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C3-Containing IgE Immune Complexes in Asthmatic Patients

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Higher levels of IgE-containing immune complexes (IC) have been reported in sera from patients with allergic diseases than in sera from controls. To evaluate the possibility of an IC-mediated mechanism in the pathogenesis of bronchial asthma, we measured circulating C3-containing IgE IC (C3-IgE IC) using anti-C3 ELISA from 20 house dust mite (HDM)-sensitive asthmatics, 20 non-atopic asthmatics, and 14 non-atopic controls. C3-IgE IC levels were significantly higher in HDM-sensitive asthmatics (mean±S.D.: 12.2±7.8 AU/ml) than in non-atopic asthmatics (6.5±7.5 AU/ml) or controls (5.8±4.4 AU/ml). C3-IgE IC levels were significantly correlated with HDM-specific IgE levels ($r=0.50$, $p<0.05$), but not with total IgE levels ($r=0.36$, $p>0.05$) in HDM-sensitive atopic asthmatics. C3-IgE IC levels in sera did not significantly change during HDM-bronchoprovocation test in six HDM-sensitive asthmatics who showed positive reaction. Part of C3-IgE IC could be precipitated by protein G coupled beads. In conclusion, C3-IgE IC levels were elevated in sera from HDM-sensitive asthmatics; moreover IgG antibodies might be a component of C3-IgE IC. Our results suggest that an IgE IC-mediated mechanism could be involved in the pathogenesis of atopic asthma.

Key Words : *IgE-Immune complex, Complement 3, Bronchial asthma, House dust mite.*

INTRODUCTION

Higher levels of IgE-containing immune complexes (IC) have been reported in sera from patients with allergic diseases than in sera from controls by ultracentrifugation method (Brostoff et al., 1977) and polyethylene glycol (PEG) precipitation method (Stevens and Bridts, 1984). The increase of specific IgE IC was detected after food challenge in atopic patients (Brostoff et al., 1979). In patients with food allergy, IgE IC were shown to be able to activate the complement system (Brostoff et al., 1979) and bind to conglutinin column after the addition of fresh human sera, suggesting the possible participation of C3 in IgE IC (Carini and Brostoff, 1987). However, measurement of C3-containing IgE IC (C3-IgE IC) in serum from patients with bronchial asthma has not been tried.

Recent studies on the pathogenesis of asthma revealed that asthma is a chronic inflammatory disease of the bronchial mucosa characterized by an infiltration of various inflammatory cells including eosinophils (McFadden and Gilbert, 1992). Immune complexes containing IgE have been shown to activate inflammatory cells including monocytes, neutrophils (De Clerck et al., 1991), and eosinophils (Tomassini et al., 1991). C3a fragment can degranulate mast cells and C3b fragment can activate monocytes, neutrophils, and eosinophils (Fearon,

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1983). We hypothesized that an IgE IC-mediated mechanism could be involved in the pathogenesis of atopic asthma.

To investigate the possibility of an IC-mediated mechanism in the pathogenesis of bronchial asthma, we measured circulating C3-containing IgE IC (C3-IgE IC) using anti-C3 ELISA in 20 house dust mite (HDM)-sensitive asthmatics, 20 non-atopic asthmatics, and 14 non-atopic controls.

MATERIALS AND METHODS

Subjects

Twenty adult house dust mite(HDM)-sensitive asthmatics who showed a positive skin prick test to *Dermatophagoides farinae (D. farinae)*, and 20 adult non-atopic asthmatic patients who showed all negative responses to 50 common allergens including *D. farinae*, cat and dog dander, Aspergillus, grass and tree pollen mixture, egg, and cow milk (Bencard Co, U.K.) were included. All patients had history of asthma, nonspecific bronchial hyperreactivity, and airway obstruction reversible with treatment. Fourteen control subjects were included from healthy adults who showed a negative skin test to 50 common allergens. Blood from patients was allowed to clot at room temperature, centrifuged, and sera were collected and stored at -20℃.

