

Original article

Different airway responsiveness profiles in atopic asthma, nonatopic asthma, and Sjögren's syndrome

Background: Different mechanisms may underlie bronchial hyperresponsiveness (BHR) in different diseases. The aim of this study was to investigate the bronchial responsiveness profile produced by three different challenge tests, methacholine, a direct stimulus, and two indirect stimuli, adenosine 5'-monophosphate (AMP) and cold air, in subjects with asthma and patients with Sjögren's syndrome.

Methods: The study population comprised 40 adult patients with asthma, 18 subjects with Sjögren's syndrome, and 20 controls. Blood samples were collected before each challenge for measurements of serum eosinophil peroxidase (S-EPO) and eosinophil cationic protein (S-ECP). The investigated subjects recorded peak expiratory flow and kept a symptom diary.

Results: Atopic subjects with asthma were significantly more hyperresponsive to AMP than nonatopic subjects with asthma ($P=0.01$) and subjects with Sjögren's syndrome ($P=0.02$). No difference was seen between atopic and nonatopic subjects with asthma in the case of challenges with methacholine or cold air. In atopic subjects with asthma, a significant correlation was found between challenges with methacholine and AMP ($r=0.91$, $P=0.0001$) and methacholine and cold air ($r=0.83$, $P=0.004$), but, in nonatopic subjects with asthma, no significant correlation was seen between methacholine and AMP or cold air challenges. In atopic subjects with asthma, the dose-response slope for AMP was correlated to S-EPO ($r=-0.56$; $P=0.01$) and S-ECP ($r=-0.51$, $P=0.02$), while no correlation between BHR and inflammation markers was found in the two other patient groups.

Conclusions: The results of this study suggest that patients with asthma and subjects with Sjögren's syndrome display different bronchial responsiveness profiles for different challenge agents. Atopic subjects with asthma are more hyperresponsive to AMP than nonatopic subjects and patients with Sjögren's syndrome. More than one challenge may be required to detect different aspects of bronchial responsiveness.

D. Lúdvíksdóttir, C. Janson, E. Björnsson, G. Stålenheim, G. Boman on behalf of the BHR Study Group*

Department of Respiratory Medicine and Allergology, Asthma Research Centre, Akademiska sjukhuset, Uppsala University, Uppsala, Sweden

H. Hedenström

Department of Clinical Physiology, Asthma Research Centre, Akademiska sjukhuset, Uppsala University, Uppsala, Sweden

P. Venge

Department of Clinical Chemistry, Asthma Research Centre, Akademiska sjukhuset, Uppsala University, Uppsala, Sweden

B. Gudbjörnsson, S. Valtýsdóttir

Department of Rheumatology, Asthma Research Centre, Akademiska sjukhuset, Uppsala University, Uppsala, Sweden

Key words: airway hyperresponsiveness; airway responsiveness; asthma; atopy; bronchial hyperresponsiveness; bronchial responsiveness; Sjögren's syndrome.

Prof. Gunnar Boman
Department of Respiratory Medicine and Allergology
Akademiska sjukhuset
SE-751 85 Uppsala
Sweden

Accepted for publication 4 November 1999

* Anders Ahlander,⁵ Kawa Amin,⁵ Eythór Björnsson,¹ Gunnar Boman,¹ Britt-Marie Eriksson,⁶ Björn Gudbjörnsson,³ Monika Hall,² Göran Hedenstierna,² Hans Hedenström,² Lena Håkansson,⁴ Marieann Högman,² Christer Janson,¹ Maria Lampinen,⁴ Kerstin Lindblad,⁴ Dóra Lúdvíksdóttir,¹ Otto Nettelbladt,¹ Godfried Roomans,⁵ Lahja Sevéus,⁷ Ulrike-Spetz-Nyström,¹ Gunnemar Stålenheim,¹ Sigríður Valtýsdóttir,³ Per Venge,⁴ Charlotte Woschnagg.⁴

¹Department of Respiratory Medicine and Allergology, ²Department of Clinical Physiology, ³Department of Rheumatology, ⁴Department of Clinical Chemistry, ⁵Department of Anatomy, ⁶Department of Infectious Diseases, Asthma Research Centre, Uppsala University, ⁷Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden.

