

# Bronchial allergen challenge with isolated major allergens of *Dermatophagoides pteronyssinus*: The role of patient characteristics in the early asthmatic response

Maurits J. van der Veen, MD,<sup>a</sup> Christa E. Lopuhaä, MD,<sup>a</sup> Rob C. Aalberse, PhD,<sup>b</sup>  
Henk M. Jansen, MD, PhD,<sup>a</sup> and Jaring S. van der Zee, MD, PhD<sup>a</sup> Amsterdam,  
The Netherlands

**Background:** The use of allergen extracts will hamper studies into quantitative aspects of allergic responses because the precise amount of relevant allergen for each patient is unknown. **Objective:** We applied isolated IgE-binding components (major allergens) in the technique of bronchial allergen challenge to determine the role of patient characteristics in the early asthmatic response (EAR).

**Methods:** In 30 patients with mild-to-moderate asthma, the EAR was investigated after inhalation of an isolated major allergen of *Dermatophagoides pteronyssinus* (i.e., Der p 1 [ $n = 16$ ] or Der p 2 [ $n = 14$ ]). The degree of early-phase bronchial responsiveness to allergen (the cumulated dose of allergen causing a 20% fall in FEV<sub>1</sub> [PD<sub>20</sub>allergen]) was related to the degree of nonspecific bronchial responsiveness (the concentration of histamine causing a 20% fall in FEV<sub>1</sub> [PC<sub>20</sub>histamine]) and the level of specific IgE or allergen thresholds as found in skin tests and basophil histamine release assays.

**Results:** Twenty-seven patients with an immediate response during allergen and histamine challenges ( $\Delta$ FEV<sub>1</sub>,  $\geq 20\%$ ) were analyzed. In these patients, a strong correlation was found between PD<sub>20</sub>allergen and PC<sub>20</sub>histamine ( $r = 0.81$ ,  $p < 0.001$ ). Weak correlations were found between PD<sub>20</sub>allergen and the level of specific IgE ( $r = -0.36$ ,  $p = 0.07$ ) or allergen thresholds as found in skin tests (skin prick test,  $r = 0.36$  and  $p = 0.07$ ; intracutaneous test,  $r = 0.49$  and  $p = 0.01$ ) or basophil histamine release assays ( $r = 0.37$ ,  $p = 0.08$ ). Moreover, no significant contribution of these indices of IgE-mediated hypersensitivity to the prediction of PD<sub>20</sub>allergen by multilinear regression models with PC<sub>20</sub>histamine was found.

**Conclusion:** In asthmatic patients allergic to house dust mites the degree of nonspecific bronchial hyperresponsiveness is the main determinant of early-phase bronchial responsiveness to allergen. In these patients the degree of allergic sensitivity does not contribute to the prediction of the EAR after allergen inhalation. (*J Allergy Clin Immunol* 1998;102:24-31.)

**Key words:** Allergens, bronchial provocation test, early asthmatic response, IgE, skin test

## Abbreviations used

|                             |                                                                   |
|-----------------------------|-------------------------------------------------------------------|
| AUC:                        | Area under the curve                                              |
| BHRA:                       | Basophil histamine release assay                                  |
| EAR:                        | Early asthmatic response                                          |
| HDM:                        | House dust mite                                                   |
| ICT:                        | Intracutaneous test                                               |
| MA:                         | Major allergen                                                    |
| PBS:                        | Phosphate-buffered saline                                         |
| PC <sub>20</sub> histamine: | Concentration of histamine causing a 20% fall in FEV <sub>1</sub> |
| PD <sub>20</sub> allergen:  | Cumulated dose of allergen causing a 20% fall in FEV <sub>1</sub> |
| SPT:                        | Skin prick test                                                   |

Allergic responsiveness to inhalant allergens is found to be closely related to respiratory diseases such as asthma and rhinitis.<sup>1,2</sup> To investigate allergic responses, allergen extracts are frequently used (e.g., house dust mite [HDM] extract). Allergen extracts, however, are complex mixtures with various contents of major and minor allergens,<sup>3,4</sup> in addition to several non-IgE-binding components (e.g., endotoxin).<sup>5</sup> As a result of this complex nature, the use of allergen extracts will hamper the investigation of quantitative aspects of allergic responses after allergen challenge. The allergenic potency, for example, will be determined by the distribution of the different IgE-binding components in the allergen extract<sup>6</sup> in relation to the individual IgE antigen recognition pattern.<sup>7</sup>

Major allergens (MAs), defined as those IgE-binding components of an allergen source to which more than 50% of sensitized patients have an IgE response, can be isolated from allergen extracts.<sup>3,4,7,8</sup> The use of isolated MAs can improve the specificity of assays for diagnostic<sup>9</sup> and research<sup>7</sup> purposes. Recently, we have investigated patient- and allergen-dependent factors determining the outcome of the skin test by using highly purified MAs.<sup>8</sup>

Bronchial allergen challenge is frequently used as a model to investigate the underlying mechanisms of asthma.<sup>10-13</sup> After allergen challenge, an immediate- and a late-phase bronchial obstructive response can occur. The early asthmatic response (EAR) is found from 10 minutes after allergen inhalation and resolves spontaneously within 2

From <sup>a</sup>the Department of Pulmonology, Academic Medical Center, University of Amsterdam; and <sup>b</sup>the Department of Allergy, Central Laboratory of The Netherlands Red Cross Bloodtransfusion Service, Amsterdam.

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Reprint requests: Jaring S. van der Zee, MD, PhD, Academic Medical Center, University of Amsterdam, Department of Pulmonology, F4-208, P.O. box 22700, 1100 DE Amsterdam, The Netherlands.

