Influence of Omalizumab on Allergen-Specific IgE in Patients with Adult Asthma

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Key Words
Omalizumab · Anti-immunoglobulin E · Allergic asthma · Seroconversion

Abstract
Background: Omalizumab, an anti-immunoglobulin E (IgE) monoclonal antibody, inhibits the binding of circulating IgE to mast cells and basophils, resulting in fewer episodes of airway inflammation, asthma symptoms and exacerbations in patients with severe allergic asthma. Treatment of patients with asthma using omalizumab increases serum total IgE (tIgE) levels. However, little is known about the influence of omalizumab on allergen-specific IgE (sIgE).

Methods: tIgE and sIgE in 47 adult patients with severe asthma were measured with a fluorescent enzyme immunoassay (ImmunoCAP-FEIA) before and after omalizumab treatment. Results: Treatment with omalizumab increased tIgE and sIgE levels. The increases in sIgE by class category after omalizumab treatment were positively correlated with baseline sIgE positivity before treatment. The mean changes in sIgE levels after omalizumab treatment were also correlated with baseline sIgE levels before treatment. The mean changes in tIgE levels were positively correlated with the mean changes in IgE levels against Dermatophagoides pteronyssinus, crude house dust, Japanese cedar and moth. Omalizumab markedly influenced the negative-to-positive seroconversion rate for IgE against Japanese cedar (30.8%), Candida (29.0%) and moth (28.0%). Finally, all patients with negative-to-positive seroconversion for Japanese cedar-specific IgE had cedar pollinosis before beginning omalizumab treatment.

Conclusions: The changes in sIgE levels after omalizumab treatment may be dependent on the baseline sIgE levels. Our data may indicate the presence of undetectable but functional sIgE.

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Introduction

Immunoglobulin E (IgE), discovered by Ishizaka [1] and Johansson [2] in 1966, plays a key role in allergic reactions. Levels of total IgE (tIgE) and allergen-specific IgE (sIgE) are helpful for diagnosing allergic diseases such as
asthma, allergic rhinitis and atopic dermatitis. IgE is a critical factor for the development of bronchial hyperresponsiveness in patients with asthma [3]. Several epidemiological studies have demonstrated that the tIgE level is higher in patients with asthma than in those without asthma [4–6]. In addition, the tIgE level in children with severe asthma is significantly higher than in children with mild to moderate asthma [7]. Global multicenter studies, such as SARP (Severe Asthma Research Program) and ENFUMOSA (European Network for Understanding Mechanisms of Severe Asthma), have demonstrated that there is no correlation between the tIgE level and asthma severity [8, 9]. Serum tIgE levels peak during early adolescence and decrease with age [10–12]. However, longitudinal changes in IgE level are heterogeneous among adult patients with asthma. Recently, we found that longitudinal increases in tIgE levels are associated with poor disease control in patients with adult asthma [13]. However, the association between tIgE level and asthma control has not been fully elucidated. Moreover, the association between sIgE levels and asthma severity remains unknown.

Omalizumab, a humanized anti-human IgE monoclonal antibody, is an adjunct treatment option for severe persistent allergic IgE-mediated asthma in addition to the optimized standard therapy for patients with asthma aged ≥6 years. The clinical effects of omalizumab on patients with severe asthma revealed that IgE-mediated mechanisms, which cannot be suppressed by steroids or leukotriene receptor antagonists, play an important role in severe asthma. Omalizumab reduces free IgE levels by approximately 95% by binding to the ce3 region on free IgE, thereby blocking the binding of free IgE to its specific, high-affinity receptor on mast cells, basophils and any other cells expressing IgE receptors. Omalizumab does not interact with membrane-bound IgE. By reducing IgE levels, omalizumab leads to the downregulation of FceRI on mast cells and basophils [14]. Omalizumab has a long half-life (19–22 days), in part due to its slow removal by the hepatic reticuloendothelial system [15]. The half-life of omalizumab-bound IgE is longer than that of free IgE [16], and clinical tests cannot discriminate between free IgE and omalizumab-bound IgE. Therefore, administration of omalizumab generally increases serum IgE levels in patients with asthma [17]. Several reports have measured free IgE levels separately from omalizumab-bound IgE [18, 19], but the studies concerned did not involve a clinical setting.

