

Original article

Relationship of skin-prick reactivity to aeroallergens and hyperresponsiveness to challenges with methacholine and adenosine monophosphate

Background: Clarification of the relationship between atopy and bronchial hyperresponsiveness (BHR), both key features of asthma, is critical to our understanding of the disease. We therefore investigated the putative relationship between skin-prick reactivity to aeroallergens and BHR to direct and indirect stimuli.

Methods: We performed a retrospective analysis of data from 332 patients presenting with a diagnosis of asthma. Patients were characterized by skin prick tests (SPT), spirometry and bronchial challenge with methacholine and adenosine monophosphate (AMP).

Results: For patients who had BHR to methacholine but not AMP, the presence of atopy was associated with a lower PD_{20} (the provocative dose of methacholine producing a fall in FEV_1 of 20%), amounting to a geometric mean (95% confidence interval (CI)) of 2.3-fold (1.4–4.0) difference. Furthermore, the number of skin-prick positive (SPP) responses was related to methacholine reactivity: 0–1 SPP, $PD_{20} = 69.9 \mu g$; 2–4 SPP, $PD_{20} = 47.8 \mu g$; 5–8 SPP, $PD_{20} = 35.6 \mu g$. There was a 2.0-fold (1.1–3.6) difference between the groups with a low (0–1 SPP) and high (5–8 SPP) degree of skin-prick reactivity. A similar pattern was seen when data were analyzed including only perennial allergens. Spirometry was unrelated to the degree of skin-prick reactivity.

Discussion: These results suggest that skin-prick reactivity to aeroallergens is associated with BHR to methacholine.

Stephen J. Fowler, Brian J. Lipworth

Asthma and Allergy Research Group, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland

Key words: asthma; atopic hypersensitivity; bronchial provocation tests.

Professor Brian J Lipworth
Asthma and Allergy Research Group
Ninewells Hospital and Medical School
University of Dundee
Dundee DD1 9SY
Scotland

Accepted for publication 10 September 2002

The prevalence of both atopy and atopic diseases is increasing. IgE-mediated allergy testing by specific serum IgE or skin prick test (SPT) is positive to at least one inhaled allergen in a large proportion of asthmatics. Another key component of the asthmatic phenotype is bronchial hyperresponsiveness (BHR). The nature of any relationship between BHR and atopy has been identified as a central issue in our understanding of asthma (1).

Bronchial hyperresponsiveness is usually measured by bronchial challenge with direct or indirect stimuli. Direct bronchial challenge with methacholine results in airway narrowing through activation of cholinergic receptors on bronchial smooth muscle. The degree of hyperresponsiveness to methacholine is correlated to several markers of airway inflammation (2, 3) and asthma severity (4, 5). Furthermore, targeting bronchial responsiveness with inhaled corticosteroids over 2 years has been shown to reduce airway-remodelling (6). Adenosine monophosphate (AMP) causes bronchoconstriction indirectly, through degranulation of primed mast cells and consequent release of proinflammatory mediators, such as histamine and leukotrienes, as well as through neuronal

mechanisms (7). AMP challenge is a more sensitive marker of change during treatment with corticosteroids (8, 9), as well as being more asthma-specific than direct challenge (7).

Population-based studies have suggested that BHR to direct challenge is more common in the presence of atopy (10, 11). A subgroup analysis of the study by Cockcroft and colleagues found that a higher degree of IgE-mediated sensitization (calculated on wheal number and size) was weakly correlated to increasing bronchial reactivity to histamine. Others have suggested that this relationship becomes nonsignificant when data are weighted for asthma severity (12). With indirect challenge, a population-based study did not find a significant relationship between BHR (to cold air) and atopy (13), whereas another showed that patients with atopic asthma were more likely to have BHR to AMP (but not cold air) than nonatopic asthmatics (14). More recently BHR to AMP was found to be associated with atopic airway disease and atopic status, where BHR to methacholine was related to diminished airway calibre (15).

In view of these inconsistencies in the data we have performed a study with two main aims: to clarify the relationship between the degree of BHR and skin-prick reactivity, and to determine any differences in this relationship when investigated with direct or indirect bronchial challenge.

Material and methods

We have used nomenclature relating to allergy and allergic disease as per the guidance set out in the EAACI position paper (16).