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TABLE I. Patient characteristics

|         | Patient no. | Age (yrs) | Sex | FEV <sub>1</sub> pred (%) | PC <sub>20</sub> histamine (mg/ml) | Total IgE (IU/ml) | IgE anti-HDM (IU/ml) | Other allergies |
|---------|-------------|-----------|-----|---------------------------|------------------------------------|-------------------|----------------------|-----------------|
| Der p 1 | 1           | 45        | F   | 76                        | 0.08                               | 1118              | 100                  | C, G            |
|         | 2           | 18        | M   | 82                        | 0.2                                | 228               | 52                   | T               |
|         | 3           | 25        | M   | 103                       | 0.3                                | 291               | 58                   | C, T, G         |
|         | 4           | 27        | F   | 98                        | 0.3                                | 177               | 39                   | T, G            |
|         | 5           | 29        | M   | 74                        | 0.3                                | 149               | 12                   | C, T, G         |
|         | 6           | 24        | F   | 70                        | 0.5                                | 347               | 64                   | C, T, G         |
|         | 7           | 23        | F   | 114                       | 0.5                                | 67                | 5                    |                 |
|         | 8           | 27        | F   | 117                       | 0.6                                | 22                | 7                    | C               |
|         | 9           | 19        | F   | 115                       | 0.7                                | 1661              | 290                  |                 |
|         | 10          | 21        | F   | 101                       | 0.8                                | 710               | 94                   | C, T, G         |
|         | 11          | 43        | M   | 92                        | 1.1                                | 97                | 22                   |                 |
|         | 12          | 35        | F   | 100                       | 2.0                                | 819               | 58                   | T, G            |
|         | 13          | 32        | M   | 93                        | 2.4                                | 67                | 18                   |                 |
|         | 14          | 24        | M   | 107                       | 4.4                                | 91                | 19                   |                 |
|         | 15          | 28        | M   | 97                        | 5.0                                | 815               | 48                   | C, T, G         |
| Der p 2 | 16          | 26        | F   | 116                       | 8.3                                | 99                | 5                    | C, T, G         |
|         | 17          | 20        | F   | 100                       | 0.3                                | 309               | 24                   | T               |
|         | 18          | 23        | M   | 93                        | 1.0                                | 409               | 85                   |                 |
|         | 19          | 35        | F   | 113                       | 1.3                                | 16                | 5                    |                 |
|         | 20          | 28        | M   | 106                       | 1.7                                | 43                | 17                   |                 |
|         | 21          | 35        | M   | 88                        | 2.0                                | 73                | 15                   | C, G            |
|         | 22          | 28        | M   | 114                       | 2.5                                | 65                | 4                    | T, G            |
|         | 23          | 24        | F   | 91                        | 4.8                                | 144               | 17                   |                 |
|         | 24          | 26        | M   | 90                        | 5.2                                | 222               | 19                   |                 |
|         | 25          | 28        | M   | 75                        | 6.2                                | 1186              | 28                   | T, G            |
|         | 26          | 23        | F   | 92                        | 7.0                                | 58                | 5                    | T, G            |
|         | 27          | 23        | F   | 85                        | 10.0                               | 42                | 4                    |                 |
|         | 28          | 22        | F   | 101                       | > 32                               | 365               | 47                   | C, T, G         |
|         | 29          | 31        | M   | 113                       | > 32                               | 385               | 44                   | C, G            |
|         | 30          | 22        | M   | 99                        | 6.6                                | 241               | 1                    | T, G            |

FEV<sub>1</sub>pred, Baseline value of FEV<sub>1</sub> expressed as percentage of predicted value; C, cat dander; T, tree pollen; G, grass pollen.

hours. It is considered to be predominantly the result of IgE-mediated mast cell activation, with mediator release and subsequent bronchial smooth muscle cell stimulation.<sup>13</sup> The late asthmatic response, which develops from 4 to 6 hours after allergen challenge, is found to be more related to late inflammatory changes of the bronchial wall, with implication of activated eosinophils and T-lymphocytes.<sup>10-12</sup>

In 1959, Tiffeneau<sup>14</sup> suggested that early-phase bronchial responsiveness to allergen is related to both nonspecific bronchial hyperresponsiveness and allergic sensitivity. In a study by Cockcroft et al.,<sup>15</sup> a relation was developed for the prediction of airway responsiveness to allergen, taking into account the degree of airway hyperresponsiveness and the skin prick test (SPT) allergen threshold. However, in this study seven different allergen extracts with possible differences in allergenic potency were used. Because allergen thresholds are inversely related to the allergenic potency of the allergen extract used, the relation between skin sensitivity and airway responsiveness to allergen could have been influenced by the use of different allergen extracts.

We performed the following study to investigate in further detail the role of patient-dependent factors in the EAR after allergen inhalation. For this purpose, we used isolated IgE-binding components (MAs) in the technique of bronchial allergen inhalation challenge as an optimal quantitative

model for strictly allergen-induced, IgE-mediated bronchial responses. In a group of patients with mild-to-moderate asthma allergic to HDMs, the EAR was investigated after inhalation of an isolated MA of *Dermatophagoides pteronyssinus* (i.e., Der p 1 or Der p 2). Early-phase bronchial responsiveness to allergen was related to the degree of nonspecific bronchial responsiveness, levels of total and specific IgE, IgG and allergen thresholds in skin tests, and basophil histamine release assays (BHRAs).<sup>7,8</sup>

## METHODS

### Study subjects

Thirty subjects were investigated by the same investigator (15 women and 15 men; mean age, 27.1 years [range, 18 to 45 years]). All patients were sensitized to HDM (*D. pteronyssinus*) as determined by SPT and RAST and had symptoms of mild-to-moderate asthma.<sup>16</sup> Patient characteristics are shown in Table I. All subjects were investigated in a stable phase of their disease and had an FEV<sub>1</sub> of 70% or more of predicted value on all study days. Medication consisted of oral antihistamines, inhalation therapy with short acting  $\beta_2$ -adrenoceptor agonists, and/or corticosteroids and was ceased 1 week, 12 hours, and 6 weeks before allergen challenge, respectively. None of the patients had received HDM-specific immunotherapy. Patients did not have a history of respiratory tract infection or obvious changes in allergen exposure for at least 6 weeks before allergen challenge. Concomitant inhalant allergies were determined by RAST with a panel of inhalant allergens common in the Netherlands.<sup>17</sup>

