Effects of Autologous Sera on Immediate and Late Skin Reaction to the House Dust Mite in Atopic Individuals

Dong Ho Nahm*, Jung-Won Park and Chein-Soo Hong

To evaluate the in vivo effect of autologous serum including antibodies to house dust mite in atopic individuals, we observed the immediate (15mins) and late (6hours) skin reactions (ISR, LSR) on intradermal (ID) test of serially diluted Dermatophagoides farinae antigens (DFA, Allergopharma, Germany) mixed with autologous sera (DFA-S) and diluent alone (DFA-D). We tested 34 DFA-skin reactive atopic individuals including 12 asthmatics (BA), 8 asthmatics on immunotherapy with DFA (IT), and 14 healthy atopic controls (AC). We observed complete inhibition of ISR in the lowest allergen dose of DFA-S in 7 (58.3%) of 12 BA, 3 (37.5%) of 8 IT, and 2 (14.3%) of 14 AC. In BA, the inhibition of ISR was more frequent than AC (p<0.05). We observed larger late reactions in half of LSR positive cases on ID test by DFA-S than by DFA-D (≥1.5 X size; accentuation of LSR). Accentuation of LSR were shown more frequently by DFA mixed with larger amount of serum (25% in 1:1 mix; 80% in 1:3 mix, p<0.05). But there were no differences of DFA-specific IgE and IgG subclass antibodies regardless of the inhibition of ISR or the accentuation of LSR. In conclusion, some autologous sera from DFA-sensitive individuals showed the inhibition of ISR and the accentuation of LSR on DFA-ID test.

Key Words: Dermatophagoides farinae, autologous serum, immediate skin reaction, late skin reaction

House dust mites have been known as the most common allergen producing asthma and other allergic diseases (Voorhast et al. 1967; Miyamoto et al. 1968). IgE antibody response to mite allergen is essential in the pathogenesis of allergic diseases, but the clinical significance of IgG antibody response to mite allergen is not yet established. Allergen-specific IgG antibodies were known to increase after immunotherapy and thought as blocking antibodies to protect allergic reactions after allergen exposure in immunotherapy patients (Aalberse et al. 1983a; Urbanek et al. 1986). In the recent study (Hong et al. 1994), we measured the specific IgG antibody to Dermatophagoides farinae antigen (DFA) in the sera of respiratory allergic patients and healthy subjects. In all subjects significant IgG antibodies to DFA were detected. This result was consistent with the report of Kemenen et al. (1989) that all subjects were capable of recognizing and mounting an IgGl antibody response to natural exposure to house dust mites.

In present study we planned to evaluate the

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in vivo effect of autologous serum including antibodies to DFA in atopic individuals. We compared the immediate (15 mins) skin reaction (ISR) and late (6 hours) skin reaction (LSR) after intradermal (ID) injection of DFA mixed with autologous sera or diluent alone. And then we analyzed the association of skin test results with DFA-specific IgE and IgG subclass antibody levels.

MATERIALS AND METHODS

Patient

Asthmatic patients who visited the Department of Internal Medicine in Severance Hospital of Yonsei University College of Medicine were selected for the asthmatic group; 12 house dust mite-sensitive asthmatic patients (BA group) who showed positive skin prick test to DFA (Bencard Co., England) and 8 house dust mite-sensitive asthmatic patients on immunotherapy (IT group) with DFA for more than 2 years. As an atopic control, we also studied 14 healthy house dust mite-sensitive (A/H ratio ≥ 2) medical students of the Yonsei University College of Medicine (AC group). Each subjects sera were aseptically prepared and stored at −20°C until used.

Allergens

For the ID test, DFA lyophilized extract (5000 SBE/ml for injection use) obtained from Allergopharma (Germany) was used (Allergopharma-DFA). For the ELISA study, cultured mite bodies of *D. farinae* were defatted with ethylether, and then 100 ml of phosphate buffered saline (PBS) was added to 1 gm of defatted mites and stirred continuously for 48 hours at 4°C. Then the extract was centrifuged at 10,000g for one hour at 4°C and the supernatant was dialyzed in a large amount of distilled water for 48 hours. The supernatant was lyophilized and stored in a freezer until used (whole body antigen of *D. farinae*; WBA-DFA).

