

Original Article

Detection of anti-IgE and anti-FcεRI α chain auto-antibodies in patients with atopic dermatitis

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ABSTRACT

Anti-IgE and anti-FcεRI α chain auto-antibodies have been detected in the sera of atopic and non-atopic subjects; however, the biological activity of these auto-antibodies is still unclear. These two kinds of auto-antibodies were examined in atopic dermatitis (AD) patients. In results, 12 of 92 AD patients have the anti-IgE, eight have the anti-FcεRI α auto-antibodies and two have both auto-antibodies. Furthermore, biological characterization of these auto-antibodies has been performed. In results, three of 12 samples with the anti-IgE auto-antibodies exhibited inhibitory activity for IgE-FcεRI α binding. Two of the eight samples with the anti-FcεRI α auto-antibodies and one of the two samples with both auto-antibodies had histamine releasing activity. It is documented here that both anti-IgE and anti-FcεRI α auto-antibodies were detected in some AD patients, some anti-IgE auto-antibodies had inhibitory activity for IgE-FcεRI binding and also histamine releasing activity was detected in the anti-FcεRI α chain auto-antibody.

Key words: atopic dermatitis, auto-antibody, high affinity IgE receptor, immunoglobulin E.

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INTRODUCTION

Anti-IgE auto-antibodies have been detected in patients with atopic and non-atopic diseases.^{1–5} However, the biological activities of these auto-antibodies are still unclear. There are actually controversial reports on the histamine releasing activity of the anti-IgE auto-antibodies.^{4,6,7}

Although these auto-antibodies have been considered anaphylactogenic, low levels of anti-IgE auto-antibodies have also been found in normal individuals.^{8–10} Vogel *et al.* have documented that the epitopes of anti-IgE auto-antibodies are mostly idiotypic determinants and few react specifically with the CH3 domain of IgE, in which the receptor binding region is located.¹¹

In contrast, anti-FcεRI α chain auto-antibodies have been detected in the sera of patients with chronic urticaria.¹² These anti-FcεRI α chain auto-antibodies function as histamine-releasing factors in these patients. Both the anti-IgE and anti-FcεRI α chain auto-antibodies have been reported in chronic urticaria and the anti-FcεRI α chain auto-antibodies exert higher histamine-releasing activity. These reports led us to examine the occurrence of the anti-IgE and anti-FcεRI α auto-antibodies in patients with atopic dermatitis (AD). These antibodies may modulate clinical symptoms of AD patients according to their biological activities.

In general, auto-antibodies are polyclonal and recognize various epitopes of the same antigen. The different epitopes may contribute to different biological activities of the auto-antibodies. The histamine-releasing activity of anti-IgE and anti-FcεRI α chain auto-antibodies possibly influences the severity of AD. Another possible activity of these auto-antibodies is the inhibitory activity against IgE-FcεRI α binding. The anti-IgE auto-antibodies that

recognize the CH3 domain of IgE may inhibit IgE binding to FcεRI α. This type of anti-IgE auto-antibodies may attenuate the symptoms of AD by blocking IgE binding to the FcεRI α chain.

In the present study, we investigated both auto-antibodies in AD patients to make a detailed analysis for pathophysiology of AD.

METHODS

Patients and sera

Serum samples from 92 patients with AD and six normal volunteers were collected and stored at -20°C until use. When subjected to enzyme-linked immunosorbent assay (ELISA), all samples were 1:20 diluted with phosphate-buffered saline (PBS, pH 7.4) containing 0.03% human serum albumin (HSA).

