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IgA and IgG Antibodies to *Streptococcus Pneumoniae* in Induced Sputum from Asthmatic Patients

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Eosinophils are known to be main effector cells in airway inflammation of bronchial asthma and contribute to epithelial damage by degranulation of cytotoxic granular proteins. The mechanism of eosinophil degranulation in asthmatic airway is still poorly understood. There have been some *in vitro* data suggesting immunoglobulins as a stimulus for eosinophil degranulation. In order to evaluate a possible role of specific antibodies to bacterial organism on eosinophil degranulation within airway secretion from atopic asthmatic patients, we measured IgA and IgG antibodies to *Streptococcus pneumoniae* polysaccharide antigen in the induced sputum from 16 atopic asthmatic patients and 12 non-atopic non-asthmatic controls by enzyme-linked immunosorbent assay (ELISA). Eosinophil cationic protein (ECP) levels were measured in induced sputum from 16 atopic asthmatic patients. Levels of specific IgA antibodies to *S. pneumoniae* in the induced sputum from mite-sensitive asthmatic patients were significantly higher than those from controls ($p < 0.005$). No significant difference was found in the levels of IgG antibodies to *S. pneumoniae* in induced sputum between asthmatics and controls. ECP levels in induced sputum from mite-sensitive asthmatics correlated significantly with the levels of specific IgA antibodies to *S. pneumoniae* ($r=0.56$, $p < 0.05$), but not with IgG antibodies to *S. pneumoniae*. These results suggest that IgA antibodies to bacterial antigen could participate in eosinophil degranulation in the airway secretion from atopic asthmatics patients.

Key Words: Asthma, Induced sputum, *Streptococcus pneumoniae*, Antibody, Eosinophil cationic protein

INTRODUCTION

Eosinophils play a major role in airway inflammation of bronchial asthma[1]. Cytotoxic granular proteins released from eosinophils were suggested to induce epithelial damage in asthmatic airway[2]. Various chemoattractants and cytokines such as PAF, IL-3, IL-5, and GM-CSF are known to affect eosinophil migration, proliferation, and survival, but they act as a weak degranulation inducer for eosinophils[3]. The mechanism of eosinophil degranulation in asthmatic airway is still poorly understood.

Immunoglobulins (IgA, IgE, and IgG) are known to be potent stimuli for eosinophil degranulation, resulting in the release of cytotoxic proteins such as eosinophil cationic protein (ECP), eosinophilic peroxidase, and eosinophil-derived neurotoxin[4,5]. *In vitro* study suggested that IgG antibodies to allergen can degranulate eosinophil in the presence of immobilized allergen on solid phase, and suggested that allergen-specific IgG antibodies may be responsible for degranulation of eosinophils in brochial asthma[6]. We previously showed that total IgA antibody levels correlated significantly with ECP levels in induced sputum from patients with atopic asthma, and suggested that IgA antibodies in tracheo-bronchial secretion may be involved in eosinophil degranulation in bronchial mucosa of asthmatic

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patients[7]. We also recently presented data showing a close correlation between the levels of specific IgA antibodies to house dust mite and ECP in induced sputum from mite-sensitive asthmatics, and suggested that allergen-specific IgA antibodies might be involved in eosinophil degranulation in air way secretion from patients with atopic asthma[8].

Infection of bacteria and viruses usually aggravate the course of bronchial asthma. Thus, the role of bacteria and viruses has been studied by many investigators[9,10]. The association between bacterial infection and elevated circulating ECP was reported[11], and recently colonization of *Streptococcus pneumoniae* has also been suggested to be associated with increased ECP levels in bronchial lavage fluid from patients with chronic bronchitis[12]. However, the role of bacterial organism on eosinophilic inflammation of bronchial asthma is still uncertain.

To evaluate the possible contribution of IgA and IgG antibodies to bacterial organism in eosinophil degranulation in the airway secretion from patients with asthma, we measured IgA and IgG antibodies to *S. pneumoniae* polysaccharide antigen in induced sputum from patients with atopic asthma and controls, attempting to find any correlation with ECP level.

