

Autologous Serum Skin Test for Autoantibodies Is Associated with Airway Hyperresponsiveness in Patients with Asthma

An-Soo Jang Jong-Sook Park June-Hyuk Lee Sung Woo Park Do-Jin Kim
Choon-Sik Park

Asthma and Allergy Research Group, Division of Allergy and Respiratory Diseases, Soonchunhyang University, Bucheon Hospital, Gyeonggi-do, Republic of Korea

© S. Karger AG, Basel

**PROOF Copy
for personal
use only**

ANY DISTRIBUTION OF THIS
ARTICLE WITHOUT WRITTEN
CONSENT FROM S. KARGER
AG, BASEL IS A VIOLATION
OF THE COPYRIGHT.

Key Words

Autoantibodies against FcεRI • Immunoglobulin E • Asthma

Abstract

Background: Autoimmune diseases have been implicated as a cause of intrinsic asthma; however, there is little data on the role of autoimmunity in the pathogenesis of asthma. **Objective:** The purpose of this study was to investigate circulating functional autoantibodies against the high-affinity IgE receptor FcεRI or IgE in patients with asthma. **Methods:** Twenty-eight patients with asthma and 19 control subjects were included. All subjects were skin tested with autologous serum to assess for the potential presence of receptor FcεRI or IgE autoantibodies. If the serum-induced wheal diameter was 1.5 mm larger than the histamine-induced wheal diameter and that was 3 mm larger than the saline-induced wheal diameter at 30 min, the reaction was defined positive. **Results:** Of the 47 total subjects (both asthma patients and control subjects), 13 (27.7%) had a positive autologous serum skin test (ASST). Of the 28 asthma patients, 8 (28.6%) were regarded as having autoimmune origin. Autoantibodies against FcεRI or IgE were found in asthma patients, irrespective of atopic status (atopy+ 3/13 vs. atopy– 5/15). The wheal diameter related to ASST was not related to atopy. Asthma patients with ASST-positive results as compared with patients with ASST-negative results exhibited a significant increased airway hyperresponsiveness (PC₂₀ methacholine,

2.70 ± 1.27 vs. 9.08 ± 2.35; p < 0.026). **Conclusion:** Our data demonstrate that aberrant autoantibodies against the high-affinity IgE receptor FcεRI or IgE are related to airway hyperresponsiveness in patients with asthma.

Copyright © 2006 S. Karger AG, Basel

Introduction

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role [1]. There is good evidence indicating that asthma is a heritable disease [2–5]. Atopy appears to be an important host factor that predisposes individuals to developing asthma. The available epidemiological evidence suggests that the population-based proportion of asthma cases attributable to atopy is about 50% [6].

Despite intensive efforts and advances in molecular biology and genetics, no gene (or genes) involved in the heritability of atopy or asthma has been identified with any certainty [7, 8]. The results of several studies provide an indication that multiple immune reactions may be involved in the pathogenesis of asthma [5, 7, 8].

The other mechanisms apart from atopy remain to be clarified. Allergy and autoimmune disease are two potential outcomes of dysregulated immunity. Both are characterized by localized inflammation that leads to injury and/or destruction of target tissues. Until recently, it

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2006 S. Karger AG, Basel
0025-7931/06/0000-0000\$23.50/0

Accessible online at:
www.karger.com/res

Dr. An-Soo Jang
Division of Allergy and Respiratory Diseases, Department of Internal Medicine
Soonchunhyang University Hospital, 1174, Jung-dong, Wonmi-gu, Bucheon-si
Gyeonggi-do, 420-767 (Republic of Korea)
Tel. +82 32 621 5143, Fax +82 32 621 5016, E-Mail jas877@schbc.ac.kr

Table 1. Characteristics of asthma patients and control subjects

	Asthma	Controls
Patients	28	19
Sex, males/females	12/16	9/10
Age, years	50.2 ± 15.9	48.6 ± 12.3
FEV ₁ , % predicted	84.8 ± 26.6*	111.8 ± 12.2
FEV ₁ /FVC	72.5 ± 21.0	78.2 ± 19.2
Duration of asthma, years	2.73 ± 3.16	ND
Atopy, %	13/28, 46.4%	5/19, 26.3%
ASST	8/28, 28.6%	5/19, 26.3%
PC ₂₀ , methacholine, mg/ml	7.30 ± 9.06	>25
Total IgE	423.7 ± 532.7	405.7 ± 592.1

ND = Not determined.

