IgE to Gly m 5 and Gly m 6 is associated with severe allergic reactions to soybean in Japanese children

To the Editor:

Soybean is 1 of 8 foods believed to cause a majority of food-induced allergic reactions in children. However, the prevalence of soybean allergy in Japan might be higher than in Europe and the United States, with soybean reported as the fifth most common food allergen causing anaphylaxis. Soybeans contain about 40% protein, the majority of which is composed of the 2 storage proteins β-conglycinin and glycgin, which have been recently designated Gly m 5 and Gly m 6. Four other proteins are officially accepted as allergens, and at least an additional 12 have been reported as IgE-reactive proteins. Data regarding soybean allergens associated with clinical symptoms in children are limited. In this study we have examined the IgE reactivity pattern to 5 soybean and 3 cross-reactive allergens in a group of children with and without soybean allergy. Furthermore, we have investigated the clinical usefulness of analyzing specific IgE antibodies to Gly m 5 and Gly m 6.

There were 74 subjects (range, 0.6-16.3 years), of whom 33 were given diagnoses of soybean allergy (symptomatic group) based on challenge outcome (n = 29) or clinical history after intake (n = 4; 3 experienced apparent skin symptoms and 1 experienced anaphylaxis). The symptomatic group was further divided into subjects with severe symptoms (n = 14) and mild symptoms (n = 19). Severe symptoms were defined as a combination of skin, respiratory, or gastrointestinal symptoms, whereas mild symptoms were defined as isolated skin symptoms, oral symptoms, or both (Table I). The remaining 41 subjects were sensitized to soybean without any symptoms from soybean (non-symptomatic group). Tolerance in the non-symptomatic group was either confirmed by means of food challenge (n = 22) or a history of daily ingestion of soybean products (n = 19). Food challenges were conducted in accordance with the Japanese guidelines.

IgE reactivity to 8 different allergens was tested in an in-house, qualitative multiplexed immunoassay, essentially as reported elsewhere. The 8 allergens included in the setup were Gly m 5, Gly m 6, rGly m 4, soybean Kunitz trypsin inhibitor (Sigma-Aldrich, St Louis, Mo), soybean agglutinin (Vector Laboratories, Peterborough, United Kingdom), Cross-reactive carbohydrate determinants (CCDs) purified from digested bromelain (essentially MUXF3), profilin from timothy pollen (rPhl p 12), and lipid transfer protein from peach fruit (rPrf p 3). Native Gly m 5 and Gly m 6 were essentially purified according to the method of Thanh and Shibasaki. All recombinant allergens, as well as the CCD reagent, were produced at Phadia AB (Uppsala, Sweden).

IgE antibody levels to soybean, Gly m 5, and Gly m 6 were analyzed in serum by using ImmunoCAP (Phadia AB), all of which were commercially available. The lower limit of quantification of the tests was 0.10 kU/L. The Fisher exact test was used to determine differences regarding the prevalence of IgE reactivity analyzed by using the multiplex assay (categorical data). The Spearman rank correlation test was used in the analysis of associations between IgE concentrations. The relationship between IgE concentrations and clinical status outcome was analyzed by using logistic regression analysis. Odds ratios were estimated by using regression models, and 95% CIs were generated according to the Wald test.

Among the children in the symptomatic group with mild symptoms, all had skin symptoms, and 3 had oral symptoms (Table I). Respiratory symptoms, mostly coughing and wheezing, were the most frequent symptoms (n = 12) in the severe group. The multiplex immunoassay showed that among the children in the symptomatic group, 67% had IgE reactivity to Gly m 5 (49% in the nonsymptomatic group), 58% to Gly m 6 (39% in the nonsymptomatic group), 21% to Gly m 4 (20% in the nonsymptomatic group), and 6% to soybean agglutinin and soybean trypsin inhibitor (7% and 10%, respectively, in the nonsymptomatic group). The number of subjects with IgE reactivity to lipid transfer protein, profilin, and CCDs varied between 12% and 15% (7% to 17% in the nonsymptomatic group). No significant difference in the frequency of IgE reactivity between the symptomatic and nonsymptomatic groups was observed for any of the allergens included in the study. However, a tendency toward a higher frequency of IgE reactivity in the symptomatic group was noted for both Gly m 5 and Gly m 6 (P = .16 for both). Therefore quantitative analysis of IgE to Gly m 5 and Gly m 6 was performed to investigate the true prevalence.