Antibodies and reagents

Immunoglobulin E myeloma protein was purified from the serum of an IgE myeloma patient as described previously.¹³⁻¹⁵ Two types of mouse antihuman FcεRI α chain monoclonal antibodies, CRA2 and biotinylated CRA1, were used for ELISA assays. CRA2 competes with IgE for binding to the FcεRI α, while CRA1 does not (described elsewhere).¹⁶

A recombinant soluble form of the human FcεRI α chain ectodomain (soluble α) was prepared as previously described.¹⁷⁻¹⁹

Monoclonal mouse antihuman IgE and horseradish peroxidase (HRP)-labeled goat antihuman IgG antibodies were purchased from Cappel (Durham, NC, USA) and HSA from Sigma Chemical Co. (St Louis MO, USA). Diaminobenzidine (DAB) color development was performed by using VECTASTAIN-ABC kit[®] (Funakoshi, Tokyo, Japan), according to the manufacturer's instruction. Secreted histamine was measured by using Histamine Release Test[®] (HRT; Miles/Hollister-Stier, Spokane, WA, USA) following the manufacturer's instructions.

Detection of anti-IgE and anti-FcεRI α auto-antibodies by ELISA

Anti-IgE and anti-FcεRI α chain auto-antibodies were evaluated by ELISA.

For the detection of anti-IgE auto-antibodies, each well of the ELISA plate (Costar, Cambridge, MA, USA) was coated with human IgE by incubation with 50 μL IgE-containing buffer (2 μg/mL in 0.01 mol/L PBS, pH 7.4) at

4°C for 12 h. The plate was washed 3 times with 0.05% Tween 20-PBS and 0.3% HSA-PBS solution was added to each well to block non-specific binding of the serum immunoglobulins. After incubation at 37°C for 4 h, the plate was washed 3 times and then 50 μL of sample sera (1:20 diluted with 0.03% HSA-PBS) was added to each well in triplicate. The plate was incubated at 37°C for 1 h and washed 3 times. Horseradish peroxidase-labeled antihuman IgG (diluted 1:2000) was added to each well and the plate was incubated at 37°C for 1 h and washed 3 times. *o*-Phenylenediamine dihydrochloride (OPD; 100 μL of 1 mg/mL; Wako pure chemical industries, Osaka, Japan) in citrate buffer containing 0.0075% (w/v) H_2O_2 was added to each well. After 15 min color development, the enzymatic reaction was stopped by adding 100 μL sulfuric acid (2 mol/L). Optical density (OD) at 492 nm wavelength was measured by an ELISA microplate reader (Bio-Rad, Richmond, CA, USA).

Anti-FcεRI α chain auto-antibody was evaluated basically in the same way as described above, except that the coated antigen was the soluble α (50 μL of 3.6 μg/mL solution in 50 mmol/L bicarbonate buffer, pH 9.4) for this ELISA.

Second screening for the anti-FcεRI α chain auto-antibodies by ELISA

To exclude the effects of IgE-anti-IgE auto-antibody complexes on measuring the anti-FcεRI α chain auto-antibodies at the first screening, another ELISA system was adopted for the second screening.

Each well of the ELISA plate was coated with 50 μL CRA2 (2 μg/mL in 50 mmol/L bicarbonate buffer, pH 9.4) at 4°C for 12 h, washed 3 times and blocked with 0.3% HSA. Then, 50 μL of the soluble α (1.8 μg/mL in 0.03% HSA-PBS) was added to each well and the plate was incubated at 37°C for 1 h. After 3 washes, 1:20 diluted sera (which showed substantially higher OD titer at the first screening of anti-FcεRI α chain auto-antibody) were added to each well in triplicate and the plate was incubated at 37°C for 1 h. Finally, HRP-labeled antihuman IgG antibody was added to each well and the reaction mixture was developed with substrate as described earlier.

Preparation of the serum IgG fraction

The auto-antibody-positive sera were heat inactivated at 56°C for 2 h to inactivate serum IgE.²⁰ After heat inactivation, IgG fractions of the serum samples were

semipurified by salt fractionation with 50% saturated ammonium sulfate (Wako pure chemical industries). After salt fractionation (twice), protein concentrations of the samples were adjusted to 10 mg/mL with 0.03% HSA-PBS. Purity of IgG was checked by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Histamine-releasing assay

Histamine-releasing activity of the auto-antibody positive samples was assessed by measuring the released histamine from peripheral blood leukocytes with these auto-antibodies using HRT[®] test (Miles/Hollister-Stier).

