesome and interfered with normal daily activity or sleep). The total symptom score (TSS) was the sum of the scores for the individual symptoms. TSS values (0-12) were categorized as mild (0-4), moderate (5-8), or severe (9-12).

SPT

The SPT was performed with a wide panel of the most prevalent inhalant allergens, including house dust mite (DP, *dermatophagoides farinae*, *lepidoglyphus destructor*, and *blomia tropicalis*), pollens (*poa*, *phleum*, *lolium*, *cassuarina*, *eucalyptus*, *cupresus*, *platanus*, *olea*, *helianthus*, *chenopodium*, *plantago*, *artemisia*, *parietaria judaica*, *salsola kali*, *rumex* and *ricinus*), molds (*alternaria*, *aspergillus*, *cladosporium* and *penicillium*), latex, and animal epithelia (dog, cat, and hamster) (ALK-Abelló, Spain).

Intradermal skin test

An intradermal skin test was performed in all PNAR patients with freshly reconstituted freeze-dried allergen solutions of Der p 1 (0.4 and 4 µg/mL) (ALK-Abelló).

Nasal lavage and sample processing

After a standard anterior rhinoscopy, nasal lavage was performed as described,^{18,19} using a modified 14G latex Foley catheter (C.R. Bard Inc., Covington, Ga) and 10 mL of physiologic saline. To increase cell viability, sample processing was kept at 4°C. Samples were centrifuged at 2000 rpm (1069 g) for 7 minutes at 4°C. The supernatant was stored at -20°C until the final analysis and the pellet was studied by flow cytometry.

Flow cytometry measurements

The cell fraction was resuspended in 0.9% NaCl and incubated with DTT 10mmol/L (Sigma-Aldrich, St Louis, Mo) for 15 minutes. After 3 washes, the cells were resuspended in 0.9% NaCl for counting and viability assessment. Cells were incubated in the dark, at 4°C, for 30 minutes with the following fluoresceinated monoclonal antibodies and their isotype controls: CD45-APC (Caltag), CD33-PE (Pharmingen, BD), CD16-FITC (Pharmingen, Becton-Dickinson), CD3-PerCP (Pharmingen), CD4-PE (Pharmingen), and CD8-FITC (Pharmingen); markers of leukocytes, myeloid series, NK cells and myeloid series, T cells, T helper cells, and T cytotoxic cells, respectively. A FACSCalibur was used and the results were analyzed using Cell Quest Software (Becton-Dickinson).

Albumin, ECP, total IgE, and specific IgE

Albumin was measured by nephelometry (Boehringer) in serum and nasal lavage supernatant. The serum and nasal lavage fluid level of ECP, total IgE, and specific IgE were measured by fluoroenzyme immunosorbent assay (UNICAP; Pharmacia Diagnostics, Uppsala, Sweden). For serum specific IgE, we used the same allergen panel described for the skin prick test. In nasal lavage, we measured specific IgE to DP (sIgE-DP). The cutoff value of the specific IgE assay was 0.35 kU/L.

NAPT

Saline challenge was performed to exclude nasal hyperactivity. If negative to saline challenge, NAPT was performed after a week with freshly reconstituted freeze-dried allergen solutions of Der p 1 (.04-

TABLE I. Epidemiologic and clinical data of the study subjects

Groups	PNAR*	PAR	CG
Subjects (N)	50	30	30
Age (y)	39 ± 16	31 ± 12	35 ± 11
Disease duration (y)	7 ± 5	8 ± 6	NA
Onset age (y)	32 ± 18	21 ± 15	NA
TSS	9 ± 5	8 ± 3	0.5 ± 0.2
Sex, N (%):			
Male	17 (34%)	11 (37%)	10 (33%)
Female	33 (66%)	19 (63%)	20 (67%)
FHA, N (%)	23 (46%)	13 (43%)	14 (47%)
Dwelling, N (%):			
City	31 (62%)	20 (67%)	19 (63%)
Country	19 (38%)	10 (33%)	11 (38%)
Comorbidity, N (%):			NA
Conjunctivitis	24 (48%)	13 (43%)	
Asthma	16 (32%)	9 (30%)	
Angioedema	0 (0%)	1 (3%)	
Atopic dermatitis	1 (2%)	0 (0%)	

CG, Control group; FHA, family history of atopy; N, number of subjects; NA, not applicable; PAR, persistent allergic rhinitis; PNAR, persistent nonallergic rhinitis; TSS, total symptom score.

