

Bronchial Challenge Test in Asthmatics Sensitized to Mites: Role of Particle Size in Bronchial Response

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ABSTRACT

Although major house dust mite allergen (Der p 1) is carried mainly on large particles ($>10\ \mu\text{m}$), standard bronchial challenge tests (BCT) use nebulizers that deliver smaller particles (sizes from 1 to 5 μm) and may therefore not reflect actual domestic exposure. The objective of this study was to evaluate the influence of particle size of *Dermatophagoides pteronyssinus* extract on bronchial response. Specific BCT were performed with different mass median aerodynamic diameters (MMAD): 1.1, 5.6, and 9.7 μm . Each of the 19 mite-sensitized patients underwent mite BCT three times, once with each nebulizer. IL-5 levels were assessed in induced sputum and blood samples. The PD₂₀ for Der p 1 differed substantially with particle size, with less Der p 1 (11.2 ng) needed to produce a PD₂₀ with the largest particles (9.7 μm), compared to 18.1 ng for the 5.6 μm particles and 142.5 ng for the 1.1 μm particles ($p < 0.0001$). Large particles also induced an early phase response significantly more often than small particles (100% vs. 63%). Although the late phase reaction (LPR) frequency was similar with all three particle sizes, lower mean oral corticosteroid doses were needed to treat LPR with the largest particles (23 mg), compared to the smaller particles, with 34 mg for the 5.6 μm particles and 51 mg for the 1.1 μm . The 1.1 μm particles produced a significantly greater increase in IL-5 concentrations in sputum and blood compared to the larger particles. Large particles clearly play a role in the immediate bronchial response in asthmatics sensitized to mites and, therefore, should be included in pharmacological studies in humans.

Key words: bronchial challenge test, particle size, house dust mite, asthma

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INTRODUCTION

THE ALLERGEN BRONCHIAL CHALLENGE TEST (BCT) is a useful clinical model to investigate allergic asthma and to assess drug effectiveness on bronchial reactivity. However, its clinical relevance is controversial.^(1,2) Indeed, it is important that a BCT reflects as closely as possible patient's natural allergen exposure. However, the final site of allergen deposition in the airways during natural exposure is not known with certainty, and it has been assumed that allergen is deposited at the same site during BCT.

International guidelines offer no indications about the size of the nebulized aerosol droplets to be used in BCT.^(3,4) Since Tiffeneau,⁽⁵⁾ bronchial challenge testings are conducted with particles of diameter varying from 1 to 4 μm , which are thought to be deposited in the peripheral airways.^(3,6) We know, however, that allergens, including cat and house dust mite allergens, are inhaled on particles of a wide range of size, including large ($\geq 10 \mu\text{m}$) as well as small particles ($\leq 5 \mu\text{m}$).⁽⁷⁻⁹⁾ The particles used for BCT, therefore, may not correspond to natural allergen exposure, and responses to aeroallergens during BCT may differ greatly from that occurring naturally. In agreement with this hypothesis, the dose of cat allergen required to induce symptoms during a standard BCT [with a nebulizer releasing particles with a mass median aerodynamic diameter (MMAD) ranging from 3 to 5 μm] was reported to be 5 to 300 times higher than the dose needed to induce symptoms at home.⁽¹⁰⁾

To determine whether particle size may influence the bronchial response to allergen, cat BCTs were performed in intermittent asthmatics sensitized to cats using three different aerosols delivering particles with MMAD of 1.4, 4.8, or 10.3 μm . Our results showed that the dose of Fel d 1 (major cat allergen) inducing an immediate bronchial response was inversely correlated with the size of particles, and that the Fel d 1 PD₂₀ obtained with particles of 10.3 μm diameter (large particles) closely approached the allergen levels that provoke symptoms at home.⁽¹¹⁾ These results suggested that large particles play an important role in the immediate response to cat allergens. They are, however, in conflict with the idea that the rapidity of bronchial symptoms to cat allergens are due to the fact that natural cat allergens are carried by a high proportion of small particles ($40\% < 5 \mu\text{m}$), which can enter deeply in the airways.⁽¹²⁾

House dust mites (HDMs) are another major indoor allergen, whose airborne behavior differs greatly from that of cat allergen. Indeed, the major HDM allergen, Der p 1, is associated with large particles; after disturbance, more than 80% of the allergen is associated with particles with a MMAD greater than 10 μm .^(8,9) We therefore sought to determine whether HDM allergen, despite its different aerodynamic properties when compared to cat allergens, would confirm the importance of large particles in the immediate response to allergens. To do so, we evaluated, in the present study, the influence of allergen particle size on the immediate and late bronchial response to *Dermatophagoides pteronyssinus* (Dpt) in intermittent asthmatics sensitized to HDMs.