**TABLE II.** Results of bronchial challenges, RASTs, skin tests, and histamine release assays

|         | Patient no. | PD <sub>20</sub> allergen<br>( $\mu$ g) | IgE anti-MA<br>(IU/ml) | ICT-threshold<br>(pg/ml) | SPT-threshold<br>(ng/ml) | BHRA-threshold<br>(ng/ml) |
|---------|-------------|-----------------------------------------|------------------------|--------------------------|--------------------------|---------------------------|
| Der p 1 | 1           | 0.03                                    | 27.1                   | 9.8                      | 41.5                     | 1.6                       |
|         | 2           | 0.04                                    | 17.2                   | 10.5                     | 10.0                     | 0.8                       |
|         | 3           | 0.17                                    | 19.9                   | 1.6                      | 5.6                      | 0.2                       |
|         | 4           | 0.05                                    | 10.7                   | 11.5                     | 25.4                     | 0.4                       |
|         | 5           | 0.49                                    | 6.0                    | 9.6                      | 26.3                     | 0.2                       |
|         | 6           | 0.11                                    | 37.3                   | 12.0                     | 18.6                     | 1.3                       |
|         | 7           | 0.17                                    | 4.1                    | 83.5                     | 152.9                    | 2.2                       |
|         | 8           | 0.20                                    | 3.3                    | 9.0                      | 18.9                     | <0.09                     |
|         | 9           | 0.19                                    | 75.5                   | 8.0                      | 13.1                     | <0.09                     |
|         | 10          | 0.09                                    | 10.5                   | 9.8                      | 54.8                     | 1.6                       |
|         | 11          | 0.09                                    | 6.6                    | 15.1                     | 10.2                     | 1.2                       |
|         | 12          | 0.21                                    | 19.5                   | 9.8                      | 12.9                     | 60.1                      |
|         | 13          | 0.31                                    | 5.5                    | ND                       | 189.6                    | NR                        |
|         | 14          | 0.61                                    | 5.9                    | 105.3                    | 16.2                     | 0.5                       |
|         | 15          | 5.49                                    | 20.6                   | 65.6                     | 103.7                    | 18.7                      |
|         | 16          | 2.73                                    | 4.0                    | 24.7                     | 595.0                    | 3.3                       |
| Der p 2 | 17          | 0.04                                    | 23.0                   | 5.6                      | 20.4                     | 1.1                       |
|         | 18          | 0.03                                    | 66.7                   | 5.3                      | 11.7                     | <0.05                     |
|         | 19          | 0.17                                    | 4.1                    | 51.3                     | 28.4                     | 1.1                       |
|         | 20          | 0.06                                    | 13.0                   | 4.8                      | 7.3                      | 0.1                       |
|         | 21          | 0.17                                    | 7.5                    | 5.2                      | 2.8                      | 1.7                       |
|         | 22          | 0.09                                    | 3.9                    | 28.1                     | 13.4                     | 6.5                       |
|         | 23          | 0.17                                    | 19.6                   | 4.9                      | 18.7                     | <0.05                     |
|         | 24          | 0.12                                    | 8.9                    | 5.1                      | 5.3                      | NR                        |
|         | 25          | 0.22                                    | 44.9                   | 5.1                      | 5.8                      | 0.1                       |
|         | 26          | 1.28                                    | 5.7                    | 6.1                      | 8.2                      | NR                        |
|         | 27          | 0.90                                    | 3.0                    | 59.3                     | 22.7                     | 350.0                     |
|         | 28          | 0.05                                    | 36.9                   | 0.7                      | 1.3                      | 0.1                       |
|         | 29          | >5.2                                    | 25.6                   | 6.5                      | 5.9                      | 0.9                       |
|         | 30          | >5.2                                    | 1.4                    | 4567.8                   | 507.4                    | 959.9                     |

IgE anti-MA, Specific IgE directed to the MA used; ICT-threshold, ICT allergen threshold; SPT-threshold, SPT allergen threshold; BHRA-threshold, BHRA allergen threshold; ND, not done; NR, non-responder.

Patients sensitized to grass or tree pollen were investigated outside the relevant pollen season. The study was approved by the Academic Medical Center Medical Ethical Committee, and written informed consent was given by all patients.

### HDM MAs

The MAs Der p 1 and Der p 2 were isolated from an acetone-precipitated HDM extract derived from a *D. pteronyssinus* culture (ALK, Hørsholm, Denmark). Hydrophobic interaction chromatography on Phenyl-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) was performed with a sample of the HDM extract dissolved in 0.05 mol/L acetate buffer (pH 5.0)/1 mol/L  $(\text{NH}_4)_2\text{SO}_4$ . The MAs Der p 1 and Der p 2 were subsequently eluted. Der p 1 was eluted with 0.05 mol/L carbonate buffer (pH 10.0) and 0.05 mol/L carbonate buffer (pH 10.0)/50% ethylene glycol. Der p 2 was eluted with 0.05 mol/L acetate buffer (pH 5.0)/1 mol/L  $(\text{NH}_4)_2\text{SO}_4$ , fractions were pooled, and rechromatography was performed on the same reequilibrated column. In this run Der p 2 was eluted with 0.05 mol/L acetate buffer (pH 5.0)/0.5 mol/L  $(\text{NH}_4)_2\text{SO}_4$  and further purified by gel filtration on FPLC (Superdex 75). The MA fractions were dialyzed and tested with crossed immunoelectrophoresis with rabbit anti-HDM antibody in which no detectable other MAs were found. Hereafter, the MA preparations were freeze-dried and diluted under sterile conditions in phosphate-buffered saline (PBS) to be stored at  $-70^\circ\text{C}$ . MA content was determined by using radioimmunoassays as described before,<sup>8</sup> read from a calibration curve of the World Health Organization standard. The estimation of weight concen-

