ORIGINAL ARTICLE

Nonallergic Airway Hyperresponsiveness and Allergen-Specific IgE Levels Are the Main Determinants of the Early and Late Asthmatic Response to Allergen

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■ **Abstract**

Background: Conflicting results have been reported in studies of predictive factors for airway responsiveness to allergens during bronchial challenges.
Objective: The aim of this study was to assess determinants of airway responsiveness to 3 different allergens during standardized bronchial challenges.
Methods: Data were collected from asthmatic patients who participated in allergen challenge trials between 2000 and 2006 (cat, n=37; house dust mite [HDM], n=35; grass pollen, n=27). PD_{20} (provocative dose causing a 20% fall in forced expiratory volume in the first second) methacholine, PD_{20} allergen, allergen skin test endpoint, allergen-specific immunoglobulin (Ig) E levels, and late asthmatic response were analyzed for each allergen group.
Results: During the early asthmatic response, a significant relationship was found between PD_{20} allergen and PD_{20} methacholine ($P<.01$ for cat, HDM, and grass pollen), as well as between PD_{20} allergen and allergen-specific IgE levels ($P<.05$ for cat and HDM). No relationship was observed between PD_{20} allergen and allergen skin test endpoint ($P>.05$). Late asthmatic response was significantly more frequent after HDM challenge than after cat or grass pollen challenges (57.1% vs16.2% and 33.3%, $P<.01$). Dual responders during HDM challenges had significantly higher allergen-specific IgE levels ($P<.05$) and higher nonallergic airway responsiveness ($P<.05$).
Conclusion: Nonallergic airway hyperresponsiveness and allergen-specific IgE levels were the main determinants of early and late asthmatic responses. HDM challenges were the most interesting model with regard to the occurrence of late asthmatic response. In contrast to previous publications and to the official statement on standardized challenge testing with sensitizing stimuli, skin sensitivity appears to be a poor predictor of the early asthmatic response.

Key words: Bronchial allergen challenge. Non-specific airway hyperresponsiveness. Allergen skin sensitivity. Allergen-specific IgE levels. Early asthmatic response. Late asthmatic response.

■ **Resumen**

Introducción: Hay una gran controversia sobre los posibles factores predictivos de la respuesta a la provocación bronquial con alérgenos.
Objetivos: El objetivo del estudio fue analizar factores determinantes de la reactividad bronquial frente a tres diferentes alérgenos durante la provocación bronquial estandarizada.
Métodos: Se estudiaron los datos de pacientes asmáticos participantes en diversos estudios de provocación con alérgenos, realizados entre los años 2000 al 2006 (gato, n=37, ácaros del polvo, n=35, polen de gramíneas, n=27). Se analizaron para cada grupo de alérgeno la PD_{20} metacolina , la PD_{20} alérgeno, la titulación a punto final de la prueba cutánea, los niveles de IgE específica y la respuesta asmática tardía.
Resultados: En relación con la respuesta inmediata, se observaron correlaciones significativas entre la PD_{20} alérgeno y la PD_{20} metacolina (gato, ácaros del polvo, polen de gramíneas; $p<0.01$), y también entre la PD_{20} alérgeno y los niveles de IgE específica (gato y ácaros del polvo; $p<0.05$). No encontramos correlación entre la PD_{20} alérgeno y la titulación a punto final de la prueba cutánea. Se observaron respuestas tardías de significativamente mayor frecuencia tras la provocación bronquial con ácaros del polvo que las observadas tras la provocación con gato o polen de gramíneas (57.1% vs. 16.2% y 33.3%; $p<0.01$). Los pacientes que presentaron respuestas duales

durante la provocación bronquial con ácaros del polvo presentaban niveles más elevados de IgE específica (*p*<0.05) junto con una mayor reactividad bronquial frente a metacolina(*p*<0.05).
Conclusion: La reactividad bronquial no relacionada con alérgeno y los niveles de IgE específica frente al alérgeno fueron los principales determinantes de la respuesta asmática inmediata y tardía. La provocación bronquial con ácaros presentaba frecuencias mayores de respuestas tardías. En contra de lo referenciado en la literatura, incluyendo un protocolo oficial de estandarización de la provocación bronquial, la reactividad cutánea parece un pobre factor predictivo de la respuesta asmática inmediata

Palabras clave: Provocación bronquial. Reactividad bronquial inespecífica. Sensibilidad cutánea frente al alérgeno. Nivel de IgE específica. Respuesta asmática inmediata. Respuesta asmática tardía.