Briefly, 35 μ L of leukocytes from peripheral blood from one AD patient (auto-antibody negative by ELISA), were added to each well, to which 15 μ L of IgG fractions of the positive or normal samples were added. After 30 min incubation at 37°C, the released histamine from basophils in the peripheral blood was precipitated by addition of the indicated concentration of the HRP-labeled antihistamine antibody. The HRP-labeled antihistamine antibody that exceeded the amount of histamine was adsorbed by the histamine-coated plastic pegs. This excess HRP-labeled antihistamine antibody was color developed. High histamine release is shown as low OD titer and low histamine release as high OD titer by measuring OD at 410 nm wavelength.

Evaluation of inhibitory activity of the anti-IgE auto-antibodies against IgE binding to the Fc ϵ RI

The ELISA plate was coated with 50 μ L human IgE (4 μ g/mL in 0.03% HSA-PBS) at 4°C for 12 h. After 3 washes and blocking with 0.3% HSA-PBS, the diluted samples (1:50–1:500 diluted with 0.03% HSA-PBS) were added to each well and the plate was incubated at 37°C for 1 h. After 3 washes, 50 μ L of the soluble α (900 ng/mL in 0.03% HSA-PBS) was added to each well and the plate was incubated at 37°C for 1 h. After 3 washes, 50 μ L biotinylated-CRA1 (4 μ g/mL in PBS) was added to each well and the plate was incubated at 37°C for 1 h. After washes, the avidine-peroxidase solution was added to each well and DAB color development was performed using the VECTASTAIN-ABC kit (Funakosi) according to the manufacturer's instructions.

RESULTS

Serum anti-IgE auto-antibodies in AD patients

We examined sera of 92 AD patients and six normal volunteers for their serum IgG activity for binding to human IgE. The levels of the anti-IgE auto-antibodies for each group are shown as OD values. The OD values of AD samples were distributed over a wide range; however, those of normal volunteers showed a narrow distribution (Fig. 1). The average of each group demonstrated no statistical difference. Samples that showed a higher OD titer than the mean + 2 SD were classified as positive. Twelve of 92 (13%) were evaluated as positive.

Anti-Fc ϵ RI α chain auto-antibodies in the sera of AD patients

The same 92 AD samples were subjected to the first screening for anti-Fc ϵ RI α chain auto-antibodies. The values of the anti-Fc ϵ RI α chain auto-antibodies for each group are shown in Fig. 2. After the first screening, 15 samples were picked up as positive (higher OD titer than the mean + 2 SD) for binding to the soluble α . However, by the ELISA assay, using the soluble α as antigen, not only anti-Fc ϵ RI α chain auto-antibodies but also the IgG in IgE-anti-IgE auto-antibody complex was detected by

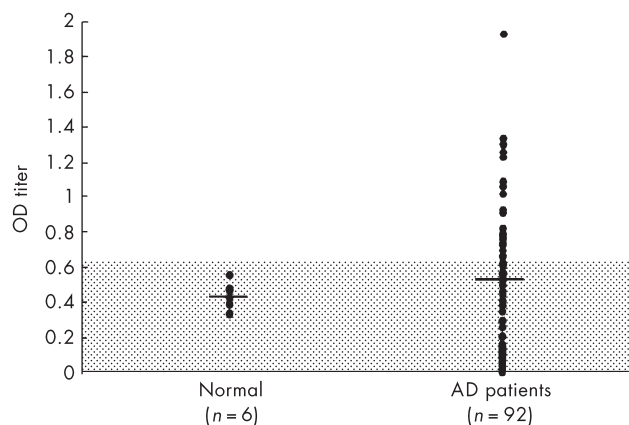


Fig. 1 Levels of anti-IgE auto-antibody. Sera were assessed by enzyme-linked immunosorbent assay using human IgE-coated plates and horseradish peroxidase-labeled anti-human IgG antibody as described in Methods. The values of anti-IgE auto-antibody are indicated as optical density (OD) titer. The shaded area represents values under the mean + 2 SD OD titer of healthy individuals. The horizontal bars are the means of each group. No significant differences between the normal group and the atopic dermatitis (AD) group were found by *t*-test.

the HRP-labeled anti-IgG antibody. Therefore, the second screening was performed for 15 of 92 positive samples as described in Methods.