trations of Der p 1 and Der p 2 in this standard were used as described by Platts-Mills et al.<sup>18</sup> (0.125 ng Der p 1/U and 0.005 ng Der p 2/U). The contamination of the Der p 1 preparation with Der p 2 and the Der p 2 preparation with Der p 1 was found to be 0.1% and 0.01%, respectively. Endotoxin concentrations were measured (Limulus amoebocyte lysate; Endosafe, Charleston, S.C.)<sup>19</sup> and found to be 7.1 pg/ $\mu$ g Der p 1 and 2.0 pg/ $\mu$ g Der p 2 for the Der p 1 and Der p 2 preparations, respectively. For use in allergen challenge procedures, the MA preparations were diluted to a 1:10 stock solution in PBS, 0.03% human serum albumin, and 0.5% phenol (ALK Benelux, Groningen, The Netherlands) and stored for a maximum of 3 months at  $4^\circ\text{C}$ . On the day before each allergen challenge, series of twofold dilutions of the allergen preparations were made from this stock solution in the same buffer.

Possible differences in biologic activity between Der p 1 and Der p 2 were determined by parallel line regression of the relation between skin test allergen thresholds (intracutaneous test [ICT]-threshold and SPT-threshold; see below) and the level of specific IgE (see below).<sup>8,20</sup> By using the common slope, Der p 2 was found to be a factor 1.9 and 3.5 (mean, 2.7) more biologically active on a weight basis than Der p 1 as calculated with ICT-threshold and SPT-threshold data sets, respectively.

### Bronchial allergen challenge

Bronchial allergen challenge (Der p 1,  $n = 16$ ; Der p 2,  $n = 14$ ) was performed as described by Sterk et al.<sup>21</sup> by using a reservoir aerosol delivery system.<sup>22</sup> An allergen aerosol was generated from a 0.5 ml sample with a highly efficient nebulizer (Mallinckrodt Diagnostica, Petten,

**TABLE III.** Multiple linear regression of early-phase bronchial responsiveness to allergen\*

| Independent variables                                                            | B (SE)       | $\beta$ | p Value |
|----------------------------------------------------------------------------------|--------------|---------|---------|
| (model: $R^2 = 0.65$ , $R^2_{\text{adjusted}} = 0.62$ , $p < 0.001$ , $n = 27$ ) |              |         |         |
| Constant                                                                         | 0.01 (0.22)  |         |         |
| log PC <sub>20</sub> histamine                                                   | 0.90 (0.14)  | 0.89    | <0.001  |
| Allergen type†                                                                   | -0.60 (0.15) | -0.53   | <0.001  |
| + log IgE anti-MA                                                                | -0.17 (0.18) | -0.12   | 0.34    |
| (model: $R^2 = 0.67$ , $R^2_{\text{adjusted}} = 0.62$ , $p < 0.001$ , $n = 27$ ) |              |         |         |
| + log ICT-threshold                                                              | 0.16 (0.17)  | 0.12    | 0.36    |
| (model: $R^2 = 0.67$ , $R^2_{\text{adjusted}} = 0.63$ , $p < 0.001$ , $n = 26$ ) |              |         |         |
| + log SPT-threshold                                                              | 0.18 (0.15)  | 0.16    | 0.26    |
| (model: $R^2 = 0.67$ , $R^2_{\text{adjusted}} = 0.63$ , $p < 0.001$ , $n = 27$ ) |              |         |         |
| + log BHRA-threshold                                                             | 0.06 (0.07)  | 0.11    | 0.42    |
| (model $R^2 = 0.69$ , $R^2_{\text{adjusted}} = 0.65$ , $p < 0.001$ , $n = 24$ )  |              |         |         |

Optimal regression model and resulting models after addition of an index of IgE-mediated immediate hypersensitivity.

IgE anti-MA, Specific IgE directed to the MA used; ICT-threshold, ICT allergen threshold; SPT-threshold, SPT allergen threshold; BHRA-threshold, BHRA allergen threshold.

\*Dependent variable: log PD<sub>20</sub>allergen.

†Dummy coding: Der p 1 = 1, Der p 2 = 2.

the Netherlands) into a collapsible reservoir of approximately 30 L made of static field dissipative material (RCAS 1206, Richmond Redlands, Calif.) and filled with dry air. Subsequently, the entire volume of the reservoir was inhaled by tidal breathing in approximately 3 minutes through a three-way valve system with the nose clipped. Recovery of aerosol from the reservoir as determined with <sup>99m</sup>Tc-tagged human albumin was found to be 80% to 85%. The aerosol mass median diameter of 4  $\mu$ m was similar to an aerosol generated by a DeVilbiss 646 nebulizer.