Asthma is a chronic inflammatory airway disease characterized by bronchial hyperresponsiveness (BHR) and variable airflow obstruction (1). The pathogenesis of BHR is not completely understood but involves interaction between multiple mechanisms including the constriction of airway smooth muscle, edema of bronchial mucosa, and increase in the amount of secretion in the airway wall. The presence of inflammatory cells in the airways appears not to be a necessary factor for BHR (2), but it is likely to be an important stimulus for structural changes such as airway remodeling or autonomic dysfunction (3).

Damage to the airway epithelium, changes in epithelial permeability, and mediator release by the airway epithelial cells may also contribute to increased bronchial responsiveness.

BHR is not unique to asthma and can be demonstrated in a number of other inflammatory airway diseases, such as chronic obstructive lung disease (4), cystic fibrosis (5), and bronchiectasis (6). Patients with Sjögren's syndrome (SS), which is a chronic autoimmune disease characterized by dry mucous membrane, also often exhibit BHR (7, 8).

Bronchial challenge agents have been divided into direct and indirect stimuli on the basis of their mechanism of action. Direct stimuli, such as methacholine, cause airway narrowing by affecting the airway smooth muscle, while indirect stimuli, such as cold air and adenosine 5'-monophosphate (AMP), cause airflow limitations as a result of mediator release from inflammatory cells, and it has therefore been suggested that they produce a better correlation with the underlying airway inflammation (9).

In this study, we investigated the bronchial responsiveness profile produced by three different challenge tests, methacholine, AMP, and cold air, in subjects with asthma and patients with SS, and its relationship to atopy, serum markers of eosinophil activation, respiratory symptoms, and peak flow variability.

Material and methods

Subjects

Seventy-eight subjects aged 17–69 years participated in the study. The clinical characteristics of the subjects are shown in Table 1. Patients with asthma were recruited from the Department of Respiratory Medicine and Allergology at our hospital. Subjects were defined as having asthma if they had physician-diagnosed asthma (1) and had previously demonstrated increased responsiveness to inhaled methacholine (10). The patients with asthma had current respiratory symptoms and a forced expiratory volume in 1 s (FEV₁) above 70% of the predicted value. Patients with asthma who had at least one positive skin prick test were defined as having atopy. Skin prick tests were carried out with Solu Prick

(ALK, Denmark) standardized allergen extract against the following allergens: birch, timothy grass, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus*, *Cladosporium*, and *Alternaria*. A positive test was a wheal with a mean diameter of ≥ 3 mm and no dermatographism.

Fourteen (70%) of the atopic patients with asthma and 15 (75%) of the nonatopic patients with asthma were on regular treatment with inhaled glucocorticoids (budesonide 200–1600 $\mu\text{g/day}$ [$n=25$] or beclometasone 100–400 $\mu\text{g/day}$ [$n=4$]). There was no statistical difference in the steroid treatment between the two groups. Inhaled corticosteroid therapy was continued with no changes. All the patients but one with asthma were taking inhaled β_2 -agonists as required. None of the patients were being treated with theophylline compounds. One atopic subject with asthma and eight nonatopic subjects with asthma were ex-smokers (stopped smoking 2–20 years previously).

The patients with primary SS were outpatients at the Department of Rheumatology at our hospital (Table 1). The diagnosis of SS was based on the Copenhagen criteria (11). Six subjects with SS were ex-smokers (had stopped smoking 4–17 years previously). Three of the SS patients were taking β_2 -agonists as required, and one was taking inhaled corticosteroids. Six subjects with SS were atopic.

The control group comprised subjects who responded to a request for volunteers or were recruited from the staff at our hospital; none had asthmatic symptoms or were receiving antiasthmatic treatment, and all were nonatopic (Table 1).

All the investigated subjects were nonsmokers and free from respiratory infections for at least 6 weeks prior to the study, and none had a history of cardiovascular disease. All the participants received a questionnaire on the occurrence of airway symptoms that was based on the European Community Respiratory Health Survey questionnaire (12). The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Medical Faculty at Uppsala University. Verbal informed consent was obtained prior to the study.

