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A comparative study of human IgE binding to proteins of a genetically modified (GM) soybean and six non-GM soybeans grown in multiple locations

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ABSTRACT

Prior to commercialization, genetically modified (GM) crops are evaluated to determine the allergenicity of the newly expressed protein. Some regulators require an evaluation of endogenous allergens in commonly allergenic crops including soybean to determine if genetic transformation increased endogenous allergen concentrations, even asking for IgE testing using sera from individual sensitized subjects. Little is known about the variability of the expression of endogenous allergens among non-GM varieties or under different environmental conditions. We tested IgE binding to endogenous allergenic proteins in an experimental non-commercial GM line, a non-GM near-isoline control, and five non-GM commercial soybean lines replicated at three geographically separated locations. One-dimensional (1D) and two-dimensional (2D) immunoblotting and ELISA were performed using serum or plasma from eleven soybean allergic patients. The results of immunoblots and ELISA showed no significant differences in IgE binding between the GM line and its non-GM near-isoline control. However, some distinct differences in IgE binding patterns were observed among the non-GM commercial soybean lines and between different locations, highlighting the inherent variability in endogenous allergenic proteins. Understanding the potential variability in the levels of endogenous allergens is necessary to establish a standard of acceptance for GM soybeans compared to non-GM soybean events and lines.

1. Introduction

Many genetically modified (GM) crops have been developed by the insertion of a gene, which encodes a protein that confers the traits of interest (NASEM, 2016). Common traits in GM crops include herbicide resistance, drought tolerance, delayed ripening, bacterial disease resistance, high oleic acid levels, and resistance to insect pests (Chrispeels, 2014; Delmer, 2014; Metcalfe et al., 1996; Newell-McGloughlin, 2014; Taylor and Hefle, 2001). Farmers in the United States (U.S.) have rapidly adopted the use of GM soybean with herbicide tolerance and GM maize with herbicide tolerance and insect resistance since they were first commercialized in 1996 (Fernandez-Cornejo et al., 2014; James, 2008). The use of new GM traits has grown remarkably since then. New GM crops intended for food and/or feed use undergo a rigorous safety assessment to ensure no biologically relevant intended or unintended changes occurred in their nutrients, anti-nutrients, toxins, and allergens (Goodman, 2014; Goodman et al., 2013; König et al., 2004).

Soybean [*Glycine max* (L.) Merr.] ranks as one of the most common allergenic foods recognized by regulatory authorities worldwide. The use of soybean must be declared on labels of processed food in the U.S. and the European Union (EU) (EU, 2011; FDA, 2004). However, soybean allergy only affects approximately 0.1–0.2% of the general population although higher prevalence is found in infants and children under 10 years of age, although substantially lower than the incidence of allergy from other commonly allergenic foods (Gupta et al., 2011; Roehr et al., 2004; Sicherer and Sampson, 2010; Taylor et al., 2015). Because soybean is a common allergenic food, there is an additional regulatory requirement for developers of GM soybean compared to those developing GM-maize and other GM crops that are not considered

Abbreviations: 1D, one-dimensional; 2D, two-dimensional; 2-DE, two-dimensional gel electrophoresis; CCD, cross-reactive carbohydrate determinants; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; EU, European Union; EFSA, European Food Safety Authority; GM, genetically modified; HRP, horseradish peroxidase; IgE, immunoglobulin E; LS-Means, least squares means; NFDM, non-fat dry milk; OD, optical density; PBS, phosphate buffered saline; PBST, PBS plus 0.05% Tween 20; vdc, volts of direct current; w/v, weight to volume

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major sources of food allergy. Based on regulatory guidance, all GM crops are assessed for allergenicity by considering the source of the gene, comparison of the amino acid sequence of the newly expressed protein to known allergens, evaluation of the stability of the newly expressed protein to pepsin digestion at acidic pH, and determination of abundance of the protein in the plant tissues used as food. However, for soybean and other commonly allergenic crops, the additional measure is the relative level of known endogenous allergens to ensure that the genetic modification has not increased the level of endogenous allergens to an extent that would adversely impact human health. A number of human serum IgE binding studies have been performed with new GM soybeans (Burks and Fuchs, 1995; Panda et al., 2013). The European Food Safety Authority (EFSA) requested specific serum IgE screening, to evaluate whether the GM crop has approximately equivalent IgE binding to its closest non-GM counterpart, the near-isoline, and other non-GM commercial varieties of the crop (EFSA, 2006, 2011). Recently the European Union (EU) began requiring comparison of IgE binding using individual soy allergic subjects or analytical methods to measure certain endogenous allergens in the GM plant compared with its non-GM comparator(s) (EU, 2013).