The starting dose for allergen challenge was determined with the relation between SPT-threshold and the concentration of histamine causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>histamine), as described by Cockcroft et al.<sup>15</sup> Doubling doses of allergen were inhaled in 10-minute intervals until a fall in FEV<sub>1</sub> of 20% or greater (best of two measurements) relative to baseline FEV<sub>1</sub> (mean of three measurements within 5%) occurred 10 or 20 minutes after inhalation, as measured with a dry rolling-seal spirometer (SensorMedics BV, Bithoven, The Netherlands). If the FEV<sub>1</sub> dropped between 15% and 20%, the last dose of allergen was administered again. After the final dose of allergen, FEV<sub>1</sub> was measured every 10 minutes until 60 minutes ( $t = 60$  minutes) and at 90 minutes ( $t = 90$  minutes). On the day before allergen challenge, a control bronchial challenge was performed with three doses of diluent (PBS, 0.03% human serum albumin, and 0.5% phenol; ALK Benelux, Groningen, The Netherlands). The functional bronchial response to allergen was expressed as the percentage change from baseline FEV<sub>1</sub> corrected for variation in FEV<sub>1</sub> as found after control challenge. The area under the curve (AUC)<sup>21</sup> was calculated from a time of 1 minute to a time of 60 minutes (AUC<sub>1-60 min</sub>). The dose of inhaled allergen was expressed as the MA content (Der p 1 or Der p 2), and the cumulated dose of allergen causing a 20% fall in FEV<sub>1</sub> (PD<sub>20</sub>allergen) was calculated by interpolation of the logarithmic converted dose-response curve.<sup>21</sup>

### Bronchial histamine challenge

Bronchial histamine challenge was performed 48 hours before allergen challenge according to the 2-minute tidal breathing method described before.<sup>21</sup> A DeVilbiss 646 nebulizer was used, attached to a three-way valve box and calibrated to an output of 0.13 mg/minute (flow, 5.8 L/sec). Nonspecific bronchial responsiveness was expressed as the concentration of histamine diphosphate in PBS (range, 0.015 to 32 mg/ml) causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>histamine) as calculated by log-linear interpolation.

### Total and specific IgE

Serum samples were collected by venepuncture 24 hours before allergen challenge. Levels of total IgE were quantified by a binding assay and an inhibition assay as described before.<sup>8</sup> The results from the binding assay were used in case of low IgE levels (<150 IU/ml) and from the inhibition assay in case of high IgE levels ( $\geq 150$  IU/ml). Levels of specific IgE were measured with RASTs as described previously.<sup>8</sup> In the RAST, 20  $\mu$ g HDM extract and 1.0  $\mu$ g MA per test was bound to the solid phase (CNBr-activated Sepharose 4B; Pharmacia, Uppsala, Sweden). The amount of specific IgE was calculated by using a correction for possible differences in the amount of allergen bound.<sup>8</sup> In short, sera were tested in a dilution resulting in approximately half maximal binding of radioactivity (<sup>125</sup>I-sheep anti-human IgE) and with two additional dilutions of the solid phase (i.e., 1:3 and 1:9). RAST results were expressed as the mean of duplicate measurements in international units per milliliter by using an in-house standard with mouse/human chimeric antibodies directed to Der p 2 and HDM solid phase calibrated against the World Health Organization standard (detection level, 0.1 IU/ml).<sup>23</sup>

### IgG and IgG4

Levels of IgG (i.e., IgG1, IgG2, and IgG4) and IgG4 antibodies directed to Der p 1 and Der p 2 were determined in the serum samples mentioned above as described previously.<sup>24</sup> In short, 1  $\mu$ l serum samples were incubated overnight with protein-A (for determination of IgG1, IgG2, and IgG4) or anti-IgG4 solid phase (CNBr-activated Sepharose 4B; Pharmacia, Uppsala, Sweden) and radiolabeled Der p 1 or Der p 2. The amount of radioactivity bound was measured and read from a standard curve. Results were expressed in arbitrary units (AU) per milliliter (detection level, 1 AU/ml).

### Skin tests

On the day before bronchial allergen challenge, SPTs were performed in duplicate in a standardized fashion as described before.<sup>17</sup> Series of twofold dilutions of the allergen preparation used for bronchial allergen challenge were tested. Wheal-and-flare reactions were determined at 15 minutes. The SPT allergen threshold concentration (SPT-threshold) eliciting a wheal diameter of 3 mm was calculated by log-linear interpolation.<sup>17</sup>

ICTs with series of tenfold dilutions of the allergen preparation used for bronchial challenge were performed in 29 patients on the day before allergen challenge as described before.<sup>8</sup> The longest (A)

**TABLE IV.** Simple and multiple linear regression of early-phase bronchial responsiveness to allergen: Der p 1 versus Der p 2\*

| Independent variables          | Der p 1                           |         |         | Der p 2                           |         |         |
|--------------------------------|-----------------------------------|---------|---------|-----------------------------------|---------|---------|
|                                | B (SE)                            | $\beta$ | p Value | B (SE)                            | $\beta$ | p Value |
| (model:)                       | $(R^2 = 0.66, p < 0.001, n = 16)$ |         |         | $(R^2 = 0.62, p = 0.004, n = 11)$ |         |         |
| Constant                       | -0.59 (0.09)                      |         |         | -0.73 (0.30)                      |         |         |
| log PC <sub>20</sub> histamine | 0.90 (0.17)                       | 0.81    | <0.001  | 0.89 (0.23)                       | 0.79    | 0.004   |
| + log IgE anti-MA              | 0.005 (0.27)                      | 0.004   | 0.98    | -0.39 (0.23)                      | -0.34   | 0.12    |
| (model:)                       | $(R^2 = 0.66, p = 0.001, n = 16)$ |         |         | $(R^2 = 0.72, p = 0.006, n = 11)$ |         |         |
| + log ICT-threshold            | 0.07 (0.27)                       | 0.06    | 0.79    | 0.27 (0.24)                       | 0.23    | 0.30    |
| (model:)                       | $(R^2 = 0.67, p = 0.001, n = 15)$ |         |         | $(R^2 = 0.67, p = 0.01, n = 11)$  |         |         |
| + log SPT-threshold            | 0.18 (0.20)                       | 0.16    | 0.39    | 0.29 (0.35)                       | 0.18    | 0.43    |
| (model:)                       | $(R^2 = 0.68, p = 0.001, n = 16)$ |         |         | $(R^2 = 0.65, p = 0.02, n = 11)$  |         |         |
| + log BHRA-threshold           | -0.007 (0.14)                     | -0.09   | 0.62    | 0.12 (0.06)                       | 0.39    | 0.07    |
| (model:)                       | $(R^2 = 0.68, p = 0.001, n = 15)$ |         |         | $(R^2 = 0.83, p = 0.005, n = 9)$  |         |         |

The optimal regression model and resulting models after addition of an index of IgE-mediated immediate hypersensitivity as found in groups of patients challenged with Der p 1 or Der p 2.