Although omalizumab is known to increase clinically detectable tIgE levels, changes in sIgE levels (ΔsIgE) have not been investigated thus far. Therefore, we observed ΔsIgE after omalizumab treatment and the influence of omalizumab treatment on these changes.

Materials and Methods

Study Participants

Eighty-nine patients with severe adult asthma were treated with omalizumab for at least 4 months in the Showa University Hospital, Japan, from April 2009 to March 2014. After excluding 42 patients, owing to lack of data regarding levels of tIgE and/or sIgE, 47 were included in the study. Thirty patients with asthma who were not receiving omalizumab were included in the control group. The data on tIgE and sIgE levels were retrospectively collected. Asthma was diagnosed according to the criteria of the Global Strategy for Asthma Management and Prevention by the Global Initiative for Asthma (GINA) guideline [20]. Japanese cedar pollinosis was diagnosed according to clinical symptoms and the presence of Japanese cedar-specific IgE. Patients with chronic obstructive pulmonary disease or other lung diseases, a smoking history of >20 pack-years, vocal-cord dysfunction or neurological disease were excluded. Diagnoses for perennial allergic rhinitis and seasonal cedar pollinosis were based on a clinical history and positive serum sIgE test results. The baseline data included demographic details (age, sex and basal BMI), clinical features (age at onset), smoking status (ex-smoker, current smoker or never-smoker), Asthma Control Test score, spirometry and fractional exhaled nitric oxide (FeNO). The study protocol was reviewed and approved by the Showa University Ethics Committee, and written informed consent was obtained from each subject.

Study Design

Omalizumab was administered subcutaneously at 2- or 4-week intervals depending on the patient’s body weight and tIgE level at screening. Blood samples were obtained before and after omalizumab treatment. tIgE levels ranged from 30 to 700 IU/ml. The clinical effectiveness of omalizumab was assessed by means of the Global Evaluation of Treatment Effectiveness (GETE) by the physician at 16 weeks [21, 22]. The GETE has 5 categories: (1) complete control of asthma, (2) a marked improvement of asthma, (3) a discernible but limited improvement in asthma, (4) no appreciable change in asthma and (5) worsening of asthma. We used the first 2 levels of the GETE to define a treatment response.

Data Collection

Blood was collected from the patients at 2 time points: before beginning treatment with omalizumab, and shortly before the omalizumab injection at least 16 weeks or a maximum of 30 weeks after treatment with omalizumab. The blood collection period among control patients ranged from 20 to 40 weeks. The serum tIgE and sIgE levels were measured with a fluorescent enzyme immunoassay (ImmunoCAP-FEIA, Phadia AB, Uppsala, Sweden). sIgE levels were determined for 11 aeroallergens: cocksfoot, ragweed, Cryptomeria japonica (Japanese cedar), Dermatophagoides pteronyssinus (Der p), crude house dust, Aspergillus fumigatus (aspergillus), Candida albicans (candida), Alternaria alternate (alter-
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sIgE levels were classified: <0.35 kU/l (class 0), 0.35–0.69 kU/l (class 1), 0.70–3.49 kU/l (class 2), 3.50–17.49 kU/l (class 3), 17.50–49.99 kU/l (class 4), 50.00–100.00 kU/l (class 5) and >100.00 kU/l (class 6). The changes in tIgE (ΔtIgE) or ΔsIgE were calculated as follows: tIgE after omalizumab – tIgE before omalizumab or sIgE after omalizumab – sIgE before omalizumab.