*Data expressed as means ± SD.

.4-1-2-4 mcg/mL) at 15-minute intervals (ALK-Abelló). Two puffs (.05 mL each) of the solution at room temperature were applied to each nasal passage with the use of a metered pump spray. A positive NAPT-DP was considered to be both an increase higher than 30% in the total visual analog scale (VAS) and a decrease higher than 30% in the Volume 2-6 cm in acoustic rhinometry compared with the baseline test. These changes in VAS and acoustic rhinometry were previously validated in symptomatic persistent allergic rhinitis patients and using mediator release (histamine).

Symptoms of nasal obstruction, rhinorrhea, itching, sneezing, and ocular symptoms were recorded by placing a vertical mark on a horizontal 100-mm line or VAS. The total VAS was the sum of the 5 VAS scores (total range 0-500).²⁰ Measurements were performed before NAPT (baseline VAS), every 15 minutes during the NAPT and 1, 2, and 24 hours after the challenge. Acoustic rhinometry was carried out with the use of the SRE 2000 rhinometer (Rhinometrics, Lyngø, Denmark) according to the guidelines of the Standardization Committee on Acoustic Rhinometry.²¹ The mean value of the volume (cm³) in the anterior nasal segment (Volume 2-6 cm) was measured before NAPT (baseline test), every 15 minutes during the NAPT and 1, 2, and 24 hours after the challenge. A positive response was categorized as immediate (15 minutes to 1 hour after challenge), late (2-24 hours after the challenge), or dual (both immediate and late).

To exclude a nonspecific effect of house dust mite in the nasal response, we used a group of 20 symptomatic patients with intermittent allergic rhinitis (IAR) with positive skin test to olive and/or grass pollen and negative to house dust mite with no immunotherapy treatment during the previous 10 years.

Statistical analysis

Differences in percentages between the groups were compared by χ^2 analysis, numeric demographic data by Student *t* test, and the flow cytometry results, total IgE and specific IgE, albumin, and ECP by the Mann-Whitney U test. All statistical analyses were carried out using the software package SPSS for Windows 11.5.1 (SPSS Corporation,

TABLE II. Frequency and severity of symptoms

Symptom	PAR	PNAR	P value
Itching			NS
Frequency	87.5	75.0	
Mild	9.5	23.1	
Moderate	63.7	28.8	
Severe	14.3	23.1	
Sneezing			NS
Frequency	95.8	96.4	
Mild	9.5	30.8	
Moderate	62.5	38.7	
Severe	23.8	26.9	
Rhinorrhea			NS
Frequency	95.8	92.9	
Mild	0.0	19.2	
Moderate	57.7	31.4	
Severe	38.1	42.3	
Nasal obstruction			<.05
Frequency	83.3	78.6	
Mild	14.3	3.8	
Moderate	54.7	21.0	
Severe	14.3	53.8	
TSS			NS
Mean ± SD	7.88 ± 2.7	7.75 ± 2.8	
Mild	16.7	10.7	
Moderate	45.8	46.4	
Severe	37.5	42.9	

PAR, Persistent allergic rhinitis; PNAR, persistent nonallergic rhinitis; NS, not significant; TSS, total symptom score.

Values are percentages unless otherwise indicated.

Chicago, Ill). A *P* value less than .05 was considered statistically significant.

RESULTS

Study groups

Clinical and epidemiologic data are shown in Table I. No significant differences were found between patients with PAR and patients with PNAR. In all 3 groups, there was a predominance of women, a lack of a family history of atopy (FHA), and city dwellings. The most frequent presentation was rhinitis, followed by rhino-conjunctivitis and rhinitis-bronchial asthma.