Introduction

Bronchial allergen challenge tests (BCTs) are of great value for understanding the underlying mechanisms of allergic airway inflammation in asthma and for studying pharmacological agents. These tests, which consist of the aerosol delivery of specific allergens, have now been well characterized and can be standardized [1,2]. For safety purposes, the severity of the bronchial constriction induced during the allergen challenge needs to be estimated.

Different factors influencing the magnitude of the asthmatic response following allergen challenges have been reported. Nonspecific airway hyperresponsiveness (AHR) has been shown to contribute to the severity of the early asthmatic response (EAR) induced by the allergen [3]. In addition, since the early 1970s, it has been thought that the immediate bronchial response is also related to the degree of allergen sensitization [4]. Cockcroft et al [5], for instance, studied the relationship between skin sensitivity to allergens, bronchial sensitivity to histamine, and bronchial sensitivity to allergens during inhalation challenges. Based on data from a population of 51 asthma patients, they proposed an equation to predict PC_{20} allergen (provocative concentration of allergen causing a 20% fall in forced expiratory volume in the first second [FEV_1] from baseline) from PC20 histamine and the allergen skin test endpoint to within 2 to 3 doubling concentrations during a bronchial allergen challenge [6]. Although the prediction equation proposed by Cockcroft et al [6] appears in the official statement on standardized challenge testing with sensitizing stimuli of the European Respiratory Society (ERS) [1] and the European Academy of Allergology and Clinical Immunology (EAACI) [2], conflicting data have been reported. Indeed, variable relationships between airway response to allergens and allergen skin sensitivity were found, and other studies have reported that cutaneous and bronchial reactivity may not parallel each other [7,8].

Late asthmatic response (LAR) to an allergen challenge is a validated model for investigating the pathogenesis of asthmatic disease and new treatments for asthma in the laboratory [9]. However, different factors influencing the occurrence and magnitude of the LAR following allergen challenges have been reported, with conflicting results [10,11].

One explanation for the different results could be that earlier allergen inhalation studies used methods that were not standardized across research laboratories, making it difficult to interpret and compare results. Great progress has been made in the past decade in terms of standardization of bronchial allergen challenges, with the emergence of well-reproducible inhalation procedures and high-quality allergen extracts [1,2].

The aim of this study was to reevaluate the influence of different factors on airway responsiveness during BCTs using standardized inhalation procedures for 3 different allergens.

Methods

Individuals

Data were collected from individuals aged between 18 and 50 years participating in bronchial allergen challenge research studies for which ethical approval had been granted and written informed consent obtained. In all the studies, the patients selected had a clinical history of intermittent asthma to the allergen to which they were challenged (cat, house dust mite [HDM], or grass pollen). Sensitization to the allergen in question was determined by a positive skin prick test (mean wheal diameter ≥75% of the mean diameter of the wheal induced by histamine (10 mg/mL, Stallergènes) and by specific immunoglobulin (Ig) E levels higher than 0.75 kU/L (Phadia). Individuals were nonsmokers, had clinically stable asthma (baseline FEV_1>80%), used only short-acting β_2-agonists, and had no history of a recent (<3 weeks) respiratory tract infection or other relevant disease. None of the individuals had significant exposure to the allergen to which he/she was challenged or concomitant exposure to other relevant respiratory allergens (home exposure to HDM and cat allergens was measured in floor and mattress dust and patients were challenged outside the pollen season).

Study Design

Data were extracted from 6 bronchial allergen challenge research studies performed at the Allergy Division of Strasbourg University Hospital [12-17]. All BCTs were performed by the same technician and physician. Data were extracted exclusively for individuals who had undergone a placebo-medicated allergen challenge [13,14,16,17]. For the research studies that evaluated the influence of particle size on bronchial response, data were recorded for individuals from challenge tests that used the same nebulizer device as in all the other research studies [12,15]. For individuals who had participated in multiple investigations, the relevant data were retrieved from the first study to avoid any possibility

of selection bias. Individuals were included if they had a positive EAR and if the following data was available: PD_{20} (provocative dose causing a 20% fall in FEV_1) methacholine, PD_{20} allergen, allergen skin test endpoint, specific IgE levels, and presence or not of an LAR. For each allergen group (cat, HDM, and grass pollen), relationships between the variables were analyzed, and mean differences between single and dual responders were studied.

