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CASE STUDY

Positive basophil activation test with soymilk protein identifies Gly m 4–related soymilk allergy

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Abstract

Soymilk allergy is caused by cross-reaction of either birch Bet v 1–specific or alder Aln g 1–specific IgE to soybean Gly m 4, which belongs to pathogenesis-related protein-10 (PR-10) family. Gly m 4–specific IgE test identifies soybean allergy patients with high sensitivity. In this case study, we found that CD203c expression–based basophil activation test (CD203c-BAT) with soymilk protein became positive in the three cases of Gly m 4–related soymilk allergy at the concentrated of 1 μg/ml, but not in the control subjects. CD203c-BAT may possibly be useful as a diagnostic tool to identify patients with soymilk allergy.

KEYWORDS
CD203c expression–based basophil activation test, Gly m 4, pollen-food allergy syndrome, soybean allergy, soymilk allergy

1 | INTRODUCTION

Pollen allergy causes pollen-food allergy syndrome (PFAS) by cross-reaction of the pollen-specific IgE to fruit and/or vegetable.[1] Recently, soymilk or soy drink allergy associated with birch or alder pollinosis increases due to increased consumption of soymilk or soy drink all over the world.[2–5] Soymilk allergy is caused by cross-reaction of either birch Bet v 1–specific or alder Aln g 1–specific IgE to soybean Gly m 4, a major allergen of soymilk allergy, because Bet v 1, Aln g 1, and Gly m 4 have similar structures belonging to pathogenesis-related protein-10 (PR-10) family.[6,7] Gly m 4–specific IgE test is now utilized in Japan and identifies soybean allergy patients with higher sensitivity compared with soybean-specific IgE test.[8] However, the specificity of this allergen-specific IgE test is not always satisfactory, because the test becomes positive even in more than half of alder pollen–sensitized cases which are not allergic to soymilk.[8] Since IgE cross-linking is essential to elicit allergic reactions, the low specificity of this test may be partly due to an existence of Gly m 4–specific IgE which have no ability to be cross-linked on mast cells/basophils. CD203c, an ectoenzyme belonging to a family of ectonucleotide pyrophosphatases and phosphodiesterases, is expressed on the cell membrane of human peripheral basophils and mast cells. Since cross-linking of the high-affinity IgE receptor upregulates CD203c expression on the cell membrane, CD203c expression–based basophil activation test (CD203c-BAT) better reflects the actual allergic symptoms. We have reported that the patients with active wheat allergy displayed over 10% activation rate in CD203c-BAT with wheat allergens, whereas the patients with tolerant to wheat displayed activation rates lower than 10%.[9] The objective of the study was to obtain a preliminary information for usefulness of CD203c-BAT with soymilk as a diagnostic tool to identify patients with soymilk allergy.

1.1 | Cases

Case 1 is a 64-year-old man who was admitted to our hospital because of anaphylaxis after soymilk intake. Thirty minutes had passed since the ingestion of soymilk, which had caused itching eyes, cough,

congested nose, urticaria, and dyspnea. He had pollinosis during spring. We suspected soymilk allergy and performed serum-allergen-specific IgE test (CAP-FEIA) and skin prick test. Soybean-specific IgE was <0.34 Ua/ml, alder pollen–specific IgE was 13.30 Ua/ml, and Gly m 4–specific IgE was 5.61 Ua/ml. A skin prick test was performed using commercial soybean extract (Torii) and the soymilk he had ingested. Soybean extract and soymilk showed positive reactions (Figure 1A). Case 2 is a 27-year-old man who presented to our hospital with a face swelling and throat tightness after unhomogenized soymilk ingestion. Soybean-specific IgE was 0.10 Ua/ml, alder pollen–specific IgE was 2.30 Ua/ml, and Gly m 4–specific IgE was 2.64 Ua/ml. A skin prick test was performed using soybean extract, the homogenized and unhomogenized soymilk which he had ingested. He showed positive reactions to all tests (Figure 1B). He had no previous history of pollinosis. Case 3 is a 62-year-old woman who presented to our hospital with urticaria and a dull feeling in the throat. She had cedar and cypress pollinosis. Soybean-specific IgE was 0.47 Ua/ml, alder pollen–specific IgE was 33.30 Ua/ml, and Gly m 4–specific IgE was 34.0 Ua/ml. A skin prick test was performed using soybean extract and the soymilk she had ingested, to which she showed positive reactions (Figure 1C).

Soymilk allergy was diagnosed by allergic reaction after ingesting soymilk, positive skin prick test with soymilk, and positive Gly m 4–specific IgE test (ImmunoCAP™, Thermo Fisher Diagnostics) as described previously.[8] Table 1 summarizes clinical features of these patients.