METHODS

Nebulizers

Laser particle size analysis (MasterSizer X; Malvern Instruments Ltd, Worcestershire, UK) was used to measure the MMAD of Dpt extract (Allerbio, Varennes, France, lot 352 A 02) with the three air-driven nebulizers (Microcirrus and Cirrus by Intersurgical Laboratories, Fontenay-sous-bois, France, and Pari Tia by the Pari Aerosol Research Institute, Munich, Germany) as previously described.⁽¹³⁾ The input airflow pressure was adjusted to obtain the chosen MMAD (2.5 bars for Microcirrus nebulizer and 1.2 for the two others). The three air-driven nebulizers, Microcirrus, Cirrus, and Pari Tia, delivered particles with a MMAD of 1.1 (geometric Standard Deviation 1.95), 5.6 (gSD 2.00), and 9.7 μm (gSD 2.05), respectively; $n = 30$ assays for each model).

Subjects

We studied 19 volunteers with a history of intermittent asthma (step 1 of the GINA – Global Initiative for Asthma) having a positive skin prick test to Dpt extract and specific IgE to Dpt higher than class 2. Only short-acting β -agonists used as needed were allowed during the study period. No subject had had a respiratory tract infection in the preceding 2 weeks. Subjects sensitized to seasonal (pollens) allergens were studied outside the relevant pollen season and those sensitized to cat or dog allergens were included only if there was no pet at home. Home exposure to Der p 1 was measured in dust from subjects' mattress and floor.

Study design

This randomized, crossover study was approved by the local ethics committee. During the screening day, skin prick tests to the most common aeroallergens, specific IgE measurement to Dpt, clinical examination, and pulmonary function tests were performed. Each volunteer underwent HDM-BCT on three separate occasions, each with a nebulizer generating a different particle size. The nebulizer sequence was randomly selected. A wash-out period of 3 weeks was observed between the HDM-BCTs. The volunteers were clinically observed until 6 h post-HDM-BCT, during which time pulmonary function tests were performed every hour with a spirometer (Spiro[®]dyn'R, Dyn'R, Muret, France) to measure the FEV₁, FVC, PEF, and FEF₂₅₋₇₅. Appropriate medication was given if it was judged necessary by a physician who was blinded for the nebulizer used for BCT. Three hours after HDM-BCT, if a decrease in FEV₁ $\geq$ 15% was measured, subjects took 200 μ g of salbutamol with a metered dose inhaler (MDI). After 15 min, the response was determined. After 1 h, if a fall in FEV₁ $\geq$ 15% was observed again, subjects were first given 2 puffs of salbutamol MDI, and if no improvement of at least 10% in FEV₁ was obtained, then 40 mg of oral corticosteroid (prednisolone) was given. Subjects were allowed to go home if the drop in FEV₁ was less than 15%. If they had symptoms during night time with a fall in PEF of more than 15% and which persisted even after taking 200 μ g of salbutamol, volunteers were asked to contact the physician who decided whether to repeat the dose of corticosteroid. The Borg scale to assess dyspnea was completed before each HDM-BCT, at the end of the test, and 6 and 24 h later. Peak flow measurements were recorded by subjects when they went to bed and when they awoke the day after the HDM-BCT.

The Methacholine Bronchial Challenge Test was performed 24 h before each HDM-BCT to assess the nonspecific bronchial hyper-reactivity (NSBHR) as previously described.⁽¹⁴⁾ A doubling cumulative dose of methacholine solution (1%), from 25 to 3200 μ g, was delivered after the saline solution with a dosimeter (Mediprom FDC 88, Paris, France).

Inhalation took place through a mouthpiece, with the nose clipped, using a nebulizer (Micro Mist[®], Hudson Respiratory Care Inc., Temecula, CA). FEV₁ was measured 2 min after the saline

inhalation and 2 min after each concentration of methacholine. The test stopped when FEV₁ had decreased by at least 20% from its postsaline value. The results were expressed as the challenge dose inducing an FEV₁ decrease of at least 20% after the dose-response curve to methacholine was constructed (PD₂₀).