Symptom score

The most common symptoms were sneezing and rhinorrhea (95.8% each) in PAR and sneezing (96.4%) in PNAR. The comparison between PAR and PNAR patients showed that the symptoms with the highest severity score were nasal obstruction in PNAR (53.8% vs 14.3%, *P* < .05) and rhinorrhea in PAR (38.1% vs 42.3%, *P* = not significant) (Table II).

Skin prick test

All patients in the PAR group were positive to DP, 17 were mono-sensitized (57%), and the remaining were also sensitized to pollen (12/30), epithelia (10/30), mold (3/30), and latex (1/30).

TABLE III. Laboratory data I (serum and nasal levels of ECP, total IgE, and specific IgE)

	PNAR*	PAR	CG
Serum			
ECP, $\mu\text{g/L}$	21 \pm 12	24 \pm 16	18 \pm 13
Albumin, mg/dL	4566 \pm 237	4533 \pm 294	4475 \pm 401
Total IgE, IU/mL	71 \pm 188†	206 \pm 263‡	47 \pm 59
sIgE-DP, kU/L	<.35†	14 \pm 29‡	<.35
Nasal lavage			
ECP, $\mu\text{g/L}$	6 \pm 5‡	15 \pm 17‡	2.1 \pm 2.2
Albumin, mg/dL	25 \pm 25	48 \pm 57	9 \pm 11
Total IgE, IU/mL	0.5 \pm 4†‡	86 \pm 207	8 \pm 19
sIgE-DP, kU/L	0.1 \pm 0.3†	0.6 \pm 1.3‡	<.35

CG, Control group; ECP, eosinophil cationic protein; FHA, family history of atopy; N, number of subjects; NA, not applicable; PAR, persistent allergic rhinitis; PNAR, persistent nonallergic rhinitis; sIgE-DP, specific IgE to DP; TSS, total symptom score.

*Data expressed as mean \pm standard deviation.

†Significant differences with PAR patients.

‡Significant differences with CG.

ECP, albumin, total IgE, and sIgE-DP in serum

Table III shows that patients with PAR presented significantly higher levels of serum total IgE compared with PNAR ($P < .001$) and CG ($P < .01$). No significant differences were observed between total IgE in PNAR versus CG. Serum sIgE-DP was positive in 26/30 PAR patients (73%) and negative in 4/30 (27%). All PNAR and CG subjects had negative serum sIgE-DP. Similar, nonsignificant values were observed in levels of serum albumin and ECP in the 3 groups.

Flow cytometry measurements

Total cell counts and cell populations are shown in Table IV. In all 3 groups, the number of leukocytes approached or was higher than 50% of the total cell count. The remaining cells were mostly of epithelial origin. PNAR showed significantly increased levels of eosinophils and CD3⁺ T cells compared with CG ($P < .001$, $P < .05$) and decreased levels of total lymphocytes ($P = .01$), CD3⁺CD4⁺ T cells, and CD4/CD8 T cell ratio ($P < .05$) compared with PAR ($P < .05$). The PNAR and PAR groups presented similar levels of CD45⁺ cells, neutrophils, eosinophils, basophils, and CD3⁺CD8⁺ T cell populations. Compared with the CG, the PAR group showed significantly increased levels of eosinophils ($P < .0001$), total lymphocytes ($P = .003$), CD3⁺ T cells ($P = .001$), and CD3⁺CD4⁺ T cells ($P = .01$).

Although no significant differences were observed in neutrophils among the 3 groups, the values in the CG were almost double those of the other groups. No differences were observed in the basophil levels among the 3 groups.

PNAR patients with positive nasal sIgE-DP and/or NAPT-DP showed a similar leukocyte phenotype to the PAR group, with increased levels of eosinophils ($P < .001$), CD3⁺ T cells ($P < .005$), and CD3⁺CD4⁺ T cells ($P < .05$) compared with CG. These patients also showed increased levels of basophils and CD3⁺ T-cell populations compared with the PNAR group ($P < .05$).