1.2 | Results and Discussion

The CD203c-BAT was performed as previously described using a commercial kit (Allergenicity Kit, Beckman Coulter).[10] The CD203c

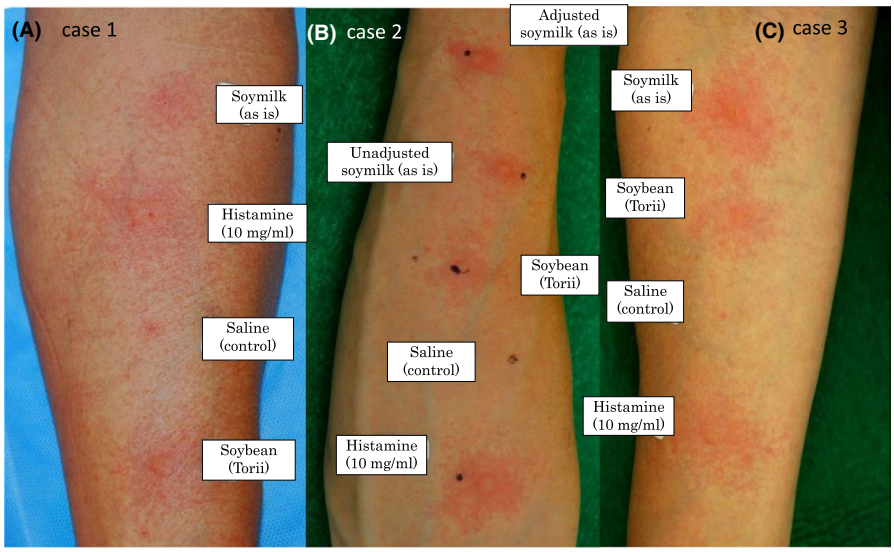

FIGURE 1 The results of skin prick test. Commercial soybean extract and soymilk showed positive reactions (Case 1). Soybean extract and the prepared and pure soymilk showed all positive reactions (Case 2). Soybean extract and the ingestion soymilk showed positive reactions (Case 3)

TABLE 1 Background of the subjects

Subject	Case 1	Case 2	Case 3	Healthy 1	Healthy 2	Healthy 3
Gender/Age	Male/64	Male/27	Female/62	Female/48	Female/36	Female/59
Symptoms after ingesting soymilk	Itching on eyes, congested nose, cough, dyspnea, generalized urticaria	Face swelling, discomfort in throat	Discomfort in throat, urticaria	–	–	–
Pollinosis (allergens)	Spring	–	Japanese ceder, cypress	Japanese ceder	–	–
Skin prick test						
Soybean extract	2+	2+	2+	–	–	2+
Soymilk	2+	2+	2+	–	–	2+
ImmunoCAP (UA/ml)						
Soybean	<0.34	0.1	0.47	<0.1	<0.1	0.14
Alder	13.3	2.3	33.3	<0.1	<0.1	3.34
Gly m 4	5.61	2.64	34	<0.1	<0.1	<0.1
Gly m 5	<0.1	<0.1	<0.1	NT	NT	NT
Gly m 6	<0.1	<0.1	<0.1	NT	NT	NT

NT, not tested.

