

Letter to the Editor

Role of the basophil activation test in the diagnosis of local allergic rhinitis

To the Editor:

In clinical practice, distinguishing between allergic rhinitis (AR) and nonallergic rhinitis (NAR) can be difficult.¹ Recent publications have demonstrated that a significant number of patients previously given a diagnosis of NAR have local allergic rhinitis (LAR) or “entopy,”^{2–4} with an estimated prevalence of 25.7% of all cases of rhinitis evaluated in a 1-year period in our department.⁵ LAR is characterized by a positive response to the specific nasal provocation test (NPT) with allergens, with a T_H2 response in the absence of systemic atopy (negative skin test/serum specific IgE).² The NPT is a key tool for diagnosis of LAR. This test has high sensitivity and specificity and is very reproducible, although it requires well-trained personnel and is time consuming. Measurement of local specific IgE levels is noninvasive and very specific, but the sensitivity is rather low.² Therefore without the correct diagnostic approach, a large number of patients with LAR can receive a misdiagnosis.

Peripheral basophils are key cells in the allergic response and are involved in immediate IgE-mediated allergic reactions. The basophil activation test (BAT) is a validated technique for *in vitro* diagnosis of sensitization to inhalant and food allergens, Hymenoptera venom, and several drugs.⁶ The aim of this study was to evaluate the presence of specific allergen activation of

basophils by using the BAT in a group of patients with confirmed LAR caused by *Dermatophagoides pteronyssinus*.

BATs with 5, 20, and 50 ng/mL of *D pteronyssinus* extract were performed in 55 subjects: 16 with LAR (positive NPT with *D pteronyssinus*, negative skin test, and specific IgE to *D pteronyssinus*), 14 with AR (positive NPT with *D pteronyssinus*, positive skin test, and specific IgE to *D pteronyssinus*), 10 with NAR (negative NPT, skin test, and specific IgE to *D pteronyssinus*, *Alternaria*, *Phleum*, and olive tree pollen), and 15 healthy control subjects (negative NPT, skin test, and specific IgE to *D pteronyssinus*). BATs with wortmannin pretreatment were performed in 4 patients with LAR to demonstrate a specific IgE mechanism of basophil activation (for more information, see the **Methods** section in this article’s Online Repository at www.jacionline.org).

The clinical characteristics of all patients are shown in **Table E1** in this article’s Online Repository at www.jacionline.org. The results of BAT with *D pteronyssinus* are summarized in **Fig 1**. One patient with AR and 1 healthy control subject were not included in the analysis because of negative results with the anti-IgE–positive control and allergen (nonresponders). BAT results with *D pteronyssinus* were positive in 11 (85%) of 13 patients with AR (**Fig 1, A**). In patients with LAR, 8 (50%) of 16 had a positive BAT result with *D pteronyssinus* (**Fig 1, B**). One (7%) of 14 healthy control subjects had a positive result (**Fig 1, C**), and 1 (10%) of 10 patients with NAR had positive results (**Fig 1, D**). BAT sensitivity was 85% in patients with