Because the IgE-binding site of the soluble α was occupied by CRA2, IgE-anti-IgE auto-antibody complexes could not bind to the soluble α and false positive IgE-anti-IgE auto-antibody complexes could be excluded by this ELISA assay (second screening).

At the second screening, seven of the 15 samples that were positive at the first screening ELISA diminished in their ability to bind to the soluble α , as shown in Fig. 3. These results indicate that the anti-IgE auto-antibodies (IgG class) formed the IgE-anti-IgE complexes and that some IgE-anti-IgE complexes in the sera of AD patients actually bound to the soluble α . In other words, some anti-IgE auto-antibodies did not affect the α chain binding site that is located in the CH3 domain of IgE.

From the positive sera in anti-IgE and/or anti-Fc ϵ RI α chain auto-antibodies, IgG fractions were semipurified and subjected to further biochemical assays.

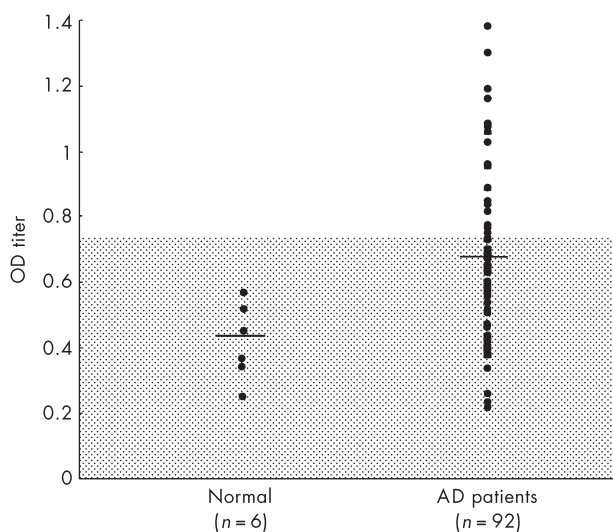


Fig. 2 Levels of anti-Fc ϵ RI α chain auto-antibody. Sera were assessed by enzyme-linked immunosorbent assay using the soluble α -coated plates and horseradish peroxidase-labeled anti-human IgG antibody as described in Methods. The values of anti-Fc ϵ RI α chain auto-antibody are indicated as optical density (OD) titer. The shaded area represents values under the mean + 2 SD OD titer of healthy individuals. The horizontal bars are the means of each group. No significant differences between the normal group and the atopic dermatitis (AD) group were found by *t*-test.

Histamine-releasing activity of the auto-antibodies in AD patients

All semipurified IgG fractions of samples that contained either auto-antibody were examined for their histamine-releasing activity from peripheral blood basophils of AD patients.

A mouse antihuman IgE monoclonal antibody was used as a positive control for the histamine-releasing assay (Fig. 4). Compared with the positive control, three samples (Fig. 4) presented histamine-releasing activity and showed a lower OD titer than the control.

Inhibitory activity of anti-IgE auto-antibodies against IgE binding to Fc ϵ RI

Anti-IgE auto-antibodies were also evaluated for their inhibitory activity against the IgE-Fc ϵ RI α binding.

To catch anti-IgE auto-antibodies, an ELISA plate was coated with IgE and semipurified IgG fractions were added to the wells.