Data collection

This was a retrospective analysis of the screening database of patients attending the Asthma and Allergy Research Group for assessment and possible inclusion in clinical trials. As such, this was a heterogeneous group of patients in whom the diagnosis of asthma was usually made by a primary-care physician, and hence was based on any combination of symptoms, physical examination, peak expiratory flow, or response to treatment. On attending the department patients filled in a health questionnaire, and their airways and IgE-mediated allergic status were objectively assessed using a battery of tests that included spirometry, SPT, and (on separate days) standardized bronchial challenge with methacholine and AMP.

Spirometry

Spirometry was carried out using a Vitalograph Compact spirometer (Vitalograph, Buckinghamshire, UK), calibrated daily, according to current American Thoracic Society guidelines (17). Before performing spirometry and bronchial challenge, patients were asked to withhold short-acting β_2 -agonists for 6 h, anticholinergics for 12 h, and long-acting β_2 -agonists, theophyllines, and leukotriene receptor antagonists for 48 h. Laboratory personnel verbally rechecked that all participants had followed these restrictions at the time of testing.

Skin prick tests

Patients were tested for skin-prick reactivity to eight common inhaled allergens (grass, tree and weed pollens, house-dust mite, *Aspergillus*, feathers, cat, and dog) along with positive (1 mg/ml histamine) and negative (0.9% w/v saline) controls, using solutions supplied by Diagenics Ltd (Newark, Nottinghamshire, UK). Standard procedures were followed (18) and wheal diameters of at least 3 mm greater than those of the negative control (15 min after administration) were taken as positive. For 4 days before performing the SPT or AMP bronchial challenge, patients were asked to stop oral antihistamine treatment.

Methacholine bronchial challenge

The methacholine bronchial challenge was performed by using a standardized computer-assisted dosimetric method as previously described (19). Fresh solutions of methacholine chloride were made up daily, in doubling concentrations from 0.0625 to 32 mg/ml. We used equipment identical to that described by Beach and colleagues (19). The doses were given via ten sequential Turbo jet nebulizers (Medic Aid, Pagham, West Sussex), each calibrated to deliver 10 μ l

($\pm 10\%$) of aerosol per inhalation. Inhalation via a mouthpiece was triggered by a pressure transducer during inspiration from functional residual capacity for approximately 2 s, at a driving pressure of 138 kPa. The patient continued to inhale (or hold breath at inspiratory capacity) for 5 s, marked by an electronically controlled sound. A total of five inhalations were given for each dose.

The forced expiratory volume in 1 s (FEV_1) was calculated as the mean of the best three out of six consecutive manoeuvres for each dose. Two or more baseline FEV_1 recordings were taken 5 min apart until the variability was less than 3%. Then cumulative doubling doses (from 3.125 to 1600 μ g) of methacholine were given at 5-min intervals until a 20% fall in the FEV_1 was recorded. The provocative dose of methacholine producing a fall in FEV_1 of 20% (PD_{20}) was then calculated by a computer-assisted interpolation from the dose-response curve. If a subject did not react after the highest dose had been given, they were assigned a censored PD_{20} of 3200 μ g for purposes of analysis. For comparison, a PD_{20} value of 500 μ g approximates to a PC_{20} value of 5 mg/ml (19). This value for PD_{20} is used in our laboratory as a cut-off for defining a biologically significant degree of bronchial hyperresponsiveness to methacholine.

Adenosine monophosphate bronchial challenge

The technique of AMP bronchial challenge was modified from the protocol previously described (20). Fresh solutions of AMP were made up daily in doubling concentrations from 0.39 mg/ml to 800 mg/ml in sterile water for injections (to a total volume of 4 ml). A Nebicheck nebulizer controller (PK Morgan Ltd, Rainham, Kent) was used with a System 22 Acorn nebulizer (Medicaid, Pagham, West Sussex) with a driving pressure of 138 kPa. The nebulizer was activated for 1.2 s per inhalation. The nose was clipped and nebulized solution was inhaled via a mouthpiece. For each manoeuvre the subject first exhaled to near-residual capacity, then inhaled steadily for 1–2 s, and held their breath for 3 s. Five consecutive inhalations were given per concentration. Ninety seconds after each dose, three forced expiratory manoeuvres were performed as above, and the best FEV_1 recorded. For the first inhalation, sterile water for injections was administered in increasing concentrations until either the FEV_1 had fallen by at least 20% from the post-water baseline, or the highest dose had been given. The PC_{20} was calculated using a computer-assisted curve-fitting package (BIOLAB ASSISTANT Version 1.1, University of Dundee, Dundee, UK). If the FEV_1 had not fallen by at least 20% at the maximum dose, then the patient was assigned a censored PC_{20} of 1600 mg/ml for the purposes of analysis. A cut-off value for PC_{20} of 200 mg/ml is used in our laboratory for defining biologically significant bronchial hyperresponsiveness to AMP.