Analysis with ImmunoCAP demonstrated that all children had IgE levels to soybean, Gly m 5, and Gly m 6 of greater than 0.1 kU/L, except one in the nonsymptomatic group. The IgE levels to both Gly m 5 and Gly m 6 correlated with the IgE levels to soybean (rS = 0.89 and rS = 0.86, respectively). The IgE levels to soybean and Gly m 5 were significantly higher in the symptomatic group than in the nonsymptomatic group (P < .01). With respect to the specific IgE levels in the 2 groups, the risk of being allergic to soy increased significantly with increasing levels of IgE. For IgE to soybean, the odds increased 1.51-fold (95% CI, 1.10-2.08), and for IgE to Gly m 5, the odds increased 1.48-fold (95% CI, 1.08-2.02) per logarithmic unit increase, respectively. Significant differences were noticed between the severe and non-symptomatic groups in IgE levels to soybean, Gly m 5, and Gly m 6 (Fig 1). The IgE responses to soybean, Gly m 5, and Gly m 6 were not statistically different between the children with mild symptoms and the nonsymptomatic children. Significant differences in the IgE levels to soybean were detected between the mild and severe symptom groups but not in the IgE levels to Gly m 5 and Gly m 6.

Knowledge about specific soybean allergens associated with clinical symptoms is restricted to a few publications. Many studies demonstrating IgE reactivity to soybean proteins in sera from soybean-sensitized subjects have been published, but the patient material has generally been small and often with an unclear diagnosis. In this study we have examined IgE reactivity to 5 soybean and 3 cross-reactive allergens in sera from 74 Japanese children. To the best of our knowledge, this group, consisting of symptomatic and nonsymptomatic subjects, is the largest defined clinical sample tested with the aim of identifying important soybean allergens.
Of the 5 soybean allergens included in the multiplex assay, only the 2 storage proteins Gly m 5 and Gly m 6 were defined as major allergens. In fact, when using the more sensitive ImmunoCAP system, it was found that all children in the symptomatic group had IgE to Gly m 5 and Gly m 6. Holzhauser et al also found a large number of subjects with IgE antibodies to the same 2 proteins in European children and adults with soybean allergy, but they were not considered to be major allergens in their study group. The reason for Gly m 5 and Gly m 6 being found as major allergens in the present study might be the study group composition of children only or might depend on Japanese eating habits, with soybean being part of the daily food intake.

We found that IgE levels to Gly m 5, but not to Gly m 6, were significantly higher in the symptomatic group when compared with those in the nonsymptomatic group. Because of the significant overlap of individual values between the symptomatic and nonsymptomatic groups, it was not possible to decide on a predictive IgE level for clinical symptoms. In earlier studies Sampson showed that the positive predictive level for specific IgE to soybean was estimated at 30 kU/L, and Komata et al showed an association between the level of IgE to soybean and positive challenge outcomes for soybean. In the present study it was shown that increasing IgE levels to both soybean and Gly m 5 correlated with increasing risk for clinical reactions.

Significant differences between the IgE levels to Gly m 5 and Gly m 6 were seen between the group of children with severe symptoms and the nonsymptomatic children. A similar trend was seen in the study by Holzhauser et al, in which severe symptoms correlated with the presence of IgE to Gly m 5 and Gly m 6.

It is worthwhile noting that measurement of IgE levels to soybean extract provides the best differentiation between the symptomatic and nonsymptomatic groups. This is also true after dividing the symptomatic group into subjects with severe and mild symptoms. The major constituents in the soybean extract are the 2 storage proteins Gly m 5 and Gly m 6, and there was also a very good correlation between the IgE levels to soybean and those 2 proteins. Nevertheless, this might reflect that there are other nonidentified components present in the soybean extract to which IgE might have a predictive value. However, the well-recognized problem with IgE analysis based on soybean extract is the poor sensitivity, probably because of the presence of cross-reacting IgE antibodies primarily induced to allergens from other allergen sources, such as pollen, resulting in many sensitized subjects without symptoms from

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**TABLE I.** Demographic, serologic, and clinical characterization of study subjects

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
<th>Symptomatic (n = 33)</th>
<th>Nonsymptomatic (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male/female</td>
<td>20/13</td>
</tr>
<tr>
<td>Age</td>
<td>Median (y [range])</td>
<td>2.3 (0.7-16.3)</td>
</tr>
<tr>
<td>Total IgE</td>
<td>Median (kU/L [range])</td>
<td>1,282 (29-22,300)</td>
</tr>
<tr>
<td>Specific IgE to soybean</td>
<td>Median (kUA/L [range])</td>
<td>17.1 (0.36-92)</td>
</tr>
<tr>
<td>Diagnosis of soybean allergy</td>
<td>Oral food challenge</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>History</td>
<td>4</td>
</tr>
<tr>
<td>Graded symptoms</td>
<td>Severe/mild*</td>
<td>14/19</td>
</tr>
<tr>
<td>Symptoms after challenge or intake (severe/mild)</td>
<td>Skin</td>
<td>11/19</td>
</tr>
<tr>
<td></td>
<td>Mucosal</td>
<td>2/3</td>
</tr>
<tr>
<td></td>
<td>Respiratory</td>
<td>12/0</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal</td>
<td>3/0</td>
</tr>
</tbody>
</table>

Symptoms after challenge or intake are specified in the symptomatic children.

