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Occupational sensitization to soy allergens in workers at a processing facility

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Summary

Background Exposure to soy antigens has been associated with asthma in community outbreaks and in some workplaces. Recently, 135 soy flake processing workers (SPWs) in a Tennessee facility were evaluated for immune reactivity to soy. Allergic sensitization to soy was common and was five times more prevalent than in health care worker controls (HCWs) with no known soy exposure.

Objective To characterize sensitization to soy allergens in SPWs.

Methods Sera that were positive to soy ImmunoCAP ($n = 27$) were tested in IgE immunoblots. Wild-type (WT) and transgenic (TG) antigens were sequenced using nanoscale Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (nanoUPLC MS/MS). IgE reactivity towards 5-enolpyruvylshikimate-3-phosphate synthase (CP4-EPSP), a protein found in TG soy, was additionally investigated. De-identified sera from 50 HCWs were used as a control.

Results Immunoblotting of WT and TG soy flake extracts revealed IgE against multiple soy antigens with reactivity towards 48, 54, and 62 kDa bands being the most common. The prominent proteins that bound SPW IgE were identified by nanoUPLC MS/MS analysis to be the high molecular weight soybean storage proteins, β-conglycinin (Gly m 5), and Glycinin (Gly m 6). No specific IgE reactivity could be detected to lower molecular weight soy allergens, Gly m 1 and Gly m 2, in soybean hull (SH) extracts. IgE reactivity was comparable between WT and TG extracts; however, IgE antibodies to CP4-EPSP could not be detected.

Conclusions and Clinical Relevance SPWs with specific IgE to soy reacted most commonly with higher molecular weight soybean storage proteins compared with the lower molecular weight SH allergens identified in community asthma studies. IgE reactivity was comparable between WT and TG soy extracts, while no IgE reactivity to CP4-EPSP was observed. High molecular weight soybean storage allergens, Gly m 5 and Gly m 6, may be respiratory sensitizers in occupational exposed SPWs.

Keywords allergy, asthma, dust, exposure, IgE, IgG, occupational, respiratory, soy, workplace

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Introduction

Glycine max (soy) is one of the most profitable agricultural crops in North America [1]. As a result of the increased utilization of soy in agriculture and manufacturing sectors, occupational exposure to inhalant soy allergens has become a recent clinical concern and occupational hazard.

Soybean and soybean hull (SH) allergens have been immunologically characterized, and sensitization to these proteins has been associated with food allergy and respiratory disease in some settings [2–6]. Environmental exposure to low molecular weight SH allergens, Gly m 1 and Gly m 2, has been primarily associated with respiratory morbidity in community settings [7–13]. In contrast, occupational soy exposure and allergic sensitization are less well characterized. Occupational soy exposures are diverse and range from preprocessed soybeans encased in an outer SH [14], deoiled and dehulled soybean flakes, processed soybean flakes, and soybean flour [5, 15–24]. Occupational sensitization to soy antigens was first reported in soy mill workers with asthma symptoms in 1934 [5]. Subsequent studies of processing and mill workers have also identified soy sensitization; however, the allergens in these studies were not identified [16, 20–23]. Immunoblotting studies of bakers and food processors showed IgE reactivity to higher molecular weight soybean allergens and not to the SH allergens associated with community asthma epidemics [15, 19, 24–26] or SH processors [14].

In a recent study of 147 soy flake processing workers (SPWs) at one Tennessee facility, we found SPWs to be at increased risk of respiratory symptoms and allergic sensitization to soy [17]. The prevalence of wheeze and asthma diagnosis among SPWs was higher than expected in comparison with the US adult population. Twenty-one percent of SPWs had specific IgE to soy, and all had detectable IgG to soy [17]. The prevalence of specific IgE to soy in SPWs was five times higher than healthcare worker controls (HCWs) not known to be occupationally exposed to soy [17]. Furthermore, participants with soy-specific IgE had threefold greater odds of current asthma or asthma-like symptoms, and sixfold greater odds of work-related asthma-like symptoms [17].