IgE anti-MA, Specific IgE directed to the MA used; ICT-threshold, ICT allergen threshold; SPT-threshold, SPT allergen threshold; BHRA-threshold, BHRA allergen threshold.

\*Dependent variable: log PD<sub>20</sub>allergen.

and orthogonal (B) wheal-and-flare diameters were determined at 15 minutes, and mean diameters were calculated as  $(A+B) \div 2$ . The ICT allergen threshold concentration (ICTthreshold) eliciting a flare diameter of 10 mm was calculated by log-linear interpolation.<sup>8</sup>

## BHRA

Isolation and incubation of human leukocytes was performed as described before.<sup>8</sup> Standard series of 10-fold dilutions of Der p 1 or Der p 2 and sheep polyclonal anti-human IgE (100 ng/ml) (CLB SH25P01, Amsterdam, The Netherlands) were used. Histamine release in the supernatant of the samples was measured with an automated fluorometric method. After subtraction of spontaneous release, histamine release was calculated as the percentage of total cellular histamine content. The allergen threshold concentration (BHRA-threshold) for a positive response was the concentration eliciting 10% histamine release as found by log-linear interpolation.<sup>8</sup> BHRA-thresholds below measurement limits were extrapolated from the dose-response curve, with resulting values not exceeding one additional dilution step ( $n = 4$ ).

## Statistical analysis

Data analysis was performed with SPSS 6.1 (Chicago, Ill.). Where applicable, log-transformation was used to obtain normal distribution of data. Relations were determined by using correlation (Pearson's  $r$ ) and multiple linear regression. All  $p$  values are two-tailed.

## RESULTS

All patients tolerated the allergen inhalation procedure without complication. Three patients were excluded from analysis because no immediate response with a fall in FEV<sub>1</sub> of 20% or greater was found after inhalation of the highest dose of histamine (patients 28 and 29) or allergen (patients 29 and 30), and thresholds could not be calculated accurately. In the remaining 27 patients variability in the dimension of the EAR was found to be small as a result of the standardized allergen challenge procedure (mean  $\Delta$ FEV<sub>1max</sub>, -31.9% [SD, 8.9%]; mean AUC<sub>EAR</sub>, 24.0 [SD, 9.5]). The level of IgE directed to the MA used for bronchial challenge was 3 IU/ml or greater in all patients analyzed (median IgE

anti-MA, 10.5 IU/ml [range, 3.0 to 75.5 IU/ml]) (Table II). Low levels of IgG and IgG4 directed to Der p 1 were detected in 12 of 27 and three of 27 patients, respectively (mean IgG, 1.5 AU/ml [SD, 0.4 AU/ml]; mean IgG4, 1.3 AU/ml [range, 1.1 to 1.6 AU/ml]). No detectable levels of IgG and IgG4 directed to Der p 2 were found.

## Bivariate correlations

For analysis of bivariate correlations, all allergen thresholds as found in the bronchial challenge test, skin tests, and the BHRAs were corrected for the difference in biologic activity on weight basis between Der p 1 and Der p 2 (factor 2.7). A highly significant correlation was found between the bronchial responsiveness to allergen (log PD<sub>20</sub>allergen) and the level of nonspecific bronchial hyperresponsiveness (log PC<sub>20</sub>histamine;  $r = 0.81$ ,  $p < 0.001$ ,  $n = 27$ ; Fig. 1, A). In contrast, rather weak correlations were found between PD<sub>20</sub>allergen and the various indices of IgE-mediated immediate hypersensitivity as analyzed in this study: the amount of specific IgE directed to MA (log IgE anti-MA,  $r = -0.36$ ;  $p = 0.07$ ,  $n = 27$ ; Fig. 1, B), the ICT threshold (log ICT-threshold,  $r = 0.49$ ;  $p = 0.01$ ,  $n = 26$ ; Fig. 1, C), and the SPT threshold (log SPT-threshold,  $r = 0.36$ ;  $p = 0.07$ ,  $n = 27$ ; Fig. 1, D). In the BHRA, three of the 27 patients analyzed were found to be nonresponders (Table II).<sup>25</sup> In the remaining patients a weak relationship was found between PD<sub>20</sub>allergen and the allergen threshold in this assay (log BHRA-threshold,  $r = 0.37$ ;  $p = 0.08$ ,  $n = 24$ ; Fig. 1, E).

Associations between the allergen thresholds as found in skin tests and BHRAs and levels of specific IgE directed to MA (log IgE anti-MA) were analyzed for all 30 patients tested. The resulting bivariate correlations were found to be in the same order of magnitude as described in the previous study by Witteman et al.<sup>8</sup> (log ICT-threshold:  $r = -0.62$ ,  $p < 0.001$ ,  $n = 29$ ; log SPT-threshold,  $r = -0.52$ ,  $p = 0.003$ ,  $n = 30$ ; and log BHRA-threshold,  $r = -0.52$ ,  $p = 0.006$ ,  $n = 27$ ).

