

## Increased levels of Circulating Autoantibodies to Cultured Human Bronchial Epithelial Cell in Adult Patients with Nonatopic Asthma

The pathogenetic mechanism of nonatopic asthma has not yet been defined. The idea of a possible involvement of autoimmunity in the pathogenesis of nonatopic asthma has been proposed by earlier studies. To evaluate the possible involvement of autoimmune response against bronchial epithelial cell in the pathogenesis of nonatopic asthma, we measured circulating autoantibodies to cultured human bronchial epithelial cell (BEAS-2B cell line) using enzyme-linked immunosorbent assay. We used stored serum samples from 38 age-matched healthy controls, 26 adult patients with atopic asthma, 16 adult patients with nonatopic asthma, and 12 adult patients with systemic lupus erythematosus. Levels of IgG autoantibodies to bronchial epithelial cell were significantly higher in patients with nonatopic asthma (mean  $\pm$  SD of absorbance values;  $0.135 \pm 0.030$ ) and systemic lupus erythematosus ( $0.293 \pm 0.181$ ) than in healthy controls ( $0.112 \pm 0.016$ ) and patients with atopic asthma ( $0.116 \pm 0.031$ ) ( $p < 0.05$ ). This study showed that levels of circulating IgG autoantibodies to bronchial epithelial cell were increased in adult patients with nonatopic asthma. Further studies are needed to evaluate the possible involvement of autoimmune mechanism in the pathogenesis of nonatopic asthma.

Key Words : Asthma; Autoantibodies

Dong-Ho Nahm, Min-Jung Shin,  
Hyunee Yim<sup>\*</sup>, Yup Kang<sup>1</sup>, Dong-Chul Choi<sup>1</sup>,  
Jin-Kyoo Kim<sup>3</sup>, Sun-Sin Kim, Soo-Keol Lee,  
Hae-Sim Park

Department of Allergy and Clinical Immunology;  
Department of Pathology<sup>2</sup>, The Laboratory of  
Endocrinology<sup>1</sup>, Ajou University School of Medicine,  
Suwon; Department of Medicine, Samsung Medical  
Center, Sungkyunkwan University College of Medicine<sup>2</sup>,  
Seoul; Department of Microbiology<sup>3</sup>, Changwon  
National University, Changwon, Korea

Received : 19 February 2001  
Accepted : 10 May 2001

### Address for correspondence

Dong-Ho Nahm, M.D.  
Department of Allergy and Clinical Immunology,  
Ajou University School of Medicine, San 5, Won-  
chon-dong, Paldal-gu, Sunwon 442-721, Korea  
Tel : +82.31-219-5152, Fax : +82.31-219-5109  
E-mail : dhnahm@madang.ajou.ac.kr

\*This study was supported by KOSEF Grant 1999-  
2-212-002-2 to Dong-Ho Nahm.

## INTRODUCTION

Allergic response to common environmental agents (allergens) has been regarded as an important pathogenetic mechanism of bronchial asthma (1). However, allergic sensitization (atopy) cannot be detected in a significant number of adult asthmatic patients (2). Traditionally, asthma has been classified as atopic and nonatopic forms on the basis of the presence or absence of immediate skin-test reactivity to common aeroallergens (3). Atopic asthma usually starts during childhood and is characterized by allergen-dependent, often seasonal, symptoms (3, 4). In contrast, nonatopic asthma usually begins in adulthood and is perennial and more severe (3, 4). In addition, it is often associated with sinusitis and nasal polyposis (3). Nonatopic asthma has been referred to as "intrinsic asthma" based on a suggestion that there must be an etiologic agent in the patient's own body (3). However, the "intrinsic" etiology has not yet been defined.

The idea of a possible involvement of autoimmunity in the pathogenesis of nonatopic asthma has been proposed by previous studies which demonstrated higher incidences of

various autoantibodies including antinuclear and antithyroid antibodies in nonatopic asthmatics, as compared to atopic asthmatics and healthy controls (5, 6). However, the pathogenetic significance of these autoantibodies in nonatopic asthma remains unexplained.

Bronchial epithelium has been suggested as a target for inflammatory response in asthma (7). Presence of circulating autoantibodies against lung and nasal mucosa tissues has been reported in asthmatic patients by earlier studies (8, 9). However, autoantigenic component of respiratory mucosa tissue reacting with circulating autoantibodies is not still defined. Recently, we showed the presence of circulating IgG autoantibodies against bronchial epithelial cell in a small portion of nonatopic asthma by indirect immunofluorescence staining of human bronchial mucosa tissue (10).

To evaluate the possible involvement of autoimmune response against bronchial epithelial cell in the pathogenesis of nonatopic asthma, we examined circulating autoantibodies to cultured human bronchial epithelial cell in adult patients with nonatopic asthma by enzyme-linked immunosorbent assay (ELISA).