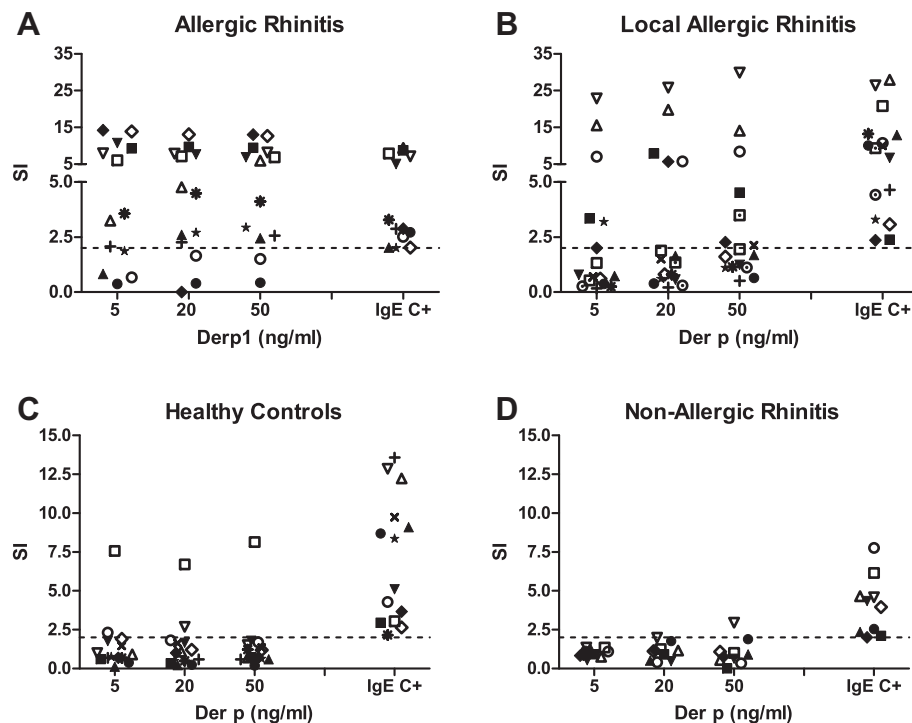


FIG 1. BAT after adding *D pteronyssinus* at 5, 20, and 50 ng/mL. The horizontal line indicates the cutoff point of the assay. *Der p*, *D pteronyssinus*; *IgE C+*, positive IgE control; *SI*, stimulation index. **A**, Patients with AR ($n = 13$). **B**, Patients with LAR ($n = 16$). **C**, Healthy control subjects ($n = 14$). **D**, Patients with NAR ($n = 10$).

TABLE I. Sensitivity, specificity, and positive and negative predictive values of BATs with *D pteronyssinus* in the AR and LAR groups

	Positive BAT result (%)	Sensitivity	Specificity	PPV	NPV	κ Index
AR (n = 14)*	11/13 (85%)	85%	93%	92%	87%	0.78†
LAR (n = 16)	8/16 (50%)	50%	93%	89%	62%	NA
CG (n = 15)*	1/14 (7%)	NA	NA	NA	NA	NA
NAR (n = 10)	1/10 (10%)	NA	NA	NA	NA	NA

CG, Control group; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

*One subject from the AR group and 1 subject from the healthy control group were excluded from the analysis (nonresponders in the BAT assay).

† $P = .0001$.

AR and 50% in patients with LAR, with a specificity of 93% for both groups. Positive predictive value for the AR group was 0.92, and that for the LAR group was 0.89. Negative predictive value was 0.87 for the AR group and 0.62 for the LAR group. The analysis of concordance⁷ of BAT results with NPT results revealed a substantial concordance in the AR group (κ index = 0.78, $P = .0001$; results are summarized in Table I). The specific IgE mechanism of basophil activation was demonstrated by performing BATs with wortmannin pretreatment in 4 randomly selected patients with LAR, showing negativization of results with the IgE-mediated positive control and persistence of positivity with N-formyl-methionine-leucine-phenylalanine (fMLP) (non-IgE-mediated positive control). Also, positive responses to *D pteronyssinus* became negative in all patients with LAR when wortmannin was added to the assay (see Fig E1 in this article's Online Repository at www.jacionline.org).

Since the first study of Huggins and Brostoff,⁸ several investigators have reported local production of IgE antibodies in patients with NAR.^{2,3} Others have shown that a percentage of patients with NAR respond to NPTs with allergens, being defined as having "local allergic rhinitis" or "entopy."^{2,4} The current investigation represents the first evidence of detection of specific basophil activation in peripheral blood in patients with LAR who did not have positive responses to conventional tests, such as skin testing and serum specific IgE measurement. Our results show that at least 50% of the patients with LAR to *D pteronyssinus* can receive a correct diagnosis by using this approach in contrast with the low sensitivity of other determinations, such as measurement of specific IgE levels in nasal lavage fluid (22% of positive responses to *D pteronyssinus*),² and it is less time-consuming than the NPT. Moreover, BAT wortmannin assays demonstrated that the activation of basophils in these patients was IgE mediated. In a recent review Dullaers et al⁹ stated that the mucosa might be the primary site of allergen-specific IgE production and suggested that some nonatopic subjects are in fact atopic but that there is a lack of spillover of the mucosally produced allergen-specific IgE into the circulation. In our study we detected allergen-specific IgE on the surfaces of peripheral basophils of patients with LAR, suggesting that after local production of specific IgE, basophils might be the first or only target cells for specific IgE before the detection of free serum specific IgE and skin mast cell sensitization. It is possible that BAT might be a useful tool for supporting the diagnosis of LAR.

