

Increased IgG Antibody-Induced Cytotoxicity Against Airway Epithelial Cells in Patients with Nonallergic Asthma

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Abstract

Background IgG autoantibodies to airway epithelial cell proteins have been detected in patients with nonallergic asthma.

Objective and Methods To evaluate the functional significance of these autoantibodies, we examined the presence of IgG antibody-induced cytotoxicity against airway epithelial cells (A549) by the microcytotoxicity assay using IgG antibodies purified from patients with nonallergic asthma.

Results IgG antibody-induced cytotoxicity (expressed as percent cell lysis) was significantly increased in nine patients with nonallergic asthma (mean $\pm$ standard deviation; $30.6 \pm 7.3\%$) as compared with eight healthy controls ($13.9 \pm 5.1\%$) and nine patients with allergic asthma ($20.3 \pm 10.4\%$; $p < 0.05$). In addition, IgG antibody-induced cytotoxicity was significantly inhibited when IgG antibodies from patients with nonallergic asthma were pre-incubated with recombinant human airway epithelial cell autoantigens (cytokeratin 18 or alpha-enolase proteins; $p < 0.05$).

Conclusion These results suggest a possible involvement of IgG autoantibody-induced cytotoxicity against airway epithelial cells in the pathogenesis of nonallergic asthma.

Keywords Asthma · autoantibody · IgG · cytotoxicity · airway epithelial cell

Introduction

Asthma is currently defined as a chronic inflammatory disorder of the airways and characterized by airway hyperresponsiveness, reversible airway obstruction, and typical respiratory symptoms [1, 2]. Many attempts to precisely define asthma have been frustrated by a lack of complete understanding of the etiological mechanisms [2]. Several studies have suggested that asthma is not a single disease, but a clinical manifestation of several different distinct diseases [3, 4].

Traditionally, asthma has been regarded as the clinical manifestation of allergic responses to environmental agents [1]. However, available epidemiological evidences suggest that less than half of all asthma cases are attributable to allergic sensitization (atopy) [5]. Nonallergic asthma generally begins at an older age and is clinically more severe than allergic asthma [6–8]. Still, the pathophysiological mechanisms responsible for the development of nonallergic asthma are poorly understood.

It has been repeatedly suggested that autoimmune mechanism may be involved in the pathogenesis of asthma [3, 8]. The “autoimmune hypothesis” of nonallergic asthma is based on the frequent detection of circulating autoantibodies to lung or bronchial mucosa tissue in patients with asthma, particularly nonallergic asthma [9–11]. The titers of autoantibodies to lung tissue in serum samples from asthmatic patients were correlated with asthma severity and paralleled serial changes in disease state [12]. However, a direct demonstration of a causal relationship between autoimmunity and asthma has not been possible [3].

The airway epithelium has been suggested as a major target tissue for inflammatory responses in asthma, regardless of allergic sensitization [13]. The mechanism responsible for inflammatory damage of the airway epithelium in

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nonallergic asthma has not been elucidated yet. Previously, we explored the hypothesis that an autoimmune response against airway epithelial cell proteins might be involved in the pathogenesis of nonallergic asthma. While exploring the hypothesis that an autoimmune response to airway epithelial cell proteins might be involved in the pathogenesis of asthma, we have identified cytokeratin 18 and alpha-enolase as airway epithelial cell autoantigens associated with nonallergic asthma and severe asthma, respectively [14, 15].

To evaluate a functional significance of circulating IgG autoantibodies against airway epithelial cell proteins in nonallergic asthma, we examined the presence of IgG antibody-induced cytotoxicity against airway epithelial cells using IgG antibodies purified from patients with nonallergic asthma.

Materials and Methods

Subjects

The subjects of the present study were nine adult patients with nonallergic asthma, nine adult patients with allergic asthma, and eight healthy controls (Table 1). All asthmatic subjects had a typical clinical history of asthma and either a 20% decrease in forced expiratory volume in one second (FEV₁) following the inhalation of less than 8 mg methacholine/ml, or a documented reversibility of FEV₁ greater than 15% after inhalation of a bronchodilator. All asthmatic subjects underwent a skin-prick test with 50 common aeroallergens (Allergy Therapeutics, West Sussex, UK). Asthmatic patients were classified as having allergic asthma when the wheal diameter of any one allergen was greater than 3 mm over the negative control (normal saline), and there was a definite clinical history or objective evidence of asthmatic responses induced by allergen exposure as previously described [14]. In this study, patients with allergic asthma displayed typical clinical histories of mild-to-moderate asthma, and the majority of the patients were