Data analysis

A database search was performed, and a spreadsheet produced, which identified all patients who had had SPT. Those who had also been characterized by spirometry and bronchial challenge had these data included. Statistical analysis was performed using the Statgraphics package (STSC, Software Publishing Group; Rockville, MD). The number of skin prick positive (SPP) results, FEV_1 , and forced midexpiratory flow rate (FEF_{25-75}), were all normally distributed. Methacholine PD_{20} and AMP PC_{20} values (for patients with BHR) were logarith-

mically converted to normalize their distribution before analysis. Analysis of variance, followed by multiple range testing using the Bonferroni correction (95% CI, two-tailed), were employed to explore the relationship between the degree of SPT reactivity vs. BHR and spirometry.

Results

Three hundred and thirty-two patients from the database had documented SPT. Their demographic data are shown in Table 1. The distributions of the number of positive SPT, and of the specific allergens tested, are shown in the Fig. 1.

For patients who exhibited BHR, methacholine PD₂₀ and AMP PC₂₀ were compared to degree of skin-prick reactivity. SPT positive patients with BHR to methacholine had a geometric mean (95% CI) 2.3-fold (1.4–4.0) lower PD₂₀ than patients who were unreactive to SPT. Furthermore, when the total number of positive SPT per patient was divided into three groups, the group with greater than four positive responses had a 2.0-fold (1.1–3.6) lower methacholine PD₂₀ than the group with less than two. There was no such difference in AMP PC₂₀

vs. skin-prick reactivity (Table 2). Patients responsive to AMP, but not methacholine, had a higher mean number of positive SPT than unresponsive patients (3.1 vs. 2.4 positives, *P* < 0.05).

The same analysis was performed on the degree of skin-prick reactivity to perennial allergens, excluding grass, weed, and tree pollens. We obtained similar results, with SPT-positive patients having a 2.1-fold (1.3–3.2) lower methacholine PD₂₀ than those who were SPT-negative. Again, patients were allocated one of three groups according to the number of positive SPT recorded. Patients in group 1 (0 SPP) had a 2.2-fold (1.2–3.9) and 2.0-fold (1.2–3.6) higher methacholine PD₂₀ values than patients in group 2 (1 SPP) and group 3 (greater than 1 SPP), respectively. No such relationship was demonstrated for the patients with BHR to AMP (Table 3). Methacholine-responsive patients had a mean of 1.6 positive tests (out of five) vs. 1.3 for methacholine-negative ones (*P* < 0.05). AMP-responsive patients had a mean of 1.8 positive tests vs. 1.4 for AMP-negative patients (*P* < 0.05).

Similar trends were seen for the steroid naïve patients. Methacholine-responsive patients who were skin prick positive to at least one of the allergens tested had a 4.7-fold (1.3–17.1) lower methacholine PD₂₀ vs.

Table 1. Demographic data

Number of patients	332
Mean age in years (range)	37 (19–69)
Gender (male /female)	138 /194
Smokers	29
Nonsmokers	303
Daily inhaled corticosteroid	
Yes	208
No	115
Not known	9
Median dose µg (Interquartile range)	250 (0–400)
Spirometry (mean (SD))	
FEV ₁ litres	2.02 (0.80)
FEV ₁ % predicted	91.0 (15.7)
FEF _{25–75} litres per second	2.69 (1.09)
FEF _{25–75} % predicted	66.6 (23.4)
Skin-prick positives	
Positive	297
Negative	25
Median (Interquartile range)	2 (1.74–4.0)
Bronchial challenge with methacholine	
Total number	291
Positive	219
Negative	72
PD ₂₀ µg (geometric mean (SE))	126.2 (14.7)
Bronchial challenge with adenosine monophosphate	
Total number	289
Positive	123
Negative	166
PC ₂₀ mg /ml (geometric mean (SE))	311.0 (36.2)