In conclusion, BAT was able to diagnose at least 50% of cases of LAR to *D pteronyssinus* and was more sensitive than detection of nasal specific IgE and less time-consuming than NPTs. IgE-specific activation of basophils was demonstrated by using BAT wortmannin assays. The allergen concentration range for proper basophil stimulation in patients with LAR has to be defined because the low amount of specific IgE in these patients might require the use of higher doses of allergen stimulation to achieve better sensitivity. Although this pilot study is promising, future studies with larger populations and replication of these results with other allergens are needed to fully assess the diagnostic performance of BAT in patients with LAR.

We thank Ian Johnstone for his help with the final English version of this manuscript and Lidia Meléndez and Jose Rodríguez-Bada for technical support.

Enrique Gómez, PhD^{a*}
 Paloma Campo, MD, PhD^{b*}
 Carmen Rondón, MD, PhD^b
 Esther Barrionuevo, MD^b
 Natalia Blanca-López, MD, PhD^c
 María José Torres, MD, PhD^b
 Rocío Herrera, RN^b
 Luisa Galindo, RN^b
 Cristobalina Mayorga, PhD^{a,†}
 Miguel Blanca MD, PhD^{b,†}

From ^athe Allergy Research Laboratory and ^bU.G.C. Allergy, Instituto de Biomedicina de Málaga (IBIMA), Hospital Regional Universitario de Málaga, Málaga, Spain; and ^cthe Allergy Service, Infanta Leonor Hospital, Madrid, Spain. E-mail: campomozo@yahoo.com.

*†These authors contributed equally to this work.

Supported by grants from the Spanish Health Ministry Fund for Health in Spain (FIS) network RIRAAF (RD07/0064), PI11/02619, PI12/00900 and the Andalusian Health Ministry (PI0542-2010).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

- Molgaard E, Thomsen SF, Lund T, Pedersen L, Nolte H, Backer V. Differences between allergic and nonallergic rhinitis in a large sample of adolescents and adults. *Allergy* 2007;62:1033-7.
- Rondón C, Campo P, Togias A, Fokkens WJ, Durham SR, Powe DG, et al. Local allergic rhinitis: concept, pathophysiology and management. *J Allergy Clin Immunol* 2012;129:1460-7.
- Powe DG, Jagger C, Kleinjan A, Carney AS, Jenkins D, Jones NS. "Entopy": localized mucosal allergic disease in the absence of systemic responses for atopy. *Clin Exp Allergy* 2003;33:1374-9.
- Powe DG, Bonnin AJ, Jones NS. "Entopy": local allergy paradigm. *Clin Exp Allergy* 2010;40:987-97.
- Rondón C, Campo P, Galindo L, Blanca-Lopez N, Cassinello MS, Rodríguez-Bada JL, et al. Prevalence and clinical relevance of local allergic rhinitis. *Allergy* 2012;67:1282-8.
- Kleine-Tebbe J, Erdmann S, Knol EF, MacGlashan DW, Poulsen LK, Gibbs BF. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. *Int Arch Allergy Immunol* 2006;141:79-90.
- Landis J, Koch G. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159-74.
- Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. *Lancet* 1975;2:148-50.
- Dullaers M, De Bruyne R, Ramadani F, Gould HJ, Geavert P, Lambrecht BN. The who, where and when of IgE in allergic airway disease. *J Allergy Clin Immunol* 2012;129:635-45.