sensitized to house dust mites. Patients were classified as having nonallergic asthma when there was no positive skin reaction to any of the 50 common aeroallergens in the presence of a positive histamine control, and serum total IgE concentration was within the normal range (less than 180 IU/ml). All patients with nonallergic asthma that were included in this study had typical clinical features of severe asthma, including a past history of hospital admission and/or emergency room visits due to acute severe exacerbations of asthma. Patients with occupational asthma were excluded from this study. All serum samples obtained from the subjects were aliquoted and stored at -20°C. All subjects provided informed consent, and an institutional review board approved this study.

Cell Culture and Protein Extraction

The human airway epithelial cell line (A549) was obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured as recommended. Cells were lysed by the addition of buffer containing 10 mM Tris/HCl, pH 7.2, 2% sodium dodecyl sulfate (SDS), 158 mM NaCl, and 10 mM dithiothreitol.

Immunoblot Analysis

Proteins were separated by discontinuous SDS/polyacrylamide gel electrophoresis (SDS-PAGE). IgG autoantibodies against airway epithelial cell proteins were detected by immunoblot analysis using a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories, Hercules, CA, USA) as previously described [14]. The immunoreactivity of each serum sample was assessed by two independent investigators. Positive standard and negative control serum samples were included in each experiment. A serum sample was defined as positive for autoantibody detection if the observed immunoreactivity was equal to or greater than that of the positive standard serum sample. Monoclonal antibodies against human cytokeratin 18 (clone CK5, Sigma Chemical Co., St. Louis, MO, USA) and goat-specific antibodies against human alpha-enolase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were included in each experiment to confirm the co-localization of IgG autoantibodies and corresponding autoantigens in the immunoblot analysis.

Recombinant Human Autoantigens

The recombinant human cytokeratin 8, cytokeratin 18, and alpha-enolase proteins were produced and purified from *E. coli* using commercially available full open reading frame cDNA clones for each protein (RZPD, Heidelberg, Germany) as previously described [16, 17].

Table 1 Characteristics of Study Subjects

	N	Age (years)	Sex (F/M)	FEV ₁ (% predicted)
Healthy controls	8	39.6±19.4	3/5	108.0±16.2
Allergic asthma	9	24.8±5.6*	4/5	77.3±20.0**
Nonallergic asthma	9	42.2±18.5	5/4	57.2±18.5**,***

Data are expressed as mean ± standard deviation

* $p < 0.05$, significantly different as compared to healthy controls or patients with nonallergic asthma; ** $p < 0.05$, significantly different as compared to healthy controls; *** $p < 0.05$, significantly different as compared to patients with allergic asthma

Purification of IgG Antibodies from Serum Samples

IgG antibodies were purified from the serum samples of eight healthy controls, nine patients with allergic asthma, and nine patients with nonallergic asthma by affinity chromatography using a Protein A-coupled centrifugal column (Pro-Chem Inc., Littleton, MA, USA).

Microcytotoxicity Assay to Assess IgG Antibody-Induced Cytotoxicity

IgG antibody-induced cytotoxicity against cultured human airway epithelial cells (A549) was examined by the microcytotoxicity assay using a Terasaki plate as previously described [18]. Trypsin-treated A549 cells were washed twice with phosphate-buffered saline, and cell counts were adjusted to 2×10^6 /ml with DMEM-F12 medium without

bovine serum. The cell suspension (1 μ l/well) was incubated with 1 μ g of purified IgG (1 μ l/well) for 2.5 h at room temperature in a Terasaki microtiter plate (Nunc GmbH & Co. KG, Wiesbaden, Germany). Mineral oil (4 μ l/well, Sigma Chemical Co.) was added to the mixture to prevent drying. Following the above incubation, 5% eosin dye (5 μ l/well) and formalin (5 μ l/well) were added to stain and fix the cells. The number of cells in each well with intact cell membrane outlines was counted using an inverted light microscope (Olympus, Tokyo, Japan; Fig. 4). The IgG antibody-induced cytotoxicity against airway epithelial cells was expressed as the percent cell lysis by comparing the mean number of cells in experimental wells containing the purified IgG antibody with the mean number of cells in the negative control wells with DMEM/F12 medium only according to the following equation:

$$\text{Cytotoxicity (\% cell lysis)} = \left[\frac{(\text{mean number of intact cells in negative control wells} - \text{mean number of intact cells in wells with added IgG})}{\text{mean number of intact cells in negative control wells}} \right] \times 100.$$

Microcytotoxicity tests were performed in quadruplicate for each sample and then expressed as a mean value.