## PATIENTS AND METHODS

### Patients

We used stored serum samples from 16 adult patients with nonatopic asthma, 26 adult patients with atopic asthma, 38 age-matched healthy controls, and 12 adult patients with systemic lupus erythematosus (Table 1). Asthmatic subjects had typical clinical history of asthma and a 20% decrease in forced expiratory volume in one second (FEV1) following the inhalation of <8 mg methacholine/mL or documented reversibility of FEV1 greater than 15% after inhalation of bronchodilator. All subjects underwent skin-prick test with 50 common aeroallergens including house dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), grass pollens, tree pollens, weed pollens, cat, dog, and molds (Bencard Co., Brentford, UK). The wheal diameters were measured after 15 min. Positive result of skin-prick test was defined when wheal diameter of any one allergen was greater than 3 mm over negative control (normal saline). Atopic asthma was defined when skin-prick test to any one allergen was positive. Nonatopic asthma was defined when skin-prick test to any of the 50 common aero-allergens was negative in the presence of a positive histamine control. Patients with occupational asthma were excluded from the study subjects. Patients with systemic lupus erythematosus classified according to the criteria of the American Rheumatic Association were included as a positive control group for the measurement of autoantibodies (11). Healthy controls had normal baseline lung function and no clinical symptoms of respiratory diseases. All sera were aliquoted and stored at -20°C. Serum total IgE level was measured by radioimmunoassay using AlaS-TAT kit (Diagnostic Product Corporation, Los Angeles, CA). The institutional review board approved this study.

### Cell culture

Human bronchial epithelial cell line (BEAS-2B) was obtained from American Type Culture Collection (Rockville, MD). BEAS-2B cells were cultured as previously described (12).

### Cell-ELISA

Circulating autoantibodies to cultured human bronchial epithelial cell were measured by Cell-ELISA as previously described with modification (13). BEAS-2B cells were cultured in a 96-well tissue culture plate (Falcon, Franklin Lakes, NJ). When monolayer of cells covered 50% of the well, ELISA was conducted. After aspiration of culture medium, the plates were incubated with duplicated serum samples (100  $\mu$ L) diluted in culture medium at 1:500 (v/v) dilution for 2 hr at 37°C in CO<sub>2</sub> incubator. The plates were washed three times with Dulbecco's phosphate-buffered saline. Then, the plates were incubated with peroxidase-conjugated goat anti-human IgG antibodies (Sigma Chemical Co., St. Louis, MO) at 1:5000 dilution for 2 hr at 37°C in CO<sub>2</sub> incubator. The plates were washed three times again. In the final step, the substrate solution consisting of 0.04% (w/v) o-phenylenediamine dissolved in 24.3 mM citric acid, 51.4 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 5.0) and 0.03% H<sub>2</sub>O<sub>2</sub> was added. After 30 min, the reaction was stopped by adding 2.5 N H<sub>2</sub>SO<sub>4</sub>. Absorbance at 490 nm was measured by a microplate reader. In each plate, 5 different serum samples were included for the measurement of variation. Coefficients of intraplate variation and interplate variation were below 12%.

### Statistical analyses

Data were expressed as mean  $\pm$  standard deviation (SD). When multiple comparisons were made between groups, significant between-group variability was first established using the Kruskal-Wallis test. The Mann-Whitney *U* test was then used to assess differences between two groups. The Fisher's exact test was used to compare the frequencies of autoantibodies between two groups. A *p* value of less than 0.05 was considered significant.

## RESULTS

Levels of IgG autoantibodies to bronchial epithelial cell were significantly higher in patients with nonatopic asthma (mean  $\pm$  SD of absorbance values; 0.135  $\pm$  0.030) and systemic lupus erythematosus (0.293  $\pm$  0.181) than in healthy

Table 1. Characteristics of the study subjects

Category	n	Age (yr)	Sex (F/M)	Total IgE (IU/mL)	FEV1 (% predicted)
Healthy controls	38	38 $\pm$ 10	22/16	275 $\pm$ 329	104 $\pm$ 18 <sup>†</sup>
Atopic asthma	26	24 $\pm$ 9*	10/16	402 $\pm$ 312*	78 $\pm$ 19
Nonatopic asthma	16	42 $\pm$ 15	11/5	73 $\pm$ 82	76 $\pm$ 18
Systemic lupus erythematosus	12	27 $\pm$ 11	11/1	NM	NM

Data are expressed as mean  $\pm$  standard deviation. NM: not measured. \*Significant difference compared to healthy controls and nonatopic asthma ( $p < 0.05$ ); <sup>†</sup>Significant difference compared to atopic and nonatopic asthma ( $p < 0.05$ )