METHODS

Subjects

We collected sputum samples from 16 asthmatic patients who showed positive skin prick test to *Dermatophagoides farinae* (Bencard Co, U.K.). Positive skin test was defined when the mean wheal diameter of allergen was 3 mm greater than that of negative control (normal saline). All patients had typical clinical history of asthma and documented reversibility of forced expiratory volume in one second (FEV1) greater than 15% in response to inhaled bronchodilator or treatment and/or positive response on methacholine brochial challenge test, performed as previously described[13]. None had received inhaled or oral corticosteroids in three months prior to the study. As a control, 12 non-asthmatic healthy subjects who showed negative skin prick test to common inhalant allergens were selected (Table 1).

Table 1. Characteristics of the study subjects

	Healthy controls (n=12)	Asthmatic subjects (n=16)
Age, years(range)	35(25~62)	36(14~60)
Sex, M/F	5/7	10/6
FEV1(%)*	101.4(1.7)	87.3(4.5)
Sputum ECP(ng/ml)	ND**	231.6(44.3)

Values are presented as mean (standard error).
* Significantly lower in asthmatic subjectes than healthy controls.
**ND: Not measured.

Induction of sputum

Sputum was induced using a previously described method[14]. Immediately before the sputum expectoration, each subject was pretreated with 200 μg salbutuamol, administered by means of a metered dose inhaler. The subjects then inhaled nebulized sterile 3% saline solution for 20 minutes through an ultrasonic nebulizer (Omron Co., Japan). After being instructed to spit out the saliva in the mouth first, then the subjects were asked to cough and expectorate sputum into a clean plastic container. In six atopic asthmatics, saliva samples were collected before the induction of sputum.

Sputum and saliva processing

Collected sputum and saliva were immediately processed. The volume of the saliva and induced sputum were determined, and an equal volume of phosphate buffered saline (PBS) was added. The samples were then mixed by vortex mixer and centrifuged for 20 minutes at 3000 rpm. The supernatants were aspirated and frozen at −20°C.

Measurement of specific IgA and IgG antibodies by ELISA

Measurement of specific antibodies to pneumococcal polysaccharide antigen were done according to the method previously reported[15]. Microtiter plates (Immulon 2; Dynatech Laboratories, Inc., Chantilly, VA) were coated with pneumococcal polysaccharides (Pasteur Mérieux Sérums et Vaccins, Lyon, France) at a concentration of 10 μg/ml in 0.1M carbonate, pH 9.6, overnight at 4°C. Plates were washed and blocked by 3% bovine serum albumin in phosphate buffered saline with 0.05% Tween-20 (PBST) and then plates are incubated

with duplicated samples (50 μl) of induced sputum and saliva at 1 : 50 dilutions overnight at 4°C. After washing, plates were incubated with peroxidase conjugated affinity-purified goat anti-IgA or anti-IgG antibodies (Sigma Chemical Co., St. Louis, MO) at 1 : 2500 diution for 3 hours at room temperature. After washing, the substrate solution consisting of 0.04% (w/v) orthophenylenediamine dissolved in 24.3 mM citric acid, 51.4 mM NaH_2PO_4 (pH 5.0) and 0.03% H_2O_2 was added. After 15 minutes, the reaction was stopped by adding 2.5 N H_2SO_4. Amounts of specific antibodies in samples were calculated from control curves made by serial dilutions of positive control samples which showed high titer anibodies.

ELISA inhibition

ELISA inhibition was performed using highly positive sputum samples. Serially 2-fold diluted *Dermatophagoides farinae* extracts and *S. pneumoniae* polysaccharide antigen were added to positive sputum samples, and the assay were performed as above after overnight incubation at 4°C. The results of inhibition study of HDM-specific IgE was calculated as the percent inhibition according to the following formula:

Percent inhibition (%)=[(absorbance by addition of buffer only- absorbance by additon of antigen)/absorbance by addition of buffer only]×100.

ECP measurement

In 16 asthmatic patients, ECP level in induced sputum was measured using the Pharmacia CAP system (Pharmacia Diagnostics Inc., Uppsala, Sweden). The lowest detection limit for ECP measurement was 2 ng/ml. When ECP levels were below the lowest detection limit, they were treated as zero in value for statistical analysis.