Inhibition of IgG Antibody-Induced Cytotoxicity Against Airway Epithelial Cells with Recombinant Human Airway Epithelial Cell Autoantigens

The role of IgG autoantibodies in IgG antibody-induced cytotoxicity against airway epithelial cells was evaluated using the following inhibition experiments. IgG antibody-induced cytotoxicity was examined using purified IgG antibodies that were pre-incubated with human serum albumin, recombinant human cytokeratin 8, recombinant human cytokeratin 18, or recombinant human alpha-enolase proteins immobilized on PVDF membranes of 3×3 mm. In the above inhibition experiment, 100 μ g of purified IgG antibody was incubated with 10 μ g of human protein for 16 h at 4°C.

Statistics

The data are expressed as mean $\pm$ standard deviation. Kruskal–Wallis test or Mann–Whitney *U* test was used for comparisons of the age, baseline FEV₁, and cytotoxicity between the groups. Mann–Whitney *U* test was used to assess the inhibition of cytotoxicity with recombinant human airway epithelial cell autoantigens. The chi-square test was used to compare the frequency of autoantibodies

between the groups. Values of *p* less than 0.05 were regarded as statistically significant.

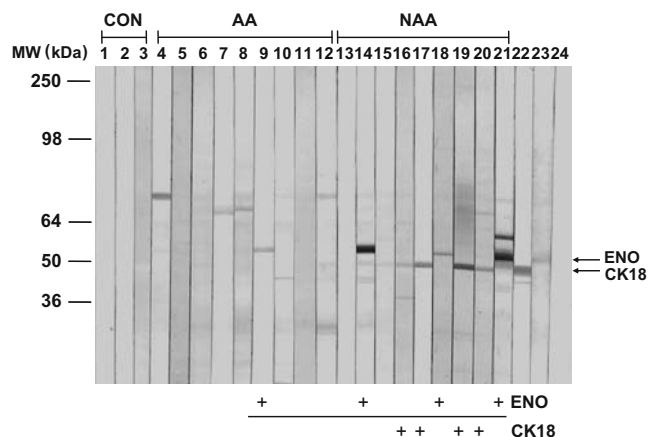


Fig. 1 Immunoblot analysis of circulating IgG autoantibodies against airway epithelial cell (A549) proteins in serum samples from study subjects. Results from serum samples of healthy controls (CON; lanes 1–3), patients with allergic asthma (AA; lanes 4–12), patients with nonallergic asthma (NAA; lanes 13–21), monoclonal antibody to cytokeratin 18 (lane 22), goat antibody to alpha-enolase (lane 23), and dilution buffer only (negative control; lane 24) are shown. Arrows indicate cytokeratin 18 protein (CK18) detected by the monoclonal antibody and alpha-enolase protein (ENO) detected by goat antibody. The plus sign symbol means positive detection of IgG autoantibody

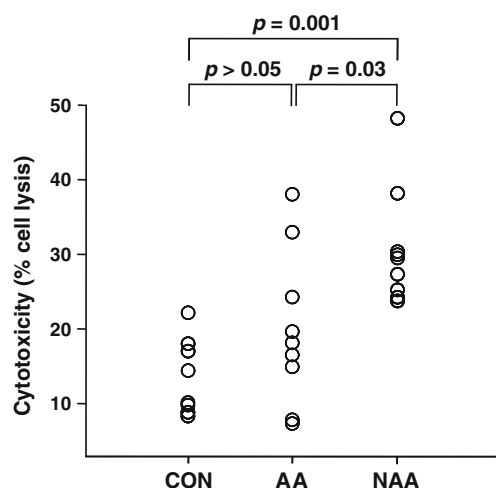


Fig. 2 Comparison of IgG antibody-induced cytotoxicity against airway epithelial cells (expressed as percent cell lysis) by purified IgG antibodies from eight healthy controls (*CON*), nine patients with allergic asthma (*AA*), and nine patients with nonallergic asthma (*NAA*)