However, ΔsIgE were assessed only in the participants whose sIgE levels were >0.35 kU/l after omalizumab treatment. Sensitization was defined as an sIgE level ≥0.35 kU/l.

Spirometry was performed using an AS-302 spirometer (Mintaro Medical Science Co. Ltd., Osaka, Japan) in accordance with American Thoracic Society/European Respiratory Society guidelines [23] to determine FEV1, forced vital capacity (FVC) and FEV1/FVC (FEV1%). FeNO was measured using a portable device (NIOX MINO®, Aerocrine AB, Solna, Sweden) at an expiratory flow rate of 50 ml/s for 10 s.

Statistical Analysis

The results are expressed as mean ± standard deviation or standard error of the mean for continuous variables. Statistical analyses were performed using JMP v10 (SAS Institute Inc., Cary, N.C., USA). The Pearson’s correlation coefficient (r) and Spearman rank order coefficient (R) were used to measure the correlation between the sIgE positivity before omalizumab treatment and the percent increase in sIgE levels by class after treatment, the correlation between the baseline levels of sIgE before treatment and the mean ΔsIgE and the correlation between the mean ΔtIgE and the mean ΔsIgE. The differences in the continuous variables were analyzed using the Wilcoxon rank-sum test or the Kruskal-Wallis test, and the differences in the categorical variables were analyzed using the Pearson χ² test. A p value <0.05 was considered significant for all statistical assessments.

Results

Study Participants

The patient demographics are shown in table 1. All participants in the omalizumab group underwent treatment step 4 and above, according to the GINA guidelines before and after omalizumab treatment. In the control group, 20 patients underwent treatment step 3, 7 underwent treatment step 4 and 3 underwent treatment step 5. There were significant differences in the Asthma Control Test score, %FVC and %FEV1 between the control group and the omalizumab group. No difference was observed in peripheral eosinophils and tIgE levels. No patient was diagnosed with perennial conjunctivitis. Twenty-six patients (55.3%) were classified as displaying ‘complete control of asthma’ or ‘a marked improvement of asthma’ at 16 weeks of omalizumab treatment, thus indicating the omalizumab responders. Ten patients owned dogs, 2 owned cats and 1 owned a bird.

ΔsIgE after Omalizumab Treatment

The prevalence of sIgE is shown in figure 1. Sensitization was defined as an sIgE level ≥0.35 kU/l. Der p-specific IgE (76.6%), crude house dust-specific IgE (76.6%) and cedar-specific IgE (71.7%) showed high positivity. ΔtIgE and ΔsIgE after omalizumab treatment are shown in table 2. There were significant differences in ΔtIgE and ΔsIgE except for Alternaria-specific IgE between the

<table>
<thead>
<tr>
<th>Table 1. Patient demographics</th>
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<tbody>
<tr>
<td>Controls (n = 30)</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Gender M:F (of M)</td>
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<tr>
<td>BMI</td>
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<tr>
<td>Age at onset of asthma, years</td>
</tr>
<tr>
<td>Smoking history current:ex-smoker:never</td>
</tr>
<tr>
<td>Pet ownership</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
</tr>
<tr>
<td>Cedar pollinosis</td>
</tr>
<tr>
<td>Asthma Control Test</td>
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<tr>
<td>Peripheral eosinophils, /μl</td>
</tr>
<tr>
<td>tIgE, U/l</td>
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<tr>
<td>%FVC, %</td>
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<tr>
<td>%FEV1, %</td>
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<tr>
<td>FEV1.0%, %</td>
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<tr>
<td>FeNO, ppb</td>
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Values are expressed as means ± SD or n (%).
control group and the omalizumab group. sIgE levels for alternaria were <0.35 kU/l in 41/44 patients before omalizumab treatment. The raw data for tIgE and sIgE before and after omalizumab treatment are shown in online supplementary table S1 (for all online suppl. material, see www.karger.com/doi/10.1159/000442668). As the durations of omalizumab treatment varied widely, we determined the differences in ΔtIgE and ΔsIgE between the early time point group (at 16–22 weeks) and the late time point group (at 23–30 weeks). No difference was observed in ΔtIgE and ΔsIgE for Der p, crude house dust, Japanese cedar, aspergillus, candida, dog and moth between the early time point group and the late time point group (online suppl. table S2). The percent increase in sIgE levels by ≥1 classes after omalizumab treatment is shown in figure 2. The percent increase in Der p-specific IgE (38.3%), crude house dust-specific IgE (36.2%), cedar-specific IgE (38.6%), candida-specific IgE (33.3%) and moth-specific IgE (26.7%) was considerably high.