<http://dx.doi.org/10.1016/j.jaci.2013.07.016>

METHODS

Study subjects

The study included 55 patients: 16 patients with LAR, 14 patients with AR, 10 patients with NAR, and 15 healthy control subjects recruited from the Allergy Departments in Carlos Haya Hospital and Infanta Leonor Hospital. The study was performed from July through September 2011, when the patients were symptom free and without treatment.

LAR inclusion criteria. These included a history of persistent LAR to *D pteronyssinus* for at least 2 years, a positive NPT with *D pteronyssinus*, a negative skin test to *D pteronyssinus* (intradermal test/skin prick test [SPT]), and negative serum specific IgE levels to *D pteronyssinus* and a battery of common aeroallergens.

AR inclusion criteria. These included a history of persistent AR to *D pteronyssinus* for at least 2 years, a positive NPT with *D pteronyssinus*, a positive SPT and serum specific IgE to *D pteronyssinus*.

NAR inclusion criteria. These included a history of persistent symptoms of rhinitis for at least 2 years; a negative NPT with *D pteronyssinus*, *Alternaria* grass, and olive tree pollen using a multiple-allergen NPT^{E1}; a negative skin test to *D pteronyssinus*; and a battery of common aeroallergens (intradermal test/SPT), and negative serum specific IgE levels to *D pteronyssinus* and a battery of common aeroallergens.

Healthy control subject inclusion criteria. The control subjects had no allergic or nasal diseases and negative SPT/intradermal test negative serum specific IgE levels to aeroallergens, and negative NPT with *D pteronyssinus*.

Exclusion criteria. Exclusion criteria includes patients with chronic rhinosinusitis evaluated by means of a computed tomographic scan; patients with massive polyposis, septal perforation, total nasal blockage, upper respiratory tract infection, or any other condition that does not allow performance of a nasal challenge; pregnant or breast-feeding patients; patients who had autoimmune and/or any other severe disease that contraindicated NPT and patients with psychosomatic disorders or who were unable to follow the instructions.

The study was conducted according to the principles of the Declaration of Helsinki and approved by the local ethics committees. All participants and parents of patients less than 16 years old were informed and signed the corresponding informed consent form.

Skin testing

SPTs were performed with a panel of the most prevalent aeroallergens, including *D pteronyssinus*, *Dermatophagoides farinae*, *Phleum*, *Lolium*, *Cupressus*, *Platanus*, *Olea europaea*, *Chenopodium*, *Artemisia*, *Parietaria*, *Salsola kali*, *Alternaria alternata*, *Aspergillus*, *Cladosporium*, *Penicillium*, and dog and cat epithelia (ALK-Abelló, Madrid, Spain).^{E2} Intradermal skin tests were performed with freshly reconstituted freeze-dried allergen with *D pteronyssinus* (0.4 µg/mL, ALK-Abelló), as previously described.^{E3}

Total and specific IgE measurement

Serum total and specific IgE measurement to the same aeroallergens in the SPT panel were done with the fluoroenzyme immunosorbent assay (UniCAP; Thermo Fisher, Waltham, Mass). The positive cutoff value of the specific IgE was greater than 0.35 kU/L.

NPT with *D pteronyssinus*

NPTs with *D pteronyssinus* were performed according to previously described methods.^{E1} Subjects had to stop taking leukotriene modifiers, antihistamines, nasal corticosteroids 1 week before the study, and systemic corticosteroids 4 weeks before the study. Nasal hyperresponsiveness was ruled out by performing a nasal challenge with 0.9% saline before applying the allergen in the nose. NPTs were performed by applying 2 puffs (100 µL) of freshly reconstituted *D pteronyssinus* extract (Der p 1, 4 µg/mL; ALK-Abelló) through a metered pump. Responses were monitored based on results of

acoustic rhinometry (SRE 2000 rhinometer; Rhinometrics, Lynge, Denmark) and symptom scores. Acoustic rhinometry was performed according to the guidelines of the Standardization Committee on Acoustic Rhinometry,^{E4} measuring the volume of the nasal cavity that corresponds to the lower turbinate (volume, 2-6 cm) in each nostril. Nasal obstruction, rhinorrhea, itching, sneezing, and ocular symptoms were recorded by using a visual analog scale (VAS) of 100 mm. The total VAS score was the sum of the 5 VAS scores (total range, 0-500 mm). NPTs were performed in symptom-free patients (total VAS, <60 mm).^{E1} A positive NPT result was considered an increase of 30% or greater in total nasal symptoms and a decrease of 30% or greater in total volume of 2 to 6 cm of both nasal cavities compared with the baseline test.