Despite these findings implicating soy sensitization in this facility's occupational asthma, the aetiological agents of sensitization remain uncharacterized. In this study, we aimed to identify and characterize soy proteins that SPWs were reacting to at this Tennessee processing facility. Occupational sensitization to 5-enolpyruvylshikimate-3-phosphate synthase (CP4-EPSP) from transgenic soy (TG) was additionally explored.

Materials and methods

Participants

Current employees of a Tennessee soy flake processing facility were invited to participate in a study that included allergy testing; specifically skin prick testing (SPT) and measurement of total and specific IgE levels. SPT using the GreerPick system (Greer Laboratories, Lenoir, NC, USA) included: soy, birch mix, cat hair, cockroach mix, Eastern 10 tree mix, house dust mite mix (*Dermatophagoides farinae* and *D. pteronyssinus*), ragweed mix, and nine Southern grass mix as described previously [17]. Birch mix was included because of the known immunological cross-reactivity between soy and birch allergens [27]. Participants provided written informed consent. De-identified sera from a population of 50 HCWs [28] not occupationally exposed to soy were used as controls. The NIOSH Human Subjects Review Board approved the study.

Determination of total and specific immunoglobulin E and G

Blood samples (20 mL) were collected from each participating SPW by venipuncture and serum was separated *in vitro* for quantitative measurement of total IgE, specific IgE, and specific IgG. Serum samples were transferred to NIOSH and stored at $-80\,^{\circ}$C until analysis. Samples were analyzed for total IgE by fluoroenzymeimmunoassay using an ImmunoCAP 100 (Phadia AB, Uppsala, Sweden). Total IgE was considered to be elevated when the titre exceeded 100 kU/L. Specific IgE levels were measured using ImmunoCAP for soy (f14), peanut (f13), and storage mite (d71) (Phadia AB). Specific IgE was considered detectable when titres exceeded 0.35 kU/L. Specific IgG levels were also measured using ImmunoCAP for soy (Gf14), and specific IgG <0.02 mg/L was considered undetectable. Peanut and storage mite were included in the analysis to address possible immunological cross-reactivity between soy and peanut antigens as well as sensitization to other aeroallergens [29]. Additionally, levels of soy-specific IgE and IgG were determined in HCWs not occupationally exposed to soy [28].

Soy protein extraction

Bulk pre-processed wild-type (WT) and TG soy flakes derived from the Tennessee processing facility were extracted in phosphate-buffered saline (PBS; pH 7.4) at 10% (w/v) for 3 h at $4\,^{\circ}$C under constant agitation on a rotary shaker. Crushed SH collected from a farming operation in Indiana was extracted using the same procedure. Following extraction, particulate matter was removed by centrifugation for 10 min at 4100 g. The WT, TG, and SH extracts were then lyophilized and resuspended in 6 mL of distilled H_2O (dH_2O). Protein concentrations were estimated using a NanoDrop ND1000 Spectrometer at 280 nm (Thermo Scientific, Wilmington, DE, USA). The supernatant fluid was then aliquoted and stored at $-80\,^{\circ}$C until used for further analysis.

Soy immunoglobulin E inhibition ImmunoCAP

IgE inhibition assays were conducted using pooled sera from 13 participants with the highest titres of soy-specific IgE. Equal volumes of pooled sera and soy extracts were mixed before addition to the soybean (F1) disc and ImmunoCAP analysis. Serial dilutions of the bulk WT soy flake extract (see 'Soy protein extraction') were used to construct a standard inhibition curve and allergen levels in unknowns were determined by comparison with the bulk soy flake extract using an assignment of 2600 arbitrary units (AU). IgE inhibition assays were used to determine the relative soy allergen content of bulk samples of pre-processed and processed soy flakes.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and western blot analysis

Soy extracts (200 μg) were separated by SDS-PAGE on 12% and 16% acrylamide gels according to the method of