Spirometry and bronchial challenges

The three bronchial challenges were performed in random order at least 3 days apart and within 3 weeks for each individual. The bronchial challenges took place from 8:30 am to 11 am. The subjects were instructed not to take any short-acting β_2 -agonists 8 h before testing or any long-acting β_2 -agonists 24 h before testing. Functional residual capacity (FRC), vital capacity (VC), airway resistance (R_{aw}), specific airway conductance ($sGaw$), forced vital capacity (FVC), and FEV₁ were measured after each challenge

Table 1. Characteristics of patients with asthma, Sjögren's syndrome, and healthy controls as median (interquartile range)

	Atopic asthma <i>n</i> =20	Nonatopic asthma <i>n</i> =20	Sjögren's syndrome <i>n</i> =18	Controls <i>n</i> =20
Age (years)	32 (26–39)	42 (24–50)	55 (45–61)	37 (25–45)
Sex (M/F)	9/11	1/19	1/17	5/15
FVC (% pred.)	96 (90–100)	89 (83–98)	92 (83–99)	97 (90–101)
FEV ₁ (% pred.)	93 (87–96)	87 (82–93)	92 (84–98)	97 (86–102)
<i>sGaw</i> (% pred.)	73 (54–82)	70 (49–89)	86 (65–98)	82 (72–115)
Inhaled corticosteroids	14	15	1	0
Disease duration (years)	10.5 (1.5–25)	3.5 (2–7.5)	7 (6–9)	

with a Masterlab Trans spirometer and a Masterlab body plethysmograph (Eric Jaeger AG, Würzburg, Germany). FVC, FEV₁, and sGaw were used for the study. Lung-function values are presented as a percentage of reference values (13, 14).

Dosimeter method

An automatic, inhalation dosimeter, Spira Elektro 2 (Respiratory Care Centre, Hameenlinna, Finland), was used in this study (15). The dosimeter was adjusted with a nebulization onset of 50 ml and a nebulization duration of 1.0 s. Inspiratory flow rate reached, but did not exceed, 0.5 l/s. The dosimeter output was 16 ± 5 µl/breath. Each individual took five tidal breaths of each concentration and sGaw, FVC, and FEV₁ were measured after 90 s. The next nebulizing concentration started 3 min after the previous concentration had stopped. Baseline values were measured after an inhalation of saline.

Methacholine and AMP challenges

After the nebulization of saline, methacholine chloride (National Corporation of Swedish Pharmacies) or AMP (Sigma Chemicals, USA) was delivered. The starting concentration of methacholine was 0.0625 mg/ml, while that of AMP was 0.39 mg/ml. The challenge was continued with inhalation of doubling concentrations of methacholine or AMP until the FEV₁ had decreased by 20% or the highest concentration (32 mg/ml of methacholine or 400 mg/ml of AMP) was reached. The quantity of inhaled methacholine or AMP was calculated by weighing the amount of methacholine or AMP that was left in the dosimeter after each concentration and subtracting it from the starting dose. Patients with a provocative concentration of ≤ 32 mg/ml of methacholine or ≤ 400 mg/ml of AMP and a decrease in FEV₁ of 20% or more (PC₂₀) were defined as having BHR (20).

Cold air

The cold air hyperventilation challenge was performed with a heat exchanger and was driven by a supply of compressed air containing 5% carbon dioxide (Turboair Challenger, Equilibrated Bio Systems, Melville, NY, USA) (16). Ventilation was recorded with an Oxycon Sigma device (Jaeger AG, Würzburg, Germany). After baseline measurements of static and dynamic lung function variables, maximal isocapnic ventilation was performed with -20°C cold air for 3 min (17). The temperature was recorded every 15 s. Measurements of lung function variables were made directly after the challenge and then repeated every 5 min until the FEV₁ started to increase again. A 10% fall in the FEV₁ after the cold air challenge was regarded as a positive test (17).

Dose-response slope

After calculations of the provocative concentration and provocative dose causing a 20% decrease in FEV₁, the least-squares slope for methacholine and AMP was calculated from the regression equation for percentage decline in FEV₁ on the cumulative dose of methacholine or AMP with all the measured points (18).