Currently, there is a lack of understanding of the range of variation of endogenous allergens between different non-GM varieties with different genotypes and the natural variability of endogenous proteins in non-GM varieties of a crop. Yet, this information is required by some regulators to evaluate whether differences found between a GM crop and its non-GM counterpart(s) are biologically relevant (Doerrer et al., 2010). When the EU mandated quantitative analysis of individual proteins identified as allergens in the comparative compositional analysis of soybean (EU, 2013), they included a number of proteins that had not been characterized or proven to be allergens. Additionally the natural variation of the expression of the individual allergens among the non-GM commercial soybean lines had not been determined. A recent proteomics study showed the variation for some soybean allergens can be large across soybean lines using proteomics methods (Houston et al., 2010).

The objectives of this study were to evaluate the differences in IgE binding between an experimental non-commercial GM line, its non-GM near-isoline control and five non-GM commercial soybean lines of different genetic backgrounds. The intent was to determine whether the transformation or the transgene might cause differences or whether the different genetic backgrounds or other factors were likely sources of variation in IgE binding. Since the environmental conditions, agronomic practices, soil conditions may change the expression of endogenous allergens (Fernandez et al., 2013), natural variability was also taken into account by including soybean lines replicated in multiple different geographical locations in this study.

2. Materials and methods

2.1. Soybean samples

Fifteen individual full-fat, ground samples of soybean representing multiple geographical replicates of the GM soybean line, the non-GM near-isoline control soybean, and five other non-GM commercial soybean lines from replicated field trials were tested (listed in Table 1). Non-soybean control samples including raw Spanish peanuts, raw navy bean and maize meal that were purchased at a grocery store in Lincoln, NE.

2.2. Human serum and plasma

A total of 11 human sera and plasma samples were acquired from two sources (Table 2). Human sera from seven soy-allergic patients were obtained from the University Hospital Zürich, Switzerland and plasma from four soy-sensitized subjects were purchased from PlasmaLab International, Everett, WA, U.S. Diagnosis of the seven soy-allergic patients from Switzerland was confirmed by double-blind placebo-control food challenge or very clear clinical histories and diagnostic tests. The four soy-sensitized subjects collected by PlasmaLab were identified based on self-reported allergies to soybean using a standard questionnaire and positive detectable ImmunoCAP® IgE binding to soybean. Human sera from five individuals without reported allergy to soybean who did not experience symptoms in an open food challenge with soy milk and with very low soybean-specific IgE (< 0.35 kU/L ImmunoCAP® value) were provided by the University Hospital of Zürich as negative controls (clinical data not shown). All serum and plasma samples were collected from subjects who provided voluntary consent following specific institutional review board approval (University Hospital in Zürich) or collections by a U.S. Food and Drug Administration licensed facility (PlasmaLab).

Table 1
List of soybean samples with multiple geographical replicates.

Soybean lines	Test sites	Number of individual soybean lines
GM	Iowa (IA), Indiana (IN), Kansas (KS)	3
Non-GM near-isoline control	IA, IN, KS	3
Non-GM commercial line 1	IA, IN	2
Non-GM commercial line 2	KS	1
Non-GM commercial line 3	IA, IN, KS	3
Non-GM commercial line 4	IN	1
Non-GM commercial line 5	IA, KS	2

Table 2
Soybean allergic human serum and plasma samples.

Human Subject ID	ImmunoCAP® (kU/l)			Symptoms as reported by history[a]
	Soy	Gly m 4	Total IgE	
Soy-allergic Human Sera from University Hospital Zürich				
Neb-3	1.18	34.4	N/A[b]	OAS[c]
Neb-4	2.06	N/A	N/A	OAS, nausea, emesis
Neb-5	9.23	N/A	N/A	OAS, dyspnea, drop blood pressure
Neb-6	4.55	N/A	N/A	OAS, dyspnea, angioedema, conjunctivitis, tightness throat
Neb-7	< 0.35	26.2	N/A	OAS
Neb-8	6.42	1.58	N/A	OAS, nausea, emesis, diarrhea, gastrointestinal pain
Neb-9	2.10	9.12	N/A	OAS, urticaria, angioedema
Soy-sensitized Human Plasma from PlasmaLab International				
19392-CS	71.60	N/A	896	Angioedema, vomiting
20197-BH	3.00	N/A	N/A	Itchy throat with nuts and raw vegetables.
22329-JE	5.00	N/A	1953	N/A
9735-RE	5.00	N/A	> 5000	Anaphylaxis to peanut. Soybean causes sore throat, itchy mouth, and queasy stomach.

[a] The reported symptoms from soy-allergic subjects were soy-allergic symptoms only. The reported symptoms from soy-sensitized subjects were not strict to soy-related symptoms.
[b] N/A, not available.
[c] OAS, oral allergy syndrome.

2.3. One-dimensional (1D) IgE immunoblotting

The 15 individual full-fat soybean flour samples, as well as ground peanut, navy bean and maize samples were extracted with phosphate buffered saline (PBS) buffer containing protease inhibitor cocktail (Thermo Fisher Scientific). The protein content of the clarified extracts were determined using a Lowry DC protein assay kit (BioRad). Equal