House Dust Mite Bronchial Challenge Tests (HDM-BCT) were performed with an automatic inhalation-synchronized jet-nebulizer dosimeter (Spira Elektro2, Hameenlinna, Finland) as previously described.^(11,14) The nebulization time was set at 0.8 sec. The mean volume ($\pm$ standard deviation) delivered per puff by each device was 3.3 ± 0.3 μ L, 3.7 ± 0.3 μ L, and 15.3 ± 1.3 μ L for the 1.1 μ m, 5.6 μ m, and 9.7 μ m aerosol droplets, respectively. Accordingly, the house dust mite allergen was diluted 1:4 for the 9.7- μ m device to obtain the same amount of Der p 1 per puff with the three nebulizers. The Dpt extract contained 2.43 μ g of Der p 1 per mL (Indoor Biotechnology, Charlottesville, VA). The starting concentration for HDM-BCT was one-tenth of the cutaneous end-point titration obtained by the prick test for each volunteer. The subject inhaled from residual volume to total lung capacity and held their breath for 2 sec. A separate flow meter monitored subject's inspiratory flow rate. The dose of inhaled allergen was progressively increased for each concentration by doubling the number of inhalations (2, 4, 8, and 16 puffs). After administering 16 puffs, the concentration was increased 10-fold. The procedure was repeated until the 100 Reactivity Index [RI] concentration of mite allergen was reached for the 1.1- and 5.6- μ m nebulizers and the 25RI concentration (1:4 dilution of the 100RI solution) for the 9.7 μ m nebulizer. The lung function test was performed with a spirometer according to the criteria for reproducible values of the ATS/ERS before BCT, after inhalation of the saline solution and 15 min after inhalation of increasing doses of the mite allergen solution. The test was stopped when the FEV₁ decreased by at least 20% of the baseline value or when the full dose (16 puffs of the highest concentration) was administered without such a drop. The provocation dose inducing a 20% decrease in FEV₁ (PD₂₀) was calculated by linear interpolation on the dose-response curve. After the end of HDM-BCT, flow volume curves were recorded hourly for 6 h and at 24 h. The late phase reaction (LPR) was defined as a fall $\geq$ 15% in the FEV₁, the PEF, or both⁽¹⁵⁾ after 4-6 h following BCT.

Induced sputum was obtained 24 h before and 24 h after the HDM-BCT according to the method described by Pin and coworkers.⁽¹⁶⁾ Cell plugs were selected from the sample, separated from saliva, weighed, and dispersed by addition of 4 volumes of 0.1% dithiothreitol (DTT, Sigma Pharmaceuticals, Saint Quentin Fallavier, France) and processed as already described.⁽¹⁶⁾ Filtered sputum was centrifuged at 1200 rpm for 15 min to separate cells from the fluid phase. Fluid phase was stored at -80°C until eosinophil cationic protein (ECP) and interleukin (IL)-5 assays could be carried out.

Cell counts in sputum: after cytocentrifugation (Shandon III cytocentrifuge, Shandon Ltd, Runcorn, Cheshire) of 60 μL of cell suspension at 450 rpm for 4 min, slides were prepared with May Grünwald Giemsa stain. Differential cell count was performed on two slides by counting at least 400 nucleated nonsquamous cells per slide.

Eosinophil cationic protein assay in fluid phase of induced sputum and serum was obtained using a commercially available specific double-antibody radioimmunoassay (Pharmacia Diagnostics, Saint-Quentin, France), as previously described.⁽¹⁴⁾ The interassay coefficient was 2.3% for 5 $\mu\text{g L}^{-1}$ and 5.8% for 15 $\mu\text{g L}^{-1}$.

Interleukin-5 assay in induced sputum and serum was obtained with a Cytomics FC 500 flow cytometer (Beckman CoulterTM), Palo Alto, CA) and the BD CBA Analysis Software. IL-5 secreted by Th2 cells, eosinophils and mast cells has been implicated in allergic inflammation, asthma and airway remodeling.⁽¹⁷⁾ IL-5 concentrations were measured using a specific capture antibody adsorbed on microspheres and a secondary PE-conjugated detection antibody to form sandwich complexes (BD Human Th1/Th2 Cytokine CBA Kit, BD Biosciences Pharmingen, San Diego, CA). Quantification was obtained by comparison to a