**Correlation between sIgE before Omalizumab and ΔsIgE after Omalizumab**

We investigated the correlations between sIgE before omalizumab treatment and ΔsIgE. Figure 3a shows a positive correlation between sIgE positivity before omalizumab treatment and the percent increase in sIgE by class after treatment (R = 0.833, p = 0.001). A positive correlation was also observed between baseline sIgE levels before omalizumab treatment and ΔsIgE (fig. 3b; R = 0.825, p = 0.001). In addition, there was no significant difference in the percent increase between each individual sIgE (online suppl. fig. S1). Neither ΔtIgE nor ΔsIgE were associated with the cumulative doses of omalizumab (data not shown).
Next, we investigated the correlation between ΔtIgE and ΔsIgE. Analyses were performed in patients with detectable sIgE levels (>0.35 kU/l) after omalizumab treatment. A positive correlation was observed between ΔtIgE and mean changes in IgE levels against Der p, crude house dust, cedar and moth (table 3). However, no correlation was detected between ΔtIgE and mean changes in IgE levels against aspergillus, candida and dog dander (table 3), despite the high correlation coefficient between ΔtIgE and mean changes in aspergillus-IgE.

**Negative-to-Positive Seroconversion in sIgE**

Finally, we investigated the negative-to-positive seroconversion rate for sIgE, which was class 0 at baseline, after omalizumab treatment. The number of samples in class 0 at baseline was 11 for Der p, 11 for HD, 13 for Japanese cedar, 28 for cocksfoot, 36 for ragweed, 31 for aspergillus, 31 for candida, 41 for alternaria, 36 for cat dander, 33 for dog dander and 25 for moth. Highly marked negative-to-positive seroconversion rates for IgE against Japanese cedar (30.8%), candida (29.0%) and moth (28.0%) were observed (fig. 4). Intriguingly, all patients with negative-to-positive seroconversion for Japanese cedar-specific IgE had cedar pollinosis even before beginning omalizumab treatment. The blood was collected in the pollen season from 1/4 patients who showed cedar-specific IgE seroconversion after omalizumab treatment.

**Discussion**

This study examined ΔsIgE in patients with adult asthma after omalizumab treatment. We found that ΔsIgE after omalizumab were positively correlated with the level of sIgE before omalizumab treatment but not the allergen specificity. To the best of our knowledge, this study is the first to elucidate the influence of omalizumab on sIgE in patients with severe adult asthma.

We found positive correlations between ΔtIgE and mean changes in the IgE levels against Der p, crude house dust, and Japanese cedar. However, no correlation was detected between ΔtIgE and mean changes in IgE levels against aspergillus, candida and dog dander.