Skin test

Allergopharma-DFA (5000 SBE/ml) was diluted at three different concentrations of 20, 200, 2000 SBE/ml with diluent (0.4% phenol-0.9% saline) as stock antigen solutions. For the intradermal tests, the stock Allergopharma-DFA solution was diluted at 10, 100 and 1000 SBE/ml by 1:1 mixture with autologous serum (DFA-S1) or diluent (DFA-D1). The stock Allergopharma-DFA solution was also diluted at 2.5, 25 and 250 SBE/ml by 1:3 mixture with autologous serum (DFA-S3) or diluent (DFA-D3). ID tests were done at the lateral surface of the upper arm of the patients to make 4~5 mm sized blebs by epicutaneous injection of the antigen solutions. The reactions were measured at 15 minutes for immediate skin response (ISR) and at 6 hours for late skin response (LSR). 17 subjects were tested with DFA-S1 and DFA-D1. And 17 subjects were tested by DFA-S3 and DFA-D3. In each test histamine solution (0.1 mg/ml) was used as a positive control and autologous serum and the diluent was used as negative controls.

ELISA

DFA-specific IgE and IgG subclass antibodies were measured with ELISA. Wells of microtiter plates were coated overnight with allergens (WBA-DFA 2 µg/well) in 50 µl of 0.05M carbonate buffer (pH 9.6) at 4°C. The plates were washed with PBS with Tween 20 (PBS-T) and blocked with 1% BSA/PBS-T for 1 hour. 50 µl of patient’s serum (1:10 diluted serum for IgG1 and IgG2 measurement, and undiluted serum for IgG3, IgG4 and IgE measurement) were added to wells of the plates and incubated for 1 hour at room temperature and the plates were washed three times with PBS-T. The plates were incubated with 50 µl of biotinylated monoclonal anti-human IgG subclass antibodies (1:500 v/v, Sigma, USA) or biotinylated polyclonal anti-human IgE antibodies (1:500 v/v, Vector Laboratories, Inc., Burlingame, CA) for 1 hour and washed 3 times with PBS-T. The plates were incubated with 50 µl of streptavidin-peroxidase (1:500 v/v, Sigma, USA) for 30 min and were washed five times with PBS-T. 100 µl ABTS solution [55 mg 2,2-azino-di-3-ethylbenzthiazoline-6-sulfonic] acid in 100 ml citrate phos-
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phate buffer (pH 4.2) with 100 μl of 30% H₂O₂ was added to wells and after 5 min at room temperature the reaction was stopped by the addition of 100 μl of 2 mM sodium azide. The absorbance was read at 410 nm by an automated microplate reader (Dynatech Lab, Alexandria, Virginia, USA).

Statistical analysis

The statistical significance was determined by paired t-test and non-parametric analysis using Mann-Whitney U test and Chi-square test.

RESULTS

Influences of autologous sera on the immediate skin reaction (ISR)

Intradermal injection of the mixture of Allergopharma-DFa and autologous serum (DFa-S1 and DFa-S3) showed significant decreases in the size of ISR in lowest allergen dose (10 SBE/ml or 2.5 SBE/ml) compared to Allergopharma-DFa diluted with diluent alone (DFa-D1 and DFa-D3) when compared with data from 10 SBE/ml and 2.5 SBE/ml (paired t-test, p<0.05) (Fig. 1). But sizes of ISR from DFa-S1 were not significantly different from those of DFa-D1 while sizes of ISR form DFa-S3 were significantly decreased compared to those of DFa-D3 (paired t-test, p<0.05). In 7 (58.3%) from 12 of the BA group, 3 (37.5%) from 8 of IT group, and 2 (14.3%) from 14 of the AC group, complete inhibition of ISR by autologous sera was observed while the presence of ISR was observed by Allergopharma-DFa alone in the lowest DFa dose (10 SBE/ml or 2.5 SBE/ml) (Fig. 2). In the BA group the inhibition of ISR was more frequent than the AC group (Chi-square test, p<0.05) (Table 1).

![Graph](image1)

**Fig. 1.** Effect of autologous sera on immediate skin reaction (25 minutes). Intradermal injection of D. farinae extract and autologous serum mixture (2.5 SBE/ml) showed significant decrease in size of ISR in lowest allergen dose compared to D. farinae extract alone (*P<0.05 by paired t-test).**

![Graph](image2)

**Fig. 2.** An immediate skin reaction by injection of D. farinae extract was completely inhibited by autologous sera in the lowest allergen dose (10 SBE/ml) while the presence of skin reaction by D. farinae extract alone.