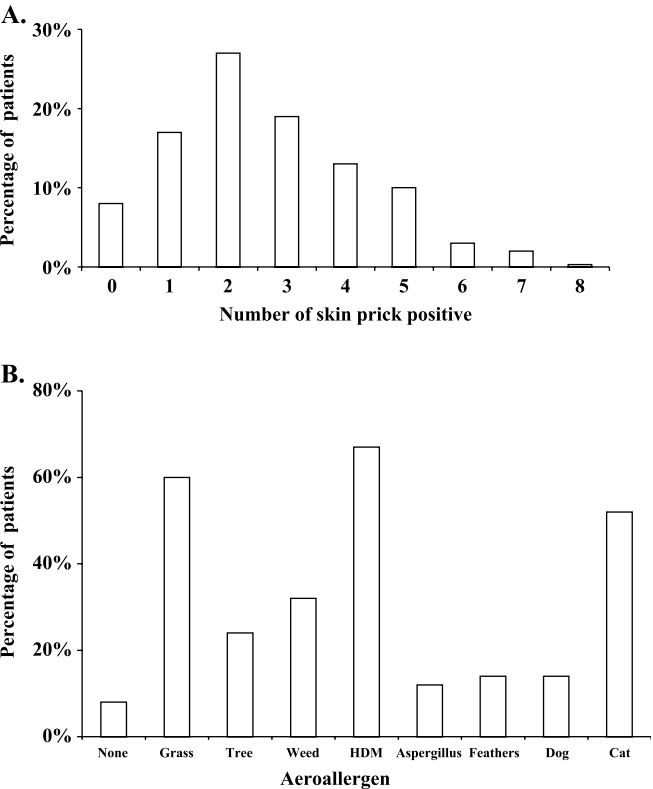


Figure 1. Histogram showing distribution of skin-prick reactivity to inhaled allergens by total number of positive tests per patient (A) and by aeroallergen (B) in our sample. (HDM, house-dust mite)

Table 2. Bronchial responsiveness (geometric mean; 95% CI) of patients with bronchial hyperresponsiveness to methacholine or adenosine monophosphate vs. degree of skin-prick reactivity to all eight allergens

Degree of reactivity	All patients: perennial allergens				Steroid naïve patients: perennial allergens			
	Methacholine		Adenosine monophosphate		Methacholine		Adenosine monophosphate	
	<i>n</i>	PD ₂₀ µg (range)	<i>n</i>	PC ₂₀ mg /ml (range)	<i>n</i>	PD ₂₀ µg (range)	<i>n</i>	PC ₂₀ mg /ml (range)
Skin-prick negative	20	107.5 (64.6–179.0)	7	17.5 (7.3–41.8)	3	259.1 (74.2–904.7)	0	N / A
Skin-prick positive	199	46.2 (39.4–54.4)*	116	45.6 (34.4–52.7)	52	54.7 (40.5–73.9)*	30	38.1 (23.9–60.9)
Group 1 (0–1 positive)	54	69.9 (51.2–95.5)	25	37.4 (23.4–59.9)	12	69.8 (36.3–134.1)	9	45.9 (19.6–107.7)
Group 2 (2–4 positive)	128	47.8 (39.0–58.6)	67	41.6 (31.2–55.4)	32	64.9 (43.5–96.8)	10	26.5 (11.8–59.4)
Group 3 (5–8 positive)	37	35.6 (24.4–51.9)†	31	40.6 (26.7–61.9)	11	39.0 (19.7–77.1)	11	45.5 (21.1–98.4)

N / A, not applicable.

* $P < 0.05$ from skin-prick negative patients.† $P < 0.05$ from group 1.

Table 3. Bronchial responsiveness (geometric mean; 95% CI) of patients with bronchial hyperresponsiveness to methacholine or adenosine monophosphate vs. the degree of skin-prick reactivity to the five perennial allergens