TABLE 1. VOLUNTEER CHARACTERISTICS

Subject no.	Age (year)	Sex	Smoking habit	Prick-tests to common aeroallergens	Specific IgE to HDM	Der p 1 level in floor dust ($\mu\text{g g}^{-1}$) ^a	Der p 1 level in mattress dust ($\mu\text{g g}^{-1}$) ^a	Preinclusion FEV ₁ (% of predicted value)	Preinclusion FEF ₂₅₋₇₅ (% of predicted value)
1	31	F	No	Dp, Df	42.0	<0.03	0.62	95	61
2	24	M	No	Dp, Df, cat, dog, grass pollens	100.0	7.41	IV	76	52
3	18	M	Two a day	Dp, Df, grass pollens	100.0	<0.03	<0.03	79	87
4	18	F	No	Dp, Df, grass pollens	28.6	<0.03	IV	108	82
5	21	F	No	Dp, Df, cat, grass pollens	52.8	0.037	IV	92	68
6	23	F	No	Dp, Df, grass pollens	11.0	<0.03	IV	106	88
7	34	F	Five a day	Dp, Df, cat, dog, grass pollens	9.9	<0.03	<0.03	91	66
8	22	F	No	Dp, Df	32.5	IV	IV	98	69
9	20	M	Five a day	Dp, Df, grass pollens	33.2	<0.03	0.52	83	62
10	27	F	No	Dp, Df, cat, dog	25.1	0.09	<0.03	77	49
11	25	F	No	Dp, Df	15.7	IV	IV	111	79
12	22	M	No	Dp, Df, cat, tree pollens	4.4	<0.03	<0.03	88	56
13	23	M	No	Dp, Df, cockroach	48.0	<0.03	<0.03	89	73
14	29	M	No	Dp, Df, cat, tree pollens	13.4	IV	0.20	96	77
15	34	F	No	Dp, Df	68.7	<0.03	<0.03	88	65
16	20	F	No	Dp, Df, cat	22.9	9.44	1.29	112	88
17	48	M	No	Dp, Df	2.7	0.45	2.49	85	54
18	23	M	No	Dp, Df, cat, dog, grass and tree pollens	18.9	0.06	0.38	93	63
19	20	F	No	Dp, Df, cat, dog, grass and tree pollens	100.0	<0.03	0.72	78	48
Mean (SD)	25.4 (7.3)				38.4 (32.2)	0.928 (2.665)	0.332 (0.629)	91.8 (11.3)	67.7 (13.0)
Median	23				28.6	<0.03	<0.03	91	66

Dp, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*; F, female; M, male; ND, not done; IV, insufficient volume of dust to carry out Der p 1 dosage.

^a0.03 $\mu\text{g g}^{-1}$ was the detection limit for dust Der p 1 concentration.

standard curve. This test was sensitive above 2.4 pg of IL-5 per mL. The intraassay coefficient was 5.6%.

Statistical analysis

Repeated-measures ANOVA compared the baseline FEV₁, FEF_{25–75}, PEF, dyspnea scale, eosinophil count, IL-5, and ECP concentrations in blood and sputum before the three mite BCTs (SPSS, version 11.01). It also compared FEV₁, FEF_{25–75} and PEF results, eosinophil counts, IL-5, and ECP concentrations at the different time points (3, 6, and 24 h) after BCT between the three aerosol particle sizes. The PD₂₀ for methacholine and Der p 1 after logarithmic transformation were also compared using ANOVA. Comparison between IL-5 levels before and after HDM-BCT in induced sputum was performed using a Wilcoxon's signed ranks test. The frequencies of negative HDM-BCT (i.e., when the 20% in FEV₁ fall was not reached after inhalation of the highest dose of allergen) were compared between the three particle sizes with the Yates chi-square test. The significance level was set at $p \leq 0.05$.

RESULTS

Subjects

The study included 19 intermittent asthmatic subjects allergic to *Dermatophagoides pteronyssinus*: 11 women and 8 men, median age 23 years (Table 1). All volunteers gave a written informed consent. Among the 19 subjects, 16 were non-smokers and 3 smoked less than five cigarettes a day. They did not differ from the other non-smokers in pulmonary functions (FEV₁, $p = 0.931$) or NSBHR ($p = 0.646$). The mean value ($\pm$ standard deviation) of subjects' HDM exposure at home was 0.928 (± 2.665) $\mu\text{g g}^{-1}$ for floor dust and 0.332 (± 0.629) $\mu\text{g g}^{-1}$ for mattress dust.

Baseline spirometry, eosinophil count, and inflammatory markers

The subjects did not differ significantly either for any of the respiratory variables measured at baseline: dyspnea (Borg scale $p = 0.359$), PEF ($p = 0.790$), FEV₁ ($p = 0.231$), FEF_{25–75} ($p = 0.589$) or for the eosinophil counts ($p = 0.253$) and ECP concentrations ($p = 0.485$) in blood.