Methacholine Inhalation Test

A methacholine inhalation test was performed 24 hours prior to the allergen challenge using a standardized technique, as previously described [12]. Doubling doses of a nebulized 1% methacholine solution were delivered using a dosimeter (Mediprom FDC 88) until FEV_1 decreased by 20% or more from the postsaline baseline value, or until a cumulative dose of 3200 µg was delivered. PD_{20} was calculated as the dose causing a 20% fall in FEV_1 from the postsaline baseline value using log-linear interpolation of 2 adjacent data points on the dose-response curve.

Allergen Extract

For each protocol, skin test endpoint titrations and BCTs were performed with an aqueous allergen extract from the same batch. Furthermore, the same batch was used for all patients and the concentration of the major allergen (µg/mL) was known in all cases: cat (Stallergènes, 100 index of reactivity [IR]/mL) Fel d 1 8.2 µg/mL [12] and 13 µg/mL [13]; HDM (Allerbio, 100 IR/mL) Der p 1 11 μg/mL [14] and 2.4 µg/mL [15]; and grass pollen (Allerbio, 100 IR/mL) Dac g 1 6.2 µg/mL [16] and 1.5 µg/mL [17].

Allergen Skin Test Endpoint

The allergen skin test endpoint titration was carried out using an aqueous allergen extract from the same batch to be used for inhalation. All the skin prick tests were conducted by the same expert physician across the different research studies, limiting variability. Duplicate skin prick tests were performed with 10-fold dilutions of the allergen preparation. Skin prick test solution was used as a negative control. The wheal diameter was measured in 2 perpendicular directions after 15 minutes, and the mean wheal diameter was determined for each dilution. The skin test endpoint was defined as the threshold concentration producing a 2-mm wheal. In most cases, this endpoint was determined 1 to 2 weeks before the allergen challenge.

Bronchial Challenge Tests

Allergen BCTs were performed with an automatic inhalation-synchronized dosimeter jet-nebulizer (Spira Elektro 2) and an air-driven nebulizer (Pari Tia; Pari Aerosol Research Institute), as previously described [12]. Particles delivered by the nebulizer had a mass median aerodynamic diameter of 10 µm. The starting concentration for the bronchial challenge was defined as one-tenth of the skin test endpoint of each individual's skin prick test. Prior to the allergen challenge, the diluent (0.9% sodium chloride solution) was inhaled to ensure FEV_1 stability

(<10% decrease). BCTs were carried out by inhalation of increasing concentrations (10^{-4} to 10 IR) of allergen extracts at 10-minute intervals (2, 4, and 8 breaths). The results of the bronchial response were expressed as the percentage change in FEV_1 from the postsaline baseline value. EAR was considered positive if a decrease in FEV_1 of 20% or more from postdiluent baseline was reached and was expressed as the dose of allergen extract triggering a 20% decrease in FEV_1 (PD_{20} allergen) using a log-linear interpolation of 2 adjacent data points on the dose-response curve. After the EAR, FEV_1 was recorded at 15-minute intervals up to 60 minutes and then every 30 minutes up to 6 hours. LAR was defined as a 15% or greater reduction in FEV_1 between 3 and 6 hours after the EAR.