Degree of reactivity	All patients: perennial allergens				Steroid naïve patients: perennial allergens			
	Methacholine		Adenosine monophosphate		Methacholine		Adenosine monophosphate	
	<i>n</i>	PD ₂₀ µg (range)	<i>n</i>	PC ₂₀ mg /ml (range)	<i>n</i>	PD ₂₀ µg (range)	<i>n</i>	PC ₂₀ mg/ml (range)
Skin-prick negative	32	93.5 (62.6–139.9)	11	29.9 (14.8–60.5)	6	132.4 (53.3–329.3)	1	109.0 (8.3–1432.5)
Skin-prick positive	187	44.9 (38.0–53.0)*	112	41.7 (33.4–52.0)	48	53.7 (38.9–74.1)	28	36.1 (22.2–58.8)
Group 1 (0 positive)	32	93.5 (62.5–139.9)	11	29.9 (14.8–60.6)	6	132.4 (53.1–330.5)	1	109.0 (7.9–1509.0)
Group 2 (1 positive)	68	43.3 (32.8–57.0)†	43	38.3 (26.8–54.7)	17	45.2 (26.2–77.7)	15	34.7 (17.6–68.4)
Group 3 (2–5 positive)	119	45.8 (37.2–56.5)†	69	44.0 (33.2–58.2)	31	59.0 (39.5–88.2)	13	37.9 (18.3–78.5)

* $P < 0.05$ from skin-prick negative patients.† $P < 0.05$ from group 1.

SPT-negative patients (Tables 2 and 3). No steroid naïve patients were both SPT-negative and AMP-responsive. In these steroid naïve patients, there were no differences in the mean number of positive SPT in those with BHR vs. those without BHR, for either methacholine or AMP.

When allergen-specific responses were analyzed, the presence of a positive reaction to house-dust mite was associated with a 2.4-fold (1.0–5.9) decrease in methacholine PD₂₀ (Table 4).

Spirometric values (FEV₁ or FEF_{25–75}) were unrelated to the degree of atopy as determined by SPT (Table 5).

Discussion

Our study has shown a link between BHR to methacholine and skin-prick reactivity to aeroallergens. We believe our data are novel in two aspects.

Firstly, we have demonstrated that this relationship is “dose-dependant” whereby patients with more SPT-positive reactions have airways that are significantly

more reactive to methacholine. In other words, we have shown that not only the presence, but also the degree, of IgE-mediated allergy is related to increased BHR to methacholine.

Secondly, we have shown this relationship only with direct bronchial challenge with methacholine, not with indirect challenge with AMP. Our results are in contrast to those of de Meer et al. (15) who reported a prevalence ratio of 3.87 (95% CI 1.76–8.52) for the relationship of BHR to AMP and atopy. In their study, the presence of atopy was defined as at least one positive SPT; this occurred in 38% of patients. In particular, they did not report on the relationship between the number of positive SPT responses and BHR.

Previous work in a population-based study has shown that the presence of BHR to methacholine is associated with high levels of specific-IgE (Class 3, > 3.5 kU/l) to inhaled allergens (odds ratio (95% CI) 5.1 1.9–13.3) for indoor allergens; 9.6 (2.0–45.0) for outdoor allergens) (21). This finding was supported by a study of 82 patients with adult-onset wheeze, where having three positive SPT or specific-IgE tests (house-dust mite, cat, and mixed grass pollen) was associated with BHR to methacholine

Table 4. Bronchial responsiveness (geometric mean; 95% CI) of all patients with bronchial hyperresponsiveness to methacholine or adenosine monophosphate vs. presence of skin-prick reactivity to individual allergens

	Methacholine		Adenosine monophosphate	
	<i>n</i>	PD ₂₀ µg (range)	<i>n</i>	PC ₂₀ mg/ml (range)
None	20	107.5 (64.6–178.8)	7	17.5 (7.6–40.4)
Grass pollen	127	44.3 (36.2–54.2)	82	40.8 (31.9–52.1)
Tree pollen	48	43.1 (31.1–60.0)	37	38.7 (26.9–55.6)
Weed pollen	64	45.8 (34.5–60.9)	46	41.2 (29.7–57.0)
House-dust mite	149	44.0 (36.6–53.1)*	87	46.7 (36.8–59.1)
<i>Aspergillus</i>	30	38.8 (25.6–58.9)	25	54.3 (34.9–84.5)
Feathers	31	45.9 (30.5–69.0)	21	48.5 (30.0–78.7)
Dog	31	48.0 (31.9–72.3)	15	24.2 (13.6–42.8)
Cat	116	45.4 (36.8–56.1)	72	40.9 (31.5–53.0)

* *P* < 0.05 from skin-prick negative patients.

