Letter to the Editor

Interleukin 2 receptor-α expression after lymphocyte stimulation for non-IgE-mediated gastrointestinal food allergies

Dear Editor,

Non-IgE-mediated gastrointestinal food allergies (non-IgE-GI-FAs) are often found in neonates and infants and are mainly caused by cow’s milk (CM). Some cases show nonspecific symptoms, such as fever, poor sucking, and poor weight gain, but with minor gastrointestinal (GI) symptoms. Additionally, approximately 10% of patients develop severe complications, such as mechanical ileus, perforation of the GI wall, shock, and developmental retardation, owing to delayed start of treatment. Oral food challenge (OFC) is necessary for definitive diagnosis, but may induce severe symptoms; accordingly, OFC should not be performed until patients are healthy enough, resulting in delayed diagnosis.

The pathology of non-IgE-GI-FAs involves antigen-presenting cells, allergen-specific lymphocytes, and eosinophils, although the specific mechanisms are still unclear. The lymphocyte stimulation test (LST), which examines the reactivity of T lymphocytes to specific allergens in vitro, facilitates the diagnosis of non-IgE-GI-FAs because LSTs can be performed irrespective of the patient’s condition, thereby permitting early diagnosis. However, in patients with drug allergies, LSTs should not be performed until 4 weeks after an allergic reaction to prevent false-negative results.

LSTs require substantial amounts of fresh peripheral blood and lengthy culture times (5–7 days). Alternative tests that require small amounts of blood and shorter experimental times are urgently needed. In LSTs for non-IgE-dependent drug allergies, T cells increase the expression of CD25 (interleukin-2 receptor-α [IL2RA]) on their surface within several hours after activation by antigen recognition and costimulation. Subsequently, the cells proliferate and differentiate into effector and memory cells. IL2RA expression has been reported in in vitro and in vivo testing and is upregulated on natural killer (NK) cells after a positive food challenge in food protein-induced enterocolitis syndrome. Thus, we hypothesized that IL2RA mRNA may be increased early in allergen-stimulated lymphocytes from patients with non-IgE-GI-FAs and that monitoring of such an increase may be an alternative to conventional LSTs for evaluation of cell proliferation.

Patients with non-IgE-GI-FAs to CM who were treated at our hospital from June 1, 2011 to July 31, 2018 were recruited after obtaining informed consent from parents or guardians. Controls who did not have non-IgE-GI-FAs or other immune diseases were also recruited. Non-IgE-GI-FAs were diagnosed based on the criteria proposed by the Japanese Pediatric Guidelines for Food Allergy, modified from those proposed by Powell. Blood specimens were collected at onset or before OFC. Peripheral blood mononuclear cells (PBMCs) from patients and controls were incubated with α-casein, κ-casein, β-lactoglobulin, α-lactalbumin, a mixture of all four components (Pmix), or CM for 24 h, and IL2RA expression was assessed by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The same sets of samples were also subjected to conventional LSTs (Supplementary Methods).

The median age (interquartile ranges) of 33 participants (19 boys and 14 girls) was 330 days (97.5–1275 days). Sixteen patients had non-IgE-GI-FAs (Supplementary Table 1), and 17 patients were controls. The median ages (interquartile ranges) of the patients and controls were 112.5 (61.3–292.5) days and 760 (287.5–1793) days, respectively. We first addressed the diagnostic significance of early IL2RA mRNA expression. All antigens significantly increased IL2RA mRNA expression in patients’ PBMCs compared with that in controls (Table 1, Fig. 1A). The area under the curve (AUC) of the receiver operating characteristic (ROC) curve of IL2RA mRNA expression after 24-h stimulation with all antigens was above 0.7, supporting the diagnostic value of this analysis. Notably, α-casein showed the largest AUC (0.9596; p < 0.0001; Table 1, Fig. 1B). These AUCs were comparable to those of LSTs (AUC: 0.846, p = 0.0007, α-casein; Supplementary Table 2). Additionally, after stimulation with a control antigen, ovomucoid, no difference was found between non-IgE-GI-FAs and disease controls, indicating the antigen specificity of the results (four non-IgE-GI-FAs to CM, six disease controls, p = 0.3524, Supplementary Fig. 1). Since the small amount of LPS that is present in milk proteins affected the results of LST, we examined the influence of LPS on IL2RA mRNA expression. We found that stimulation with LPS (1 µg/mL and 100 pg/mL) did not affect IL2RA mRNA expression (data not shown). Furthermore, subpopulation analysis after stimulation with milk allergens showed no difference in IL2RA mRNA expression between the patients who fulfilled diagnostic criteria for food protein-induced enterocolitis syndrome (FPIES) and those who did not (Supplementary Fig. 2). Next, we assessed the correlation of IL2RA mRNA expression to the results of conventional LSTs. We found weak but significant correlations for all antigens, particularly α-casein (r = 0.5144; p = 0.0022), κ-casein (r = 0.5791; p = 0.0008), and Pmix (r = 0.5833; p = 0.0044) using Spearman correlations (Supplementary Fig. 3). AUC showed better prediction for IL2RA mRNA assay of α-casein (0.8444, p = 0.0008) and Pmix (0.9294, p = 0.0042) to LST assays compared with the other antigens (Supplementary Fig. 4). These results indicated that IL2RA mRNA expression levels were correlated with conventional LSTs.

