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# Presence of Circulating Autoantibodies against Bronchial Epithelial Cell in Patients with Nonatopic Asthma

Allergic response to common environmental agents has been regarded as a main pathogenetic mechanism of bronchial asthma. However, allergic sensitization (atopy) can not be detected in a siginificant number of adult asthmatic patients. The etiology of nonatopic asthma has not yet been defined. To evaluate the possible involvement of autoimmune response against bronchial mucosa in the pathogenesis of nonatopic asthma, we performed indirect immunofluorescence staining of fresh frozen human bronchial mucosa tissue using serum samples from patients with atopic and nonatopic asthma, healthy controls, and patients with systemic lupus erythematosus. On immunostaining, circulating IgG autoantibodies against bronchial mucosa were detected in 2 (9.1%) of 22 patients with nonatopic asthma and in none of 22 patients with atopic asthma and of 22 healthy controls. IgG autoantibodies from the two patients with nonatopic asthma predominantly stained the cytoplasmic membrane of basal cells in bronchial epithelium. Serum samples from 10 patients with systemic lupus erythematosus immunostained the nucleus of epithelial cells in whole layer of bronchial epithelium. This study showed the presence of circulating IgG autoantibodies against the bronchial epithelial cell in a small portion of patients with nonatopic asthma. Further studies may be necessary to evaluate the possible involvement of autoimmune mechanism in the pathogenesis of nonatopic asthma.

Key Words: *Asthma; Autoantibodies*

**Dong-Ho Nahm, Hyunee Yim*, Hyun-Joo Lee, Eui-Jin Yim, Eun-Ah Choi, Sun-Sin Kim, Soo-Keol Lee, Hae-Sim Park**

Departments of Allergy and Clinical Immunology, Pathology*, Ajou University School of Medicine, Suwon, Korea

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**Address for correspondence**
Dong-Ho Nahm, M.D.
Department of Allergy and Clinical Immunology, Ajou University School of Medicine, 5 Wonchon-dong, Paldal-gu, Suwon 442-721, Korea
Tel: +82.31-219-5152, Fax: +82.31-219-5109
E-mail:

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## INTRODUCTION

Allergic response to common environmental agents (allergens) has been regarded as a main pathogenetic mechanism of bronchial asthma (1, 2). However, allergic sensitization (atopy) can not be detected in a significant number of adult asthmatic patients (1, 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 (1, 2). Atopic asthma usually starts during childhood and is characterized by allergen-dependent, often seasonal, symptom and elevated total and allergen-specific serum IgE (1, 2). In contrast, nonatopic asthma usually begins in adulthood and is perennial and more severe (1, 3). In addition, it shows no elevation in serum IgE and is often associated with sinusitis and nasal polyposis (1, 3). Nonatopic asthma has been referred to "intrinsic asthma" based on a suggestion that there must be an etiologic agent in patient's own body (2). 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 earlier studies which demonstrated higher incidences of various autoantibodies including antithyroid and antinuclear antibodies in nonatopic asthmatics, as compared to atopic asthmatics and healthy controls (4, 5). Recently, the presence of circulating IgG autoantibodies against a 55-kDa antigen of endothelial cell in nonatopic asthmatics has been reported (6). However, the pathogenetic significance of these autoantibodies in nonatopic asthma remains unexplained.

The pathologic findings of bronchial mucosa appears to be similar in both atopic and nonatopic asthma (7). In the pathologic view point, asthma can be defined as desquamative eosinophilic bronchitis characterized by eosinophil infiltration and degranulation in bronchial mucosa and by shedding of bronchial epithelium (7). Bronchial epithelium has been suggested as a target for inflammatory response in asthma (8). Direct immuno-

fluorescence staining of bronchial biopsy from late-onset asthmatic patients and autopsy from fatal asthmatic patients demonstrated depositions of IgG antibodies and complement in the superficial part of the basement membrane and in the cytoplasm of the epithelial cells (9, 10). These findings had been interpreted to be the result of precipitation of antibodies from serum with respiratory antigens from the bronchial lumen (9, 10).

To evaluate the possible involvement of autoimmune response against a bronchial mucosa in the pathogenesis of nonatopic asthma, we examined circulating autoantibodies against bronchial mucosa in sera from patients with nonatopic asthma by indirect immunofluorescence staining. To our best knowledge, this study is the first trial to localize circulating autoantibodies against bronchial mucosa tissue in asthmatic patients by indirect immunofluorescence staining.