**Table 3. Correlation between ΔtIgE and ΔsIgE**

<table>
<thead>
<tr>
<th></th>
<th>Total samples, n</th>
<th>Valida samples, n</th>
<th>R value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔtIgE vs. ΔDer p-IgE</td>
<td>47</td>
<td>36</td>
<td>0.496</td>
<td>0.002</td>
</tr>
<tr>
<td>ΔtIgE vs. ΔHD-IgE</td>
<td>47</td>
<td>36</td>
<td>0.485</td>
<td>0.003</td>
</tr>
<tr>
<td>ΔtIgE vs. Δcedar-IgE</td>
<td>44</td>
<td>35</td>
<td>0.479</td>
<td>0.005</td>
</tr>
<tr>
<td>ΔtIgE vs. ΔAsp-IgE</td>
<td>43</td>
<td>13</td>
<td>0.484</td>
<td>0.094</td>
</tr>
<tr>
<td>ΔtIgE vs. Δcandida-IgE</td>
<td>42</td>
<td>23</td>
<td>-0.116</td>
<td>0.608</td>
</tr>
<tr>
<td>ΔtIgE vs. Δdog-IgE</td>
<td>45</td>
<td>14</td>
<td>0.266</td>
<td>0.404</td>
</tr>
<tr>
<td>ΔtIgE vs. Δmoth-IgE</td>
<td>45</td>
<td>27</td>
<td>0.516</td>
<td>0.012</td>
</tr>
</tbody>
</table>

a Samples in which sIgE was at a detectable level (≥0.35 kU/l) before and after or at least after treatment with omalizumab. Asp = aspergillus; HD = house dust (crude).
dust, Japanese cedar and moth. Generally, the tIgE level reflects both specific and unspecific IgE. Previous birth cohort studies showed that the evolution of tIgE was extremely heterogeneous but parallel with that of airborne sIgE [24, 25], indicating that ΔtIgE are dependent on antigen-specific IgE. Although both ΔtIgE and ΔsIgE were mostly influenced by omalizumab, our data are consistent with these previous data. However, there was no correlation between ΔtIgE and the mean changes in the IgE levels against candida and dog dander. This suggests that ΔtIgE may be dependent on ΔsIgE, for which the baseline positivity was relatively high.

In this study, specific IgE levels against several allergens, which were <0.35 kU/l before omalizumab treatment, increased to >0.35 kU/l, and these patients showed negative-to-positive seroconversion after omalizumab treatment. In particular, negative-to-positive seroconversion was frequently observed for IgE against Japanese cedar, candida and moth. IgE positivity for these specific allergens was considerably high before omalizumab treatment. Intriguingly, although Der p-specific and house dust-specific IgE showed a high positivity, similar to Japanese cedar-specific IgE, no negative-to-positive seroconversion was observed. Further studies with a large sample size are necessary to clarify these findings. Interestingly, all 4 patients with negative-to-positive seroconversion for Japanese cedar-specific IgE after omalizumab treatment were diagnosed with cedar pollinosis before the treatment, indicating the presence of functional slgE, despite the level being maintained at <0.35 IU/l. However, we assume that seroconverted slgEs do not have clinical significance in patients treated with omalizumab because they are not free to bind to mast cells and basophils.

Recently, omalizumab was found to be effective in treating patients with severe nonatopic asthma who did not respond to a skin-prick test and/or with negative sIgE results [26–28]. One reason for the effectiveness of omalizumab in patients with nonatopic asthma may be the presence of local slgE. In fact, Mouthuy et al. [29] reported that both tIgE and Der p-specific IgE in sputum were significantly increased in patients with nonatopic asthma compared to in healthy nonatopic participants. Our results indicate that the presence of undetectable slgE due to the extremely low levels could be another reason for the effectiveness of omalizumab in patients with nonatopic asthma.