Measurement of serum eosinophil markers

Whole blood was drawn in SST tubes and allowed to coagulate at room temperature for 60 min. The serum was then separated by centrifugation. Serum eosinophil peroxidase (S-EPO) and eosinophil cationic protein (S-ECP) were measured by a prototype immunofluorometric assay with the Pharmacia CAP System[™] (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). The blood samples were collected before each challenge. A mean value of the three measurements was used in this study when comparing the four subject groups.

Peak flow and symptom diaries

Peak flow and symptom diaries were kept during a 17-day period, starting on the day of inclusion in the study. The peak expiratory flow rate (best of three measurements) was recorded four times daily with a Mini-Wright peak flow meter (Clement Clarke, London, UK). Daily peak flow variability was calculated by the following formula:

$$\text{highest PEF} - \text{lowest PEF} \times 100 / \text{mean PEF value of day}$$

In the symptom diary, the subjects recorded, on awakening, whether or not they had had breathing difficulties the previous night, and wheezing in the chest, attacks of breathlessness, or attacks of coughing during the previous 24 h. A symptom score (0–4) was calculated for each individual by the number of respiratory symptoms during the 17-day period.

Statistics

Comparisons between three or more groups were made with the Kruskal–Wallis test, and comparisons between two groups with the Mann–Whitney *U*-test or the chi-square test. In comparisons within a group, the Spearman rank correlation test was used. A multiple regression analysis was performed to evaluate independent relationships of atopy, treatment with inhaled steroids, and baseline FEV₁ on the dose-response slope for AMP in patients with asthma. The log-transformed dose-response values were used for this analysis to achieve a normal distribution. A *P* value of <0.05 was regarded as statistically significant.

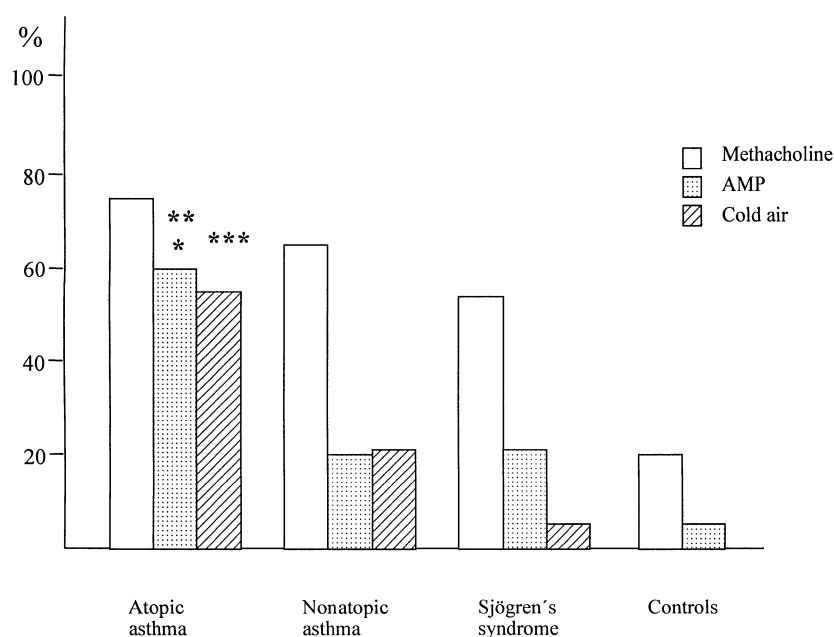


Figure 1. Positive challenge tests in atopic subjects with asthma, nonatopic subjects with asthma, patients with Sjögren's syndrome, and healthy controls as percentages. *** $P < 0.001$ compared with Sjögren's syndrome; ** $P < 0.01$ compared with nonatopic asthma; * $P < 0.05$ compared with Sjögren's syndrome.

Results

Spirometry

No differences were found between any of the groups in FEV₁, FVC, or *s*Gaw (Table 1).