* p < 0.01 compared with control subjects.

was generally accepted that the mechanisms that govern these disease processes are quite disparate. However, new discoveries suggest a possible pathogenetic linkage. Basophil granulocytes and tissue mast cells and their mediators play a role in the pathogenesis of asthma. Human basophils and mast cells (FcεRI+ cells) can be activated through immunological interaction with the IgE-FcεRI network. Anti-IgE autoantibodies are occasionally present even in normal donors and, more frequently, in a variety of allergic diseases such as chronic urticaria, atopic dermatitis and bronchial asthma, as well as in autoimmune disorders such as rheumatoid arthritis, lupus erythematosus and systemic sclerosis [9]. Therefore, to confirm the potential relationship or common mechanism, we have investigated the allergy skin test and functional autoantibodies against the high-affinity IgE receptor FcεRI or IgE in patients with asthma.

Materials and Methods

Subjects

Subjects with a cough for more than 3 weeks were recruited from the outpatient clinics of Soonchunhyang University Hospital. A clinical history was obtained for each patient using a physician-administered questionnaire. Chest posterior-anterior radiography, allergy skin prick tests, autologous serum skin tests (ASSTs) and spirometry, including bronchodilator responses after the inhalation of two puffs of 100 µg aerosolized albuterol, were performed. The blood was sampled for differential cell counts. Airway reactivity was measured in cases with forced expiratory volume in 1 s (FEV₁) percent predicted values of 70% or greater.

Asthma patients exhibited one or more asthma symptoms, and their physical examinations were compatible with the definition of asthma of the American Thoracic Society [10]. Asthma patients showed airway reversibility, as documented by a positive bronchodilator response of a greater than 15% increase in FEV₁ and/or airway hyperreactivity to less than 10 mg/ml methacholine [11]. Control subjects had no airway reversibility and/or airway hyperreactivity to greater than 25 mg/ml methacholine. Control subjects included 5 cases of sinusitis, 11 cases of bronchitis and 3 cases of bronchiectasis. The exclusion criteria included parenchymal lung disease apparent on chest radiography, a diffusing capacity of less than 80%, previous inhaled steroid or systemic steroid use within the past 4 weeks, and maintenance theophylline or leukotriene antagonist therapies. The clinical parameters are summarized in table 1. This study was performed with the approval of the Ethics Committee of the University Hospital, and informed written consent was obtained from all the study subjects.

Lung Function Tests

Baseline measurements of forced vital capacity (FVC) and FEV₁ were obtained in the absence of recent use (within 8 h) of a bronchodilator and selected according to the American Thoracic Society criteria [10]. Basal and postbronchodilator FEV₁, FVC and forced expiratory flow (FEF_{25–75%}) were measured between 1 p.m. and 4 p.m. Airway hyperresponsiveness was measured by methacholine challenge and expressed as the provocation concentration to cause a fall in FEV₁ of 20% (PC₂₀) in noncumulative units [12].

Autologous Serum Skin Test

Venous blood was collected in sterile glass tubes without clotting accelerator (Vacutainer®, Becton Dickinson, N.J., USA) and allowed to clot at room temperature for 30 min. Serum was separated by centrifugation at 500 g for 15 min, and divided aliquots were used in the ASST and other exam, as described previously [13]. Samples (0.05 ml) of autologous serum, histamine 0.01 mg/ml, and 0.9% sterile saline were separately injected into the volar aspect of the forearm. After 30 min, the mean diameters of the wheals formed by the serum were compared with those formed by histamine. If the former was 1.5 mm larger than the latter and the histamine-induced wheal diameter was 3 mm larger than that of saline, the reaction was deemed positive. When ASSTs were repeated at different sites and on different days, there was no significant difference between the measurements taken either at 2 separate sites or on 2 separate days.