Measurement of C3-IgE IC in sera

C3-IgE IC was measured according to previously described methods (Pereira et al., 1980; Hall and Lawley, 1985) with modifications. A 96-well microplate (Dynatech, Alexandria, Va, USA) was coated overnight with F(ab')2 fragments of anti-C3 antibody (Organon Teknica Corp., Durham, NC, USA) in 0.05M carbonate buffer (pH 9.6) at 4℃. After being washed with phosphate-buffered saline (pH 7.3) with 0.05% Tween 20(PBST), the wells were blocked with PBST with 1% bovine serum albumin. The wells were then incubated with undiluted sera overnight at 4℃. After washes with PBST, the wells were incubated with biotin-conjugated anti-IgE antibodies (Vector laboratories, Burlingame, CA, USA) at a dilution of 1:1000 (w/v) for 1 hour. The washing step was repeated, and streptavidin-conjugated peroxidase (Sigma, St. Louis, Mo, USA) at a dilution of 1:1000 (w/v) was added into the wells and incubated for 30 minutes. After another wash, 100μl of 0.01M ABTS (2,2'-azinobis-3 ethyl-benzthiazoline sulfonic acid) in 0.07M citrate-phosphate buffer, pH 4.2 containing 0.03% H2O2 was added to the wells. The color reaction was stopped with 100μl of 0.002 M sodium azide and the plates read at 410nm using a microtiter reader (Dynatech Laboratories, Virginia, U.S.A.). The standard for the quantification of C3-IgE immune complex was established by pooling sera with high C3-IgE levels, and arbitrarily defining the standard as 100 AU (arbitrary unit)/ml. Optical densities of test sera were compared to those from serially diluted standard and were presented as AU/ml.

Adsorption of serum C3-IgE IC by protein G coupled beads

Sera from two patients with high titer of C3-IgE IC were incubated with protein G-coupled Sepharose bead (Sigma, USA). After incubation for 16 hours at 4℃, the above mixture was centrifuged and the supernatants were measured for C3-IgE IC by ELISA.

Total IgE antibody and *D. farinae*-specific IgE antibody

Total IgE antibody and specific IgE antibody to *D. farinae* were measured by radioimmunoassay using AlaSTAT kit (DPC, Los Angeles, CA, USA).

Change of C3-IgE IC in sera during bronchoprovocation test with *D. farinae* antigen

Bronchoprovocation tests were performed, using *D. farinae* antigen (Allergopharma, Germany) in 15 HDM-sensitive asthmatic patients. One ml of phenolized saline solution was aerosolized by nebulizer 646 (Devilbiss Co., Somerset, Pennsylvania, USA) connected to a compressed air source (5 L/mlin). The patient was asked to breathe the aerosol for 2 minutes with tidal breathing. The forced expiratory volume in one second (FEV1) was measured as a baseline value with a spirometer 10 minutes after inhalation of the phenolized saline. Then the *D. farinae* antigen was diluted in a phenolized saline (10, 100, 1000 biological equivalent /ml) and administered to the patients by the above method, beginning with the lowest concentration. The FEV1 was measured 10 minutes after inhalation of each concentration of *D. farinae* antigen. In the absence

of a decrease of FEV1 below 80% of the baseline value, the procedure was repeated with a higher concentration of allergen. A positive reaction was recorded when FEV1 fell below 80% of baseline value. Then the FEV1 was measured every 10 minutes during the first hour, then every hour for 7 hours. In each patient, blood was collected before the test, and 30 minutes and 4 hours after inhalation of the highest concentration of *D. farinae* antigen.

Statistical analysis

Statistical analysis of results was performed by the Mann-Whitney U test to determine differences among the groups. Data from each group were expressed as a mean ± standard deviation (S.D.). A linear regression analysis was used to evaluate possible correlation between data.

RESULTS

Serum C3-IgE IC levels were significantly higher in HDM-sensitive asthmatics (12.2±7.8 AU/ml) than in non-atopic asthmatics (6.5±7.5 AU/ml) and non-atopic controls (5.8±4.4 AU/ml) ($p<0.05$, Fig. 1). With cut-off level set at 2 S.D. above the mean of controls, 7.1 % of controls, 5% of non-atopic asthmatics, and 30% of HDM-sensitive asthmatics had elevated C3-IgE IC levels. In HDM-sensitive asthmatics, the C3-IgE IC levels were significantly correlated with serum HDM-specific IgE levels ($r=0.50$, $p<0.05$, Fig. 2), but not with total IgE levels ($r=0.36$, $p>0.05$, Fig. 3). There was no significant change of serum C3-IgE IC levels during bronchoprovocation test with HDM antigen in six HDM-sensitive asthmatics, all of whom showed positive reaction (Fig. 4). C3-IgE IC could be adsorbed by protein G-coupled beads in a dose-dependent manner (Fig. 5).