Detection of the soluble α with biotinylated CRA1 revealed that low OD titer samples contained the inhibitory anti-IgE auto-antibody, because the CRA1 does

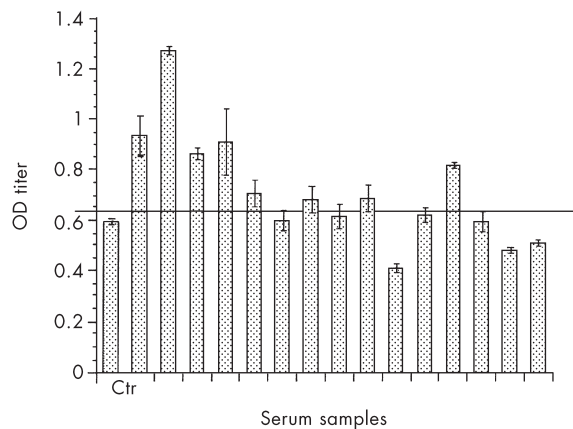


Fig. 3 Second screening for the anti-Fc ϵ RI α chain auto-antibody to exclude false positive samples. To exclude the contamination of IgE-anti IgE auto-antibody complexes, first, CRA2 (anti-Fc ϵ RI α mAb, competitive with IgE) coated plates were incubated with the soluble α . Then, sera were incubated with these plates and detection of 'true' anti-Fc ϵ RI α chain auto-antibodies was performed by addition of horseradish peroxidase-labeled anti-human IgG as described in Methods. Seven of 15 samples that showed a positive OD titer at the first anti-Fc ϵ RI α chain auto-antibody screening fell within + 2 SD of healthy control individuals (Ctr). The horizontal line indicates the + 2 SD OD titer of healthy individual serum samples.

not compete with IgE for the binding to the FcεRI and can bind even IgE-bound FcεRI.

As shown in Fig. 5, three samples actually had the inhibitory anti-IgE (lower than the mean - 2 SD of the control) and the dilution of samples made their inhibitory activity decrease in a dilution-dependent manner.

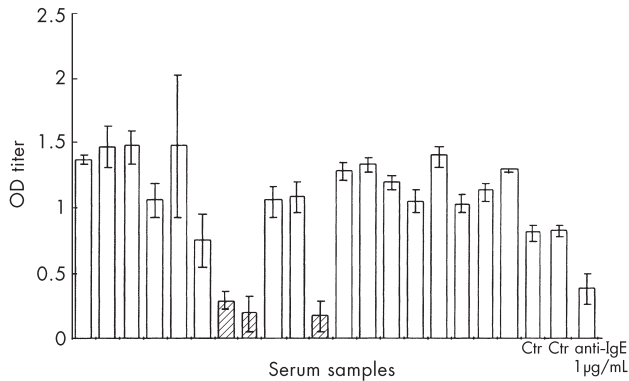


Fig. 4 Histamine-releasing activity of the auto-antibodies in atopic dermatitis patients. Histamine-releasing activity of the auto-antibodies was assessed by measuring the released histamine from peripheral blood leukocytes with these auto-antibodies using Histamine Releasing Test (HRT®). Low optical density (OD) titer means high histamine-releasing activity. Three samples (▨) represent histamine-release activity. Two samples are single positive for the anti-FcεRI α chain auto-antibody and one sample is positive for the both auto-antibodies. Ctr, healthy individual sample.

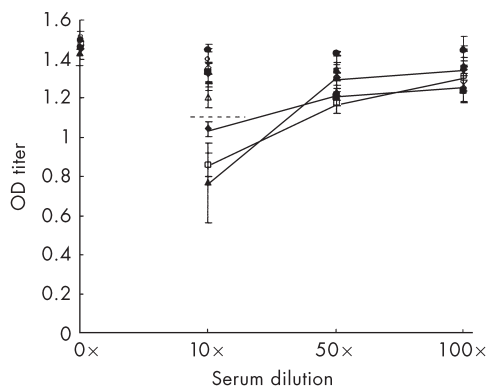


Fig. 5 Inhibitory activity of anti-IgE auto-antibodies against IgE binding to the FcεRI α chain. A human IgE-coated plate was incubated with semipurified sera, then the soluble α was added to the plate. Detection of the captured soluble α was performed by biotinylated CRA1 (anti-FcεRI α mAb, non-competitive with IgE). Three samples under the mean - 2 SD of the healthy individual (---) have inhibitory activity against IgE-FcεRI α binding. Dilution of the samples made their inhibitory activity decrease in a dilution dependent manner. Ctr, healthy individual sample.