Allergy Skin Prick Tests

Allergy skin prick tests were performed using commercially available inhalant allergens, which included dust mites (*Dermaphagoides farinae* and *Dermatophagoides pteronyssinus*; Ben-card Co., UK) and histamine (1 mg/ml). None of the subjects had received antihistamines orally in the 3 days preceding the study. All of the tests included positive (1 mg/ml histamine) and negative (diluent) controls. After 15 min, the mean diameters of the wheals formed by the allergens were compared with those formed by histamine. If the former was the same or larger than the latter (A/H ratio ≥ 1.0), the reaction was deemed positive. Atopy was determined by the presence of an immediate skin reaction to one or more aeroallergens, as described previously [14].

Statistical Analysis

Comparisons of two groups were assessed using the Mann-Whitney U test, the χ^2 test, or Wilcoxon's test using SPSS (version 10.0; SPSS Inc., Chicago, Ill., USA). Sensitivity was calculated according to the formula: number of patients who are both asthma positive and ASST positive $\times$ 100/total number of subjects. Similarly, specificity was calculated according to the formula: number of patients who are both asthma negative and ASST negative $\times$ 100/total number of subjects. A *p* value <0.05 was considered statistically significant.

Results

Of the 47 total subjects (both asthma patients and control subjects), 13 (27.7%) had a positive ASST. Of the 28 asthma patients, 8 (28.6%) were positive on ASST. Autoantibodies against the high-affinity IgE receptor Fc ϵ RI or IgE were found in asthma patients, irrespective of atopic status (3/13 vs. 5/15). Autoantibodies against Fc ϵ RI or IgE were detected in the skin of control subjects. There were no differences in atopy and ASST between asthma patients and control subjects.

A wheal diameter related to ASST was not related to atopy. Although there were no differences in PC₂₀ according to atopy in asthma patients, asthma patients with ASST-positive results as compared with patients with ASST-negative results exhibited a significantly lower methacholine PC₂₀ (2.70 ± 1.27 vs. 9.08 ± 2.35 ; *p* <0.26) (fig. 1).

The sensitivity of ASST was 28.5% (8/28), specificity 73.6% (14/19), and accuracy 46.8% (22/47).

Discussion

Our findings provide further insight into asthma immunologic basis against the high-affinity IgE receptor Fc ϵ RI or IgE in patients with asthma. Among the patients with asthma, we found that functional autoantibodies against Fc ϵ RI or IgE were observed in more than 28.6%, and especially in patients with severe airway hyperresponsiveness. Those data suggest that autoantibodies against Fc ϵ RI or IgE may be involved in severe asthma.

Asthma mechanisms apart from atopy remain to be clarified in the development of airway responsiveness and inflammation. Autoimmune disease has been implicated as a cause of intrinsic asthma [6].

Allergy and autoimmunity result from dysregulation of the immune system. The concomitant presentation of those conditions and the potential relationship or com-