Results

Immunoblot Detection of IgG Autoantibodies Against Airway Epithelial Cell Proteins

IgG autoantibodies against cytokeratin 18 were detected in serum samples from four of nine patients with nonallergic asthma (44%), none of nine patients with allergic asthma (0%), and none of eight healthy controls (0%; $p=0.012$; Fig. 1). IgG autoantibodies against the alpha-enolase protein were detected in serum samples from three of nine patients with nonallergic asthma (33%), one of nine patients with allergic asthma (11%), and none of eight healthy controls (0%; $p>0.05$; Fig. 1).

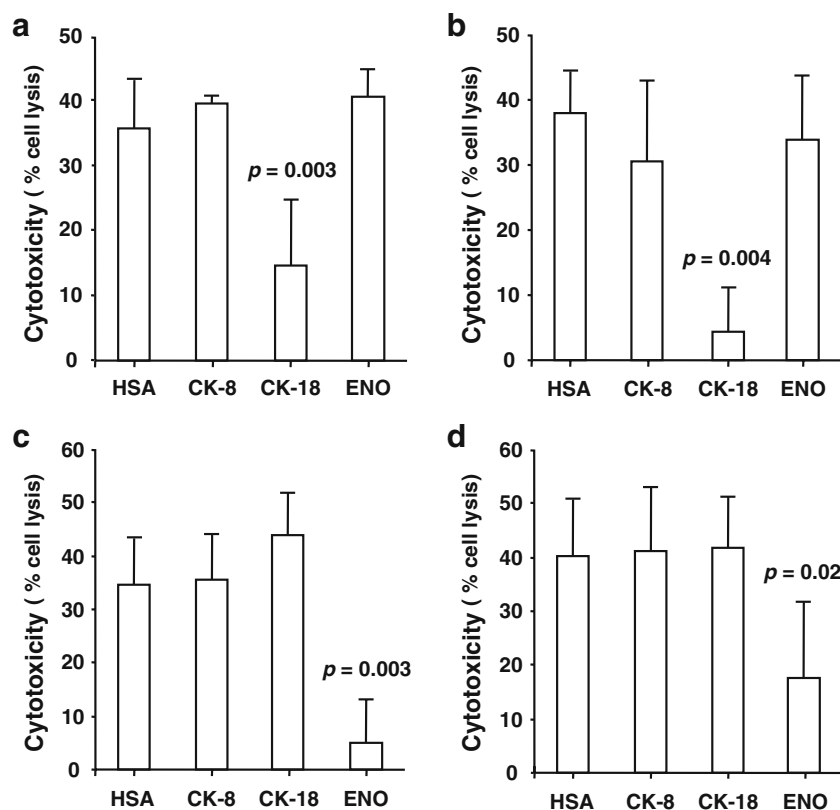


Fig. 3 Inhibition of IgG antibody-induced cytotoxicity against airway epithelial cell proteins with recombinant human airway epithelial cell autoantigens. The IgG antibodies were purified from serum samples of four patients with nonallergic asthma who have IgG autoantibodies to cytokeratin 18 protein (**a, b**) or alpha-enolase protein (**c, d**). The purified IgG antibodies were pre-incubated with human serum albumin (*HSA*), recombinant human cytokeratin 8 protein (*CK-8*), recombinant human cytokeratin 18 protein (*CK-18*), or recombinant

human alpha-enolase protein (*ENO*) to confirm that the IgG-induced cytotoxicity was caused by IgG autoantibodies to cytokeratin 18 protein (**a, b**) or alpha-enolase protein (**c, d**). IgG induced-cytotoxicity was significantly decreased by pre-incubations with cytokeratin 18 protein (**a, b**) or alpha-enolase protein (**c, d**) compared to pre-incubations with other three kinds of control proteins ($p<0.05$). Data are presented as the mean $\pm$ standard deviation of quadruplicate measurements

Assessment of IgG Antibody-Induced Cytotoxicity Against Airway Epithelial Cells by the Microcytotoxicity Assay

There was a significant inter-group difference of IgG antibody-induced cytotoxicity (expressed as percent cell lysis) among the three groups ($p=0.002$). IgG antibody-induced cytotoxicity was significantly increased in the nine patients with nonallergic asthma (mean $\pm$ standard deviation, $30.6 \pm 7.3\%$) as compared with the eight healthy controls ($13.9 \pm 5.1\%$) and the nine patients with allergic asthma ($20.3 \pm 10.4\%$; $p < 0.05$; Fig. 2).