*Severe symptoms are defined as a combination of skin, respiratory, or gastrointestinal symptoms, and mild symptoms are defined as isolated skin symptoms, oral symptoms, or both.

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**FIG 1.** Quantitative IgE measurement for soybean, Gly m 5, and Gly m 6. Comparison of IgE antibody levels between children with severe symptoms, mild symptoms, and no symptoms is shown. The Mann-Whitney U test (2-tail) was used to compare the statistical differences between the study groups, and significant differences are indicated as follows: *P < .05, **P < .01, and ***P < .001.
soybean.1,10 Analysis of IgE antibodies to Gly m 5 and Gly m 6 will therefore most likely better predict soybean allergy than an extract-based test.

Interpretation of the severity of allergic symptoms through the level of sensitization is a complex matter, but this risk assessment is of great importance for the prediction of severe and potentially fatal reactions. In this study the levels of IgE responses to Gly m 5 and Gly m 6 were found to be associated with severe clinical reactions caused by soybean in Japanese children.

We thank Magnus Sundberg for help with the purification of Gly m 5 and Gly m 6 and Fredrik Bernhardsson for help with the running of the immunoassays.

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Primary versus secondary immunoglobulin E sensitization to soy and wheat in patients with chronic granulomatous disease–induced hyperinflammation: Patient analysis and murine model

To the Editor:

Chronic granulomatous disease (CGD), a genetic deficiency in the phagocyte nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2), leads to severe recurrent infections but also to exuberant inflammatory responses. Because infections have a major effect on mortality, they have been the main focus of CGD research and therapies, resulting in markedly increased survival. Because of the improved management of infections, inflammatory complications are now an increasingly important problem. Almost any organ can be affected, with the gut being probably the most common site.1,2 Although hyperinflammation might not lead to a major increase in mortality, it is associated with high morbidity.

A breakthrough in research on CGD-induced hyperinflammation was the generation of Nox2-deficient mice with CGD, leading to the development of a skin model of inflammatory complications by Dinauer.3 Indeed, injection of sterile fungal cell wall more specifically β-glucan into the skin of mice with CGD leads to massive hyperinflammation and ultimately granuloma formation.4,5 Note that injection of sterile bacterial cell wall components did not lead to hyperinflammation.6 Underlying mechanisms are still poorly understood; however, a common observation is an increase in levels of proinflammatory cytokines, particularly TNF-α,6,7 which is often cited as a possible culprit in CGD-induced inflammatory states.3

The following lines of argument suggest that TNF-α inhibition might be a pertinent treatment approach: (1) inflammatory cells from patients with CGD release increased amounts of proinflammatory cytokines, particularly TNF-α; (2) anti–TNF-α treatments have been successfully used in other types of inflammatory diseases (eg, rheumatoid arthritis and Crohn disease); and (3) inflammatory complications in the context of other immunodeficiencies are improved by TNF-α blockers. However, it is not clear whether the increased secretion of TNF-α by leukocytes from patients with CGD is a causative mechanism in hyperinflammation. Yet despite the lack of information about the role of TNF-α in CGD-induced hyperinflammation, there is an increasing off-label use of anti–TNF-α treatments in patients with CGD. Indeed, the use of these compounds in the treatment of CGD-induced inflammatory complications has been suggested in several publications and is included in recent algorithms of CGD management. In fact, short-term treatment with infliximab has been proposed as the second-line treatment in patients with steroid-refractory chronic granulomatous colitis.8

We first performed a literature review on the treatment of CGD-induced inflammatory complications with TNF-α inhibitors (see Table E1 in this article’s Online Repository at www.jacionline.org). We found indications for off-label use of anti–TNF-α treatments in patients with CGD; indeed, we could identify a total of 17 published cases. Patients with autosomal recessive mutations are overrepresented in the collection (11/17 [65%]), and 7 of these presented with inflammatory bowel disease or arthritis as initial symptom (see patients marked by asterisks in Table E1). Note that in general autosomal recessive mutations represent approximately 30% of patients with CGD. Only in 5 patients was a clear and sustained response to treatment observed. The treatment response seemed genotype dependent: 4 (36%) of 11 autosomal...