TABLE 2. METACHOLINE PD₂₀ OBTAINED 24 H BEFORE EACH HOUSE DUST MITE BRONCHIAL CHALLENGE TEST AND DERP P 1 PD₂₀ OBTAINED DURING SPECIFIC BRONCHIAL CHALLENGE WITH THE THREE NEBULIZERS FOR THE 19 SUBJECTS

Subject no.	1.1 μm		MMAD 5.6 μm		9.7 μm	
	Metacholine		Metacholine		Metacholine	
	PD ₂₀ (μg)	Der p1 PD ₂₀ (ng)	PD ₂₀ (μg)	Der p1 PD ₂₀ (ng)	PD ₂₀ (μg)	Der p1 PD ₂₀ (ng)
1	500	233.8	291	15.2	364	19.7
2	180	299.7* (16%)	2667	63.0	136	27.8
3	343	234.6	487	31.5	467	8.1
4	138	299.7* (6%)	83	63.4	193	23.0
5	57	30.0	83	1.7	60	3.7
6	117	114.7	633	14.2	200	35.5
7	58	38.6	40	2.7	40	2.7
8	167	279.1	250	12.0	179	7.8
9	1867	299.9* (13%)	250	27.87	340	27.1
10	179	98.9	213	7.3	150	2.1
11	2800	299.7* (13%)	1959	126.4	3200	103.1
12	1842	299.7* (11%)	250	40.9	1138	25.8
13	900	299.7* (17%)	300	20.5	600	13.2
14	3200	299.7* (15%)	1416	76.6	2171	54.1
15	91	40.7	119	5.8	58	3.7
16	680	104.4	300	7.8	900	7.2
17	140	47.7	184	29.7	75	49.7
18	3200	282.9	880	48.7	1120	22.8
19	39	33.3	32	4.8	58	0.9
GM	342.5	142.5	249.1	18.1	273.1	11.2
95% CI	0–847.2	90.4–194.6	24.6–473.6	3.6–32.6	0–643.7	4.1–18.3

*Subjects with a negative HDM-BCT. The % drop in FEV₁ obtained at the end of BCT is indicated in parentheses.

Nonspecific bronchial hyperreactivity (NSBHR)

Geometric mean values in PD₂₀ methacholine evaluated 24 h before each HDM-BCT did not differ between allergen challenges ($p = 0.161$), averaging 342.5 mg, 249.1 mg, and 273.1 mg of methacholine on each of three study days, respectively (Table 2).

Early phase reaction: PD₂₀ Der p 1

The geometric mean value of PD₂₀ for Der p 1 was 142.5 ng for the 1.1 μ m aerosol, 18.1 ng for the 5.6 μ m, and 11.2 ng for the 9.7 μ m ($p < 0.0001$) (Table 2). Seven subjects (37%) had negative HDM-BCT when inhaling 1.1 μ m diameter particles but none had negative HDM-BCT after inhaling the 5.6- and 9.7- μ m particles. So, the early phase reaction occurred significantly less often with the small (1.1 μ m) than with the large particles (5.6 and 9.7 μ m) ($p < 0.001$). However, at

the end of the HDM-BCT, neither FEV₁ ($p = 0.163$), PEF ($p = 0.156$) nor FEF₂₅₋₇₅ ($p = 0.253$) differed between the three particle sizes.

Late phase reaction (LPR)

LPR (fall $\geq 15\%$ in the FEV₁ and/or the PEF) occurred in 65% of the subjects. Among the seven subjects with a negative BCT with 1.1 μ m particles, five subjects had an isolated LPR (occurrence of late phase symptoms following an allergen exposure that did not provoke an immediate response). In contrast, with 5.6- and 9.7- μ m particles, no isolated LPR was observed (Table 3). Neither the FEV₁ ($p = 0.521$), the PEF ($p = 0.407$), nor the FEF₂₅₋₇₅ ($p = 0.743$) differed significantly at 1, 2, 3, 4, 5, 6, or 24 h after the HDM-BCT between the three particle sizes. Nevertheless, after the 1.1 μ m particles, seven subjects required oral corticosteroids 5 or 6 h after HDM-BCT, com-

TABLE 3. OCCURRENCE OF LATE PHASE REACTION AFTER HOUSE DUST MITE BRONCHIAL CHALLENGE TEST AND ORAL CORTICOSTEROID NEEDED TO REVERSE IT ACCORDING TO PARTICLE SIZE INHALED DURING THE TEST