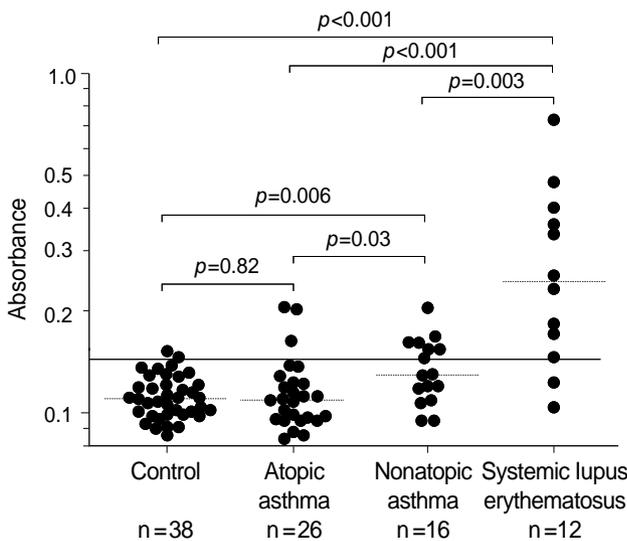


Fig. 1. Levels of circulating IgG autoantibodies to human bronchial epithelial cell in healthy controls, patients with atopic asthma, nonatopic asthma, and systemic lupus erythematosus. The horizontal line indicates mean absorbance+2 standard deviations of healthy controls. Dotted lines indicate median values.

controls ( $0.112 \pm 0.016$ ) and patients with atopic asthma ( $0.116 \pm 0.031$ ) ( $p < 0.05$ , Fig. 1). When a positive response was defined as greater than two standard deviations above the mean of the healthy controls, IgG autoantibodies to bronchial epithelial cell were positive in 7 (43.8%) of 16 patients with nonatopic asthma, 3 (11.5%) of 26 patients with atopic asthma, 2 (5.3%) of 38 healthy controls, and 10 (83.3%) of 12 patients with systemic lupus erythematosus. The positive rate of IgG autoantibodies to bronchial epithelial cell was significantly higher in patients with nonatopic asthma and systemic lupus erythematosus than in healthy controls and atopic asthma ( $p < 0.05$ , Table 2).

## DISCUSSION

This study showed that levels of circulating autoantibodies against the bronchial epithelial cell were increased in adult patients with nonatopic asthma. This result suggests a possible involvement of autoimmune response against bronchial epithelial cell in patients with nonatopic asthma. This interpretation is quite comparable with other chronic inflammatory diseases involving epithelium such as pemphigus and ulcerative colitis, in which autoantibodies against epithelial cell antigens of skin and colonic mucosa were demonstrated (14, 15).

At present, the pathogenetic importance of autoantibodies to bronchial epithelial cell is not certain. These autoantibodies could be just a secondary phenomenon due to chronic inflammation and immune response to newly exposed self-antigens in the inflammatory process. However, the sig-

Table 2. The positive rate of IgG autoantibodies to bronchial epithelial cell

Category	n	Number of positive results
Healthy controls	38	2 (5.3%)
Atopic asthma	26	3 (11.5%)
Nonatopic asthma	16	7 (43.8%)*
SLE	12	10 (83.3%) <sup>†</sup>

SLE: systemic lupus erythematosus. \*Significant difference compared to healthy controls and atopic asthma ( $p < 0.05$ ); <sup>†</sup>Significantly higher positive rate compared to remaining 3 groups ( $p < 0.05$ )

nificant difference of autoantibody levels between atopic and nonatopic asthma goes against this explanation.

Recently, we examined the presence of circulating autoantibodies to human bronchial mucosa tissue by indirect immunofluorescence staining method (10). We found autoantibodies to bronchial mucosa tissue in a small portion of patients with nonatopic asthma (10). Isotype of autoantibodies against bronchial mucosa tissue was mainly IgG and these autoantibodies reacted predominantly with cytoplasmic membrane of basal cells in bronchial epithelium (10). And the antigen-binding patterns of IgG autoantibodies in patients with nonatopic asthma were different from those patterns in patients with systemic lupus erythematosus which showed predominant binding with the nucleus of epithelial cells in whole layer of bronchial epithelium (10). However, the prevalence of autoantibodies to bronchial epithelial cell in nonatopic asthma was relatively low (9.1%) and this result was interpreted as due in part to the insensitive nature of indirect immunofluorescence staining method and to the problem of nonspecific background staining (10). In this study, we tried to quantify the levels of IgG autoantibodies to bronchial epithelial cell using cell-ELISA technique and we found a higher prevalence (43.8%) of these autoantibodies in nonatopic asthma.