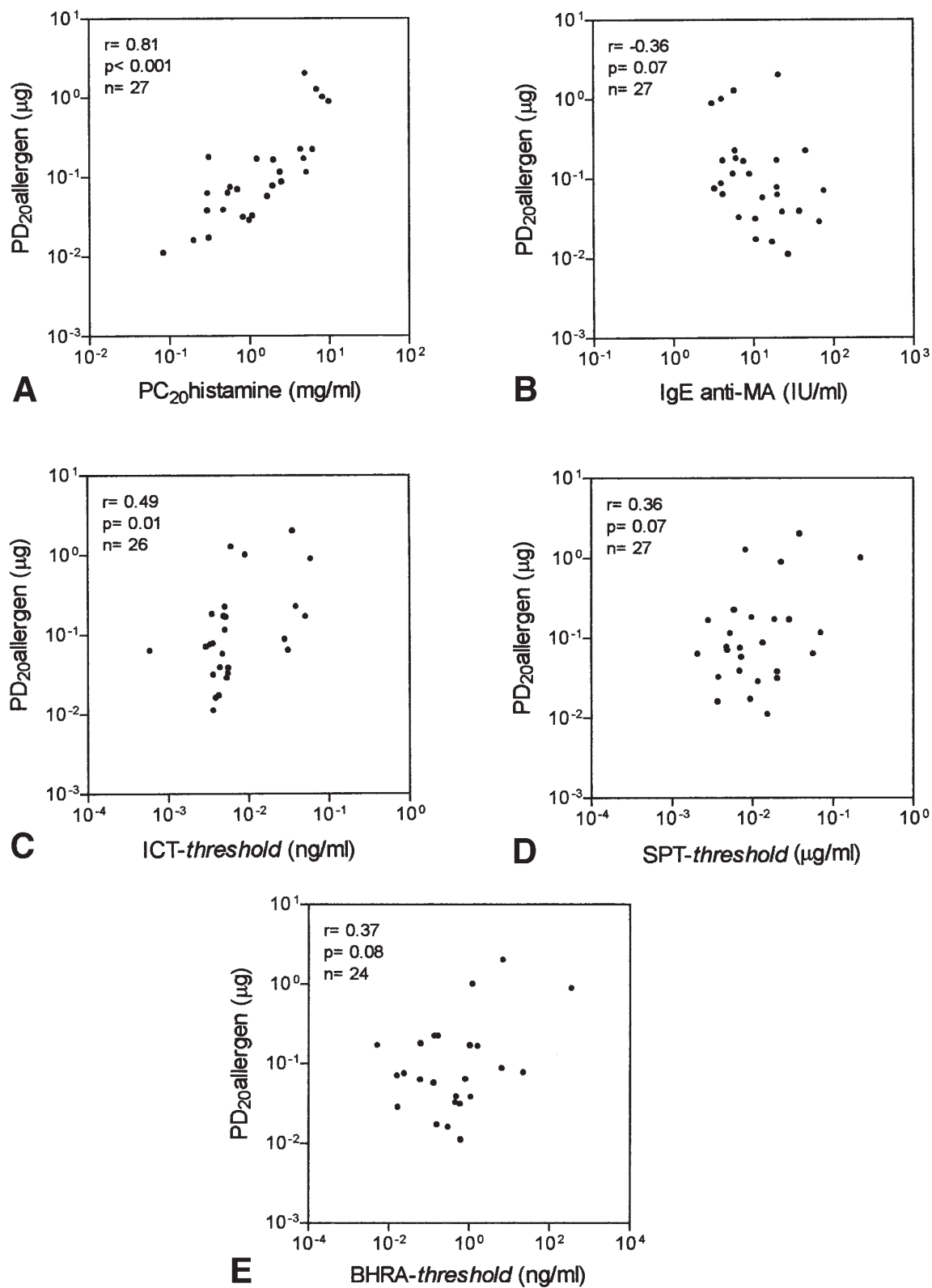


**FIG. 1.** Bivariate correlation between bronchial responsiveness to allergen ( $PD_{20}$ allergen) and nonspecific bronchial hyperresponsiveness ( $PC_{20}$ histamine) (**A**), specific IgE directed to MA (IgE anti-MA) (**B**), and thresholds for positive response in skin tests (ICT-threshold [**C**] and SPT-threshold [**D**]) and BHRA (BHRA-threshold [**E**]).  $PD_{20}$ allergen, ICT-threshold, SPT-threshold, and BHRA-threshold are corrected for difference in biologic activity between Der p 1 and Der p 2 (see text for details).

## Multiple linear regression

The results of linear regression of PD<sub>20</sub>allergen on PC<sub>20</sub>histamine are shown in Table III. As a result of the difference in biologic activity between Der p 1 and Der p 2, addition of the type of allergen to the models was found to contribute significantly to the prediction of PD<sub>20</sub>allergen. In contrast, addition of an index of allergic sensitivity in vitro or in vivo (IgE anti-MA, ICT-threshold, SPT-threshold, and BHRA-threshold) did not contribute to prediction of variation of PD<sub>20</sub>allergen (Table III). When groups of Der p 1- and Der p 2-challenged patients were analyzed separately, similar results were found (Table IV).

Inclusion of the level of total IgE, specific IgG, or baseline lung function (FEV<sub>1</sub> percent predicted) in the models presented in Tables III and IV did not contribute to the explanation of variation in PD<sub>20</sub>allergen (results not shown).

## DISCUSSION

In this study the role of patient-dependent factors in the EAR after allergen inhalation was investigated quantitatively. For this purpose, isolated MAs of *D. pteronyssinus* were applied in the technique of bronchial inhalation allergen challenge as an optimal model for strictly allergen-induced IgE-mediated bronchial reactions.