Subject no.	1.1 μ m		MMAD 5.6 μ m		9.7 μ m	
	Maximal fall in FEV ₁ (%)	Oral corticosteroid ^a (mg)	Max fall in FEV ₁ (%)	Oral corticosteroid ^a (mg)	Max fall in FEV ₁ (%)	Oral corticosteroid ^a (mg)
1	20*	40	22*	0	16**	0
2	18*, isolated LPR		20**		15*	
3	15**		22*		22**	
4	15*, isolated LPR		23**		16*	
5	21*	40	19*	40	23*	80 ^b
6	18*		12		21*	
7	11		19*		14	
8	20*	40	21*	0	12	0
9	15*, isolated LPR		11		16*	
10	29*	40	20*	0	22*	40
11	20**, isolated LPR		12		10	
12	5		13		7	
13	31*, isolated LPR	120 ^b	22*	80 ^b	17*	40
14	8		8		5	
15	2		15*		16*	
16	18*	40	28*	40	19*	0
17	7		15**		3	
18	2		6		2	
19	34*	40	15*	80 ^b	9	0
Frequency of LPR	13/19		13/19		11/19	
Total (mg)		360		240		160
Mean value (mg) (SD)		51.4 (30.2)		34.3 (36.0)		22.86 (31.5)

Late phase reaction defined as a fall in the FEV₁ (*) or PEF (**).

^aOral corticosteroid was taken 5 or 6 h after BCT.

^bA second dose of corticosteroid was taken during the night.

pared with only four and three subjects for the 5.6- and 9.7- μm particle sizes, respectively. The mean ($\pm$ standard deviation) oral corticosteroid dose administered to subjects in the 24 h following the allergen challenge was 51.4 ($\pm$ 30.2) mg with the 1.1 μm particles, which was higher than 34.3 ($\pm$ 36.0) mg with the 5.6 μm , and 23.9 ($\pm$ 31.5) mg with the 9.7 μm particles. The mean corticosteroid dose remained higher for the 1.1 μm particles, even after removing subject #13, who required 120 mg after the 1.1 μm BCT, with 40.0 $\pm$ 0.0 mg, 26.6 $\pm$ 32.7 mg, and 20.0 $\pm$ 33.5 mg, respectively. Because of the very small number of subjects, no statistical analysis could be done. Borg scale did not differ during the 24 h after BCT according to particle sizes ($p = 0.965$).

Cell counts, IL-5, and ECP levels in serum and induced sputum

In the serum, IL-5 levels differed significantly according to the three particle sizes ($p = 0.015$) (Fig. 1). A twofold increase in the IL-5 level was observed 24 h after the HDM-BCT for 1.1 μm when compared to 9.7 μm particles: 37.6 ($\pm$ 37.3) and 13.1 ($\pm$ 11.1) pg mL^{-1} , respectively, while the IL-5 level was 28.5 ($\pm$ 43.6) pg mL^{-1} for the 5.6 μm particles. Moreover, a significant increase in serum ECP levels (43.9, 34.7, 38.0 $\mu\text{g L}^{-1}$, with the 1.1, 5.6, and 9.7 μm particles, respectively) was observed after HDM-BCT ($p < 0.0001$). There was no significant difference for these ECP levels and the eosinophil count for the three particle sizes ($p = 0.278$ and 0.655, respectively).

In induced sputum, IL-5 levels increased significantly 24 h later with 1.1 μm particles ($p =$

0.022) but not with 5.6- and 9.7 μm particles. The sputum eosinophil count (19, 14, and 21% with 1.1, 5.6, 9.7 μm , respectively) and ECP levels (277.7, 139.3, 273.0 $\mu\text{g L}^{-1}$) were significantly increased 24 h after BCT ($p = 0.005$ and 0.002, respectively) but no influence of the particle size was observed ($p = 0.084$ and 0.276, respectively).

DISCUSSION

Our results demonstrate that during house dust mite bronchial challenge tests in intermittent asthmatics sensitized to *Dermatophagoides pteronyssinus*, less Der p 1 was required to induce an immediate response when the allergen was associated with large (9.7 μm MMAD) than with small (1.1 μm MMAD) particles.

Although bronchial challenge tests are considered the "gold standard" to demonstrate a relation between allergen exposure and asthma symptoms, as well as the efficacy of antiasthma medications, their clinical relevance is still controversial.^(1,2) One major reason for this debate is the lack of knowledge about the site of allergen deposition in the bronchi during natural exposure. It is important that a BCT reflects as closely as possible patient's natural allergen exposure, and it has been logically assumed that allergen is deposited at the same site during BCT. Particles varying in diameter from 1 to 4 μm , which are thought to be deposited in the peripheral airways,^(3,6) have been used during bronchial challenge testing. We know, however, that both mite and cat allergens are carried on large ($\geq 10 \mu\text{m}$) as well as small particles ($\leq 5 \mu\text{m}$).⁽⁷⁻⁹⁾ The parti-