BAT with *D pteronyssinus*

One hundred microliters of heparinized whole blood was placed in aliquots per test, and 20 µL of stimulation buffer was added and incubated for 10 minutes with agitation at 37°C in a water bath. After this, 100 µL of the washing solution was added to the negative control tube, 100 µL of anti-human IgE antibodies (BD PharMingen, San Diego, Calif) was added to the positive control tubes, and 100 µL of the allergen (*D pteronyssinus* extract, ALK-Abelló) was added at final concentrations of 5, 20, and 50 ng/mL. These concentrations were chosen based on a dose-response curve (Fig E2). The samples were incubated for 30 minutes at 37°C during agitation. The degranulation was stopped by incubating the samples on ice for 5 minutes, and then 5 µL of the mAbs anti-IgE fluorescein isothiocyanate and CD63 phycoerythrin (Caltag Laboratories, Burlingame, Calif) was added to each tube to characterize basophils and activation, respectively. After 20 minutes at 4°C in the dark, 2 mL of prewarmed lysing solution was added and centrifuged for 5 minutes at 4°C. Cells were washed and analyzed in a flow cytometer (FACSCalibur, BD Bioscience) by acquiring at least 500 basophils per sample. Results were considered positive when the stimulation index, which was calculated as the ratio between the percentage of degranulated basophils with *D pteronyssinus* and the negative control, was 2 or greater in at least 1 of the allergen concentrations mentioned above. This value was selected after the performance of a receiver operating characteristic curve comparing BAT results between patients and control subjects because this value allows adequate sensitivity and specificity for both patients with LAR and those with AR (data not shown). When the percentage of spontaneously activated basophils was less than 2.5%, we required an additional condition (ie, that the percentage of basophils activated after contact with the antigen should be ≥5%), as previously described.^{E5} Nonresponder patients, who are considered those with negative results to the anti-IgE-positive control and allergen, were not included in the study.

BAT performed with *D pteronyssinus* and wortmannin

To demonstrate a specific IgE mechanism of activation in basophils, BATs with wortmannin pretreatment (Sigma-Aldrich, Saint Louis, Mo) were performed in 4 randomly selected patients with LAR among those with a previous positive BAT response to *D pteronyssinus*. Wortmannin is an inhibitor of phosphatidylinositol-3-kinase that participates in the signal transduction pathway responsible for histamine secretion after stimulation of FcεRI,^{E6} and therefore activation should be blocked with pretreatment. In these experiments, samples are pretreated with wortmannin for 5 minutes in agitation at 37°C. As a positive control and because of the features of the inhibitor, N-formyl-methionine-leucine-phenylalanine was also included in the test. After this step, samples were processed following the protocol described above for a classic BAT.

Statistical analysis

Data were expressed as medians and ranges. The clinical and demographic data were compared between groups by using χ^2 analysis and the Mann-Whitney *U* test. The Friedman test was used to examine the overall differences. If significant differences occurred, the Wilcoxon signed-rank test was used to identify them within groups. Correlation between NPTs

and BATs was obtained by calculating the κ index, as previously described.^{E7} All *P* values of less than .05 were considered statistically significant.