Statistical Analysis

Statistical analysis was performed using SPSS 14.0 (2005, SPPS Inc). Logarithmic transformations were used for PD_{20} allergen, PD_{20} methacholine, allergen-specific IgE levels, and allergen skin test endpoint in order to obtain normal distribution. Results were presented as mean (SD). Correlations of log-transformed data were analyzed using the Pearson correlation coefficient, with separate analyses for each allergen group (cat, HDM, and grass pollen). Differences between allergen groups and between single and dual responders were tested by 1-way analysis of variance (ANOVA) and the unpaired t test, respectively. Results were verified by Bonferroni adjustment correction. Comparisons of percentages between allergen groups were conducted with the Pearson χ^2 test, with P values of less than .05 considered significant.
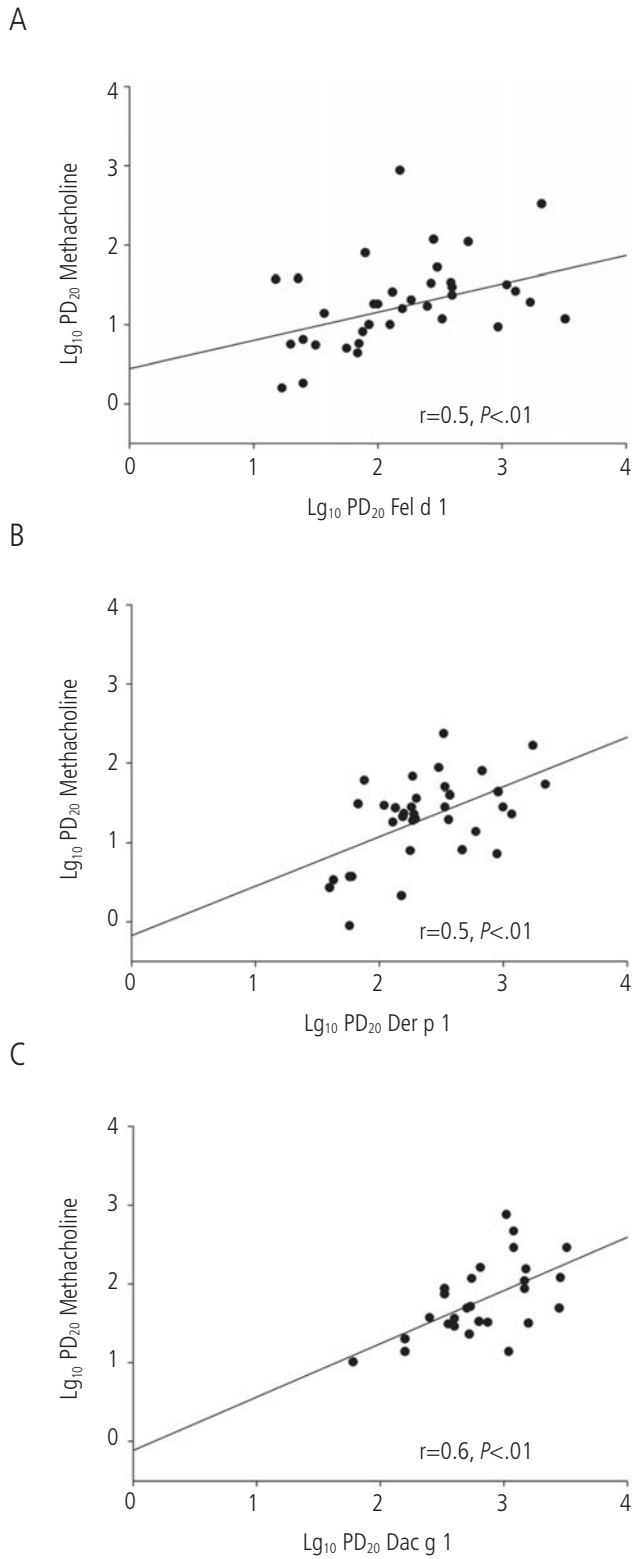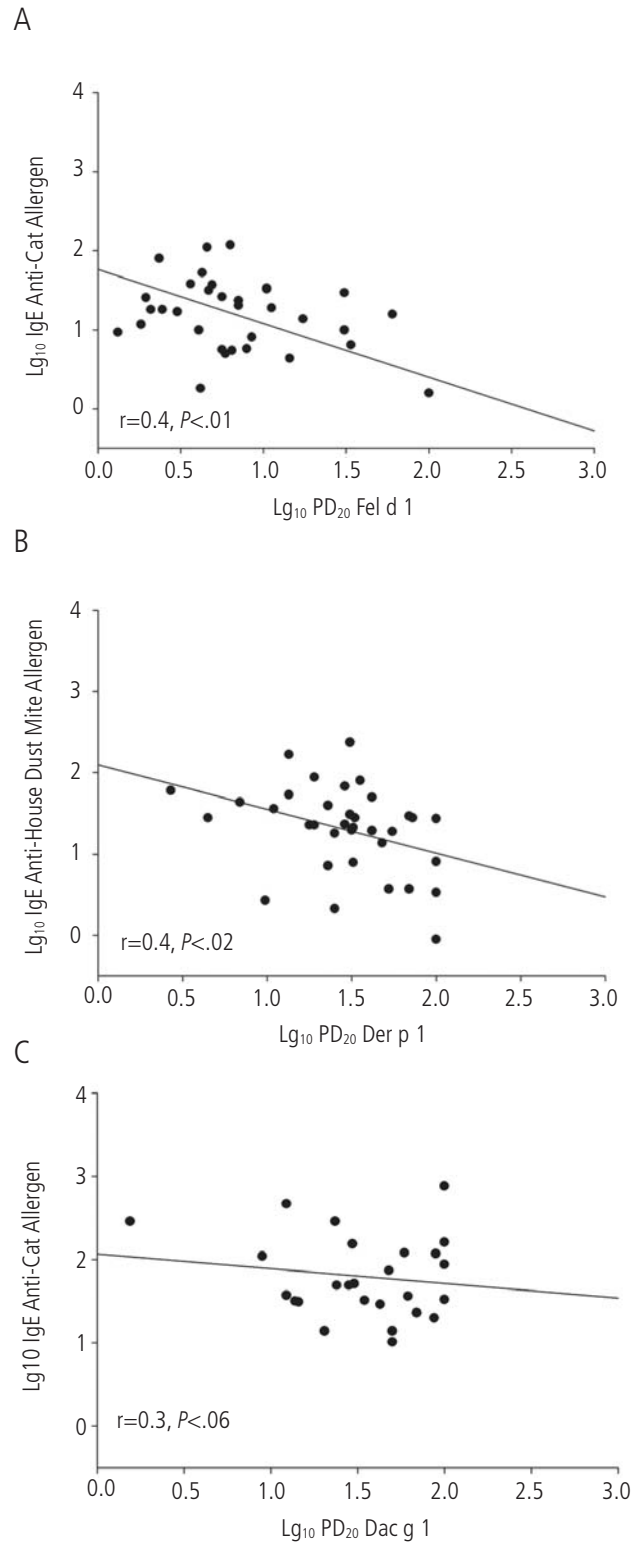
Results