Table 5. Spirometry (mean; 95% CI) of all patients vs. degree of skin-prick reactivity to all eight allergens (grass, tree and weed pollen, house-dust mite, *Aspergillus*, feathers, dog, and cat

	<i>n</i>	% Predicted	
		FEV ₁	FEF _{25–75}
Skin-prick negative	25	90.1 (83.9–96.3)	64.1 (54.9–73.3)
Skin-prick positive	307	91.0 (89.3–92.8)	66.8 (64.2–69.4)
0–1 positive	83	91.4 (88.0–94.9)	64.9 (60.0–69.9)
2–4 positive	196	91.2 (89.0–93.4)	67.9 (64.6–71.1)
5–8 positive	53	89.4 (85.2–93.7)	64.5 (58.2–70.9)

FEV₁, forced expiratory volume in 1 s; FEF_{25–75}, forced midexpiratory flow rate.

and wheezing (odds ratio (95% CI) 7.2 (2.5–20.6) (22). Another population-based study showed that children with atopy (as defined by positive SPT) had a greater degree of BHR than those with negative SPT. Furthermore, patients who changed from negative to positive over the 3.5-year study period had a corresponding increase in bronchial reactivity over that time (23). Cockcroft and colleagues found that, for patients with histamine PC₂₀ less than 16 mg/ml, and at least one positive SPT, bronchial reactivity was correlated with atopic status (*r* = – 0.36, *P* < 0.02) (11).

We have shown the same relationship between BHR to methacholine and IgE-mediated allergy when data were analyzed, both including and excluding seasonal allergens. This was a retrospective study, and therefore we were unable to factor into our analysis temporal changes in pollen counts. This is relevant, as methacholine PC₂₀ decreases 2-fold during the ragweed-pollen season in patients sensitized to ragweed (24), and repeated low-dose allergen exposure increases methacholine BHR 2–3-fold in under a week (25, 26). This may also partially explain the increased BHR in people with IgE-mediated allergy, where many are regularly exposed to allergens that they are sensitized to. Furthermore, BHR returns to its prechallenge baseline

3 days after ceasing allergen exposure (26). This seems to be the case following short-term allergen exposure. However, in a study by Benckhuijsen et al. (27), patients IgE-sensitized to house-dust mite were moved to a hypoallergenic environment; their response to indirect bronchial challenge (both AMP and exercise) was diminished by over two doubling-concentrations, but their response to methacholine remained unaltered. One could postulate from this that perennial (ever-present) allergens have a more significant long-term effect than seasonal ones, and that this is reflected by changes in direct, not indirect, BHR. Indeed, Ferrante et al. (28) found that although bronchial reactivity to methacholine was increased in the presence of a positive SPT reaction to *Dermatophagoides pteronyssinus*. This was not the case for grasses or the allergens tested as a whole.

As methacholine hyperresponsiveness has been linked to airway remodelling (6), it is conceivable that patients who are IgE-sensitized to common airborne allergens might have such changes. In one study using bronchial biopsy to examine inflammation and structural changes in atopic and nonatopic asthmatics and controls, there was an increase in a variety of inflammatory cells, as well as more epithelial damage in the atopic vs. nonatopic groups (29). Again, this indirectly reinforces the link between BHR to methacholine and IgE-mediated allergy.

Other evidence for this link comes from two large studies investigating the genetic basis of asthma. The first showed that the traits for asthma, BHR (to histamine), and atopy (serum total IgE) are coinherited (30). These findings were reinforced by a separate study looking at BHR to hypertonic saline and atopy (defined by at least one positive SPT result) (31).

There are far fewer published data on BHR to AMP and atopy apart from the de Meer study described above (15). One study found that atopic people are more reactive to AMP than those who are nonatopic (14), and another showed that BHR to a different indirect bronchial challenge (with cold air) is associated with atopy in children (32). Although we have shown that AMP-responsive patients have a statistically higher mean number of positive SPT, we found no difference in bronchial reactivity to AMP depending on IgE-mediated allergic status. This may be because, as per current guidelines (33), the majority of our patients were treated with inhaled corticosteroids, and BHR to AMP is very sensitive to such antiinflammatory treatment (9). For the reason given above, our steroid-naïve subgroup was small, thus further limiting the interpretation of our data. Finally, the patients in our study were a heterogeneous group, with only a presumptive diagnosis of asthma from primary care. However, our analysis only included patients who exhibited BHR, so we can be fairly confident that this group exhibited the asthma phenotype as such.