Bronchial responsiveness to allergen was expressed as the cumulative dose of allergen resulting in a fall of 20% of baseline lung function (FEV<sub>1</sub>) as calculated by interpolation of the logarithmic converted dose-response curve (PD<sub>20</sub>allergen).<sup>21</sup> PD<sub>20</sub>allergen was found to be strongly related to the degree of nonspecific bronchial hyperresponsiveness (PC<sub>20</sub>histamine). In contrast, marginally or nonsignificant relationships were found between PD<sub>20</sub>allergen and the level of allergen-specific IgE (IgE anti-MA) or allergen thresholds for a positive response in skin tests (ICT-threshold and SPT-threshold) and BHRAs (BHRA-threshold). Bronchial responsiveness to allergen differed by as much as a factor of 100 at the same degree of allergic sensitivity. Moreover, addition of one of the indices of IgE-mediated hypersensitivity in vitro and in vivo to a multiple linear regression model of PD<sub>20</sub>allergen on PC<sub>20</sub>histamine did not contribute significantly to the explanation of variation in bronchial responsiveness to allergen. A 10-fold difference in allergic sensitivity (e.g., specific IgE level) resulted in less than a factor 1.5 difference in PD<sub>20</sub>allergen.

Because the presence of specific IgE is a prerequisite for the EAR, these findings indicate that the influence of the amount of specific IgE in early-phase bronchial responsiveness to allergen levels off at rather low levels of antibody. In the patients studied there was a weak relationship between allergic responsiveness as measured with RAST and skin tests and bronchial responsiveness to allergen. However, in multiple linear regression, allergic parameters did not contribute to the prediction of the bronchial allergic response. This apparent paradox may be caused by the association of nonspecific bronchial hyperresponsiveness and allergic parameters. An indirect contribution of allergic responsiveness to nonspecific bronchial hyperreactivity is a likely explanation. However, there appears to be no direct

contribution of allergic parameters to the prediction of bronchial responsiveness to allergen.

In our outpatient population of asthmatic patients allergic to HDMs, 81% had 3 IU/ml or greater of specific IgE directed to HDM allergen. Hence the results shown here apply to the majority of patients with asthma allergic to HDMs. The ranges of specific IgE, skin test allergen thresholds, and degrees of specific and nonspecific bronchial hyperresponsiveness were all found to be approximately the same orders of magnitude. Therefore low variance in the data sets used cannot explain the lack of contribution of the indices of allergic sensitivity to the prediction of bronchial sensitivity to allergen.

The results of this study confirm the strong relationship between specific and nonspecific bronchial hyperresponsiveness as found by others.<sup>15,26,27</sup> Recently, it was shown, in a large group of 111 patients with asthma or rhinitis, that differences in specific bronchial reactivity to *D. pteronyssinus* extracts were almost completely attributable to differences in nonspecific hyperresponsiveness.<sup>28</sup> In a study by Cockcroft et al.,<sup>15</sup> a relationship was developed for the prediction of bronchial responsiveness to allergen, taking into account both the degree of nonspecific bronchial hyperresponsiveness and skin sensitivity to allergen (i.e., SPT allergen threshold). In this study, however, no contribution of an index of allergic sensitivity to the model consisting of PC<sub>20</sub>histamine was found. This discrepancy can be explained by differences in methodology (i.e., the use of different types of allergen extracts with unknown allergenic potency vs well-characterized isolated MAs). In addition, as described above, the selection of atopic individuals could have influenced the results.

Allergen thresholds are inversely related to the potency of the allergen source (i.e., the concentration of relevant allergen).<sup>7,8</sup> When using various allergen sources with different allergenic potency, the range of allergen thresholds will increase for both skin tests and bronchial allergen challenges. As a result, the association between skin sensitivity and bronchial responsiveness to allergen will be improved, per se, independent of patient characteristics. The prediction of bronchial responsiveness to allergen from skin test results may still be valuable when an allergen extract with unknown potency is used. The information that is provided by this relation, however, will predominantly be determined by characteristics of the allergen extract and less so by patient-dependent factors such as allergic sensitivity. The influence of allergenic potency on the relation between allergen thresholds was indicated in this study by the significant contribution of the type of allergen to the regression model for prediction of PD<sub>20</sub>allergen by PC<sub>20</sub>histamine. From the relation between specific IgE and skin test results, we found a significant difference in allergenic potency between the MAs used, with Der p 2 being a factor 1.9 to 3.5 more biologically active on a weight basis, which is in accordance with our previous report.<sup>8</sup> It should be emphasized, however, that the comparison of biologic activity of Der p 1 and Der p 2 is highly dependent on the estimation of the weight concentrations of MAs in the HDM standard used. In this study we applied the estimations for the World

Health Organization standard as reported by Platts-Mills et al.<sup>18</sup> Recently, however, alternative estimations were published by Yasueda et al.,<sup>29,30</sup> which would have resulted in a smaller difference in biologic activity between Der p 1 and Der p 2. Moreover, it remains to be established whether weight concentrations as opposed to molar concentrations are more appropriate for expressing biologic activity. In view of Der p 1 being approximately twice as large compared with Der p 2, the findings in this and our previous study<sup>8</sup> may suggest that molar concentrations are more relevant for expressing biologic activity.

As a consequence of the insignificant role of the level of allergic sensitivity in the EAR as found in the group of patients analyzed in this study, the role of factors like allergen-specific IgG<sup>24</sup> or the level of total IgE,<sup>8</sup> which potentially interfere with the activity of specific IgE antibodies, remains obscured. Moreover, in this study the levels of IgG directed to Der p 1 and Der p 2 induced by natural exposure were low compared with the levels of IgG directed to Fel d 1 that were found to inhibit early-phase allergic responses in the skin.<sup>24</sup>

In summary, the quantitative relationship between patient characteristics and the airway responsiveness to allergen was investigated by using bronchial allergen inhalation challenges with isolated MAs of *D. pteronyssinus*. We found that the allergen threshold for the EAR was highly dependent on the degree of nonspecific bronchial hyperresponsiveness. In contrast, no significant contribution of the level of specific IgE or the level of allergenic sensitivity in the skin to the degree of early-phase bronchial responsiveness to allergen was found. We conclude that in asthmatic patients allergic to HDMS, the degree of allergic sensitization is not an important determinant of the EAR after allergen inhalation.

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