Patient Characteristics

We identified 99 patients who met the inclusion criteria (cat, n=37; dust mite, n=35; and grass pollen, n=27). The patients' characteristics are summarized in Table 1. There were no significant differences with respect to sex, age, or baseline lung function between the 3 allergen groups (P>.05). Baseline nonallergic AHR, measured by a methacholine inhalation test, differed between the 3 groups and was significantly lower in the grass pollen group than in the cat and HDM groups ($P\leq.01$).
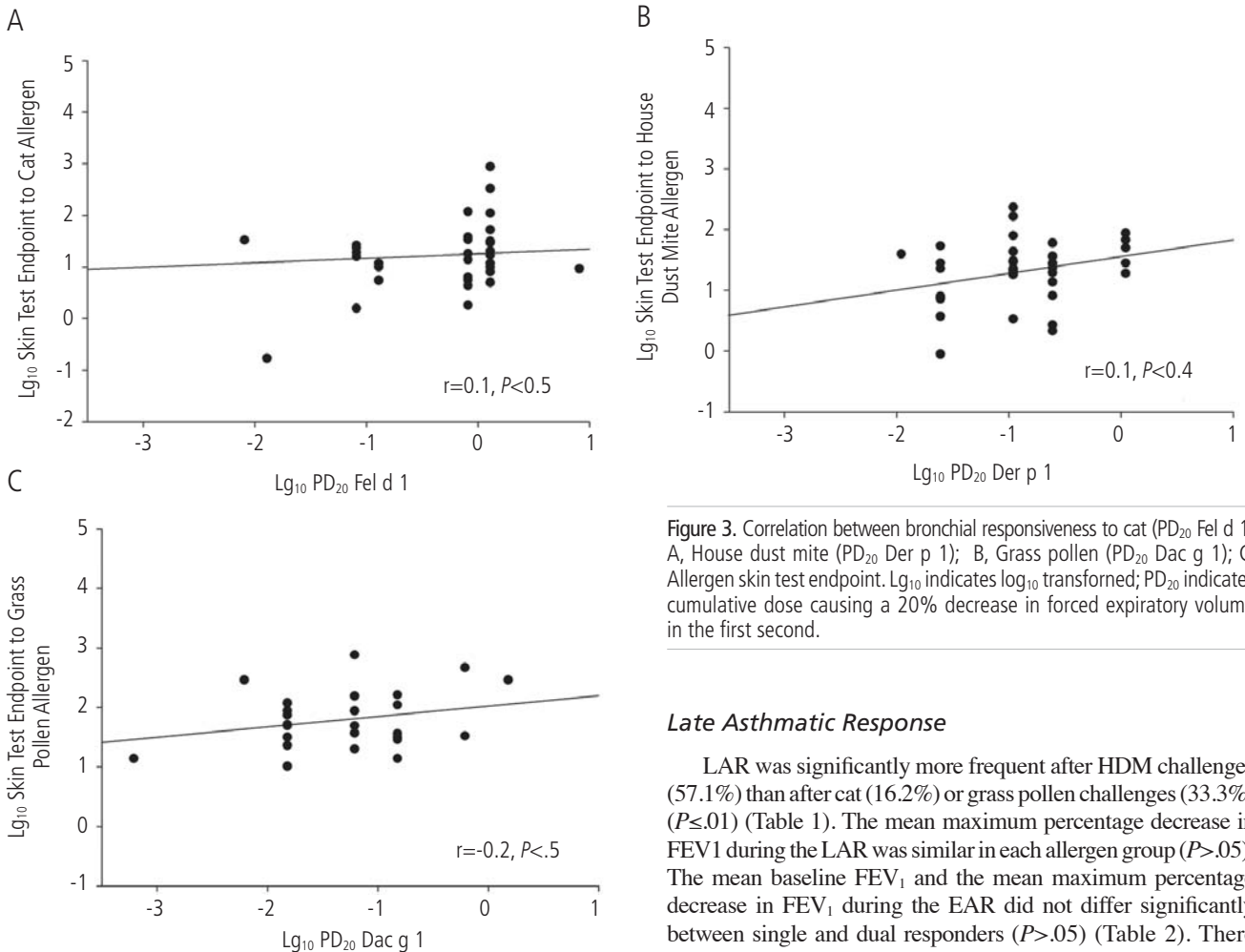
Early Asthmatic Response