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Influences of autologous sera on the late skin reaction (LSR)

Twenty two (6 BA, 6 IT and 10 AC) among

<table>
<thead>
<tr>
<th>I-ISR</th>
<th>BA</th>
<th>IT</th>
<th>AC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>12(41.7%)</td>
<td>8(25.3%)</td>
<td>14(65.7%)</td>
<td>34</td>
</tr>
<tr>
<td>Positive</td>
<td>7(58.3%)*</td>
<td>3(37.5%)</td>
<td>2(14.3%)</td>
<td>12(35.3%)</td>
</tr>
</tbody>
</table>

ISR: Immediate skin reaction at 15 minutes by intradermal injection of D. farinae extract
I-ISR: inhibition of Immediate skin reaction defined as a complete inhibition of immediate skin reaction by autologous sera
BA: mite-sensitive asthmatics
IT: asthmatics on immunotherapy with mite
AC: mite-sensitive healthy controls
*BA compared to AC, Chi-square test, p<0.05

34 subjects showed LSR at the highest dose of Allergopharma-DFa (both 1000 SBE/ml and 250 SBE/ml). Intradermal injection of the mixture of Dfa and autologous serum (DFa-S1 and DFa-S3) showed significant increases in the size of LSR compared to those of DFa-D1 and DFa-D3 (paired t-test, p<0.05 for both doses)(Fig. 3). Significant accentuation of LSR (defined as increases in size of induration more than 1.5 times by Dfa- serum compared with Dfa-diluent) were observed in 3(50%) of 6 LSR-positive BA, 3(50%) of 6 LSR-positive IT, and 5(50%) of 10 LSR-positive AC (Fig. 4) (Table 2).

Association between the inhibition of ISR and accentuation of LSR

Among the 22 subjects who did not show in-

![Fig. 3. Effect of autologous sera on late skin reaction (6 hours). Intradermal injection of D. farinae extract and autologous serum mixture showed significant increase in the size of LSR compared to D. farinae extract alone (*P<0.05 by paired t-test for both 1000 SBE/ml and 250 SBE/ml).](image)

![Fig. 4. A late skin reaction by injection of D. farinae extract and autologous serum mixture into skin showed significant increase of the late skin reaction in the highest allergen dose (250 SBE/ml) compared to D. farinae extract alone.](image)
Table 2. Accentuation of late skin reaction by autologous sera

<table>
<thead>
<tr>
<th></th>
<th>BA</th>
<th>IT</th>
<th>AC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSR</td>
<td>12</td>
<td>8</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>A-LSR*</td>
<td>3 (50%)</td>
<td>3 (50%)</td>
<td>5 (50%)</td>
<td>11</td>
</tr>
</tbody>
</table>

LSR: late skin reaction at 6 hours by intradermal injection of D. farinae extract
A-LSR: accentuation of late skin reaction defined as an increase of late skin reaction (>1.5 X of allergen alone) by autologous sera *% is rate of A-LSR among LSR positive individuals

Table 3. Association between the inhibition of ISR and accentuation of LSR

<table>
<thead>
<tr>
<th>LSR</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSR(−)</td>
<td>7 (31.8%)</td>
<td>5 (41.7%)</td>
<td>12</td>
</tr>
<tr>
<td>LSR(+) A-LSR(−)</td>
<td>9 (40.9%)</td>
<td>2 (16.7%)</td>
<td>11</td>
</tr>
<tr>
<td>LSR(+) A-LSR(+)</td>
<td>6 (27.3%)</td>
<td>5 (41.7%)</td>
<td>11</td>
</tr>
</tbody>
</table>

I-ISR: inhibition of immediate skin reaction
A-LSR: accentuation of late skin reaction
*No statistically significant differences of LSR or A-LSR between ISR positive and negative groups

Inhibition of ISR, 15 subjects (68.2%) were LSR-positive and 6 (27.3%) showed accentuation of LSR. And among the 12 subjects who showed inhibition of ISR, 7 (58.3%) were LSR-positive and 5 subjects (41.7%) showed accentuation of LSR. No significant association was observed between the inhibition of ISR and the accentuation of LSR (Table 3).