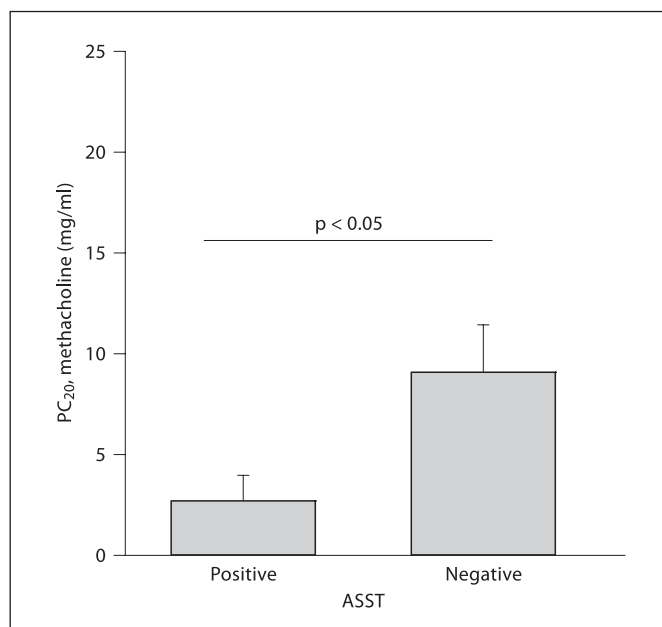


Fig. 1. Differences in airway hyperresponsiveness according to ASST. *p* <0.05 compared with negative ASST.

mon mechanisms are revealed in some cases by looking at the key elements that regulate the immune response in both asthma and autoimmune conditions: mast cells, antibodies, T cells, cytokines, and genetic determinants. The presence of autoantibodies in some allergic diseases suggested an autoimmune basis for those conditions [15].

The possible involvement of autoimmunity in the pathogenesis of asthma has been proposed by few studies [15–19]. Fc ϵ RI is a multimeric surface receptor that is expressed exclusively as a tetramer on rodent cells, but exists as a tetramer or trimer on human cells. On mast cells and basophils, Fc ϵ RI is essential for IgE-mediated acute allergic reactions. Cross-linking of the IgE-loaded high-affinity IgE receptor by multivalent antigens results in mast cell activation and subsequent release of multiple proinflammatory mediators [20]. The autoantibodies against Fc ϵ RI or IgE may be involved in the pathogenesis of asthma [17–19]. We found that 28.6% of asthma patients revealed positive autoantibodies against Fc ϵ RI or IgE, suggesting that autoimmunity may be involved in the pathogenesis of asthma. Additionally, 48.6% of asthma patients had a positive allergy skin test against common allergen, suggesting that aberrations of the immune system have a common pathophysiologic mechanism of asthma.

Palmer et al. [21] found a narrow-sense heritability of total serum IgE levels of 47.3%, i.e. additive genetic effects

contributed just under half of the total variance, using maximum likelihood analysis of variance component estimates in an Australian population-based sample of 232 Caucasian nuclear families. In this study, the involvement of the autoimmune mechanism in asthma pathogenesis contributes to the explanation of possible additive genetic effects.

Autoimmunity has been more often implicated in the pathogenesis of nonallergic asthma than in allergic asthma [16]. In our study, autoantibodies against the high-affinity IgE receptor FcεRI or IgE is not related to atopy, indicating that autoimmunity may be involved in asthma pathogenesis regardless of allergen sensitization, and asthma patients with positive autoantibodies against the high-affinity IgE receptor FcεRI or IgE have increased airway hyperresponsiveness, suggesting that autoimmunity may be involved in severe asthma pathogenesis.

Recently, attenuating allergic disease by specifically inhibiting IgE and the development of the monoclonal anti-IgE antibody were major breakthroughs in asthma management [22–24]. Although anti-IgE therapy was a significant addition to current asthma treatments, the ef-

ficacy of anti-IgE therapy in a few patients with asthma should be further explored. In our study, patients with asthma had autoantibodies against IgE receptor FcεRI or IgE, indicating that anti-IgE therapy may be a limitation to treatment in patients with asthma.

The limitation of this study was that control groups had a variety of respiratory diseases, e.g., bronchiectasis, bronchitis and sinusitis. Therefore, further studies including a health control group are needed to clarify the role of autoantibodies against IgE receptor FcεRI or IgE in asthma.

In conclusion, among asthma patients, we found that functional autoantibodies against FcεRI or IgE were observed, suggesting that autoantibodies against FcεRI or IgE may be involved in the pathogenesis of asthma regardless of the appearance of atopy.

Acknowledgement

This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (01-PJ3-PG6-01GN04-003 and 0412-CR03-0704-0001).

