Role of Circulating Immune Complex in Aspirin-sensitive Asthma

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Background & objectives: The pathogenic mechanism of aspirin-sensitive asthma (ASA-BA) remains to be further defined. To evaluate the role of circulating immune complex (CIC) in ASA-BA.

Subjects & Methods: We measured IgG- and IgA-IC level by ELISA using anti-C3 antibody in 33 ASA-BA patients whose sensitivity was confirmed by lysine-aspirin bronchoprovocation test, and compared with those of 14 allergic, 14 intrinsic asthma patients and 7 healthy controls.

Results: There was no significant difference in IgG-IC level among the four groups (p>0.05), while IgA-IC levels of aspirin-sensitive asthma were higher than those of other groups (p=0.0035). Patients with nasal polyp had significantly higher IgG-IC than those without it (p=0.02). No differences were found according to medication and symptom scores, and presence of atopy, rhino-sinusitis, articular or concurrent sensitivity to sulphite (p>0.05). Insignificant correlation was found between IgG-IC level and asthma duration, total IgE level, or circulating eosinophil count.

Conclusion: These findings suggest a possible contribution of IgG-IC to the development of nasal polyp in ASA-BA. Further study will be needed to clarify the role of IgA-IC in the pathogenesis of ASA-BA.

Key words: circulating immune complex, aspirin-sensitive asthma, nasal polyp

INTRODUCTION

ASA and non-steroidal anti-inflammatory drugs (NSAIDs) can induce bronchoconstriction in 10-20% of adult asthmatic patients. The mechanism of ASA-induced bronchoconstriction is partly clarified. Appreciable numbers of aspirin-sensitive asthma had rhino-sinusitis and/or nasal polyp. Immunohistological study of the bronchial tissue from ASA-BA showed increased inflammatory cell infiltrations, including eosinophil, mast cell and lymphocyte, similarly to non-aspirin sensitive asthma. In this study, in order to further understand the pathogenic mechanism of ASA-BA, we tried to detect C3-containing IgG- and IgA-IC level in sera from ASA-BA patients and analyzed them based upon various clinical characteristics seen in ASA-BA.

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METHODS

Subjects

Thirty three ASA-BA patients, aged between 23 and 72 years, participated in this study. All showed positive responses on lysine-aspirin (L-ASA) bronchoprovocation test (BPT), and they were compared with 14 allergic asthma patients sensitive to house dust mite, 14 intrinsic asthma patients and seven healthy controls. Their sera were collected and stored at -20°C. Atopy was defined on the basis that they showed more than 2+ skin prick test reactions to more than two common inhalant allergens, such as Dermatophagoides farinae, birch, oak, rye grass, mugwort, ragweed, Aspergillus spp. (Bencard Allergy Service, Bredford, Middlessex, UK).

L-ASA bronchoprovocation test

L-ASA BPT was performed according to the method...
with some modifications. Pulmonary functions were measured with a spirometer (Chest), the FEV₁ and maximum mid-expiratory flow (MMEF), before and during the provocation. The test solution was delivered by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Penn., USA) and connected to a compressed air source (5 l/min). Normal saline was inhaled as a placebo solution. The patients were asked to breathe the nebulized aerosol up to their vital capacity, 10 times L-ASA (Young Jin Pharmaceutical Co. Korea), as a powder containing 1800 mg L-ASA was made up freshly on each challenge day. The challenges with the placebo were performed seven days before the L-ASA BPT. L-ASA BPT from 11.25 up to 180 mg/ml was performed to induce more than a 20% fall of FEV₁. The FEV₁ and MMEF were measured frequently during the first hour, and then hourly for 8 hours.

Measurement of C₃-containing IgG- and IgA immune complex (IC)

C₃ containing IgG-IC was measured according to previously described methods with some modifications. A 96-well microplate (Dynatech, Alexandria, Va, USA) in 0.05M carbonate buffer (pH 9.6) at 4°C was used. After washing with phosphate-buffered saline with 0.05% Tween 20 (PBST), the wells were blocked with 1% bovine serum albumin-PBST. The wells were then incubated with undiluted sera overnight at 4°C. After washes with PBST, they were incubated with 50 μl biotin-conjugated anti-IgG or anti-IgA 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, 50 μl of 0.01M ABTS (2,2'-azino bis-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 0.002M sodium azide and the plate was read at 410 nm using a microtiter reader (Dynatech Laboratories, Virginia, USA). The standard for quantification of C₃-IgG- or IgA-IC was established by pooling systemic lupus erythematosus patients sera with high IgG-IC levels, and arbitrarily defining the standard as 100 AU (arbitrary units/ml). Optical density of the test sera was compared to that from serially diluted standard and presented as AU/ml.

Statistical Analysis

The ANOVA and Mann-Whitney tests were applied using the SPSS version 7.0 (Chicago) to evaluate statistical significance. A p value of 0.05 or less was regarded as significant.