The mean maximum percentage decrease in FEV_1 during the EAR was similar in each allergen group (P>.05) (Table 1). A significant relationship was found between PD_{20} allergen and PD_{20} methacholine during cat (r=0.5, P<.01), HDM (r=0.5, p<.01), and grass pollen (r=0.6, P<.01) allergen inhalation challenges (Figure 1). A significant inverse relationship was found between the allergen-specific IgE titer and PD_{20} allergen for the cat (r=-0.4, P=.01) and HDM (r=-0.4, P=.02) BCTs; statistical significance was not reached for the grass pollen tests (r=-0.3, P=.06) (Figure 2). No relationship was observed between the allergen skin test endpoint and PD_{20} allergen for the cat (r=-0.1, P=.5), HDM (r=-0.1, P=.4), or grass pollen (r=-0.2, P=.5) BCTs (Figure 3).

Figure 1. Correlation between bronchial responsiveness to cat (PD_{20} Fel d 1). A, House dust mite (PD_{20} Der p 1); B, grass pollen (PD_{20} Dac g 1); C, Nonspecific airway hyperresponsiveness (PD_{20} methacholine). Lg_{10} indicates log_{10} transformed; PD_{20}, cumulative dose causing a 20% decrease in forced expiratory volume in the first second.

Figure 2. Correlation between bronchial responsiveness to cat (PD_{20} Fel d 1). A, House dust mite (PD_{20} Der p 1); B, Grass pollen (PD_{20} Dac g 1); C, Allergen-specific immunoglobulin E levels. Lg_{10} indicates log_{10} transformed; PD_{20}, cumulative dose causing a 20% decrease in forced expiratory volume in the first second.d.

A

B

Figure 3. Correlation between bronchial responsiveness to cat (PD_{20} Fel d 1) A, House dust mite (PD_{20} Der p 1); B, Grass pollen (PD_{20} Dac g 1); C, Allergen skin test endpoint. Lg_{10} indicates log_{10} transformed; PD_{20} indicates cumulative dose causing a 20% decrease in forced expiratory volume in the first second.

C

Late Asthmatic Response

LAR was significantly more frequent after HDM challenges (57.1%) than after cat (16.2%) or grass pollen challenges (33.3%) ($P \leq .01$) (Table 1). The mean maximum percentage decrease in FEV1 during the LAR was similar in each allergen group ($P > .05$). The mean baseline FEV_1 and the mean maximum percentage decrease in FEV_1 during the EAR did not differ significantly between single and dual responders ($P > .05$) (Table 2). There

Table 1. Characteristics of Patients[a]

	Allergen		
	Cat	House Dust Mite	Grass Pollen
Patients, No.	37	35	27
Male/female, No.	16/21	14/21	8/19
Age, y	27 (7.2)	25.2 (6.3)	26.1 (6.8)
Baseline FEV_1, L	4.02 (0.32)	3.99 (0.41)	3.84 (0.15)
Baseline FEV_1, % predicted	92.3 (2.3)	93.7 (1.9)	91.1 (5.5)
PD_{20} methacholine, µg	414 (675)	406 (487)	963 (850)b
EAR, % decrease in FEV_1	23.8 (4.7)	24 (5.3)	24.7 (4.1)
LAR, No. (%)	6 (16.2)	20 (57.1)b	9 (33.3)
LAR, % decrease in FEV_1	18.3 (5.9)	17.5 (2.2)	20.1 (4.1)
Specific IgE,[c] kU/L	12.9 (20.2)	38.5 (28.5)	48.6 (33.5)
Skin test endpoint,[d] µg/mL	1.28 (2.38)	0.27 (0.36)	0.16 (0.31)
PD_{20} allergen, ng	55.9 (150.6)	37.5 (46.9)	117 (166.8)