Laemmli [30]. For immunoblots, SDS-PAGE gels were electrophoretically transferred overnight to a nitrocellulose membrane (0.2 μm, BioRad, Hercules, CA, USA) as described previously [31]. Membranes were stained with Ponceau S solution (Sigma-Aldrich, St Louis, MO) to visually assess the transfer of the proteins, destained, and blocked for 1 h with 3% bovine serum albumin (BSA; Sigma-Aldrich) in PBS. After blocking, the membrane was incubated with individual participant sera diluted 1 : 5 (v/v) in 3% BSA/PBST for 3 h at room temperature (BioRad multiscreen apparatus, BioRad). After washing three times in PBST the membrane was incubated for 90 min with a 1 : 5000 dilution of alkaline phosphatase-labelled affinity-purified goat anti-human IgE (KPL Inc., Gaithersburg, MD, USA) in 3% BSA-PBST. After washing three times, bound antibody was visualized using nitroblue tetrazolium and bromo-chloro-indolyl phosphate (NBT/BCIP, Promega, Madison, WI, USA). Negative controls included (1) labelled affinity-purified goat anti-human IgE and NBT/BCIP, (2) NBT/BCIP alone, and (3) HCWs control sera not sensitized to soy [28]. Soy antigens from both WT and TG soy flakes were tested in duplicate.

5-enolpyruvylshikimate-3-phosphate synthase antibody analysis

The presence of CP4-EPSP in both WT and TG extracts was visualized by immunoblot using the anti-CP4-EPSP monoclonal antibody provided in the Quantiplate Kit (Envirologix, Portland, ME, USA). WT and TG extracts were separated by SDS-PAGE and transferred to nitrocellulose as described above. Blocked WT or TG membranes were incubated with horseradish peroxidase-conjugated anti-CP4-EPSP antibody (QuantiPlate Kit, Envirologix) diluted 1 : 20 (v/v) in 3% BSA/PBST for 1 h at room temperature. After washing three times, CP4 EPSP was identified by incubation (5 min) with one-component TMB Membrane Peroxidase Substrate (KPL Inc.).

Two-dimensional gel electrophoresis and immunoblot analysis

Soy extracts (0.5 mg/mL in dH_2O) were processed with a ReadPrep^{TM} 2D Cleanup Kit (BioRad). The protein pellet was solubilized in rehydration solution [8 M urea, 2% CHAPS, 0.5% IPG Buffer, 20 mM dithiothreitol (DTT), trace bromophenol blue] and loaded onto 7 cm IPG strips (pH 3-10, GE Healthcare, Uppsala, Sweden) and focused at 3500 V for 1 h, 3500 V for 1.5 h, and 200 V for 1 h (IPGphor instrument, GE Healthcare). IPG strips were equilibrated for 30 min at room temperature in equilibration buffer (50 mM Tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 65 mM DTT, trace bromophenol blue), placed on a 12% acrylamide separating gel, and electrophoresed for 2 h at 100 V. The gel was stained in Imperial Stain Solution (Thermo Scientific, Rockford, IL, USA) for 1 h at room temperature, destained in dH_2O overnight, and then the images were scanned and stored for further analysis. Immunoblots were performed as described above and immunostained using a different SPW serum pool ($n = 13$) to the high titre SPW pool used in the soy IgE inhibition ImmunoCap experiments. In this SPW serum pool, 13 sera were selected with a similar pattern of immunoreactivity (Fig. 2a and b, Lanes 22–34). The SPW serum pool was diluted 1 : 10 (v/v) in 3% BSA/PBST. Negative controls included (1) labelled affinity-purified goat anti-human IgE and NBT/BCIP, (2) NBT/BCIP, and (3) HCWs control sera not sensitized to soy. Soy antigens from both WT and TG soy dust were probed with SPWs serum pool at least twice.