DISCUSSION

We showed here that circulating C3-containing IgE IC were significantly higher in HDM-sensitive asthmatics than in non-atopic asthmatics or controls. In contrast to previous studies showing increased IgE IC in allergic diseases, using physical property of IC in detection methods such as the PEG precipitation (Stevens and Bridts, 1984) or ultracentrifugation (Brostoff et al., 1977), we used the

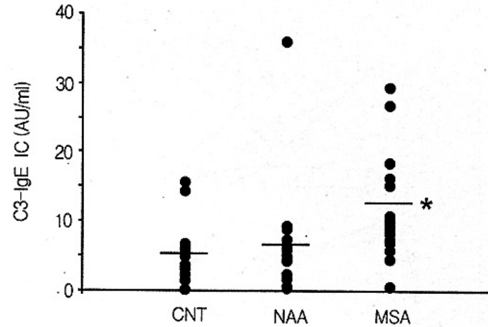

Fig. 1. C3-IgE immune complex(IC) levels in sera of asthmatics and controls. CNT : non-atopic controls, NAA : non-atopic asthmatics, MSA : HDM-sensitive asthmatics. Horizontal bars indicate mean values.
* Significantly higher levels were noted in MSA as compared with CNT, and NAA ($p<0.05$).

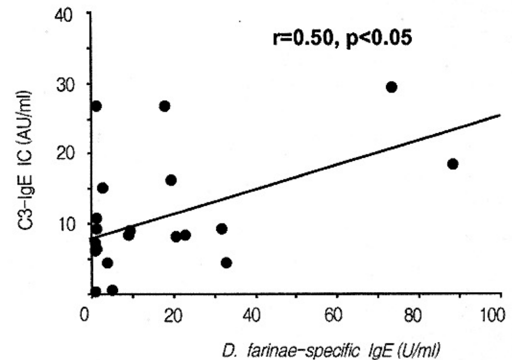

Fig. 2. Correlation between C3-IgE immune complex and HDM-specific IgE levels in HDM-sensitive asthmatics.

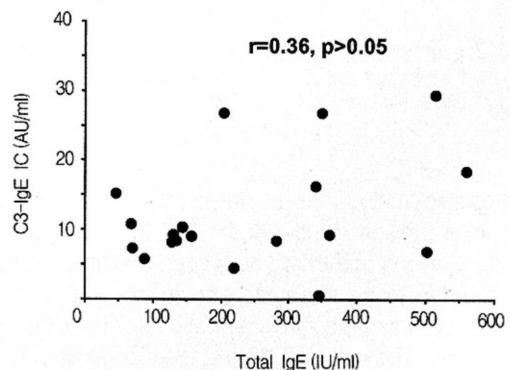

Fig. 3. Correlation between C3-IgE immune complex and total IgE levels in sera from HDM-sensitive asthmatics.

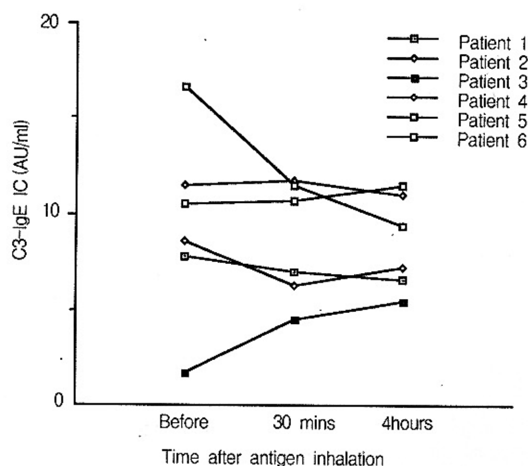

Fig. 4. Changes in C3-IgE immune complex levels during bronchoprovocation test with HDM antigen in six HDM-sensitive asthmatics. No significant changes in C3-IgE immune complexes levels were noted.