TABLE IV. Laboratory data II (Nasal flow cytometry)

	PNAR*	PAR	CG
TCC	8510 \pm 2806	8895 \pm 2006	8733 \pm 2303
CD45+	59 \pm 28	66 \pm 23	52 \pm 29
N, %	10 \pm 9	9 \pm 6	17 \pm 23
E, %	37 \pm 23‡	43 \pm 18‡	14 \pm 8
B, %	0.80 \pm 0.72	0.90 \pm 1.37	0.82 \pm 0.94
L, %	0.62 \pm 0.73†	0.95 \pm 0.90‡	0.32 \pm 0.37
T cell, %	0.26 \pm 0.39‡	0.37 \pm 0.42‡	0.11 \pm 0.24
CD4 ⁺ T cell, %	17 \pm 24†	23 \pm 30‡	7 \pm 12
CD8 ⁺ T cell, %	35 \pm 34	36 \pm 32	24 \pm 32
CD4 ⁺ / CD3 ⁺ CD8 ⁺	0.36 \pm 0.69†	0.82 \pm 1.80	0.04 \pm 0.05

B, Basophils; CG, control group; E, eosinophils; ECP, eosinophil cationic protein; FHA, family history of atopy; L, lymphocytes; N, neutrophils; NA, not applicable; PAR, persistent allergic rhinitis; PNAR, persistent nonallergic rhinitis; sIgE-DP, specific IgE to DP; TCC, total cell count.

*Data expressed as mean \pm standard deviation.

†Significant differences between PAR and PNAR patients.

‡Significant differences with CG.

No significant differences were found in the recovered volumes (mean \pm SD) of nasal lavage among the 3 study groups (PAR: 6.5 \pm 0.6 mL, PNAR: 7.0 \pm 0.8 mL, CG: 6.2 \pm 1.1 mL). The following supernatant data are presented as absolute concentrations. The total protein level in nasal lavage fluid was used as a marker of dilution. Levels of IgE, ECP, and albumin in supernatant were analyzed after normalization by concentration of total protein, and the results obtained did not differ significantly from those expressed as an absolute concentration in nasal lavage fluids (data not shown).

ECP, albumin, total IgE, and sIgE-DP in nasal lavage

The results obtained are shown in Table III. The PNAR and PAR patients all showed increased levels of nasal ECP ($P < .05$, $P = .01$) and nasal albumin compared with CG ($P = .003$, $P < .05$). Comparison between PAR and PNAR, although higher for the former, showed no significant differences.

Nasal total and sIgE-DP levels were significantly higher in PAR compared with PNAR patients ($P < .001$, $P = .003$). Nasal sIgE-DP was also significantly higher in PAR compared with CG ($P < .001$).

In the PNAR group, we detected 6 patients (12%) with selective nasal sIgE-DP (1.60, 1.26, 1.20, 0.81, 0.70, and 0.54 kU/L, respectively). These patients only had symptoms of rhinitis or rhinoconjunctivitis. In the PAR group, 25/30 patients presented sIgE-DP (83%) either in blood and/or nasal lavage. We observed a higher percentage in nasal lavage (23/30 patients, 76%) compared with serum (20/30 patients, 67%). Eight PAR patients only had sIgE-DP in nasal lavage, and 80% of them had symptoms of allergic rhinitis without asthma or conjunctivitis.

NAPT-DP

No PNAR or IAR patient had a positive response to saline nasal challenge.