Inhibition of IgG Antibody-Induced Cytotoxicity Against Airway Epithelial Cells with Recombinant Human Airway Epithelial Cell Autoantigens

The cytotoxicity against airway epithelial cells induced by IgG antibodies purified from patients with nonallergic asthma was significantly decreased by pre-incubations with specific airway epithelial cell autoantigens recognized by IgG antibodies in each serum samples compared to pre-incubations with other three kinds of control proteins ($p < 0.05$; Figs. 3 and

4). The patterns of inhibition were consistent with the specific types of airway epithelial cell autoantigens recognized by IgG autoantibodies in each serum sample (Fig. 3). These results indicate that the cytotoxicity against airway epithelial cells induced by IgG antibodies purified from patients with nonallergic asthma is caused by IgG autoantibodies against airway epithelial cell autoantigens.

Discussion

In this study, we showed that IgG antibody-induced cytotoxicity against airway epithelial cells was increased in patients with nonallergic asthma. This result suggests a possible involvement of IgG autoantibody-induced cytotoxicity against airway epithelial cells in the pathogenesis of nonallergic asthma.

It is remarkable that the exact etiopathogenetic mechanisms of adult-onset nonallergic asthma are not yet known, given that Hippocrates described asthma about 2,500 years ago [4]. Approximately 50 years ago, Bergquist and Mendes respectively suggested that autoimmunity might be an important pathogenetic mechanism underlying the