## PATIENTS AND METHODS

### Patients

We used stored serum samples from 22 patients with nonatopic asthma, 22 patients with atopic asthma, 22 age-matched healthy controls, and 10 patients with systemic lupus erythematosus (Table 1). All 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, U.K.). Atopic asthma was defined when wheal diameter of any one allergen was greater than 3 mm over negative control (normal saline). Nonatopic asthma was defined when there was no visible skin reaction to all 50 common aeroallergens in the presence of a positive histamine control and when serum total IgE levels were less than 200 IU/mL. Patients with occupational asthma were excluded from the study subjects. Ten patients with systemic lupus erythematosus classified according to the criteria of the American Rheumatic Association were included to evaluate the disease-specificity of autoantibodies against bronchial mucosa tissue (11). Twenty two age-matched healthy controls who have normal baseline lung function and no clinical symptoms of asthma were included as negative controls. All sera were aliquoted and stored at -20℃. Serum total IgE level was measured by radioimmunoassay using AlaSTAT kit (Diagnostic Product Corporation, Los Angeles, CA, U.S.A.).

### Methods

For the detection of autoantibodies to bronchial mucosa by indirect immunofluorescence staining, 5 $\mu$m fresh cryostat sections of normal human bronchial mucosa tissue were obtained from patients with lung cancer who underwent therapeutic lung resection. After air-drying for 1 hr and incubating in phosphate buffered saline (PBS) for 5 min, the tissue sections were incubated with serum samples diluted five times in PBS for 1 hr at room temperature, were then washed two times by incubating in PBS for 5 min, and were stained with fluorescein isocyanate conjugated (FITC) rabbit antibodies to human IgG, IgA, and IgM (Dako, Copenhagen, Denmark; diluted 1:100 with PBS) for 30 min at room temperature. After two more washings, the slides were mounted and observed under a fluorescence microscope with vertical illumination (Olympus Optical Co., Tokyo, Japan).

## RESULT

On immunostaining, circulating IgG autoantibodies against bronchial mucosa were detected in 2 (9.1%) of 22 patients with nonatopic asthma and in none of 22

**Table 1.** Characteristics of the study subjects

| | n | Age (yr) | Sex (F/M) | Total IgE (IU/mL) | FEV1 (% predicted) |
|---|---|---|---|---|---|
| Healthy controls | 22 | 44±17 | 8/14 | 239±330 | 105±14[§] |
| Atopic asthma | 22 | 25±8* | 7/15 | 406±317[†] | 81±19 |
| Nonatopic asthma | 22 | 40±14 | 13/9 | 74±56 | 74±56 |
| SLE | 10 | 32±12 | 9/1[†] | NM | NM |

Data are expressed as mean±standard deviation
SLE: systemic lupus erythematosus, NM: not measured
*Significant difference compared to healthy controls ($p<0.05$)
[†]Significant difference compared to healthy controls, atopic asthma, and nonatopic asthma ($p<0.05$)
[†]Significant difference compared to healthy controls and nonatopic asthma ($p<0.05$)
[§]Significant difference compared to atopic and nonatopic asthma ($p<0.05$)