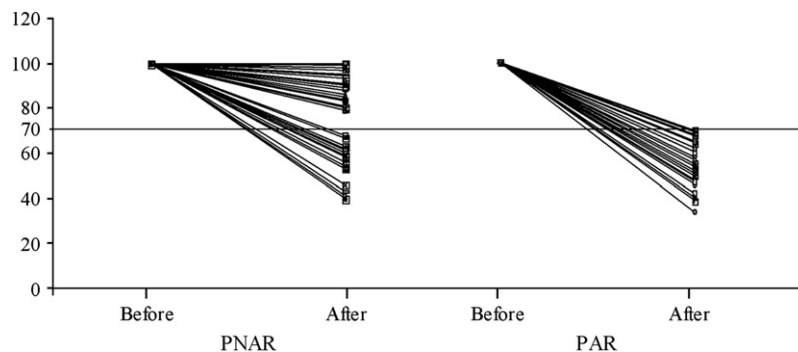


FIG 1. Percent volume 2-6 cm in acoustic rhinometry before and after NAPT-DP.

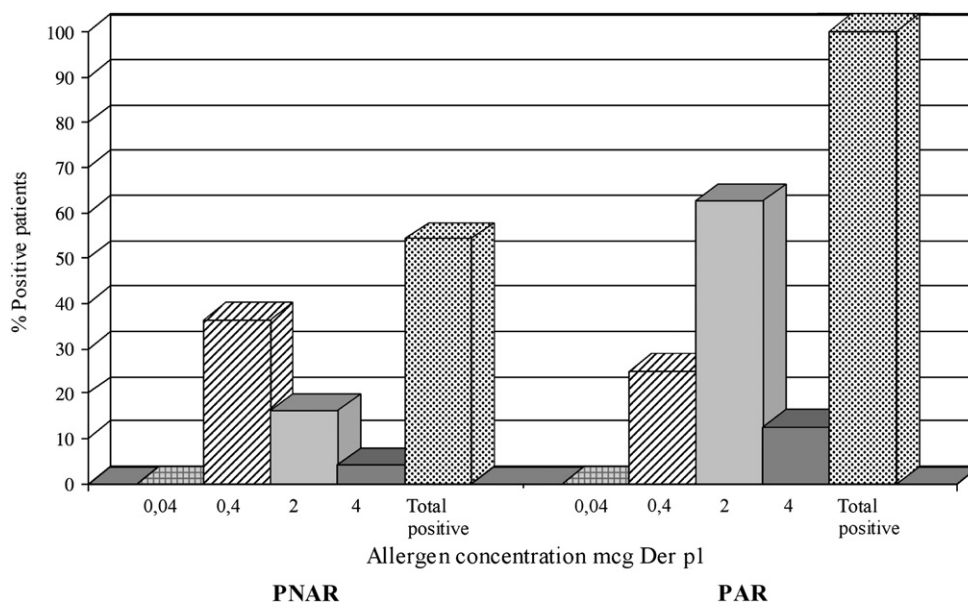


FIG 2. Der p1 concentration and positive response to NAPT-DP in PNAR and PAR groups.

In the PNAR group, 27/50 patients (54%) presented a positive response to NAPT-DP with an increase in the VAS and a decrease higher than 30% in the %Volume 2-6 cm (Fig 1). The majority of these patients, 16/27 patients (59%), had a positive response with 0.4-mcg Der p1 (Fig 2), with 15/27 (56%) having a bilateral response, 12/27 (44%) a unilateral response in acoustic rhinometry, 27/27 an immediate response, and 10/27 (37%) a dual response. No patient had just a late response only. Of the 27 patients with a positive NAPT-DP in the PNAR group, 6 had selective nasal sIgE-DP (22%). The nasal response to DP was bilateral in 4/6 patients, unilateral in 2/6 patients, immediate in 6/6 patients, and dual in 3/6 patients. In the PNAR patients with a positive response to NAPT-DP, we found a significant positive correlation between nasal sIgE-DP and percentage of eosinophils in nasal lavage fluid ($Rho = 0.888, P = .001$).

NAPT-DP was negative in all IAR patients, who were symptomatic to olive or grass pollen.