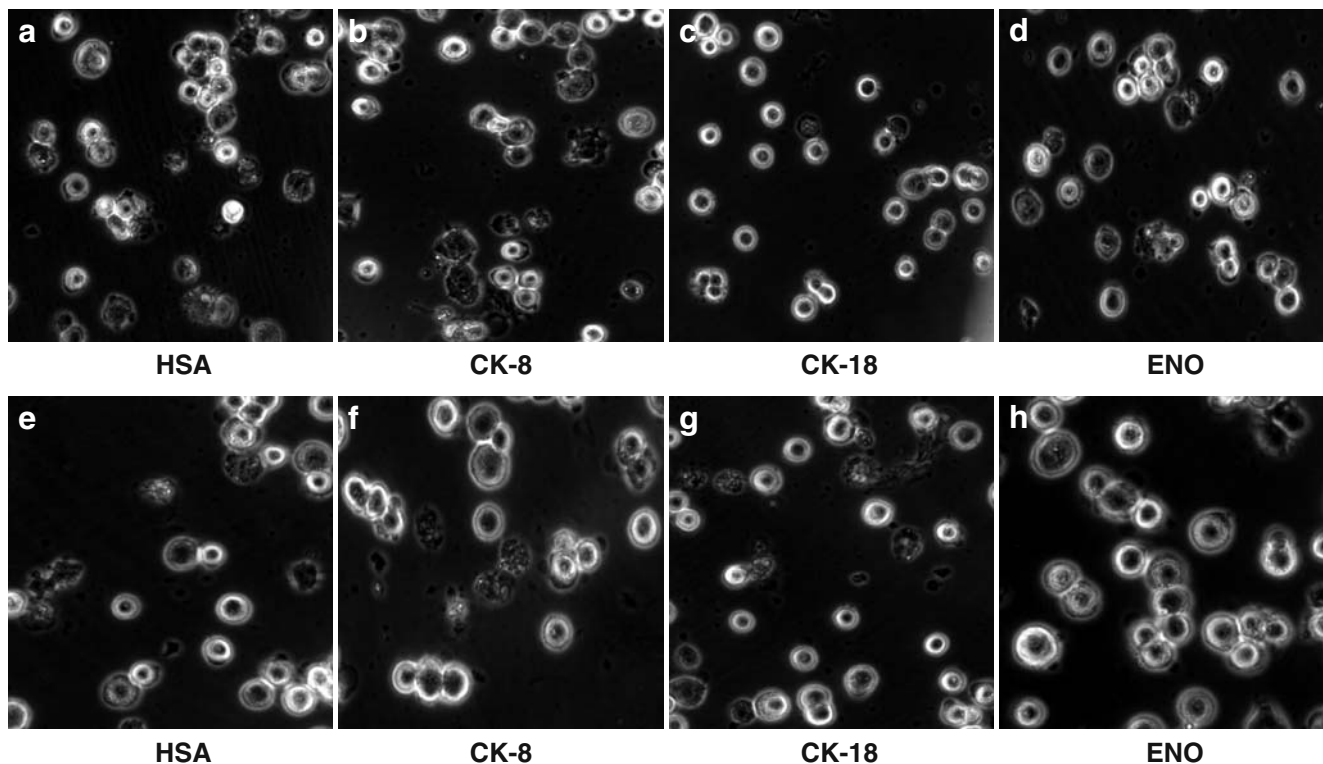


Fig. 4 Microscopic examination of cultured airway epithelial cells (A549) incubated with purified IgG autoantibodies from two patients with nonallergic asthma who have IgG autoantibodies to cytokeratin 18 protein (a–d) or alpha-enolase protein (e–h). Inhibition of IgG antibody-induced cytotoxicity against airway epithelial cells with

human serum albumin (HSA; a, e), recombinant human cytokeratin 8 protein (CK-8; b, f), recombinant human cytokeratin 18 protein (CK-18; c, g), or recombinant human alpha-enolase protein (ENO; d, h) is shown

development of asthma in addition to allergy [19, 20]. Many trials have attempted to provide supporting evidences for this hypothesis by demonstrating the presence of various autoantibodies against antigens in the bronchial mucosa, paranasal sinus, lung, and endothelial cells in patients with asthma, especially in patients with nonallergic asthma [9–11]. Analysis of T cells in the airway fluids from patients with nonatopic asthma have shown features of clonal expansion, which indicates an antigen-induced activation of T cells in the airway [21]. A significant correlation between the proliferative response of T cell to a 55-kDa endothelial autoantigen and the degree of airway obstruction in asthmatic patients has been reported [11]. Further, anti-CD4 antibody treatment provided a significant clinical improvement in patients with severe asthma [22]. Non-atopic airway hyperresponsiveness was passively transferred by CD4+ T cells in the mouse model [23]. These reports suggest a possible involvement of T and B cell responses to self-antigen in the pathogenesis of asthma.

A possible involvement of common pathophysiological mechanism in both severe asthma and chronic obstructive pulmonary disease has been suggested [1, 24]. In a rat model of autoimmune emphysema, the passive transfer of pathogenic CD4+ T cells or antibodies (developed by immunization with human endothelial proteins) caused emphysema [25]. Recently, circulating IgG autoantibodies against pulmonary epithelial cells were detected in the majority of patients with chronic obstructive pulmonary disease [26]. Autoantibodies from patients with chronic obstructive pulmonary disease induced an antibody-dependent mononuclear cell-mediated cytotoxicity response against pulmonary epithelial cells [26]. These suggest that T cell and autoantibody responses against airway epithelial cell autoantigens might be involved in the pathogenesis of both nonallergic asthma and chronic obstructive pulmonary disease.

There have been only a few trials to demonstrate a functional significance of autoimmune response in the pathogenesis of asthma [1, 3]. Autoantibodies against the beta-2 adrenergic receptor [27] and vasoactive intestinal polypeptide [28] have been found in the serum samples of asthmatic patients. The functional significance of these autoantibodies has been suggested by in vitro experiments [29, 30]. The results of the present study could provide additional in vitro evidence supporting a functional role of autoantibodies in the pathogenesis of asthma.

In this study, we showed that purified IgG antibodies from nonallergic asthma patients could directly induce cytotoxicity against airway epithelial cells without a further addition of complement. Autoantibodies against alpha-enolase protein purified from patients with cancer-associated retinopathy showed direct cytotoxic effects on retinal cells [31]. In the above study, autoantibodies against alpha-

enolase protein induced an apoptotic cell death of retinal cells without an addition of complement. Autoantibodies to vasoactive intestinal peptide purified from asthmatic patients showed a catalytic enzyme-like activity [32]. We suppose that IgG autoantibodies against airway epithelial cell autoantigens (cytokeratin 18 and alpha-enolase) from patients with nonallergic asthma could directly induce cytotoxic damage to airway epithelial cells. However, the detailed molecular and biochemical mechanisms of IgG antibody-induced cytotoxicity against airway epithelial cells in nonallergic asthma should be further studied.