Statistics

Data are expressed as the mean and standard error of the mean (SEM). The Mann-Whitney *U* test was used to assess differences between groups. A spearman's rank correlation was calculated to assess the correlation between data. The differences between induced sputum and saliva samples from the same subjects were compared by a Wilcoxon signed-rank test.

RESULTS

Levels of specific IgA and IgG antibodies to *S. pneumoniae* in induced sputum

Levels of specific IgA antibodies to *S. pneumoniae* were significantly higher in induced sputum from mite-sensitive asthmatic patients than in those from controls ($p<0.005$) (Fig. 1). No significant difference was found in the levels of IgG antibodies to *S. pneumoniae* in induced sputum between the asthmatics and the controls (Fig. 1).

ELISA inhibition

S. pneumoniae antigen dose-dependently inhibited specific bindings in specific IgG and IgA assays (Fig. 2)

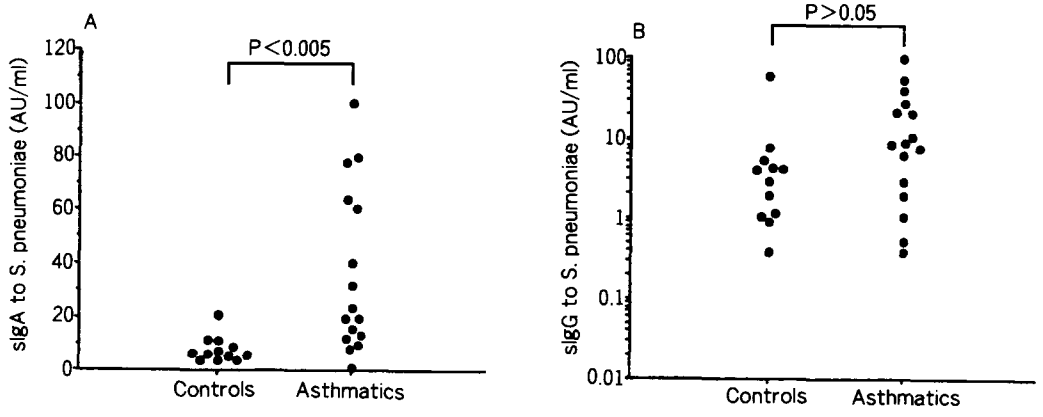

Fig. 1. Levels of specific IgA (sIgA) and IgG (sIgG) antibodies to *S. pneumoniae* in induced sputum from controls and asthmatics.

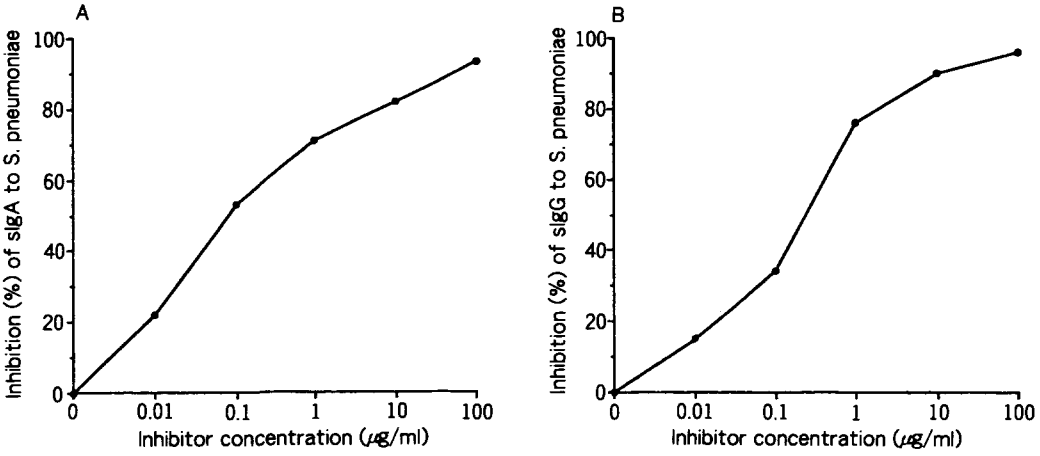

Fig. 2. ELISA inhibition of specific IgA (sIgA) and IgG (sIgG) antibodies (sIgG) to *S. pneumoniae* in induced sputum from a asthmatic patient.