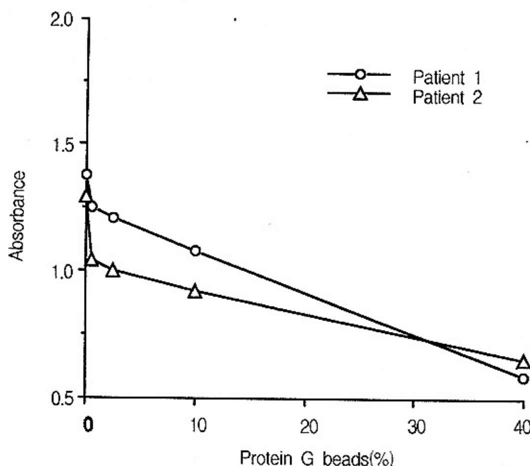

Fig. 5. Adsorption of C3-IgE immune complexes by protein G-coupled sepharose beads in two sera with high titer of C3-IgE immune complex.

immunologic method using anti-complement antibody. In a previous study by the PEG precipitation method (Stevens and Bridts, 1984), IgE containing IC was reported to be elevated (> mean of controls + 2 S.D.) in 5.7% of controls and 59.5% of allergic patients with asthma and rhinitis, and in 19.4% of nonallergic patients with asthma and rhinitis. Our result is quite consistent with those results by showing that the C3-IgE IC level was elevated (> mean of controls + 2 S.D.) in 7.1 % of controls and 5% of non-atopic asthmatics, and 30% of HDM-sensitive asthmatics. The discrepancy in the sensitivity of these 2 methods for the detection of IgE IC may be due to the possibility that each method may detect different spectrums of IC.

IgE antibody has been considered a non-complement fixing immunoglobulin isotype (Turner, 1983). However there are reports that IgE can activate complement both by the classical (Saint-Remy et al., 1983) and by the alternative pathway (Ishizaka et al., 1972). Anti-IgE autoantibody with IgG isotype and IC consisting of Anti-IgE and IgE antibodies have been reported to be elevated in sera from asthmatics (Ritter et al., 1991). To evaluate the possible contribution of IgG antibodies on the complement fixation in IgE IC, we tried to adsorb the C3-IgE IC by protein G which is known to selectively bind to IgG antibody (Peng et al., 1994). Partial adsoption of C3-IgE IC by protein G-coupled bead suggests that IgG antibody may be involved in complement fixation and that complexes consisting of IgE, C3, and IgG may be present.

The pathogenetic significance of IgE IC in allergic disease was not determined. Some in vitro data showed that IC containing IgE could activate inflammatory cells including monocytes, neutrophils (De Clerck et al., 1991), and eosinophils (Tomassini et al., 1991). Preformed allergen-IgE complexes can induce immediate erythema and wheal reaction (Ishizaka and Ishizaka, 1968). Activated C3 fragment can degranulate mast cells and activate monocytes, neutrophils, and eosinophils (Fearon, 1983). An IC-mediated mechanism has been suggested to contribute to the late allergic reaction on the basis that the late skin reaction to the HDM antigen could be accentuated when autologous serum was mixed with the HDM antigen on intradermal test in HDM-sensitive individuals (Nahm et al., 1995). Our result on elevated C3 containing IgE IC from sera of atopic asthmatics also suggests that an immune complex-mediated mechanism may be involved in the pathogenesis of atopic asthma.

That IgE IC consists of allergen and specific IgE antibody have been suggested by a report which showed an increase of specific IgE IC after food allergen challenge in patients with food allergy (Brostoff et al., 1979). Decrease in complement com-

ponent has been also demonstrated after inhalation of allergen (Arroyave et al., 1977) and the possible existence of immune complexes consisting of IgE antibodies and inhalant allergens has been suggested (Allen et al, 1979; Stevens and Bridts, 1984), but it has not been demonstrated in human sera. To evaluate this hypothesis, we measured circulating C3-IgE IC after inhalation of HDM antigen in atopic asthmatics in this study, but we could not demonstrate any significant changes in C3-IgE IC. Our result is consistent with previous reports (Stevens and Bridts, 1984) showing no significant changes in IgE IC measured by PEG precipitation method after bronchoprovocation with allergen. In this study, we showed that C3-IgE IC levels were significantly correlated with HDM-specific IgE levels, but not with total serum IgE levels in HDM-sensitive asthmatics. This result suggests a possible involvement of allergen-specific IgE antibody in the formation of C3-IgE IC in atopic asthmatics.

In conclusion, C3-IgE IC levels were elevated in sera from HDM-sensitive asthmatics; moreover IgG antibodies might be a component of C3-IgE IC. Our results suggest that an IgE IC-mediated mechanism could be involved in the pathogenesis of atopic asthma.

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