Influence of serum amount as a diluent on skin reaction

Accentuation of LSR was noted in 8 of 10 LSR-positive cases by 1:3 mixture of DFa and serum. And the accentuation of LSR noted 3 of 12 LSR-positive cases by 1:1 mixture of

Table 4. Influence of mixing ratio of autologous sera on early and late skin reactions

<table>
<thead>
<tr>
<th>Skin reaction</th>
<th>1:1 mixture</th>
<th>1:3 mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. farinae &amp; serum mixture</td>
<td>(n=17)</td>
<td>(n=17)</td>
</tr>
<tr>
<td>I-ISR</td>
<td>4 (23.5%)</td>
<td>8 (47.1%)</td>
</tr>
<tr>
<td>LSR</td>
<td>12 (70.6%)</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>A-LSR*</td>
<td>3 (25.0%)</td>
<td>8 (40.0%)*</td>
</tr>
</tbody>
</table>

I-ISR: complete inhibition of immediate skin reaction at lowest allergen concentration of allergen mixture
A-LSR: accentuation of late skin reaction at strongest allergen concentration of allergen mixture
1, D. farinae was mixed with equal volume of autologous serum and final allergen concentration was 1000 SBE/ml, 100 SBE/ml, 10 SBE/ml
2, 1 volume of D. farinae was mixed with 3 volume of autologous serum and final allergen concentration was 250 SBE/ml, 25 SBE/ml, 2.5 SBE/ml
3, % is rate of A-LSR among LSR positive individuals
*1:3 compared to 1:1, Chi-square test, p<0.05

**Fig. 5. D. farinae specific IgE, IgG1 and IgG4 antibodies in mite-sensitive asthmatics (BA), asthmatics on immunotherapy with mite (IT) and healthy atopic controls (AC).

(*Between BA and AC, p<0.05 by Mann-Whitney U test
**Between BA and IT, p<0.05 by Mann-Whitney U test)
DFa and serum. The accentuation of LSR became more prominent by increasing the amount of serum in the mixture (Chi-square test, $p<0.05$) (Table 4). The frequency of inhibition of ISR also increased two-fold by increasing the amount of serum in the mixture (4 of 17 cases by 1:1 mixture; 8 of 17 cases by 1:3 mixture), but there was no statistically significant difference (Chi-square test, $p>0.05$) (Table 4).

**Fig. 6. Comparison of D. farinae specific IgE, IgG1 and IgG4 antibodies between inhibition of immediate skin reaction (I-ISR) positive and negative subject’s sera. ($p>0.05$ by Mann-Whitney U test.)**

**Fig. 7. Comparison of D. farinae specific IgE, IgG1 and IgG4 antibodies between accentuation of late skin reaction (A-LSR) positive and negative subject’s sera. ($p>0.05$ by Mann-Whitney U test.)**

**DISCUSSION**

In 1935, Cooke et al. reported that injection of allergen mixed with serum from a patient with hay fever treated with allergen injection inhibited the immediate skin reaction and he suggested the development of blocking antibody that prevented the reaction of allergen on the sensitizing antibody. Furthermore, allergen specific IgG antibody including IgG4 antibody was increased after immunotherapy and was thought as blocking antibodies to protect allergic reaction (Aalberse et al. 1983a; Urbanek et al. 1986). But high levels of allergen specific IgG4 antibodies are present in some non-immunized individuals with symptomatic and asymptomatic atopic diseases. And high IgG4 level, measured within 3 months after initiation of immunotherapy, was reported to be associated with treatment failure after immunotherapy (Djurup and Malling, 1987). In special occupational conditions, IgG antibodies to occupational environmental agents were thought as an indicator for the degree of exposure of sensitized agents (Platts-Mills et al. 1987; Botham et al. 1989). There was also some evidence to suggest that IgG4...
antibodies were synthesized in response to chronic exposure to antigen (Aalberse et al. 1983b; Homburger et al. 1986 ). Kemeney et al. (1989) reported that all subjects were capable of recognizing and mounting an IgG1 antibody response to natural exposure to both house dust mite and pollen. The role of the allergen specific IgG antibody is not yet well determined. In this study we also noted that specific IgG4 antibody was detectable in sera from atopic individuals who did not have immunotherapy and interestingly the IgG4 antibody levels were significantly higher in the atopic asthma group than the atopic healthy controls. This result was also consistent with our previous report(Hong et al. 1994) on the ELISA study of mite-specific IgE and IgG subclass antibodies. The IgG4 production usually occurs during secondary response to protein antigens and is enhanced by repeated exposure to antigens. But the reason of an IgG4 increase in atopic patients remains unclear(Jeannin et al. 1994).