Number of positive challenge tests in all groups

The percentage of positive challenge tests (20% fall in FEV₁ for methacholine and AMP, and 10% fall in FEV₁ for cold air) is shown in Fig. 1. There was no significant difference in the number of positive methacholine challenge tests between subjects with asthma or SS. Atopic subjects with asthma more frequently displayed hyperresponsiveness to AMP than nonatopic subjects with asthma ($P = 0.01$) and patients with SS ($P = 0.02$). Furthermore, hyperresponsiveness to cold air was more common in atopic subjects with asthma than those with SS ($P = 0.001$).

All subjects who had a positive AMP or cold air challenge also had a positive methacholine challenge (Table 2). Four controls had a positive methacholine

challenge (PC₂₀ 11.5–32 mg/ml), and one of them had a positive AMP challenge (PC₂₀ 380 mg/ml) (Table 2). All were nonatopic women, of whom one was an ex-smoker. One nonatopic patient with asthma was unable to complete the cold air challenge because of breathing problems. No adverse reactions to methacholine or AMP occurred.

Dose-response slope for methacholine and AMP, and the mean fall in FEV₁ after cold air challenge

All three patient groups had a significantly steeper dose-response slope for methacholine and AMP than the controls. Atopic subjects with asthma had a significantly larger decrease in FEV₁ after the cold air challenge than the control group (Table 3). Among the three groups of patients, no significant differences were found in the dose-response slopes for methacholine. Atopic patients with asthma had a significantly steeper dose-response slope for AMP than nonatopic patients with asthma. This difference remained significant after adjusting for baseline FEV₁ and dose of inhaled steroids ($P = 0.01$). The group of atopic patients with asthma also had a significantly greater decrease in FEV₁ after the cold air challenge than the group of patients with SS (Table 3).

Table 2. Number (%) of positive AMP and cold air challenge tests in subjects with positive methacholine challenge test in all groups

	AMP	Cold air
Atopic asthma ($n = 15$)	12 (80%)**	11 (73%)*
Nonatopic asthma ($n = 13$)	4 (30%)	4 (30%)
Sjögren's syndrome ($n = 10$)	4 (40%)*	1 (10%)
Controls ($n = 4$)	1 (25%***)	0

Significantly correlated to methacholine challenge test. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Correlation between the dose-response slope for methacholine and AMP and mean fall in FEV₁ cold air challenge

In atopic patients with asthma, a significant correlation was found between the airway response to all three methods, while in nonatopic patients with asthma, a

Table 3. Dose-response slope (DRS) and PC₂₀ for methacholine and AMP and fall in % FEV₁ after cold air challenge as median (interquartile range)

	Atopic asthma n=20	Nonatopic asthma n=20	Sjögren's syndrome n=18	Controls n=20
DRS methacholine	-13.2*** (-58.0, -2.3)	-7.5*** (-14.7, -2.2)	-3.1*** (-29.8, -1.4)	-0.8 (-1.6, -0.27)
PC ₂₀ methacholine mg/ml	6.0*** (1.5, >32)	11.2** (7.0, >32)	32 (4.0, >32)	>32 (>32)
DRS AMP	-0.62***, †, ‡ (-10.6, -0.08)	-0.07** (-0.2, 0.02)	-0.06** (-0.3, -0.03)	-0.01 (-0.03, 0.1)
PC ₂₀ AMP mg/ml	161***, †, ‡ (7.8, >400)	>400 (>400)	>400 (>400)	>400 (>400)
Cold air (%)	-10**, ‡ (-18, -2)	-4 (-6, -2)	-2 (-4, -2)	-2 (-4, -1)

****P*<0.001 compared with controls; ***P*<0.01 compared with controls; **P*<0.05 compared with controls; †*P*<0.01 compared with nonatopic asthma; ‡*P*<0.01 compared with Sjögren's syndrome.

significant correlation was observed only between AMP and cold air challenges. Patients with SS showed a significant correlation between the responses to methacholine and AMP (Table 4).

Peak flow variability and symptoms

In all three patient groups, peak flow variability and the symptom score were significantly higher than in the control group, but no significant differences were found between the patient groups (Table 5).

Inflammatory markers

No significant difference in the level of S-EPO or S-ECP was found between any of the investigated groups (Table 5).