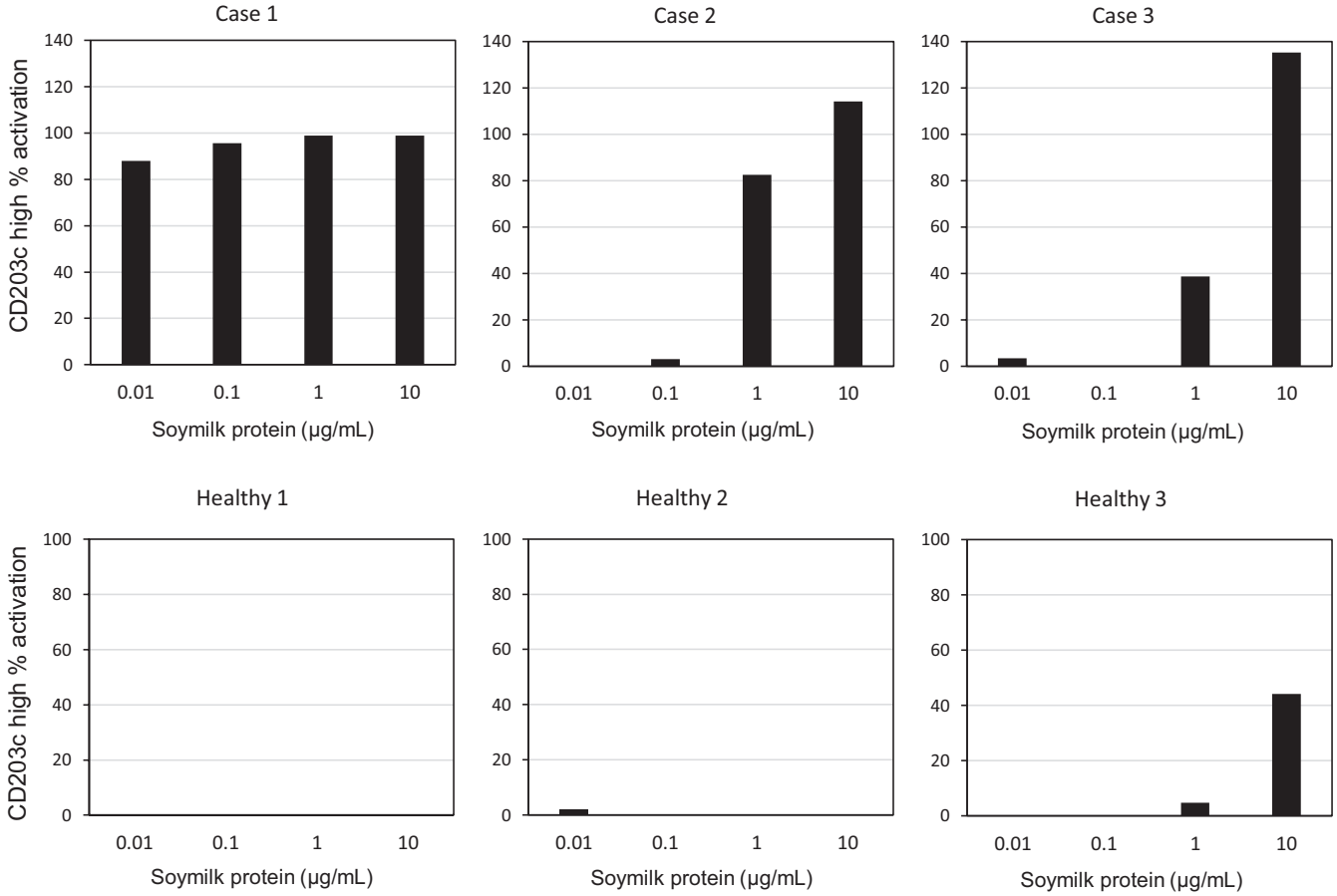

FIGURE 2 Results of CD203c expression–based basophil activation test in the patients with soymilk allergy and healthy subjects following soymilk protein stimulation at the concentrations of 0.01, 0.1, 1, and 10 μg/ml

expression on the basophil surface was monitored by fluorescence-activated cell sorting on peripheral blood basophils activated against soymilk protein at the concentrations of 0.01, 0.1, 1, and 10 μg/ml of diluted commercial soymilk. Anti-IgE antibody at 4 μg/ml as a positive control and phosphate-buffered saline as a negative control were used. As controls, three healthy subjects who had no episodes of soymilk allergy and negative Gly m 4–specific IgE test were used (Table 1). The CD203c-BAT showed positive reaction of basophils from all three patients with soymilk allergy with soymilk protein. The activation rate was examined by comparing the anti-IgE activation, and the value was expressed as a percentage against that of anti-IgE activation.[10,11] Ten percent basophil activation was achieved at the concentration of 1 μg/ml of diluted commercial soymilk protein in all cases (Figure 2). In contrast, three healthy subjects did not show the activation of CD203c-BAT in the same condition, indicating specificity of the test. The activation was elicited even at the concentration of 0.01 μg/ml of soymilk protein in Case 1, suggesting a variation in the sensitivity of basophil activation.

In this study, we present an evidence that soymilk allergy subjects show basophil activation in CD203c-BAT with soymilk at the concentration of 1 μg/ml commercial soymilk. Although this result has been obtained from small number of patients, this possibly provides that CD203c-BAT with soymilk protein is a useful as a diagnostic tool to identify patients with soymilk allergy. The basophil activation was seen with 0.01 μg/ml of soymilk protein in Case 1 which had 5.61 UA/ml Gly m 4–specific IgE, whereas significant basophil activation was observed with much higher concentration of soymilk protein (1 μg/ml) in cases 2 and 3, suggesting an existence of Gly m 4–specific IgE without cross-linking ability in these cases. We need to determine whether the CD203c-BAT with soymilk shows negative result in the patients with Gly m 4–specific IgE test positive but without episode of soymilk allergy. Further, the CD203c-BAT should be investigated in combination with soymilk provocation test for the patients with alder/birch pollen allergy.

In conclusion, CD203c-BAT with soymilk may be a useful test to identify the patients with soymilk allergy in combination with Gly m 4–specific IgE test.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors have accepted responsibility for the entire content of this manuscript and approved submission.

DECLARATION SECTION

Approval of the research protocol: This study was approved by the Ethics Committee of the Shimane University Faculty of Medicine (Approval No. 1570).

Informed consent: We obtained written informed consent from the participants at the time of enrollment.

Registry and the Registration No.: N/A.

Animal Studies: N/A.

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