RESULTS

Subjects' characteristics

A total of 55 subjects were included in the study, and the characteristics of these patients have been summarized in Table E1. In the AR, LAR, and NAR groups, all subjects had perennial rhinitis symptoms for at least 2 years (AR group, 6.4 years [2-35 years]; LAR group, 5.5 years [2-50 years]; NAR group, 5.8 years [2-48 years]; *P* = not significant). Asthma symptoms were present in 38% of patients with AR, 33% of patients with LAR, and 35% of patients with NAR. Also, 77% of patients with AR, 88% of patients with LAR, and 79% of patients with NAR had conjunctivitis. Total IgE levels were significantly higher in patients with AR than in patients with LAR, patients with NAR, and control subjects (AR group, 247 IU/mL [53-4010 IU/mL]; LAR group, 21 IU/mL [4-131 IU/mL]; healthy control group, 23 IU/mL [4-213 IU/mL], and NAR group, 25 IU/mL [4-248 IU/mL]; *P* < .05). All patients with AR had positive skin test and specific IgE levels to *D pteronyssinus*, and results were negative in patients with LAR, patients with NAR, and control subjects. NPT results with *D pteronyssinus* were positive in all patients with AR and those with LAR and negative in both control groups. All patients with LAR, patients with NAR, and control subjects had negative skin test and specific IgE levels to all allergens tested, including *D pteronyssinus*.

Dose-response curve for BATs

For performing the BAT, *D pteronyssinus* was added to basophils of all 55 participants at final concentrations of 5, 20,

and 50 ng/mL. These concentrations were chosen based on a dose-response curve, in which sequential dilutions of *D pteronyssinus* were incubated with basophils of positive subjects (6 patients with AR) and healthy control subjects. As shown in Fig E2, *D pteronyssinus* concentrations of 400 ng/mL or less did not induce activation of basophils in control subjects. Concentrations of *D pteronyssinus* of greater than 50 ng/mL (100, 200, and 400 ng/mL) did not produce any significant increase in stimulation index values in subjects with positive results compared with the 50 ng/mL concentration.

REFERENCES

- E1. Rondón C, Campo P, Herrera R, Blanca-López N, Meléndez L, Canto G, et al. Nasal allergen provocation test with multiple aeroallergens detects polysensitization in local allergic rhinitis. *J Allergy Clin Immunol* 2011;128:1192-7.
- E2. Bousquet J, Heinzerling L, Bachert C, Papadopoulos NG, Bousquet PJ, Burney PG, et al. Practical guide to skin prick tests in allergy to aeroallergens. *Allergy* 2012;67:18-24.
- E3. López S, Rondón C, Torres MJ, Campo P, Canto G, Fernandez R, et al. Immediate and dual response to nasal challenge with *Dermatophagoides pteronyssinus* in local allergic rhinitis. *Clin Exp Allergy* 2010;40:1007-14.
- E4. Hilberg O, Pedersen OF. Acoustic rhinometry: recommendations for technical specifications and standard operating procedures [published erratum in *Rhinology* 2001;39:119]. *Rhinol Suppl* 2000;16:3-17.
- E5. Gómez E, Blanca-Lopez N, Torres MJ, Requena G, Rondón C, Canto G, et al. Immunoglobulin E-mediated immediate allergic reactions to dipyrone: value of basophil activation test in the identification of patients. *Clin Exp Allergy* 2009;39:1217-24.
- E6. Yano H, Nakanishi S, Kimura K, Hanai N, Saitoh Y, Fukui Y, et al. Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL-2H3 cells. *J Biol Chem* 1993;268:25846-56.
- E7. Landis J, Koch G. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159-74.