In atopic patients with asthma, a significant correlation was found between S-EPO and S-ECP and the response to AMP ($r = -0.56$, $P = 0.01$; $r = -0.51$, $P = 0.02$), respectively. This correlation remained significant after adjusting for dose of inhaled steroids and baseline FEV₁ ($P = 0.02$). Apart from this, no significant correlations were found between the inflammatory markers and the response to the challenge tests in any group.

Discussion

The results of the present study revealed that patients with asthma and subjects with SS had different patterns of BHR when the three different challenge agents were compared. Atopic subjects with asthma were more hyperresponsive to AMP than nonatopic subjects with asthma, while the majority of both atopic and

nonatopic subjects with asthma and subjects with SS were hyperresponsive to methacholine. Therefore, atopy may be a major determinant of AMP hyperresponsiveness. Furthermore, in atopic patients with asthma, there is a significant correlation between AMP hyperresponsiveness and S-EPO and S-ECP, suggesting that AMP responsiveness may provide an indication of eosinophil activation.

To our knowledge, this is the first study to demonstrate differences in AMP responsiveness between atopic and nonatopic subjects with asthma. Previous studies in adults have found that nonsmoking patients with COPD are less responsive to inhaled AMP than nonsmoking atopic asthmatics (19). In children, challenges with AMP distinguished asthma from chronic airway obstruction of other causes with a sensitivity and specificity of 85–90% (20). Moreover, the sensitivity to AMP challenge was increased in both asthmatic and nonasthmatic atopic subjects when compared with nonatopic nonasthmatic subjects (21), suggesting that atopy and mast-cell priming may be important factors in determining airway responsiveness to inhaled AMP. However, Phillips & Holgate studied six atopic and six nonatopic subjects with asthma and found that all of them were hyperresponsive to AMP (22). The reason for this discrepancy in the results is not clear, but it suggests that factors other than atopy also determine the degree of AMP sensitivity.

AMP, an indirect stimulus, causes airflow limitations as a result of mediator release from inflammatory cells; therefore, it has been suggested that it produces a better correlation with the underlying airway inflammation. Exactly which cells are involved is unknown, but AMP may enhance release of bronchoconstrictive mediators such as histamine from activated mast cells and

Table 4. Correlation between dose-response slope methacholine and AMP and fall in FEV₁ after cold air challenge in all groups

	Methacholine and AMP	Methacholine and cold air	AMP and cold air
Atopic asthma	0.91***	0.66**	0.83***
Nonatopic asthma	0.31	0.44	0.54**
Sjögren's syndrome	0.61*	0.41	0.19
Controls	-0.29	-0.18	-0.15

****P*<0.001; ***P*<0.01; **P*<0.05.

Table 5. Peak flow (PEF) variability, respiratory symptom score, serum eosinophil peroxidase (S-EPO), and eosinophil cationic protein (S-ECP) as median (interquartile range)

	Atopic asthma	Nonatopic asthma	Sjögren's syndrome	Controls
PEF variability (%)	6 (5–10)*	7 (5–11)*	8 (5–10)*	5 (4–6)
Symptom score (0–4)	2 (1–3)***	3 (1–4)***	1 (0–2)*	0
S-EPO µg/l	15 (9–32)	13 (10–16)	12 (7–19)	12 (9–21)
S-ECP µg/l	10 (7–15)	10 (8–13)	9 (5–11)	9 (7–12)

PEF variability (highest PEF – lowest PEF × 100/mean PEF value of day).

****P*<0.001 compared with controls; **P*<0.05 compared with controls.

circulating basophils (23). Furthermore, mast cells may participate in the chronic inflammatory response by releasing multifunctional cytokines which can cause the activation of eosinophils (24).

Our data appear to be consistent with the findings of Polosa et al., who found a better correlation between AMP challenge and eosinophils in the sputum of atopic subjects than with methacholine challenge (25).