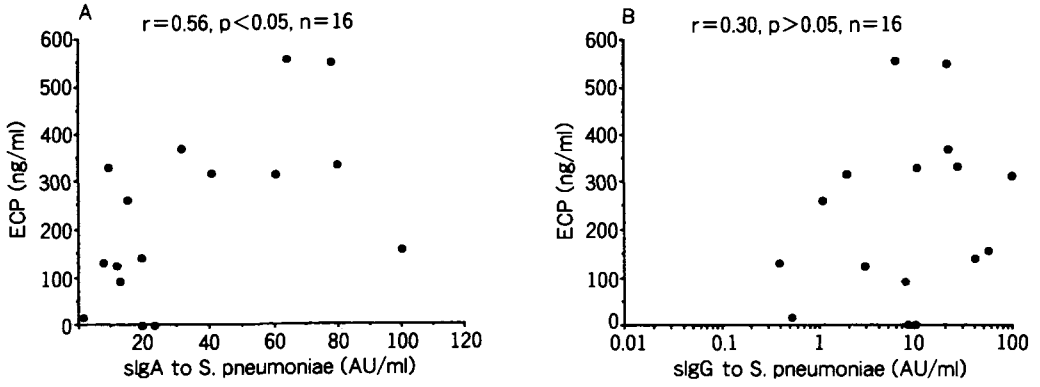

Fig. 3. Correlation between the levels of specific IgA (sIgA) and IgG (IgG) antibodies to *S. pneumoniae* and ECP in induced sputum from asthmatics.

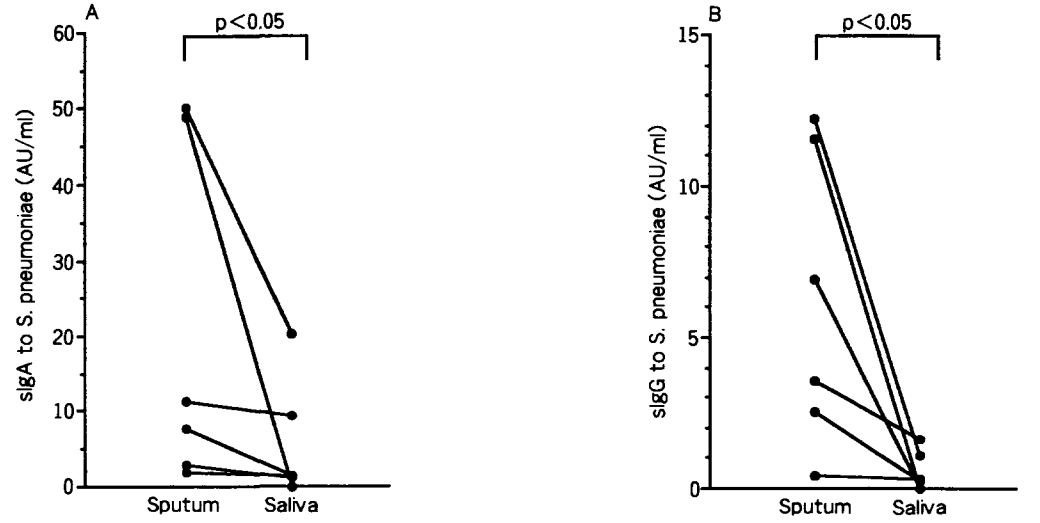

Fig. 4. Levels of specific IgA antibodies (sIgA) to *S. pneumoniae* and specific IgG antibodies (sIgG) to *S. pneumoniae* in induced sputum and saliva from six asthmatics.

Correlation between specific IgA and IgG antibodies to *S. pneumoniae* and ECP levels

Levels of ECP in induced sputum from mite-sensitive asthmatics correlated significantly with the levels of specific IgA antibodies to *S. pneumoniae* ($r=0.56$, $p<0.05$) (Fig. 3). No significant correlation was found between the levels of specific IgG antibodies to *S. pneumoniae* and ECP (Fig. 3).