DISCUSSION

The purpose of this work was to study and characterize the nasal inflammatory response, the presence of nasal specific IgE, and the intranasal response to DP in patients with PNAR, and to compare the results with PAR patients and healthy controls. We determined the leukocyte-lymphocyte phenotype by flow cytometry with monoclonal antibodies (CD16, CD8, CD4, CD33, CD3, and CD45) in nasal fluid, nasal levels of ECP and albumin, and nasal specific IgE to DP, and we performed an NAPT-DP. As no differences were observed in symptom frequency between PAR and PNAR, as may occur in many clinical situations,

additional studies are necessary to differentiate the 2 groups. We used flow cytometry to identify in the nasal lavage the neutrophils, eosinophils, basophils, and CD3⁺CD4⁺ and CD3⁺CD8⁺ lymphocyte populations in the 3 groups. The PNAR showed increased nasal levels of eosinophils, CD3⁺ T cells, ECP, and albumin compared with healthy controls and similar levels of CD45⁺ cells, neutrophils, eosinophils, basophils, and CD3⁺CD8⁺ T-cell populations compared with PAR, but with decreased levels of total lymphocytes and T cells and CD4/CD8 T-cell ratio. Similar data were found by Powe et al²² in the CD3⁺ T-cell population by performing immunohistochemical studies in nasal biopsies from patients with PAR and PNAR, although they found higher levels of CD8⁺ T cells in PNAR than PAR. Of the PNAR group, 54% showed an immediate positive response to intranasal challenge with DP, and 37% also had a late response. Considering that DP did not induce any positive response in negative atopic controls with symptomatic IAR, these data are consistent with an IgE-mediated response. However, in only 22% of this subgroup could we detect specific IgE antibodies in nasal fluids. The reasons for this low percentage may be another immunologic mechanism or low sensitivity of the technique. The former seems unlikely because all patients developed an immediate response in the NAPT-DP with no isolated late response and DP did not induce any response in the negative control group of IAR who were symptomatic to grass and/or olive pollen. The latter may be related with a dilution effect, which could explain these values. This effect needs further examination.

Several studies have addressed the mechanisms involved in PNAR.²³⁻²⁸ Nasal provocation studies in PNAR have shown that a positive response may exist despite a negative skin test and/or serum specific IgE.²⁹⁻³⁰ Additional studies have shown the presence or synthesis of IgE in the nasal mucosa.¹²⁻¹⁶ Our study provides information about inflammatory mechanisms, the presence of nasal specific IgE, and a positive response to intranasal challenge with a perennial allergen, DP, in a large group of PNAR patients.

Within the PNAR, the subgroup of patients with positive nasal sIgE-DP and/or NAPT-DP showed a similar leukocyte phenotype to the PAR group, with increased levels of eosinophils, CD3⁺ T cells, and CD3⁺CD4⁺ T cells compared with the CG. The positive association between nasal sIgE-DP and the percentage of eosinophils in nasal fluid can be explained by the fact that, the stronger the *in situ* IgE response, the higher the capacity for eosinophil recruitment. This complex mechanism is the result of the combination of mast cell activation and other tissue cells involved in the inflammatory response. We are, in fact, planning to study these relationships. This subgroup of patients also showed increased levels of basophils and CD3⁺ T-cell populations compared with the general PNAR group. These cases could represent a type of localized mucosal allergic disease in the absence of systemic atopy,^{15,17} which is also referred to as entopy by Powe et al.¹⁷ We cannot discard the possibility in the PNAR

group that IgE antibodies could be produced to other aero-allergens, because we only measured nasal specific IgE to DP. To determine whether IgE antibodies exist to other allergens, a wider panel of allergens for nasal specific IgE should be used in future studies.

In summary, the results of our study show that the nasal lavage analysis of the cell pellet by flow cytometry and the supernatant of PNAR showed similarities with PAR and clear differences with healthy controls. Within the PNAR group, a subgroup of patients with a positive response to intranasal challenge with DP was identified, with positive nasal specific IgE in 22% of the cases. Whether the remaining patients had specific IgE antibodies to other perennial allergens needs further study. We do not currently know whether these patients will progress to the classic disease, with the additional presence of IgE antibodies in peripheral blood, or whether they will remain unchanged.

We thank Ian Johnstone for help with the final English language version of this manuscript.

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