References

- Global Initiative for Asthma (GINA): Global strategy for asthma management and prevention. NHLBI/WHO workshop report. Bethesda, National Institutes of Health National Heart, Lung, and Blood Institute, 2002. NIH publication No. 02-3659. *J Allergy Clin Immunol* 2002;110:S141–S219.
- Sibbald B, Horn ME, Gregg I: A family study of the genetic basis of asthma and wheezy bronchitis. *Arch Dis Child* 1980;55:354–357.
- Holgate ST: Genetic and environmental interaction in allergy and asthma. *J Allergy Clin Immunol* 1999;104:1139–1146.
- Holgate ST: The epidemic of allergy and asthma. *Nature* 1999;402:B2–B4.
- Holloway JW, Beghe B, Holgate ST: The genetic basis of atopic asthma. *Clin Exp Allergy* 1999;29:1023–1032.
- Wiesch DG, Meyers DA, Bleecker ER: Genetics of asthma. *J Allergy Clin Immunol* 1999;104:895–901.
- Barnes KC: Evidence for common genetic elements in allergic disease. *J Allergy Clin Immunol* 2000;106:192–200.
- Pearce N, Pekkanen J, Beasley R: How much asthma is really attributable to atopy? *Thorax* 1999;54:268–272.
- Marone G, Spadaro G, Palumbo C, Condorelli G: The anti-IgE/anti-FcεRIα autoantibody network in allergic and autoimmune diseases. *Clin Exp Allergy* 1999;29:17–27.
- American Thoracic Society: Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991;144:1202–1218.
- Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease and asthma. *Am Rev Respir Dis* 1987;136:225–244.
- Junifer EF, Cockcroft DW, Hargreave FE: Histamine and Methacholine Inhalation Tests. A Laboratory Tidal Breathing Protocol, ed 2. Lund, Astra Draco, 1994.
- Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW: The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999;140:446–452.
- Park CS, Kim YY, Kang SY: Collection between RAST and skin test for inhalant offending allergens. *J Korean Soc Allergol* 1983;3:1–9.
- Rottem M, Shoenfeld Y: Asthma as a paradigm for autoimmune disease. *Int Arch Allergy Immunol* 2003;132:210–214.
- Nahm DH, Lee YE, Yim EJ, Park HS, Yim H, Kang Y, Kim JK: Identification of cytokeratin 18 as a bronchial epithelial autoantigen associated with nonallergic asthma. *Am J Respir Crit Care Med* 2002;165:1536–1539.
- Szczeklik A, Nizankowska E, Serafin A, Dyczek A, Duplaga M, Musial J: Autoimmune phenomena in bronchial asthma with special reference to aspirin intolerance. *Am J Respir Crit Care Med* 1995;152:1753–1756.
- Quintero JM, Burrell RG, Cather CH, Wymer B, Baise R: Detection of antibodies to sinus tissue in chronic bronchial asthma. *Allergy* 1966;37:84–89.
- Dinu IV, Roth L: The presence of antilung antibodies in the sera of asthma patients. *Rum Med Rev* 1965;19:26–29.
- Kraft S, Rana S, Jouvin MH, Kinet JP: The role of the FcεRI β-chain in allergic diseases. *Int Arch Allergy Immunol* 2004;135:62–72.
- Palmer LJ, Burton PR, James AL, Musk AW, Cookson WOCM: Familial aggregation and heritability of asthma-associated quantitative traits in a population-based sample of nuclear families. *Eur J Hum Genet* 2000;8:853–860.
- Buhl R: Anti-IgE antibodies for the treatment of asthma. *Curr Opin Pulm Med* 2005;11:27–34.
- Finn A, Gross G, van Bavel J, Lee T, Windom H, Everhard F, Fowler-Taylor A, Liu J, Gupta N: Omalizumab improves asthma-related quality of life in patients with severe allergic asthma. *J Allergy Clin Immunol* 2003;111:278–284.
- Corren J, Casale T, Deniz Y, Ashby M: Omalizumab, a recombinant humanized anti-IgE antibody, reduces asthma-related emergency room visits and hospitalizations in patients with allergic asthma. *J Allergy Clin Immunol* 2003;111:87–90.