Abbreviations: EAR, early asthmatic response; FEV_1, forced expiratory volume in the first second; IgE, immunoglobulin E; LAR, late asthmatic response; PD_{20} allergen: cumulative dose of allergen (Fel d 1, Der p 1 or Dac g 1 respectively) causing a 20% decrease in FEV_1; PD_{20} methacholine, cumulative dose of methacholine causing a 20% decrease in FEV_1.
[a]Data are expressed as mean (SD) unless otherwise specified.
[b]$P \leq .01$.
[c]Specific IgE were measured for *Dermatophagoides pteronysinnus*, cat, and grass pollen allergens, respectively.
[d]Skin test endpoint concentrations were derived from the content of the major allergen in the allergen extract (Fel d 1, Der p 1, or Dac g 1 respectively).

Table 2. Comparison between Single (-) and Dual Responders (+)[a]

	Allergen		
	Cat -/+	House Dust Mite -/+	Grass Pollen -/+
Baseline FEV_1, L	3.99 (0.22)/3.80 (0.12)	3.82 (0.15)/4.03 (0.11)	4.22 (0.28/3.97 (0.23)
PD_{20} methacholine, μg	363 (522)/681 (1241)	628 (645)/240 (207)[b]	1143 (959)/605 (419)
PD_{20} allergen, ng	63.7 (163.7)/15.7 (12.7)	62.4 (62.6)/18.7 (13.5)[c]	97 (82.9)/159.7 (269.9)
EAR, % decrease in FEV_1	23.2 (3.3)/26.7 (8.9)	23.6 (4)/25.6 (5.1)	24.2 (3.9)/25.7 (4.6)
Specific IgE,[d] kU/L	9.6 (14)/30.4 (36.2)[b]	30.9 (31.5)/43.6 (26.1)[b]	41.7 (34.1)/62.4 (29.3)
Skin test endpoint,[e] μg/mL	1.45 (2.59)/0.49 (0.6)	0.26 (0.35)/0.27 (0.37)	0.15 (0.34)/0.17 (0.25)

Abbreviations: EAR, early asthmatic response; FEV_1, forced expiratory volume in the first second; IgE, immunoglobulin E; PD_{20} allergen: cumulative dose of allergen (*Fel d 1*, *Der p 1* or *Dac g 1* respectively) causing a 20% decrease in FEV_1; PD_{20} methacholine: cumulative dose of methacholine causing a 20% decrease in FEV_1;.
[a]Data are expressed as mean (SD).
[b]$P<.05$.
[c]$P \le .01$.
[d]Specific IgE were measured for *Dermatophagoides pteronysinnus*, cat, and grass pollen allergens, respectively.
[e]Skin test endpoint concentrations were derived from the content of the major allergen in the allergen extract (Fel d 1, Der p 1, or Dac g 1 respectively).

was no correlation between the maximum decrease in FEV_1 during the EAR and LAR (cat: r=0.5, P=.1; HDM: r=0.4, *P*=.09; and grass pollen: r=-0.4, *P*=.2). For HDM allergen challenges, there was a significant difference in mean PD_{20} methacholine between single and dual responders (*P*<.05) (Table 2). Moreover, the mean cumulative dose of mite allergens to obtain an EAR was significantly lower in patients with a dual response (*P*≤.01). Dual responders had significantly higher specific IgE titers than single responders after HDM and cat challenges (*P*<.05). No differences in terms of mean allergen skin sensitivity between single and dual responders were found (*P*>.05).

Discussion

In this study, we sought to determine reliable factors that influence airway responsiveness during BCTs. Allergen inhalation tests were performed using highly standardized inhalation procedures, with the use of a dosimeter to accurately calculate the allergen dose delivered. Technical biases were minimized, as all the individuals were investigated by the same physician and technician in 1 center, under the supervision of a single investigator. The patients were carefully selected and had the same level of asthma severity; groups challenged with different allergens were analyzed separately.