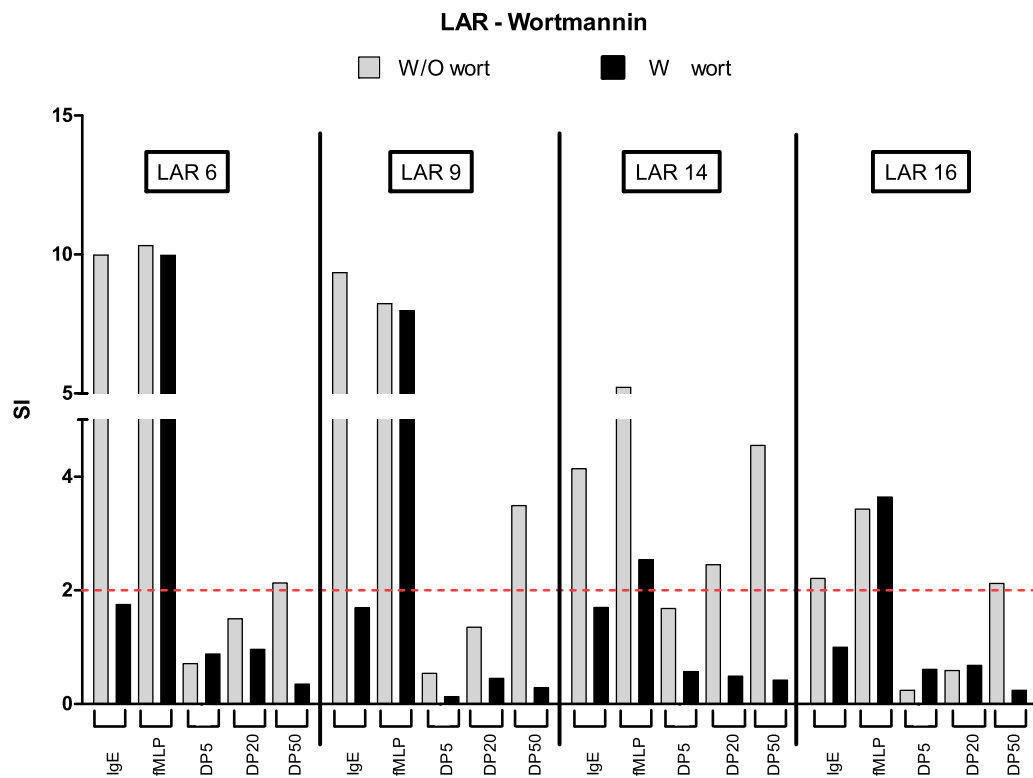


FIG E1. BATs performed in 4 patients with LAR by using both the standard assay (*gray bars*) and wortmannin pretreatment (*full bars*). Results are plotted side by side for each dilution. The *horizontal line* indicates the cutoff point of the assay. DP, *D pteronyssinus*; fMLP, N-formyl-methionine-leucine-phenylalanine; SI, stimulation index.

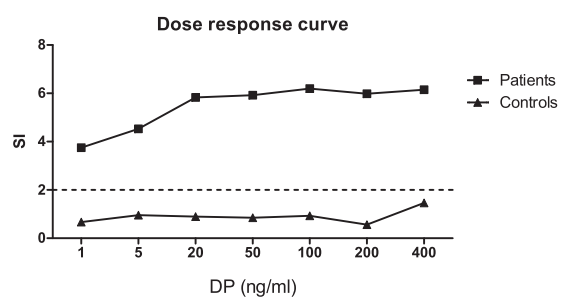


FIG E2. BAT dose-response curves with serial dilutions of *D pteronyssinus* (DP) extract (1-400 ng/mL) in 6 patients with AR and 6 control subjects. Results are expressed as the stimulation index (SI).

TABLE E1. Clinical characteristics of patients with AR, patients with LAR, patients with NAR, and healthy control subjects

	Age (y), median (range)	Sex (F/M)	Diagnosis of rhinitis (y), median (range)	Presence of asthma symptoms (%)	Presence of conjunctivitis (%)	Total IgE (IU/mL), median (range)	Specific IgE (kU/L), median (range)	Positive SPT/ID result with DP (%)	Positive NPT result with DP (%)
AR (n = 13)	33 (16-62)	7/6	6.4 (2-35)	38%	77%	247 (53-4010)*	12.6 (1.9-95.9)*	100%*	100%
LAR (n = 16)	33.5 (15-58)	12/4	5.5 (2-50)	33%	88%	21 (4-131)	<0.35	0%	100%
CG (n = 14)	36 (26-50)	12/2	NA	NA	NA	23 (4-213)	<0.35	0%	0%
NAR (n = 10)	34.2 (17-60)	8/2	5.8 (2-48)	35%	79%	25 (4-248)	<0.35	0%	0%

DP, *D pteronyssinus*; F, female; CG, healthy control group; ID, intradermal test; M, male; NA, not applicable.

* $P < .05$.