There was a significant difference in levels of IgG autoantibodies to bronchial epithelial cell between nonatopic asthma and atopic asthma or healthy controls. However, the differences of mean absorbance values between nonatopic asthma and atopic asthma or healthy controls were relatively small. This result can be interpreted as follows: 1) the bronchial epithelial autoantigens reacting with IgG autoantibodies from nonatopic asthma are expressed in only a small amount in bronchial epithelial cell; 2) the circulating IgG autoantibodies to bronchial epithelial cell are present in a very small amount in patients with nonatopic asthma; 3) lastly, the circulating IgG autoantibodies to bronchial epithelial cell are also present in healthy controls and patients with atopic asthma but these autoantibodies react with different antigens on bronchial epithelial cell. Further studies on the molecular characterization of bronchial epithelial cell antigens reacting with circulating IgG autoantibodies might be essential for evaluating the importance of these autoantibodies in the pathogenesis of nonatopic asthma.

Previous report suggested that there were fundamental immunological differences between atopic and nonatopic asthma in the patterns of T cell activation and cytokine production (16). Asthma might just be a syndrome including various heterogeneous diseases in regards to etiology, natural history, and severity, all of which have common physiological characteristics of reversible airway obstruction. Our study suggests a possible existence of asthmatic population characterized by the presence of autoantibodies against bronchial epithelial cell.

This study showed that circulating IgG autoantibodies against the bronchial epithelial cell are increased in adult patients with nonatopic asthma. Further studies are needed to evaluate the possible involvement of autoimmune response to bronchial epithelial cell in the pathogenesis of nonatopic asthma.

### ACKNOWLEDGMENTS

This study was supported by KOSEF Grant 1999-2-212-002-2 to Dong-Ho Nahm. The authors thank Professor Heather Yu for reviewing the manuscript.

### REFERENCES

1. Lemanske RF Jr, Busse WW. *Asthma*. *JAMA*. 1997; 278: 1855-73.
2. Pearce N, Pekkanen J, Beasley R. *How much asthma is really attributable to atopy*. *Thorax* 1999; 54: 268-72.
3. Rackemann FM. *A working classification of asthma*. *Am J Med* 1947; 3: 601-6.
4. Ray P, Vervloet D, Charpin D, Gautier V, Proudhon H, Redier H, Godard P. *Evaluation of atopy through an expert system: description of the database*. *Clin Exp Allergy* 1995; 25: 1067-73.
5. Hall R, Turner-Warwick M, Doniach D. *Autoantibodies in iodide goitre and asthma*. *Clin Exp Immunol* 1966; 1: 285-96.
6. Lidor Y, Topilsky M, Spitzer SA, Yehoshua H. *Autoimmune antibodies in intrinsic (non-atopic) asthma*. *Ann Allergy* 1980; 44: 296-8.
7. Montefort S, Herbert CA, Robinson C, Holgate ST. *The bronchial epithelium as a target for inflammatory attack in asthma*. *Clin Exp Allergy* 1992; 22: 511-20.
8. Dinu IV, Roth L. *The presence of antilung antibodies in the sera of asthma patients*. *Rum Med Rev* 1965; 19: 26-9.
9. Quintero JM, Burrell RG, Cather CH, Wymer B, Baise R. *Detection of antibodies to sinus tissue in chronic bronchial asthma*. *J Allergy* 1966; 37: 84-9.
10. Nahm DH, Yim H, Lee HJ, Yim EJ, Choi EA, Kim SS, Lee SK, Park HS. *Presence of circulating autoantibodies against bronchial epithelial cell in patients with nonatopic asthma*. *J Korean Med Sci* 2000; 15: 631-4.
11. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. *The 1982 revised criteria for the classification of systemic lupus erythematosus*. *Arthritis Rheum* 1982; 25: 1271-7.
12. Atsuta J, Sterbinsky SA, Plitt J, Schwiebert LM, Bochner BS, Schleimer RP. *Phenotyping and cytokine regulation of the BEAS-2B human bronchial epithelial cell: demonstration of inducible expression of the adhesion molecules VCAM-1 and ICAM-1*. *Am J Respir Cell Mol Biol* 1997; 17: 571-82.
13. Lee JC, Cevallos AM, Naeem A, Lennard-Jones JE, Farthing MJ. *Detection of anti-colon antibodies in inflammatory bowel disease using human cultured colonic cells*. *Gut* 1999; 44: 196-202.
14. Diaz LA, Roscoe JT, Eaglstein NF, Labib RS, Patel HP, Mutasim DF, Anhalt GJ. *Human pemphigus autoantibodies are pathogenic to squamous epithelium*. *Ann N Y Acad Sci* 1986; 475: 181-91.
15. Takahashi F, Das KM. *Isolation and characterization of a colonic autoantigen specifically recognized by colon tissue-bound immunoglobulin G from idiopathic ulcerative colitis*. *J Clin Invest* 1985; 76: 311-8.
16. Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC Jr. *Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage*. *Am Rev Respir Dis* 1992; 146: 109-15.