The patients with SS in this study were significantly more hyperresponsive to methacholine than to AMP and cold air. The reason for this difference is unknown. It is possible that the airway inflammation of patients with SS is different from the inflammation observed in asthma. Bronchial biopsy studies on patients with SS have reported submucosal mononuclear infiltrates in both small and large bronchi, and high-resolution computed tomography has revealed thickening of the segmental bronchi (26). In fact, treatment with inhaled corticosteroids does not appear to decrease BHR or respiratory symptoms in patients with SS as it does in asthma (27). It is also possible that factors other than inflammation may predispose to BHR in patients with SS. The chronic dryness of the airways that is commonly seen in these patients can cause hyperosmolarity of the epithelial lining and increased bronchial responsiveness, or possibly could make the epithelium more vulnerable to damage, resulting in airway wall remodeling, thickening of the airway wall, and BHR.

In the present study, four women in the control group had asymptomatic BHR. They were nonatopic and one

was an ex-smoker. Population studies have reported a higher prevalence of BHR in women (28). The clinical significance of asymptomatic BHR is not clear, but it may be a risk factor for developing asthma later in life (29).

It was a little surprising that only 75% of the atopic patients with asthma and 60% of the nonatopic patients with asthma had a positive methacholine challenge in this study. All of them had previously been positive on methacholine challenge by another method (10). This difference may possibly be explained by the different methods used or the variation in bronchial responsiveness with time.

In conclusion, the results of this study suggest that patients with asthma and subjects with SS display different hyperresponsiveness profiles for indirect and direct stimuli. Atopic subjects with asthma were more hyperresponsive to AMP than nonatopic subjects and patients with SS. Furthermore, in atopic subjects with asthma, BHR to AMP was related to signs of eosinophil activation. More than one challenge may be required to detect different aspects of BHR in different diseases associated with BHR.

Acknowledgments

We thank our research nurse Ulrike Spetz-Nyström and research technician Monica Hall for their assistance with collecting data and technical assistance with the bronchial challenges. We acknowledge the support of the Swedish Heart-Lung Foundation, the King Oscar II Jubilee Foundation, and the Bror Hjerpstedt Foundation.

References

1. American Thoracic Society. Medical Section of the American Lung Association. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225–231.
2. POWER C, SREENAN S, HURSON B, BURKE C, POULTER LW. Distribution of immunocompetent cells in the bronchial wall of clinically healthy subjects showing bronchial hyperresponsiveness. *Thorax* 1993;**48**:1125–1129.
3. CRIMI E, SPANEVELLO A, NERI M, IND PW, ROSSI GA, BRUSASCO V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;**157**:4–9.
4. YAN K, SALOME CM, WOOLCOCK AJ. Prevalence and nature of bronchial hyperresponsiveness in subjects with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1985;**132**:25–29.
5. VAN ASPEREN P, MELLIS CM, SOUTH RT, SIMPSON SJ. Bronchial reactivity in cystic fibrosis with normal pulmonary function. *Am J Dis Child* 1981;**135**:815–819.
6. PANG J, CHAN HS, SUNG JY. Prevalence of asthma, atopy and bronchial hyperactivity in bronchiectasis: a controlled study. *Thorax* 1989;**44**:948–951.
7. POTENA A, LA CORTE R, FABBRI LM, PAPI A, TROTTA F, CIACCIA A. Increased bronchial responsiveness in primary and secondary Sjögren's syndrome. *Eur Respir J* 1990;**3**:548–553.
8. GUDBJÖRNSSON B, HEDENSTRÖM H, STÅLENHEIM G, HÄLLGREN R. Bronchial hyperresponsiveness to methacholine in patients with primary Sjögren's syndrome. *Ann Rheum Dis* 1991;**50**:36–40.
9. POLOSA R, HOLGATE ST. Adenosine bronchoprovocation: a promising marker of allergic inflammation in asthma? *Thorax* 1997;**52**:919–923.
10. CARLSON M, HÅKANSSON L, KÄMPE M, STÅLENHEIM G, PETERSON C, VENGE P. Degranulation of eosinophils from pollen atopic patients is increased during pollen season. *J Allergy Clin Immunol* 1992;**89**:131–139.
11. MANTHORPE R, OXHOLM P, PRAUSE JU, SCHIÖDT M. The Copenhagen criteria for Sjögren's syndrome. *Scand J Rheumatol* 1986;**61**:19–21.
12. BURNEY PGJ, LUCZYNSKA C, CHINN S, JARVIS D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;**7**:954–960.
13. HEDENSTRÖM H, MALMBERG P, AGARWAL K. Reference values for lung function tests in females. Regression equations with smoking variables. *Bull Eur Physiopathol Respir* 1985;**21**:551–557.
14. HEDENSTRÖM H, MALMBERG P, FRIDRIKSSON HV. Reference values for pulmonary function tests in men. Regression equations which include tobacco smoking variables. *Ups J Med Sci* 1986;**91**:299–310.