Proteomic identification of soy flake proteins

Proteins of interest identified by two-dimensional (2D) immunoblot analysis were excised from Imperial stained 2D gels prepared in parallel. Excised spots were digested with porcine trypsin (Protea Biosciences, Morgantown, WV, USA) according to published methods [32, 33]. Digestions (37 °C overnight) were combined with all wash supernatants and dried in a centrifugal evaporator (DNA120, Thermo Scientific, Waltham, MA, USA) and subsequently resuspended in 20 μL 0.1% trifluoroacetic acid.

Tryptic peptides were purified and separated using a nanoACQUITY UPLC (Waters, Milford, MA). An 8 μL aliquot of each sample was trapped and desalted on a Symmetry C18 (5 μm particle size, 180 μm × 20 mm) trapping column for 5 min at 3 μL/min (97/3, 0.1% formic acid/0.1% formic acid in acetonitrile). Samples were subsequently eluted and analyzed on an XBridge BEH130 C18 (1.7 μm particle size, 100 μm × 100 mm) analytical column at 25 °C using a gradient of 97/3 (0.1% formic acid/0.1% formic acid in acetonitrile) to 60/40 (0.1% formic acid/0.1% formic acid in acetonitrile) over 120 min at a flow rate of 250 nL/min.

Eluent from the nanoscale Ultra-Performance Liquid Chromatography (nanoUPLC) was directed to the source of a SYNAPT quadrupole time-of-flight mass spectrometer (Waters) operated in positive nanoelectrospray mode (+ESI) at a potential of +3 kV. Dry nitrogen gas was generated in the laboratory (NitroFlowLab, Parker Hannifin, Cleveland, OH, USA) and supplied to both the nanoelectrospray source and the sampling cone of the mass spectrometer. Data were acquired in MSe mode [33] using argon as collision gas (low collision energy 4 eV/ high collision energy 15–25 eV). Mass spectra were externally calibrated using the doubly charged monoisotopic peak of [Glu^1]-fibrinopeptide B (785.8421 u) at 30 s intervals, supplied via a second nanoelectrospray emitter perpendicular to the source and selected via a mechanical rotating baffle.

Nanoscale UPLC Tandem Mass Spectrometry (NanoUPLC-MS/MS) data were queried against the SwissProt database using the ProteinLynx search algorithm (ProteinLynx Global Server, Ver. 4.3, Waters). Database searching was constrained using trypsin as enzyme, mass error ($m/\Delta m$) < 5 ppm and a minimum of two peptides for positive identification.

Results

Soy processing worker reactivity

Of the 147 (52%) SPWs who consented to participate, 118 participants were male and 29 were female with a median age of 46 years (range 19–66). Other demographic characteristics including race, smoking status, current work classification, and the median tenure at the soy processing facility have been described elsewhere [17]. A majority of participants underwent SPT ($n = 132$; 90%) and provided blood samples for immunological analysis ($n = 135$; 92%).

A total of eight aeroallergens were tested by SPT and 57 (43%) of the 132 participants reacted to at least one SPT extract as described previously [17]. The most common allergen extract that elicited a positive SPT were the southern grasses mix (30%), ragweed mix (18%), and house dust mite mix (17%). Nine (7%) SPWs had a positive SPT response to soybean.

Table 1 shows the total IgE and soy-specific IgE serum test results among SPWs and HCWs controls. All 135 SPWs had detectable IgG to soy and concentrations ranged between 0.5 and 2100 mg/L (mean 97.9 mg/L). Fifty-five (41%) SPWs had elevated total IgE levels and 27 (20%) had specific IgE to soy (>0.35 kU/L). In contrast, 27 of 50 (54%) HCWs with no *a priori* occupational soy exposure, had detectable IgG to soy and just two of 50 (4%) HCWs had detectable soy-specific IgE.

Allergen reactivity

Imperial stained protein profiles of WT and TG extracts demonstrated comparable profiles. The SH extract; however, displayed a distinct profile and contained additional low molecular weight proteins (Fig. 1). Immunoblotting performed using individual sera from 27 SPWs with soy-specific IgE (Fig. 2) showed IgE reactivity to multiple soy antigens ranging from 17 to 159 kDa. Several bands were recognized by most of the participants, while others appeared to be unique to an individual. The major proteins that bound SPW IgE were localized at 48, 54, and 62 kDa and recognized by 81%, 67%, and 63% of the SPWs serum IgE, respectively. Other proteins that bound 30–50% of the SPWs serum IgE and these were localized at 29, 33, 41, 69, 112, and 159 kDa. Comparable IgE reactivity patterns were observed between WT and TG soy extracts (Figs 2a and b).