Conclusion

IgG antibody-induced cytotoxicity against airway epithelial cells was increased in patients with nonallergic asthma. This result suggests a possible involvement of autoantibody-induced cytotoxicity mechanism in the pathogenesis of non-allergic asthma.

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References

- Holgate ST. Pathogenesis of asthma. *Clin Exp Allergy*. 2008; 36:872–97. doi:10.1111/j.1365-2222.2008.02971.x.
- Global Initiative for Asthma. Global strategy for asthma management and prevention. Bethesda, MD, National Institutes of Health, 2002. (NIH publication no. 02-3659.).
- Rottem M, Shoenfeld Y. Asthma as a paradigm for autoimmune disease. *Int Arch Allergy Immunol*. 2003;132:210–4. doi:10.1159/000074301.
- A plea to abandon asthma as a disease concept. *Lancet* 2006;368:705 (Editorial). doi:10.1016/S0140-6736(06)69257-X.
- Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy. *Thorax*. 1999;54:268–72.
- Rackemann FM. Intrinsic asthma. *J Allergy*. 1940;11:147–62. doi:10.1016/S0021-8707(40)90829-2.
- Greenberger PA. Asthma. In: Patterson R, Grammer LC, Greenberger PA, Zeiss CR, editors. *Allergic diseases, diagnosis and management*. 5th ed. Philadelphia: Lippincott-Raven; 1997. p. 467–542.
- Virchow JC Jr, Kroegel C, Walker C, Matthys H. Inflammatory determinants of asthma severity: mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma. *J Allergy Clin Immunol*. 1996;98:S27–40.
- Wagner V, Tomšíková A, Šach J, Janková J, Novácková D. Autoimmune mechanism in diseases of the respiratory tract. *Acta Allergol*. 1965;20:1–9.
- Girard JP, Dami J. Antigenic properties of lung tissue. Presence of specific antibodies in certain chronic respiratory disorders. *Poumon Coeur*. 1973;29:267–70.