15. NIEMINEN MM, LAHDENSUO A, KELLOMAEKI L, KARVONEN J, MUUTTARI A. Methacholine bronchial challenge using a dosimeter with controlled tidal breathing. *Thorax* 1988;**43**:896–900.
16. ELIASSON AH, PHILIPS YY, RAJAGOPAL KR, HOWARD RS. Sensitivity and specificity of bronchial provocation testing. *Chest* 1992;**102**:347–355.
17. TESSIER P, GHEZZO H, L'ARCHEVEQUE J, CARTIER A, MALO JL. Shape of the dose-response curve to cold air inhalation in normal and asthmatic subjects. *Am Rev Respir Dis* 1987;**136**:1418–1423.
18. CHINN S, BURNEY PGJ, BRITTON JR, TATTERSFIELD AE, HIGGINS BG. Comparison of PD₂₀ with two alternative measures of response to histamine challenge in epidemiological studies. *Eur Respir J* 1993;**6**:670–679.
19. OOSTERHOFF Y, de JONG JW, JANSEN MAM, KOËTER GH, POSTMA DS. Airway responsiveness to AMP in chronic obstructive pulmonary disease is determined by smoking. *Am Rev Respir Dis* 1993;**147**:553–558.
20. AVITAL A, SPRINGER C, BAR-YISHAY E, GODFREY S. Adenosine, methacholine and exercise challenges in children with asthma or paediatric chronic obstructive pulmonary disease. *Thorax* 1995;**50**:511–516.
21. CHURCH MK, FEATHERSTONE RL, CUSHLEY MJ, MANN JS, HOLGATE ST. Relationship between adenosine, cyclic nucleotides and xanthines in asthma. *J Allergy Clin Immunol* 1986;**78**:670–675.
22. PHILLIPS GD, HOLGATE ST. Absence of a late-phase response or increase in histamine responsiveness after bronchial provocation with adenosine 5'-monophosphate in atopic and nonatopic asthma. *Clin Sci* 1988;**75**:429–436.
23. CHURCH MK, HOLGATE ST, HUGHES PJ. Adenosine inhibits and potentiates IgE-dependent histamine release from human basophils from an A₂-receptor mediated mechanism. *Br J Pharmacol* 1983;**80**:719–726.
24. GALLI SJ. New concepts about the mast cell. *N Engl J Med* 1993;**328**:257–265.
25. POLOSA R, CIAMARRA I, PROSPERINI G, et al. Sputum eosinophilia correlates with bronchial hyperresponsiveness to AMP in patients with rhinitis. *Am J Respir Crit Care Med* 1997;**155**:A978.
26. PAPIRIS SA, MANIATI M, CONSTANTOPOULOS SH, ROUSSOS C, MOUTSOPOULOS HM, SKOPOULI FN. Lung involvement in primary Sjögren's syndrome is mainly related to the small airway disease. *Ann Rheum Dis* 1999;**58**:61–64.
27. STÅLENHEIM G, GUDBJÖRNSSON B. Anti-inflammatory drugs do not alleviate bronchial hyperreactivity in Sjögren's syndrome. *Allergy* 1997;**52**:423–427.
28. LEYNAERT B, BOUSQUET J, HENRY C, LIARD R, NEUKIRCH F. Is bronchial hyperresponsiveness more frequent in women than in men? *Am J Respir Crit Care Med* 1997;**156**:1413–1420.
29. LAPRISE C, BOULET LP. Asymptomatic airway hyperresponsiveness. A three-year follow-up. *Am J Respir Crit Care Med* 1997;**156**:403–409.