Currently, levels of specific IgE >0.11 kU/l can be detected using ImmunoCAP-Feia in a clinical setting in Japan. However, patients who began omalizumab treatment before 2011 showed levels of specific IgE ≥0.35 kU/l. Therefore, sensitization was defined as a specific IgE level ≥0.35 kU/l. Extremely low specific IgE levels in combination with the methods used to measure these levels could be important. ImmunoCAP is the most common method used to measure tIgE and specific IgE levels. An alternative assay is the IMMULITE 2000 3gAllergy™ (IMMULITE, Siemens Healthcare Diagnostics Inc., USA). IMMULITE employs liquid-phase kinetics in a bead format and a chemiluminescent enzyme immunoassay. The allergens are covalently attached to a soluble polymer matrix in IMMULITE and to a solid column in ImmunoCAP. The liquid-phase allergens increase the number of binding sites and their accessibility to the sIgE. In addition, IMMULITE has a wide measurement range, from 0.1 to 500 IUA/ml and a high degree of sensitivity [30–32]. Although the overall advantages of IMMULITE over ImmunoCAP have not been studied, IMMULITE seems to be a promising methodology with applicability in clinical settings. In this study, artificial increases in IgE by omalizumab shed light on the presence of clinically functional class 0 slgE levels. Therefore, advances in the current methods used to measure high-sensitive IgE and re-examination of the class 0 IgE level are necessary.

![Fig. 4. Percentage of negative-to-positive seroconversion rates for slgE. Raw data are shown above the bars. Numbers of patients appear in bold type.](image-url)
This study had several limitations that need to be acknowledged. First, the time points of blood collection were not constant. The blood collections were performed from week 16 up to week 30 after beginning omalizumab treatment. Omalizumab treatment rapidly increases and then gradually decreases tIgE levels [33]. However, there is no previous report that describes the time-course of tIgE and sIgE levels after omalizumab treatment. If samples were collected when the IgE level peaked, the tIgE and sIgE levels could have been higher. In addition, the frequency of negative-to-positive seroconversion for sIgE after omalizumab treatment could further increase. However, we demonstrated no difference in ΔsIgE between the early time point group and the late time point group. We therefore believe that even if blood was collected at the optimum time point, the positive correlation observed between ΔsIgE after omalizumab treatment and baseline levels of sIgE would not change. A previous study demonstrated that early ΔtIgE can be used as a predictor of future responders to omalizumab [34]. In this study, only a single time point was used, i.e. after omalizumab treatment. Therefore, we could not assess the relationship between ΔsIgE and the major outcome variables of omalizumab, such as pulmonary function, symptom scores and oral corticosteroid use. Measurements of sIgE at different time points would provide further elucidation of the influence of omalizumab on sIgE. Second, this study involved a small sample size. Omalizumab is an expensive medicine, and opportunities to administer it are limited, even for patients with uncontrolled asthma. Therefore, it was relatively difficult to collect enough samples in a real-life setting. In particular, it was difficult to determine the frequency of negative-to-positive seroconversion for sIgE, because only those patients sensitized to perennial allergens are indicated for omalizumab treatment. Third, this study did not consider seasonal variations in IgE levels. The prevalence of cedar pollinosis in the study participants was 61.7%. tIgE should increase in participants with cedar pollinosis in the Japanese cedar season, i.e. mostly in March and April, and just after this season. We showed that the percent increase in cedar-specific IgE, which was susceptible to seasonal variations, did not significantly differ from that observed for the other sIgEs. Thus, even though we standardized the season of omalizumab treatment and blood collection, we believe that the positive correlation observed between ΔsIgE after omalizumab treatment and baseline levels of sIgE would not change.

In conclusion, we found that ΔsIgE after omalizumab treatment were dependent on the baseline levels of sIgE. In addition, we also found that all patients with negative-to-positive seroconversion for cedar-specific IgE due to omalizumab treatment had cedar pollinosis before starting on omalizumab treatment. These findings suggest that sIgEs can play a critical role in allergic diseases despite being under the detectable level. Further larger-scale studies are needed to validate our data.

Acknowledgments

The authors thank Ms. Kyoko Inui and Ms. Manami Matsuda for their excellent assistance with the data collection and analysis.

Disclosure Statement

There were no potential conflicts of interest to declare.

References


Erratum

In the article by Mizuma et al., entitled ‘Influence of omalizumab on allergen-specific IgE in patients with adult asthma’ [Int Arch Allergy Immunol 2015;168:165–172, DOI: 10.1159/000442668], the third last author’s name was misspelt and should read Matsukura.