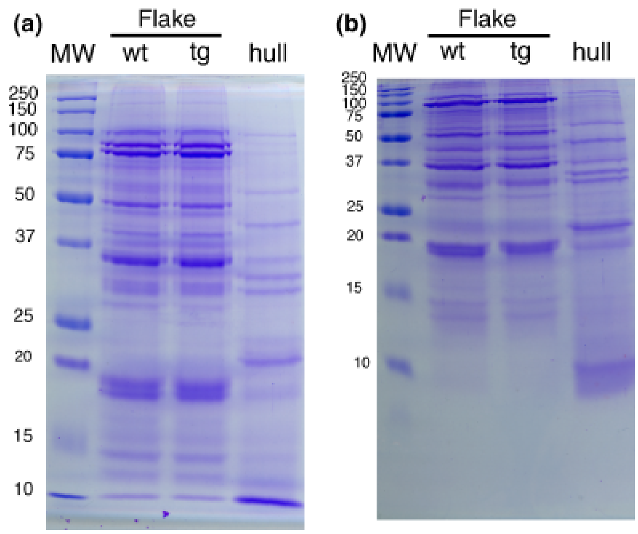

Fig. 1. One-dimensional SDS-PAGE of Imperial stained soy-flake and soybean hull (SH) proteins on 12% (a) and 16% (b) acrylamide gels. Proteins were collected from both wild-type (WT) and transgenic (TG) flake samples and crushed SH from a farming operation located in Indiana. MW, molecular weight standard.

Table 1. Total and specific IgE results among soy processing workers (SPWs) and health care worker controls (HCWs) control subjects ($n = 50$)

	SPWs		HCWs	
Total and specific IgE levels	*n* (%) Positive*	Mean† IgE kU/L (range)	*n* (%) Positive*	Mean† IgE kU/L (range)
Total IgE	55 (41)	489.07 (102–7202)	9 (18)	255.0 (113.0 to 592.0)
Soybean IgE	27 (20)	2.78 (0.36–15.7)‡	2 (4)	0.50 (0.45 to 0.54)
Peanut IgE	23 (17)	3.10 (0.37–18.7)	2 (4)	0.48 (0.35 to 0.60)
Storage mite IgE	14 (10)	1.56 (0.37–6.24)	5 (10)	0.98 (0.37 to 1.94)

*Total IgE is considered positive when titres exceed 100 kU/L. Specific IgE results are considered positive when titres exceed 0.35 kU/L. The numbers of SPWs with positive soybean IgE and peanut IgE are slightly lower than those reported previously [17]. In the previous report, SPWs with specific IgE <0.35 kU/L and an alternative scoring method quotient >90% were also classified as having positive specific IgE for epidemiologic analyses. This approach resulted in one additional SPW with positive soybean IgE and one additional SPW with positive peanut IgE than shown here.

†Mean IgE kU/L calculated by substituting 0.175 for values <0.35. Mean IgE values calculated for those SPWs and HCWs with total and specific IgE greater than 100 and 0.35 kU/L, respectively.

‡All tested SPWs ($n = 135$) had detectable IgG to soy. 54% of HCWs ($n = 50$) with no *a priori* occupational soy exposure, had detectable IgG to soy.