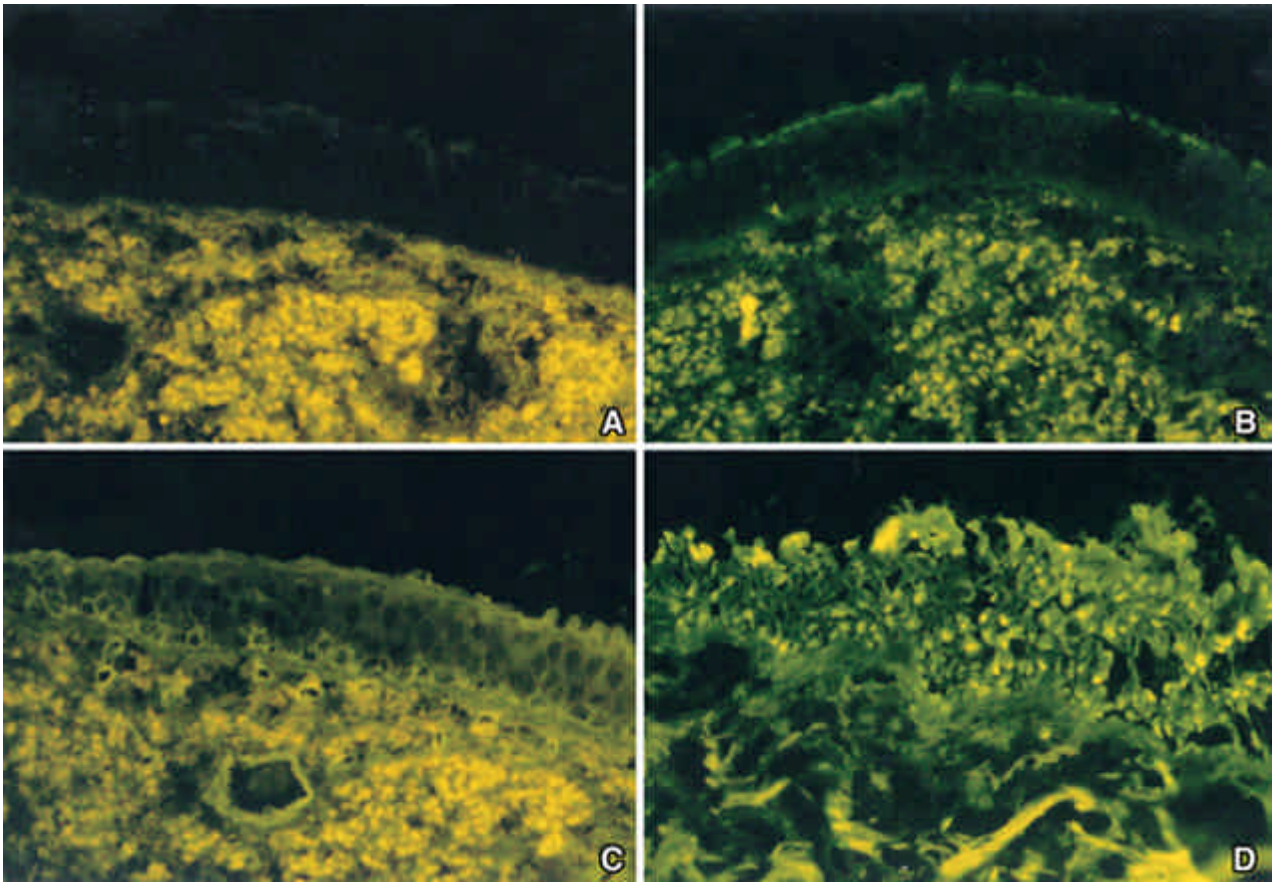


Fig. 1. Indirect immunofluorescence staining of cryostat sections of human bronchial mucosa with diluent alone (A) showing background fluorescence of submucosa, with serum from a healthy control (B), with serum from a patient with nonatopic asthma showing staining of the cytoplasmic membrane of basal cells in the epithelium (C), with serum from a patient with systemic lupus erythematosus showing nuclear staining of epithelial cells in whole layer of epithelium (D) (×200).

patients with atopic asthma and of 22 healthy controls. This result was reproducible in repeated studies using bronchial tissues from 3 different individuals. IgG autoantibodies in two patients with nonatopic asthma predominantly stained the cytoplasmic membrane of basal cells in bronchial epithelium (Fig. 1). Serum samples from 10 patients with systemic lupus erythematosus immunostained the nucleus of epithelial cells in whole layer of bronchial epithelium (Fig. 1). Circulating IgA and IgM autoantibodies against bronchial mucosa could not be detected in all 44 asthmatic patients and 22 controls.

## DISCUSSION

This study showed the presence of circulating IgG autoantibodies against the bronchial epithelial cell in a small portion of patients with nonatopic asthma. This result suggests a possible involvement of autoimmune response against bronchial epithelial cell in a portion of 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 squamous epithelium of skin and colonic mucosa were demonstrated (12, 13). Especially in pemphigus, these autoantibodies were suggested to have a primary pathogenetic importance in the development of disease because they could induce similar pathologic lesion in experimental animals (12). We suppose that circulating autoantibodies might be involved in the inflammatory damage of bronchial epithelial cells in patients with nonatopic asthma.

In this study, the prevalence of autoantibodies to bronchial epithelial cell in nonatopic asthma was relatively low (9.1%). This result can be interpreted as follows: 1) One possibility is that only a minor portion of patients with nonatopic asthma is associated with autoimmune response to bronchial epithelial cell; 2) The other possibility is that the prevalence of autoantibodies against bronchial epithelial cell is underestimated due to the insensitive nature of indirect immunofluorenscence staining method and the problem of nonspecific back-

ground staining. Further studies using more advanced immunological detection method might be essential to clarify the pathogenetic role of autoantibodies against bronchial epithelial cell in nonatopic asthma.

The possible involvement of autoimmune mechanism against respiratory mucosa in the pathogenesis of asthma has been suggested in a previous report (14). It has been also postulated that repeated injury to respiratory tissue may alter the tissue sufficiently to render them autoantigenic and elicit autoantibodies, and this mechanism might be responsible for the progressive reactions to nonspecific irritants in asthma and difficulties in treatment (15).

Recent research suggested that there may be 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 with etiology, natural history, and severity (17). Our study suggests that there is an unique population of asthmatic patients characterized by the presence of autoantibodies against bronchial epithelial cell.

The immunostaining pattern of bronchial mucosa showing predominant staining of the basal cells of epithelium by sera from nonatopic asthmatics seems to be similar to the immunostaining pattern of nasal mucosa by IgG antibodies from patients with Wegener's granulomatosis (18). To define the nature of bronchial epithelial cell antigen recognized by IgG autoantibodies from patients with nonatopic asthma, further studies are needed.

This study showed the presence of circulating IgG autoantibodies against the bronchial epithelial cell in a small portion of patients with nonatopic asthma. Further study might be essential to evaluate the possible involvement of autoimmune mechanism in the pathogenesis of nonatopic asthma.

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