11. Lassalle P, Delneste Y, Gosset P, Gras-Masse H, Wallaert B, Tonnel AB. T and B cell immune response to a 55-kDa endothelial cell-derived antigen in severe asthma. *Eur J Immunol*. 1993;23:796–803. doi:[10.1002/eji.1830230404](https://doi.org/10.1002/eji.1830230404).
12. Pasková S, Kolesár J, Siposová E. Pulmonary autoantibodies in bronchial asthma patients undergoing cave and climate therapy in Bystrá. *Allerg Immunol (Leipz)*. 1976;22:23–7.
13. 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. doi:[10.1111/j.1365-2222.1992.tb00159.x](https://doi.org/10.1111/j.1365-2222.1992.tb00159.x).
14. Nahm DH, Lee YE, Yim EJ, Park HS, Yim H, Kang Y, et al. Identification of cytokeratin 18 as a bronchial epithelial autoantigen associated with nonallergic asthma. *Am J Respir Crit Care Med*. 2002;165:1536–9. doi:[10.1164/rccm.200201-009OC](https://doi.org/10.1164/rccm.200201-009OC).
15. Nahm DH, Lee KH, Shin JY, Ye YM, Kang Y, Park HS. Identification of alpha-enolase as an autoantigen associated with severe asthma. *J Allergy Clin Immunol*. 2006;118:376–81. doi:[10.1016/j.jaci.2006.04.002](https://doi.org/10.1016/j.jaci.2006.04.002).
16. Lee KH, Chung HS, Kim HS, Oh SH, Ha MK, Baik JH, et al. Human alpha-enolase from endothelial cells as a target antigen of anti-endothelial cell antibody in Behçet's disease. *Arthritis Rheum*. 2003;48:2025–35. doi:[10.1002/art.11074](https://doi.org/10.1002/art.11074).
17. Ditzel HJ, Strik MC, Larsen MK, Willis AC, Waseem A, Kejlving K, et al. Cancer-associated cleavage of cytokeratin 8/18 heterotypic complexes exposes a neoepitope in human adenocarcinomas. *J Biol Chem*. 2002;277:21712–22. doi:[10.1074/jbc.M202140200](https://doi.org/10.1074/jbc.M202140200).
18. Smith JD, Crisp SJ, Dunn MJ, Pomerance A, Yacoub MH, Rose ML. Pre-transplant anti-epithelial cell antibodies and graft failure after single lung transplantation. *Transpl Immunol*. 1995;3:68–73. doi:[10.1016/0966-3274\(95\)80009-3](https://doi.org/10.1016/0966-3274(95)80009-3).
19. Bergquist G. A contribution to the problem of autosensitization in bronchial asthma. *Acta Allergol*. 1956;10:187–93.
20. Mendes E. Experimental asthma of the guinea pig provoked by human lung tissue. *Acta Allergol*. 1958;12:231–9.
21. Umibe T, Kita Y, Nakao A, Nakajima H, Fukuda T, Yoshida S, et al. Clonal expansion of T cells infiltrating in the airways of non-atopic asthmatics. *Clin Exp Immunol*. 2000;119:390–7. doi:[10.1046/j.1365-2249.2000.01148.x](https://doi.org/10.1046/j.1365-2249.2000.01148.x).
22. Kon OM, Sihra BS, Compton CH, Leonard TB, Kay AB, Barnes NC. Randomised, dose-ranging, placebo-controlled study of chimeric antibody to CD4 (keliximab) in chronic severe asthma. *Lancet*. 1998;352:1109–13. doi:[10.1016/S0140-6736\(97\)12261-9](https://doi.org/10.1016/S0140-6736(97)12261-9).
23. De Sanctis GT, Itoh A, Green FH, Qin S, Kimura T, Grobholz JK, et al. T-lymphocytes regulate genetically determined airway hyper-responsiveness in mice. *Nat Med*. 1997;3:460–2. doi:[10.1038/nm0497-460](https://doi.org/10.1038/nm0497-460).
24. The ENFUMOSA Study Group. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *Eur Respir J*. 2003;22:470–7. doi:[10.1183/09031936.03.00261903](https://doi.org/10.1183/09031936.03.00261903).
25. Taraseviciene-Stewart L, Scerbavicius R, Choe KH, Moore M, Sullivan A, Nicolls MR, et al. An animal model of autoimmune emphysema. *Am J Respir Crit Care Med*. 2005;171:734–42. doi:[10.1164/rccm.200409-1275OC](https://doi.org/10.1164/rccm.200409-1275OC).
26. Feghali-Bostwick CA, Gadgil AS, Otterbein LE, Pilewski JM, Stoner MW, Csizmadia E, et al. Autoantibodies in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008;177:156–63. doi:[10.1164/rccm.200701-014OC](https://doi.org/10.1164/rccm.200701-014OC).
27. Venter JC, Fraser CM, Harrison LC. Autoantibodies to beta 2-adrenergic receptors: a possible cause of adrenergic hyporesponsiveness in allergic rhinitis and asthma. *Science*. 1980;207:1361–3.
28. Paul S, Said SI, Thompson AB, Volle DJ, Agrawal DK, Foda H, et al. Characterization of autoantibodies to vasoactive intestinal peptide in asthma. *J Neuroimmunol*. 1989;23:133–42. doi:[10.1016/0165-5728\(89\)90032-5](https://doi.org/10.1016/0165-5728(89)90032-5).
29. Turki J, Liggett SB. Receptor-specific functional properties of beta 2-adrenergic receptor autoantibodies in asthma. *Am J Respir Cell Mol Biol*. 1995;12:531–9.
30. Berisha HI, Bratut M, Bangale Y, Colasurdo G, Paul S, Said SI. New evidence for transmitter role of VIP in the airways: impaired relaxation by a catalytic antibody. *Pulm Pharmacol Ther*. 2002;15:121–7. doi:[10.1006/pupt.2001.0337](https://doi.org/10.1006/pupt.2001.0337).
31. Adamus G, Amundson D, Seigel GM, Machnicki M. Anti-enolase-alpha autoantibodies in cancer-associated retinopathy: epitope mapping and cytotoxicity on retinal cells. *J Autoimmun*. 1998;11:671–7. doi:[10.1006/jaut.1998.0239](https://doi.org/10.1006/jaut.1998.0239).
32. Olopade CO, Yu J, Abubaker J, Mensah E, Paul S. Catalytic hydrolysis of VIP in pregnant women with asthma. *J Asthma*. 2006;43:429–32. doi:[10.1080/02770900600710730](https://doi.org/10.1080/02770900600710730).