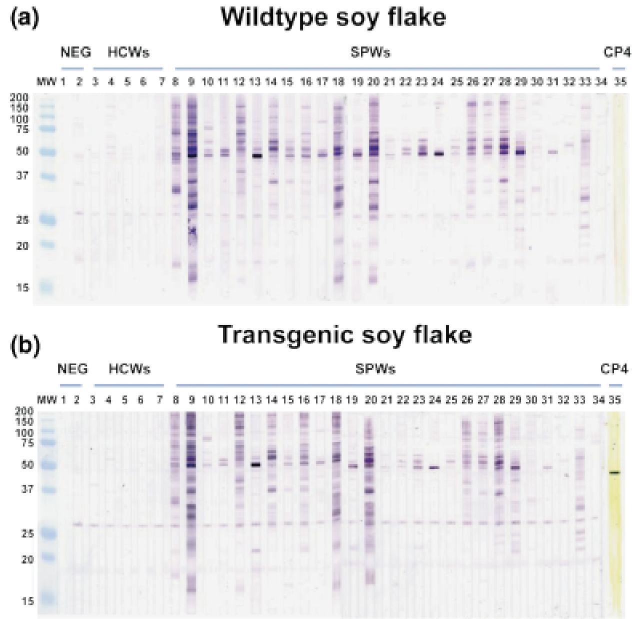

Fig. 2. Soy flake processing workers (SPWs) IgE reactivity to wild-type (WT) and transgenic (TG) soy flake extracts. Lanes 1–2: NBT/BCIP and labelled affinity purified goat anti-human IgE non-specific binding control (NEG); Lanes 3–7: HCW sera (<0.35 kU/L soy IgE); Lanes 8–34: SPW sera (>0.35 kU/L soy IgE); Lane 35: Monoclonal anti-CP4-EPSPS. MW, molecular weight standard.

CP4-EPSP was specifically identified in the TG soy extracts but not in the WT extracts (Fig. 2b). CP4-EPSP-specific IgE was not detected using individual SPWs serum (Fig. 2). Sensitization to lower molecular weight SH allergens (Gly m 1 and Gly m 2), was explored using 16% acrylamide separating gels but no IgE immunoreactivity was observed to SH-specific allergens <10 kDa (Fig. 3). Non-specific secondary antibody staining was detected in negative controls with a particularly prominent band at 27 kDa and weak reactivity at 19, 45, 82, and 93 kDa (Fig. 2).

The influence of processing steps at the plant on WT soy IgE reactivity was explored. Using sera pooled from 13 soy-sensitized workers, inhibition assays demonstrated that soy allergen levels were higher in pre-processed soy flakes than processed soy flakes (Table 2). The soy flake extract was assigned a value of 2600 AU. By comparison, the processed soy powders averaged a 56% reduction in IgE reactivity. Since the total protein content did not change quantitatively, this reduction presumably reflects structural changes in the soy protein allergens during processing. Processed soy powder extracts showed additional high molecular weight bands compared with pre-processed soy extracts, which likely represents protein aggregation (data not shown).

Pooled sensitized sera ($n = 13$) was used to immunostain 2D blots of WT or TG soy antigens (Fig. 4). IgE reactivity was greatest between ~50 and 75 kDa in the neutral pH range. Several alkaline and acidic proteins ranging from ~25 to 60 kDa were identified to bind specific IgE. Comparable IgE reactivity patterns were observed between WT (Fig. 4a) and TG (Fig. 4b) soy extracts. Immunostained proteins were identified using nanoUPLC-MS/MS. Peptide sequence analysis indicated that the prominent proteins that bound SPW IgE were primarily derived from the cupin family of proteins [34], specifically the soybean storage proteins, β-conglycinin (Gly m 5) and glycinin (Gly m 6) (Fig. 4). β-conglycinin α and β chain precursors were identified to have the greatest IgE reactivity in Western blotting. Glycinin (Gly m 6) G1, G3, and G4 subunits were also prominent and identified by proteomic analysis to bind SPW IgE. Although the majority of proteins sequenced were β-conglycinin (Gly m 5) and glycinin (Gly m 6), other proteins including sucrose binding precursor and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase were also identified in the analysis (Fig. 4). These proteins may be new soy proteins that bind IgE; however, only two peptides that covered 4% and 9% of the sequence were identified in the analysis. Weak non-specific binding of the secondary antibody was found to be to basic 7S globulin precursor (Fig. 4).