A summary is presented in Table 1. Twelve of 92 (13%) AD patient samples had anti-IgE auto-antibodies and eight of 92 (8.7%) had anti-FcεRI α chain auto-antibodies. Two samples had both anti-IgE and anti-FcεRI α chain auto-antibodies. Three of 12 (25%) anti-IgE auto-antibody positive samples showed inhibitory activity against IgE-soluble α binding and three samples positive for both anti-IgE and anti-FcεRI α chain auto-antibodies possessed histamine-releasing activity. All of the releasing assay positive samples contained anti-FcεRI α chain auto-antibodies (one sample had both anti-IgE and anti-FcεRI α chain auto-antibodies).

DISCUSSION

Anti-IgE auto-antibodies have been detected in patients with atopic diseases (asthma, atopic dermatitis, allergic conjunctivitis)²⁻⁴ and also in patients with chronic urticaria.^{5,6,9} Recently, anti-FcεRI α chain auto-antibodies have also been reported in patients with chronic urticaria.¹²

In the present study, we investigated whether the anti-FcεRI α chain auto-antibodies occurred in sera of AD patients. Thirteen percent of patients with AD had anti-IgE and 8.7% had anti-FcεRI α chain auto-antibodies.

According to previous reports, the proportion of the anti-IgE auto-antibody positive sera varied from 20 to 80% in the population.^{3,4,8,21} However, our results show a lower level of positives than previous reports. One possible explanation for this discrepancy is that the reagents used for blocking non-specific binding varied between experiments. In our study, when we used bovine serum albumin (BSA)-PBS as blocking and dilution reagents, a false positive reaction was detected. Using HSA instead of BSA, we could reduce OD titers of false positive samples to within the mean + 2 SD of those in normal volunteers. False positive reactions may be attributable to the IgG reacting with the proteins contained in the blocking reagents, such as casein. Thus, we used HSA (IgG free) as a blocking and dilution reagent in screening to exclude false positives.

The other problem in detecting auto-antibodies is the very small quantities of auto-antibodies that are approximately equimolar to IgE.^{22,23} In such cases, the anti-IgE auto-antibodies that form the IgG-IgE immune complexes in the sera or *in vitro* incubation²⁴ are underestimated by our methods.

There is a variety of autoimmune diseases in which pathogenic auto-antibodies play an important role. For

Table 1 Anti-IgE and anti-FcεRI α chain auto-antibodies in AD patients

	Anti-IgE auto-antibody	Anti-FcεRI auto-antibody	Both auto-antibodies
No. antibody positive samples	12/92*	8/92*	2/92*
No. samples with inhibitory activity against IgE binding	3/12†	ND	ND
No. samples with histamine-releasing activity	0/12†	2/8‡	1/2§

*No. auto-antibody positive samples/Total no. samples; †no. assay positive samples/no. anti-IgE auto-antibody single positive samples; ‡no. assay positive samples/no. anti-FcεRI α chain auto-antibody single positive samples; §no. assay positive samples/no both auto-antibody positive samples. AD, atopic dermatitis; ND, not done.

example, in pemphigus vulgaris, antidesmoglein III auto-antibodies attack the epidermal cell components, causing acantholysis and erosion of the skin or mucosa.²⁵ In Graves' disease, antithyroid hormone receptor auto-antibodies mimic the action of thyroid-stimulating hormone.²⁶

However, the functional roles of anti-IgE auto-antibodies are still unclear. Because of difficulty in purifying anti-IgE auto-antibodies that occur as immune complexes, estimations of the function of anti-IgE auto-antibodies still give conflicting results.^{27,28} In the present study, we could not detect the anti-IgE auto-antibodies that had histamine-releasing activity in peripheral blood basophils of AD patients, but we detected the anti-IgE auto-antibodies that possessed inhibitory activity for the IgE-FcεRI binding. One possible explanation for the difference in biochemical activities of the anti-IgE auto-antibodies may be the variation on the epitopes of IgE that were recognized by these auto-antibodies.^{29,30}

Most of the murine monoclonal anti-IgE auto-antibodies were directed against the different idiotypic determinants and some clones reacted with the CH3 domain of the Fcε chain.¹¹ The possible role of anti-idiotypic auto-antibodies to IgE may be in the regulation of the IgE system. Both the histamine-releasing activity and the inhibitory activity for IgE binding to the FcεRI are attributable to the anti-IgE auto-antibody recognizing the receptor binding site located in the CH3 domain of the Fcε chain.