We observed that during EAR, nonallergic AHR and allergen-specific IgE levels, but not allergen skin sensitivity, were correlated with PD_{20} allergen. The influence of nonallergic AHR and degree of sensitization to the allergen on induced EAR has already been reported [4,5]. Degree of allergen sensitivity is usually defined as skin sensitivity to the allergen,

and the relationship between skin and bronchial sensitivity to allergens during inhalation challenges has mainly been investigated by Cockcroft et al [6]. Cockcroft and his group proposed an equation based on histamine PC_{20} or PD_{20} and the allergen skin test endpoint to predict allergen PC_{20} or PD_{20} during the inhalation challenge. More recently, they also proposed a predictive equation based on PC_{20} methacholine and the allergen skin test endpoint [7]. Although this prediction equation appears in the official statement on standardized challenge testing with sensitizing stimuli of the ERS [1] and the EAACI [2], some authors have reported limited influence of skin sensitivity on EAR [7,8]. Ravensberg et al [18] also reported limited influence of skin sensitivity in 104 patients challenged by HDM allergens in a standardized manner.

Our results might partly be explained by the use of 10-fold dilutions rather than the 2-fold dilutions used by Cockcroft et al [6] to determine the allergen skin prick test endpoint [6]. However, other studies with 2-fold dilutions of the allergen preparations have also reported that skin and bronchial allergen reactivity may not parallel each other [7,8].

Discrepancies between studies reporting a close relationship between bronchial and skin allergen sensitivities may be explained by differences in study populations and methodologies. Earlier studies used challenge procedures that were not uniform across research laboratories and results are therefore difficult to compare. Furthermore, patients were often challenged with a wide variety of allergens (including HDM, pollen, cat, and horse) [5,6,19].

Skin and bronchial sensitivity to the same allergen may not parallel each other due to the number or sensitivity of allergic effector cells, which may differ in the target organs, such as the respiratory tract and skin [20]. Indeed, differences

in mast-cell populations between organs and different levels of mast cell–associated IgE have been described [21]. Moreover, mast cells from different sites may differ in their response to stimuli, with subsequent differences in mediator release [21].

Allergen-induced LAR is thought to be a model for chronic asthma and is often used to investigate inflammatory mechanisms and to evaluate the therapeutic potential of anti-asthma agents [9]. Contradictory results have also been reported for factors influencing the occurrence and magnitude of LARs after inhalation challenges [10,11,22]. In our study, the frequency of LARs was significantly higher after HDM challenge than after cat or grass pollen challenges. It is generally admitted that approximately 50% of individuals who have an EAR will also have an LAR. In 1 limited study, a higher frequency of LARs was observed after HDM challenges in 6 individuals who underwent inhalation allergen challenge with HMD and grass pollen allergens on 2 separate occasions [23].

We found that patients who experienced an LAR after an HDM challenge had higher nonallergic AHR, higher bronchial allergen sensitivity, and higher specific IgE levels. A lower PC_{20} methacholine at baseline in dual responders has also been reported [24]. However, the degree of baseline nonallergic AHR has also been shown to have no influence on LAR patterns [25]. Such discrepancies may be explained by differences in study populations and the use of different allergens [11,24]. Moreover, the lack of relation between immediate skin sensitivity and late response in the challenge test is to be expected, and it might be more appropriate to use intradermal tests with late quantification of results to assess possible relations. Boulet et al [26] measured late cutaneous allergic response mean diameters 6 to 8 hours after intradermal skin tests, and reported a high probablity of occurrence of an LAR when a late cutaneous allergic response is elicited for a low allergen dose. Similary, van der Venn et al [27] performed intracutaneous tests with series of 10-fold dilutions of an HDM allergen preparation and recorded late cutaneous responses. They found increased late skin reactions and more severe LARs after challenge with HDM, suggesting a correlation between these 2 variables. Therefore, late allergic skin response might be a better predictor of the occurrence and the magnitude of LARs.

Conclusions

We observed that nonallergic AHR and allergen-specific IgE levels were the main determinants of EAR and LAR. Skin sensitivity appeared to be a poor predictor of EAR. HDM challenges were the most interesting model in regard to the occurrence of LARs. Therefore, allergen sources should be considered for their role in immediate IgE-mediated bronchial allergic reactions and their capacity to induce LAR.

Conflicts of Interest

FDB has worked as a consultant for Stallergenes, Novartis, Mundipharma, and ALK laboratories, and has received speaker's fees and participated in advisory committee activities for MSD, Novartis, Stallergenes, and ALK laboratories.

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