In our patients we found no relationship between SPT positivity and spirometry (FEV_1 or FEF_{25-75}). Few comparative data are available, although one study found that the number of SPT-positive tests was weakly correlated with decreasing FEV_1 in 100 Japanese medical students (including 19 asthmatics) (34). Our findings would therefore strengthen the hypothesis that the observed changes in BHR are not due simply to altered airway geometry, but to underlying structural and/or inflammatory variations that depend on the degree of IgE-mediated allergy present.

In conclusion, our study on a large number of unselected asthmatics has shown that the degree of

skin-prick reactivity to aeroallergens was associated with BHR to methacholine. This was independent of spirometry, whether patients were treated with inhaled corticosteroids, or whether they were allergic to perennial or seasonal allergens. This suggests that the effects of BHR and atopy are intimately linked in the pathogenesis and evolution of asthma.

Acknowledgments

This study was funded by a University of Dundee Research Grant.

References

1. WOOLCOCK AJ, PEAT J. What is the relationship between airway hyperresponsiveness and atopy? *Am J Respir Crit Care Med* 2000;**161**:S215–S217.
2. JATAKANON A, LIM S, KHARITONOV SA, CHUNG KF, BARNES PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;**53**:91–95.
3. GIBSON PG, SALTOS N, BORGAS T. Airway mast cells and eosinophils correlate with clinical severity and airway hyperresponsiveness in corticosteroid-treated asthma. *J Allergy Clin Immunol* 2000;**105**:752–759.
4. WEISS ST, VAN NATTA ML, ZEIGER RS. Relationship between increased airway responsiveness and asthma severity in the childhood asthma management program. *Am J Respir Crit Care Med* 2000;**162**:50–56.
5. FOWLER SJ, DEMPSEY OJ, SIMS EJ, LIPWORTH BJ. Screening for bronchial hyperresponsiveness using methacholine and adenosine monophosphate. Relationship to asthma severity and beta(2)-receptor genotype. *Am J Respir Crit Care Med* 2000;**162**:1318–1322.
6. SONT JK, WILLEMS LN, BEL EH, VAN KRIEKEN JH, STERK PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPOL Study Group. *Am J Respir Crit Care Med* 1999;**159**:1043–1051.
7. POLOSA R, HOLGATE ST. Adenosine bronchoprovocation: a promising marker of allergic inflammation in asthma? *Thorax* 1997;**52**:919–923.
8. WILSON AM, LIPWORTH BJ. Dose-response evaluation of the therapeutic index for inhaled budesonide in patients with mild-to-moderate asthma. *Am J Medical* 2000;**108**:269–275.
9. MEIJER RJ, KERSTJENS HA, ARENDS LR, KAUFFMAN HF, KOETER GH, POSTMA DS. Effects of inhaled fluticasone and oral prednisolone on clinical and inflammatory parameters in patients with asthma. *Thorax* 1999;**54**:894–899.
10. SEARS MR, BURROWS B, HERBISON GP, HOLDAWAY MD, FLANNERY EM. Atopy in childhood. II. Relationship to airway responsiveness, hay fever and asthma. *Clin Exp Allergy* 1993;**23**:949–956.
11. CROCKCROFT DW, MURDOCK KY, BERSCHIED BA. Relationship between atopy and bronchial responsiveness to histamine in a random population. *Ann Allergy* 1984;**53**(1):26–29.
12. KELLY WJ, HUDSON I, PHELAN PD, PAIN MC, OLINSKY A. Atopy in subjects with asthma followed to the age of 28 years. *J Allergy Clin Immunol* 1990;**85**:548–557.
13. WELTY C, WEISS ST, TAGER IB, et al. The relationship of airways responsiveness to cold air, cigarette smoking, and atopy to respiratory symptoms and pulmonary function in adults. *Am Rev Respir Dis* 1984;**130**:198–203.
14. LUDVIKSDOTTIR D, JANSON C, BJORNSSON E, et al. Different airway responsiveness profiles in atopic asthma, nonatopic asthma, and Sjogren's syndrome. BHR STUDY GROUP. *Bronchial hyperresponsiveness. Allergy* 2000;**55**:259–265.
15. DE MEER G, HEEDERIK D, POSTMA DS. Bronchial responsiveness to adenosine 5'-monophosphate (AMP) and methacholine differ in their relationship with airway allergy and baseline FEV_1 . *Am J Respir Crit Care Med* 2002;**165**:327–331.
16. JOHANSSON SG, HOURIHANE J O'B, BOUSQUET J, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.
17. AMERICAN THORACIC SOCIETY. Standardization of spirometry—1987 update. Statement of the American Thoracic Society. *Am Rev Respir Dis* 1987;**136**:1285–1298.
18. PEPYS J. Skin tests for immediate, type I allergic reactions. *Proc R Soc Med* 1972;**65**:271–272.
19. BEACH JR, YOUNG CL, AVERY AJ, et al. Measurement of airway responsiveness to methacholine: relative importance of the precision of drug delivery and the method of assessing response. *Thorax* 1993;**48**:239–243.
20. TAN KS, MCFARLANE LC, LIPWORTH BJ. Modulation of airway reactivity and peak flow variability in asthmatics receiving the oral contraceptive pill. *Am J Respir Crit Care Med* 1997;**155**:1273–1277.
21. KERKHOF M, SCHOUTEN JP, DE MONCHY JG. The association of sensitization to inhalant allergens with allergy symptoms: the influence of bronchial hyperresponsiveness and blood eosinophil count. *Clin Exp Allergy* 2000;**30**:1387–1394.
22. BODNER C, GODDEN D, ROSS S, et al. Bronchial hyperresponsiveness and adult onset wheeze: the influence of atopy. *Eur Respir J* 1999;**14**:335–338.
23. FORASTIERE F, CORBO GM, DELL'ORCO V, PISTELLI R, AGABITI N, KRIEBEL D. A longitudinal evaluation of bronchial responsiveness to methacholine in children: role of baseline lung function, gender, and change in atopic status. *Am J Respir Crit Care Med* 1996;**153**:1098–1104.