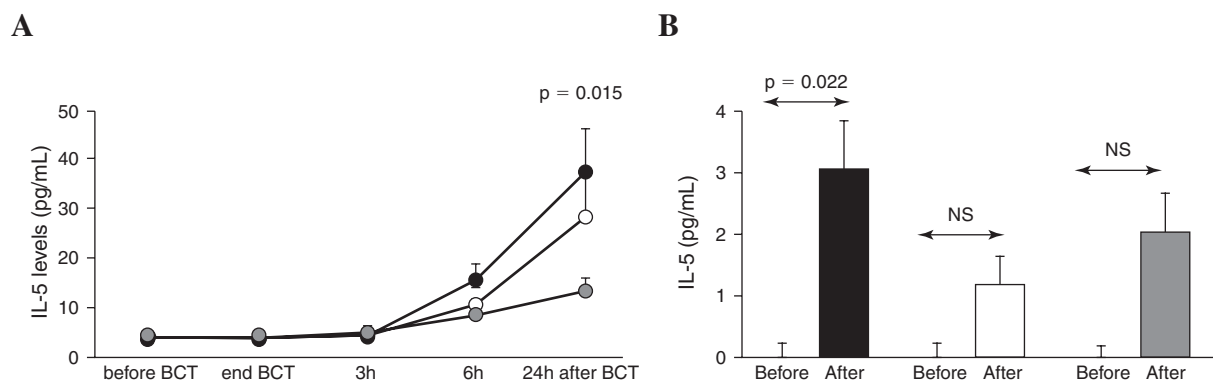


FIG. 1. (A) IL-5 levels in blood: baseline, 3, 6, and 24 h after HDM-BCT with 1.1 μm (black), 5.6 μm (white), and 9.7 μm (gray) (mean value $\pm$ SEM). (B) IL-5 levels in induced sputum 24 h before and after HDM-BCT, all samples were below the detection limit 24 h before BCT.

cles used for BCT therefore do not correspond fully to natural exposure.

The interest in large particles has always been related to their capacity to penetrate into the peripheral airways. More than 20 years ago, Michel et al. showed that deposition of inhaled pollen with diameter of 10 to 70 μm is localized mainly in the tracheobronchial tree. This observation suggests that pollen deposition was localized principally in the large airways (trachea and large bronchi) although particles larger than 10 μm could enter the small airways (distal to the seventh or eighth generation of the tracheobronchial tree) and might be responsible for pollen-associated asthma symptoms. Svartengren et al.⁽¹⁹⁾ demonstrated that 4% to 15% of large Teflon particles (8.2, 11.5, and 13.7 μm) may be deposited in the alveoli, confirming that large particles can be deposited in the peripheral airways. Thus, the role of large particles, especially in the immediate response, may be more important than has been suspected.

As previously described,⁽¹¹⁾ we carefully selected nebulizers for the HDM-BCT. These nebulizers produced polydisperse aerosols with a limited overlap in particle distribution between the 1.1 μm and the 9.7 μm MMAD. Indeed, within the 9.7 μm aerosol, only 3.9% of the total particle volume corresponded to particles with a diameter lower than 1.26 μm , whereas the 1.1 μm MMAD aerosol contained only 3% of particles with a diameter higher than 8.48 μm . Referring to the deposition data obtained with monodisperse aerosols by Heyder,⁽²⁰⁾ we can suppose that a high proportion of the particles found in the 9.7 μm MMAD aerosol are likely to have deposited in the large airways, whereas most particles of the aerosol with a MMAD of 1.1 μm likely deposited in the small airways. All 19 subjects developed an early phase response with the 9.7 μm particles but only 12 subjects (63%) did so with the 1.1 μm . To understand why the 9.7 μm particles produced immediate responses more often, two explanations could be given. The first could be that the surface area in contact with inhaled air is much smaller in the central than in the peripheral airways, and consequently, the local allergen concentration per unit area in the proximal airways would be much higher. The second could be that the seven subjects with a negative challenge with the 1.1 μm particles did not inhale a sufficient dose of allergen to reach the 20% decrease in FEV₁. This would support previous data