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Our results showed that *IL2RA* mRNA expression was increased after 24-h stimulation of PBMCs in a disease-specific manner, consistent with the results of conventional LSTs using the same set of samples. Thus, an antigen–specific early increase in the levels of *IL2RA* mRNA could reflect antigen-specific lymphocyte proliferative responses. The test of *IL2RA* expression does not require a lengthy culture time, whereas LSTs usually require 5–7 days. Furthermore, using our current test, after stimulation, cells do not have to be kept alive because the test uses mRNA. Thus, our method could be a useful, rapid complementary test for non-IgE-GI-FAs. We designated this method the instant peripheral blood allergen stimulation test (iPAST); the iPAST includes 24-h stimulation of lymphocytes with a specific allergen and analysis of mRNA levels. Further studies are needed to determine whether other genes or a combination of genes may be superior to *IL2RA* in this assay. We are currently using genome-wide analyses to identify alternative gene candidates.

Notably, α-casein showed the best diagnostic accuracy in this study. Caseins consisting of four types of proteins (αS1-, αS2-, β-, and κ-) account for approximately 80% of milk proteins. Caseins are major allergens that cause immediate-type milk allergies, and casein-specific IgE is found in most patients; hence, these proteins are considered highly immunogenic. The results of our study suggested that casein may be a major allergen in non-IgE-GI-FAs as well.

There were some limitations to this study. First, the study included a small number of patients, and the age differed between non-IgE-GI-FAs patients and controls. Notably, because non-IgE-GI-FAs develop mostly in young children, it is difficult to obtain samples from age-matched controls owing to ethical constraints and difficulties in obtaining consent. Additionally, we did not investigate which cells expressed *IL2RA* mRNA. Thus, we examined the presence of NK cells and neutrophils in PBMCs based on a recent study showing *IL2RA* expression in these cells in non-IgE-GI-FAs patients. We evaluated the expression of an NK cell-specific gene, natural cytotoxicity receptor 1 (*NCR1*), and a neutrophil-specific gene, cathepsin G (*CTSG*) in PBMCs (*Supplementary Fig. 5*). *NCR1* mRNA was detected in PBMCs from controls and patients (*Supplementary Fig. 5A*), suggesting that NK cells may promote *IL2RA* expression, as previously shown. In contrast, *CTSG* was barely detected in PBMCs from controls but faintly detected in that from patients (*Supplementary Fig. 5B*). This suggested contamination of neutrophils in patient-derived PBMCs, which could be attributable to a decrease in neutrophil density caused by inflammation. Accordingly, neutrophils may be a source of *IL2RA* expression in patients, as described previously.

### Table 1

<table>
<thead>
<tr>
<th>n</th>
<th>Fold induction of <em>IL2RA</em> mRNA&lt;sup&gt;1&lt;/sup&gt;</th>
<th>ROC curve</th>
<th>p value&lt;sup&gt;2&lt;/sup&gt;</th>
<th>AUC</th>
<th>p value</th>
<th>Cut off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
<tr>
<td></td>
<td>Patients with non-IgE-GI-FAs</td>
<td>Controls</td>
<td></td>
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<tr>
<td>α-Casein</td>
<td>3.061 (1.969–4.403)</td>
<td>1.066 (0.896–1.404)</td>
<td>&lt;0.0001</td>
<td>0.9596</td>
<td>&lt;0.0001</td>
<td>1.68</td>
<td>87.5</td>
<td>88.2</td>
</tr>
<tr>
<td>κ-Casein</td>
<td>2.640 (1.300–4.131)</td>
<td>1.218 (0.947–1.405)</td>
<td>0.0003</td>
<td>0.8493</td>
<td>0.0006</td>
<td>1.46</td>
<td>75.0</td>
<td>82.4</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>4.015 (2.973–5.808)</td>
<td>2.770 (1.787–3.704)</td>
<td>0.0061</td>
<td>0.7757</td>
<td>0.0069</td>
<td>3.21</td>
<td>75.0</td>
<td>70.6</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>5.675 (4.645–7.005)</td>
<td>3.223 (2.312–3.725)</td>
<td>0.0009</td>
<td>0.8272</td>
<td>0.0013</td>
<td>3.97</td>
<td>87.5</td>
<td>82.4</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>6.401 (4.360–10.29)</td>
<td>3.081 (1.769–5.573)</td>
<td>0.0042</td>
<td>0.7868</td>
<td>0.0050</td>
<td>4.32</td>
<td>81.3</td>
<td>70.6</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>4.411 (3.225–7.796)</td>
<td>3.243 (1.575–3.875)</td>
<td>0.0177</td>
<td>0.7522</td>
<td>0.0188</td>
<td>3.60</td>
<td>71.4</td>
<td>68.8</td>
</tr>
</tbody>
</table>

Data are expressed as medians (interquartile ranges).

<sup>1</sup> Mann–Whitney test.

<sup>2</sup> Fold induction of *IL2RA* mRNA was calculated by dividing stimulated samples with nonstimulated samples after normalization to 18S rRNA.
studies are needed to elucidate the detailed mechanisms underlying the in vitro expression of IL2RA mRNA.

In conclusion, iPAST may be useful for the supplementary diagnosis of non-IgE-GI-FAs as an alternative to LSTs owing to its high diagnostic value, rapid analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2019.11.003.

Conflict of interest

The authors have no conflict of interest to declare.

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References