24. BOULET LP, CARTIER A, THOMSON NC, ROBERTS RS, DOLOVICH J, HARGREAVE FE. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol* 1983;**71**:399–406.
25. PALMQVIST M, PETTERSSON K, SJOSTRAND M, ANDERSSON B, LOWHAGEN O, LOTVALL J. Mild experimental exacerbation of asthma induced by individualised low-dose repeated allergen exposure. A double-blind evaluation. *Respir Med* 1998;**92**: 1223–1230.
26. SULAKVELIDZE I, INMAN MD, RERECICH T, O'BYRNE PM. Increases in airway eosinophils and interleukin-5 with minimal bronchoconstriction during repeated low-dose allergen challenge in atopic asthmatics. *Eur Respir J* 1998;**11**: 821–827.
27. BENCKHUIJSEN J, VAN DEN BOS JW, VAN VELZEN E, DE BRUIJN R, AALBERS R. Differences in the effect of allergen avoidance on bronchial hyperresponsiveness as measured by methacholine, adenosine 5'-monophosphate, and exercise in asthmatic children. *Pediatr Pulmonol* 1996;**22**:147–153.
28. FERRANTE E, CORBO GM, VALENTE S, CIAPPI G. Associations between atopy, asthma history, respiratory function and non-specific bronchial hyperresponsiveness in unselected young asthmatics. *Respiration* 1992;**59**:169–172.
29. AMIN K, LUDVIKSDOTTIR D, JANSON C, et al. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. *BHR Group. Am J Respir Crit Care Med* 2000;**162**:2295–2301.
30. POSTMA DS, BLEECKER ER, AMELUNG PJ, et al. Genetic susceptibility to asthma—bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Medical* 1995;**333**:894–900.
31. CLARKE JR, JENKINS MA, HOPPER JL, et al. Evidence for genetic associations between asthma, atopy, and bronchial hyperresponsiveness: a study. *Am J Respir Crit Care Med* 2000;**162**: 2188–2193.
32. GRUBER W, EBER E, STEINBRUGGER B, MODL M, WEINHANDL E, ZACH MS. Atopy, lung function and bronchial responsiveness in symptom-free paediatric asthma patients. *Eur Respir J* 1997;**10**:1041–1045.
33. ANONYMOUS. The British guidelines on asthma management: 1995 review and position statement. *Thorax* 1997;**52** (Suppl. 1):S1–S21.
34. KUSUNOKI T, HOSOI S, ASAI K, HARAZAKI M, FURUSHO K. Relationships between atopy and lung function: results from a sample of one hundred medical students in Japan. *Ann Allergy Asthma Immunol* 1999;**83**:343–347.