The occurrence of true 'blocking' auto-antibody in patients with atopic dermatitis has also been suggested.^{24,28} Stadler *et al.* have described that these non-anaphylactogenic type anti-IgE auto-antibodies may play a role in modulating IgE biosynthesis²⁸ and serve the purpose of clearing allergen-IgE complexes from circulation.

Our data indicate that some anti-IgE auto-antibodies have inhibitory activity for IgE-FcεRI α binding. This activity may be due to the anti-IgE auto-antibody being directed against the binding site located in the CH3 domain of IgE, as it has been reported that the anti-IgE

auto-antibodies in the sera of AD patients are mainly directed to the CH3 domain.³⁰

There were no correlations between any clinical manifestations examined and the auto-antibodies (data not shown). For example, the severity of skin eruption or serum IgE levels of patients who had inhibitory anti-IgE auto-antibodies were not significantly different from those of the patients with no auto-antibodies, although the samples with the inhibitory anti-IgE auto-antibody were from only three cases.

Both anti-IgE and anti-FcεRI α chain auto-antibodies have been detected in patients with chronic urticaria.^{5,12} According to these reports, anti-FcεRI α chain auto-antibodies worked as histamine-releasing factors in patients with chronic urticaria. We also detected both auto-antibodies in patients with AD. Three of 92 sera from patients with AD showed histamine-releasing activity. All of these three sera had anti-FcεRI α chain auto-antibodies and one of the 3 also had anti-IgE auto-antibody. Most samples with anti-IgE auto-antibodies did not induce histamine release from basophils of AD patients. Our results indicate that the anti-FcεRI α chain auto-antibodies are the main factor that causes histamine release.

According to previous studies,^{31,32} detectable amounts of anti-FcεRI α auto-antibody are only in a subset of chronic urticaria patients. In contrast, in the present study, we detected anti-FcεRI α auto-antibody in AD patients. Recently, Horn *et al.*³³ have reported that the anti-FcεRI α auto-antibody is a natural auto-antibody and serves as the parental antibody for tetanus toxoid. In agreement with latter reports, our data also indicates that the anti-FcεRI α auto-antibody is not the specific auto-antibody of chronic urticaria. We could not detect anti-FcεRI α auto-antibody in normal subjects. This may be due to the number of the tested samples.

The positive rate of anti-FcεRI α auto-antibody in our results was lower than previous reports. This may be explained by the methodology we used. For coating the soluble α on the plate, we adopted the anti-FcεRI α chain

mouse antibody (CRA2), after coating the plate with soluble α . Because CRA2 competes with IgE for the binding site on the α chain, we could avoid the false positive detection of the IgE with anti-IgE auto-antibody, which recognizes epitopes of IgE other than the binding site to the α chain. However, our ELISA method mentioned earlier has the limitation that theoretically the anti-Fc ϵ R1 α chain auto-antibodies, which specifically recognize the IgE-binding site on the α chain, would be excluded. So, the positive ratio in our results was lower than that in previous reports.

In conclusion, both anti-IgE and anti-Fc ϵ R1 α chain auto-antibodies occurred in the sera of patients with AD. Anti-Fc ϵ R1 α chain auto-antibodies showed histamine-releasing activity and some anti-IgE auto-antibodies had inhibitory activity for IgE-Fc ϵ R1 α binding. So far, we have failed to detect any special relationship between the clinical parameters of AD and these auto-antibodies. To clarify the biological significance of these auto-antibodies, further biological and clinical analyses remain to be performed in future.

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