reported by Ihre et al.,⁽²¹⁾ who observed that the immediate bronchial response could be obtained only if a sufficient dose of allergen was inhaled. According to our cutoff (16 puffs of the highest concentration 100RI with the 1.1- and 5.6- μm particles or 25RI with the 9.7 μm), seven tests were negatives with the 1.1 μm particles. Nevertheless, those seven subjects demonstrated a decrease in FEV₁ (Table 2) and we suspect that extended exposure with higher doses would probably lead to a further decrease in FEV₁. Our findings that a lower dose of allergen is required to induce the immediate response with the large particles are consistent with previous studies that reported a more pronounced constriction and a decrease in PD₂₀ methacholine or histamine when the aerosol was preferentially deposited in large central airways.^(6,22,23) Those observations suggest that larger central airways contain the main choke points. On the one hand, the change in airway muscle contraction is probably a main event in the central airways during the immediate response as smooth muscle is most prominent in the conducting airways.^(22,23) On the other hand, previous studies have shown the complexity of the process used by bronchoconstrictor agents and supposed that it may involve a large variety of receptors.⁽⁶⁾ Those characteristics of the proximal airways and the fact that the local allergen concentration per unit area would be higher in the proximal airways could explain the differences observed in the immediate response during the three HDM-BCT. Furthermore, the role of tracheobronchial deposition in natural exposures seems to be supported by physiopathological data, since Carroll et al.⁽²⁴⁾ observed an increased degranulation of mast cells in proximal airways in asthmatic subjects compared to the peripheral airways. This may be due to a greater mass deposition of antigen in more proximal than in distal airways. Thus, our results demonstrated that large particles seem to play an important role in the immediate bronchial response related to the tracheobronchial deposition. As the high-dose allergen challenge is still required to study immediate and late responses in diagnosis and pharmacological trials, the use of large particles in BCT may be more appropriate, because it appears to better mimic natural exposures.

Sixty-five percent of late phase reaction (LPR), as first defined by Herxheimer,⁽¹⁵⁾ occurred after allergen exposure of our subjects. This frequency was consistent with previous publications.^(25,26)

LPR with the 1.1 μm particles seemed more severe, as assessed by the amount of oral corticosteroid needed to reverse it. Furthermore, these results coincided with cellular and mediator responses. Increased IL-5 levels in peripheral blood and induced sputum during the late phase of the allergic reaction have been previously documented,^(27,28) and such an increase was also seen in our study. A twofold increase in the mean IL-5 level was observed in the blood 24 h after the inhalation of 1.1 μm particles compared to 9.7 μm particles and this difference was statistically significant. Similarly, in induced sputum, a significant increase in IL-5 concentrations was found only with the 1.1 μm particles 24 h after HDM-BCT. No difference was observed in Borg scale results during the 24 h following HDM-BCT, probably because potential symptoms were masked by medications. This could explain that the decrease in the FEF_{25-75} and the other pulmonary functions did not differ among the three particle sizes during the 24 h after the mite challenge. Nevertheless, the severity of LPRs seemed to be associated with higher inhaled quantities of Der p 1, which could be deposited in the peripheral region of the lungs with the 1.1 μm particles, because subjects inhaled a higher total amount of Der p 1 with the 1.1 μm particles (PD_{20} 142.5 ng). This could also be related to the 1000-fold higher mass of Der p 1 per unit surface of particles with the small droplets, which allow a higher contact of allergens with the lung surface. The mass of allergen per particle unit surface area was evaluated as $\text{PD}_{20}/(\pi \times \text{MMAD}^2)$ with PD_{20} , the provocative dose of allergen and $\pi \times \text{MMAD}^2$, the surface area of the particles with the approximation that all the particles had the MMAD as the diameter. The mass of allergen per particle unit surface area were 37.5 ng Der p 1/ μm^2 for the 1.1 μm droplets and 0.038 ng/ μm^2 for the 9.7 μm . Our results concerning the LPR are consistent with our previous findings for cat allergen: FEF_{25-75} decreased significantly 24 h after cat-BCT with 1.4 μm particles.⁽¹¹⁾ Thus, LPRs seem to be concentrated in the small airways in a dose-response fashion.

In conclusion, our results demonstrated that the mean provocative dose (PD_{20}) of Der p 1 that induced immediate bronchial symptoms was 12–13 times lower with large (9.7 μm) than small (1.1 μm) allergen particles. Similar to cat allergens, large particles seem to play an important role in the early phase response to mite allergen,

while the late phase response appears to be centered in the small airways in a dose-response fashion. Large particles should be included in specific bronchial challenge tests designed to study the immediate bronchial responses to mite allergens.

ACKNOWLEDGMENTS

This work was supported by a grant of Regional PHRC from the Strasbourg University Hospital. The authors thank A. Dazy, F. Frey, M.J. Hoffner, J. Linder, L. Mahr, B. Sbinne, A. Vérot, and R. Wrobel for their excellent technical assistance. We also thank Dr. J.F. Fabriès for his contribution, and Allerbio and Pharmacia Laboratories for providing the reagents.

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Received on July 22, 2006
in final form, July 2, 2007

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