Comparison of specific antibodies between in induced sputum and saliva from six asthmatic patients

The levels of IgG and IgA antibodies to *S. pneumoniae* were significantly higher in induced sputum than those in saliva ($p<0.05$) (Fig. 4).

DISCUSSION

Although some *in vitro* studies suggested that immunoglobulins in respiratory secretion might participate in eosinophil degranuation in respiratory allergic diseases[4~6], there have been few *in vivo* data supporting this hypothesis. Several *in vitro* studies showed that immobilization of immunoglobulins on solid phase was essential in immunoglobulin induced eosinophil degranulation[16]: Beads were used to immobilize immunoglobulins[4,17]. However, a solid-phase to immobilize immunoglobulins for eosinophil degranulation is not well understood in human asthmatic airway. It has been suggested that an allergen present in the tissue bed can be potential candidate. The allergen coated on microtiter plate can degranulate eosinophils in the presence of sera from patients sensitized to the same allergen[6]. Moreover, the amounts of eosinophil degranulation correlated with the level of allergen-specific antibodies in serum[6]. In recent study, we presented data to show significant correlation between the levels of ECP and IgA antibodies to house dust mite in induced sputum from mite-sensitive asthmatic patients[8]. These results suggest that allergen-antibody immune complexes might contribute to allergic inflammation by stimulating eosinophil degranulation.

Bacterial organisms had been suggested to contribute to airway inflammation in chronic obstructive pulmonary diseases by releasing cytotoxic mediators from inflammatory cells[18]. Recently, colonization of *S. pneumoniae* has been suggested to be associated with increased ECP levels in bronchial lavage fluid from patients with chronic bronchitis[12]. However, the role of bacterial organism on eosinophilic inflammation of bronchial asthma is still uncertain. *S. pneumoniae* inhabit the upper respiratory tract of healthy adults and children, and lower respiratory tract of patients with chronic obstructive pulmonary disease are frequently colonized by the pneumococcus[18]. In this study, the levels of IgA antibodies to *S. pneumoniae* polysaccharide antigen in induced sputum from mite-sensitive asthmatic patients were shown to be higher than those from controls and correlated significantly with ECP levels. We previously demonstrated that the levels of total IgA antibodies in induced sputum from atopic asthmatic patients with sputum eosinophilia were significantly higher than those from controls and atopic asthmatic patients without sputum eosinophilia, and that the levels of IgA antibody correlated significantly with ECP levels in induced sputum from atopic asthmatic patients[7]. We suggested that an increased IgA in induced sputum from asthmatic patients with airway inflammation might be caused by increased IL-5 expression[7] which could induce both eosinophil infiltration and IgA production from B cells[19].

The purpose of this study was to further investigate the antigen-specificities of IgA antibodies in induced sputum from atopic asthmatic patients. Elevated levels of IL-5 in respiratory secretion from asthmatic patients was shown[20]. Cytokine act on adjacent cells regardless of antigen which induced secretion of the cytokine[21]. An increase in IgA antibodies to house dust mite antigen in induced sputum from mite-sentive asthmatic patients[8] together with an increase in IgA antibodies to *S. pneumoniae* polysaccharide antigen without significant increase in IgG antibodies to the same antigen might suggest polyclonal expansion of IgA antibody producing B lymphocytes, due to increased IL-5 in airway secretion from athmatic patients. Although frequent colonization of *S. pneumoniae* in respiratory tract of patients with chronic airway diseases was observed[18], elevated levels of specific IgA antibodies to *S. pneumoniae* in induced sputum could not be interpreted as an indication of current infection or colonization of *S. pneumoniae* in these asthmatics. However, these findings suggest that bacterial oranism can potentially aggravate damage of respiratory mucosa by immunoglobulin-dependent degranulation of eosinophils in patients with atopic

asthma.

In conclusion, this study suggests that IgA antibodies to bacterial antigen could participate in eosinophil degranulation in the airway secretion of atopic asthmatics patients. However, since this hypothesis is based simply on the correlation between the data, further study is neccesary to confirm the hypothesis.

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