RESULTS

This study demonstrated IgG-IC and IgA-IC levels in three asthmatic groups and seven healthy controls as shown in Fig. 1. IgG-IC levels tended to be higher than those of IgA-IC. There was no significant difference in the IgG-IC level among the four groups (p>0.05). However, IgA-IC level was significantly higher in ASA-BA than in other groups (p=0.035).

IgG-IC level was compared according to the presence of rhino-sinusitis, urticaria or nasal polyp. No significant difference was found in IgG-IC level whether the patients have rhino-sinusitis or urticaria (p=0.39, p=0.97, respectively). However, the patients having nasal polyp had significantly higher IgG-IC level than those without it (p=0.02).

No significant difference was found between IgG-IC level and atopic status or concurrent sensitivity to sulfite (p=0.32, p=0.95, respectively) as shown in Fig. 2.

Fig. 3 shows the distribution of IgG-IC level according to medication and symptom scores. Most patients had
Fig. 2. Comparison of IgG-IC levels according to the associated conditions in aspirin-sensitive asthma patients. No significant difference was found according to presence of atopy, rhino-sinusitis, urticaria or concurrent sensitivity to sulfate. Patients having nasal polyp had significantly higher IgG-IC than those without it (P = 0.02).

Fig. 3. IgG-IC levels according to medication and symptom scores in aspirin-sensitive asthma patients. No significant difference was found.

Fig. 4. Correlation between IgG-IC level and symptoms and medication scores (Class 3 or 4). No significant difference was found among the four groups (p>0.05).

Fig. 4 shows the relationship between IgG-IC level and duration of asthma symptoms, total IgE level or eosinophil count. The correlation was insignificant with asthma symptom duration (r=0.19, p=0.31), total IgE level (r=0.13, p=0.49), or total eosinophil count (r=0.18, p=0.33).

DISCUSSION

The pathogenic significance of CIC in allergic diseases...
is unclear. Several investigators have reported IC, especially IgE-IC, increased in patients with allergic asthma and food allergy, especially after the food challenge test\(^1\). In food allergy patients, IgE IC could activate the complement system and bind to the conglutinin column after the addition of fresh human sera, suggesting a participation of C3 in IgE-IC. Our previous study\(^2\) showed that C3-containing IgE-IC was more elevated in house dust mite-sensitive asthma than in non-allergic asthma. There have been some in vitro data supporting the possible involvement of IC in allergic inflammation. IC containing IgE could activate inflammatory cells, including monocyte, neutrophil\(^3\), and eosinophil\(^4\). Preformed allergen-IgE complex could induce immediate erythema and wheal reaction\(^5\). Moreover, activated C3 fragment could degranulate mast cell and activate monocyte, neutrophil and eosinophil\(^6\). Furthermore, there has been a report suggesting that an IC-mediated mechanism may contribute to late skin reaction on the basis that late reaction to the house dust mite on intanadermal test could be accentuated when autologous serum was mixed with the allergen in sensitive individuals\(^7\). These results might suggest a possible involvement of C3-containing IC in the pathogenesis of atopic asthma. However, there have been very few reports to study the role of circulating immune complex in patients with ASA-BA. In this present study, we tried to detect C3-containing IgG- and IgA-IC levels in ASA-BA, and compared them with allergic and non-allergic asthma, as well as healthy controls. IgG-IC level was higher than IgA-IC in each group. No significant difference was noted in IgG-IC level among the four groups but, interestingly, IgA-IC was significantly higher in ASA-BA than in other groups. Further studies will be needed to identify the role of IgA-IC in airway inflammation of ASA-BA.

The definitive diagnostic test for respiratory sensitivity to ASA has been oral provocation with ASA and NSAIDs. L-ASA BPT has become an alternative diagnostic test to detect ASA sensitivity in asthmatic patients\(^8\). In our previous study\(^9\), L-ASA inhalation induced late asthmatic response as well as early reaction. The mechanism to induce late asthmatic response following L-ASA inhalation has been unknown. In the present study, 15 had early asthmatic response and 18 had a late onset asthmatic response (five dual and 13 late only). No difference was found in the IgG-IC level according to the type of asthmatic response following L-ASA inhalation. These results support that L-ASA BPT is a useful method to determine lower respiratory sensitivity to ASA, and IgG-IC may not be involved in the development of the late asthmatic response.

ASA-BA has a wide clinical spectrum\(^10\). High incidence of rhino-sinusitis and/or nasal polyp was reported in ASA-BA\(^11\). The frequency of polyp formation in patients with Samter’s syndrome has ranged from 50 to 95 percent. Our recent investigation (unpublished data) on immunohistochemical analysis of nasal polyp from ASA-BA revealed that the number of mast cell and eosinophil tended to be higher than non-allergic polyp, although the statistical significance was not reached. In this study, the patients with nasal polyp had significantly higher IgG-IC level than those without it. These findings may support a possible involvement of IgG-IC in the nasal polyp formation in ASA-BA. Further investigations will be needed to detect these ICs within the nasal polyp tissue.

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