Ishizakas' (1968) report showed that preformed allergen-IgE complexes induced immediate erythema and wheal reaction but allergen-IgG complex did not. In this study we showed that the autologous serum from atopic individuals could inhibit immediate skin reaction by allergen in the same subject. This result indicates that there are some substances which can inhibit immediate skin reaction to the specific allergen in sera from atopic individuals. We think allergen-specific antibodies (probably IgG) might bind free allergen in the mixture and interfere with the binding of allergen to IgE antibodies on the mast cell and result in the inhibition of immediate skin reaction. But there were no differences in the levels of allergen specific IgE and IgG subclass antibodies among study groups regardless of the inhibition of ISR. Although mite-specific IgG4 antibody was significantly higher in the immunotherapy group than the bronchial asthma group, there was no significant difference in the frequency of inhibition of immediate skin reaction by autologous sera between the two groups. The discrepancy between IgG antibody levels and the inhibition of immediate skin reaction may be due to the epitope specificities and affinity or avidity of the antibodies. Recently we studied the antigen binding pattern of IgG antibodies from mite-sensitive atopic asthmatics and healthy controls by immunoblotting and we found that most of the mite-specific IgG antibodies (including IgG4 antibody) bound to relatively high molecular weight antigens above 30KD and not to major IgE binding allergen such as Der f I (24KD) or Der f II (15KD)(Nahm et al. 1994). Our result was quite consistent with the ELISA study of Nakada et al. (1989) which measured specific IgE and IgG antibodies to fractionated mite antigen. Another explanation is another component such as another class of immunoglobulins (IgA, IgM) or another serum component may involve in the inhibition of allergen induced immediate skin response. Recently, Shakib and Smith (1994) also reported that euoglobulin fractions from sera of asthmatic patients can inhibit allergen-induced basophil histamine release in about 50% of sera and they interpreted this result due to IgG anti-IgE autoantibodies but they also could not find any association of this modulatory effect and circulating IgG anti-IgE autoantibody levels. We think our work may be an in vivo counterpart of Shakib and Smith's work (1994). But we can not still explain the increased frequency of inhibition in the asthma group compared to the atopic healthy controls. Further studies using purified immunoglobulin from sera will be needed to explain these observations.

Although there were some reports on the clinical significances of IgG antibodies to mite antigen, roles of the allergen specific IgG antibody is not well determined in the pathogenesis of allergic disease in mite-sensitive patients. Gwynn et al. (1982) showed that patients with mite-specific IgG4 antibody developed a delayed response and patients with IgG4 and IgE antibodies against the same allergen exhibit a dual response. Ito et al. (1986) reported that there was a close correlation of the presence of high IgG1 antibodies with propensity to develop late asthmatic responses. Ito et al. (1989) also noted that IgG1 and IgG4 antibodies to mites were higher in mite sensitive steroid-independent patients.
than mite sensitive steroid-dependent patients. Our study also suggests allergen-specific IgG antibodies may be involved in the LSR accentuation because we observed that in LSR-positive cases the mixture of DFa and autologous sera accentuated the LSR than those in the same concentration of DFa alone. We think that IgG antibodies in the serum formed immune complexes with mite antigen and resulted in the accentuation of LSR through immune complex mediated reactions. And this speculation was further supported by the observation that the accentuation of late skin reaction became more frequent when the larger amounts of serum was mixed with less mite antigen.

Stevens and Bridts (1984) reported that IgG and IgE containing circulating immune complexes were increased in patients with asthma and rhinitis. Elevated IgG immune complexes were also reported in children with atopic eczema (Ferguson and Salinas 1984). IgG-immune complexes are known to stimulate eosinophil to produce leukotriene C4 (Cromwell et al. 1988) and anaphylatoxic complement fragments such as C3a, C3a, and C5a can activate basophils and mast cells to release inflammatory mediators (Fearon, 1983). Although immune complexes are elevated in the sera of patients with allergic diseases, the pathophysiological role of immune complexes are undetermined in allergic diseases. But as there are allergen specific immunoglobulins in the respiratory secretions in patients with asthma and rhinitis (Platts-Mills et al. 1979; Kitani et al. 1985), we suggest that inhaled allergens could form immune complexes and promote airway inflammation through other mechanism other than by type I hypersensitivity reaction.

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