Table 2. IgE inhibition ImmunoCap analysis of allergen content from preprocessed and processed soy flake products

Sample	Allergen concentration (arbitrary units)	Allergen reduction (% of pre-processed flakes)
Pre-processed soy flakes	2600	–
Processed soy flake (M33)	1050	60%
Processed soy flake (M35)	1276	51%
Processed soy flake (M34)	1151	56%

Discussion

In 2007, NIOSH conducted an investigation at a soy processing facility in Tennessee following initial reports by some SPWs that occupational exposure to soy caused or exacerbated various adverse health effects [17]. Unlike other facilities where workers may handle SH, soybean, or a combination of both soy products, the workers at this facility only processed dehulled and defatted soybean flakes. Environmental sampling demonstrated measurable concentrations of inhalable soy antigens that varied by work area and job title [17]. Participants had a significantly higher than expected prevalence of physician-diagnosed asthma, sinusitis, and wheeze compared with the US adult population [35]. Outcomes including current asthma, asthma-like symptoms, and work-related asthma-like symptoms were significantly associated with allergic sensitization to soy [17]. These results were not confounded by immunological cross-reactivity to other allergens such as birch, peanut, and storage mite [17]. In the

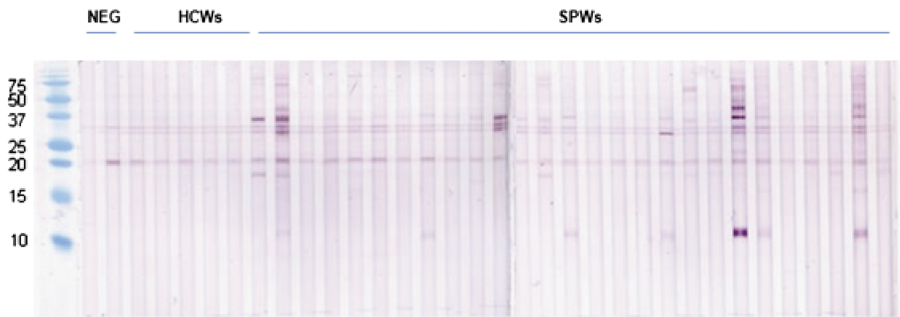

Fig. 3. Soy flake processing worker (SPW) IgE reactivity soybean hull (SH) extracts. Lanes 1–2: NBT/BCIP and labelled affinity purified goat anti-human IgE negative control (NEG); Lanes 3–7: HCW sera; Lanes 8–34: SPW sera; MW, molecular weight standard.

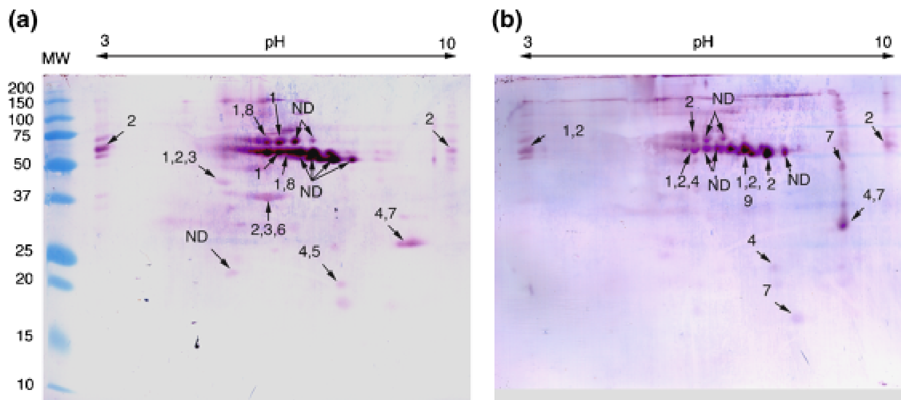

Fig. 4. Identification of IgE-binding antigens in wild-type (WT) and transgenic (TG) soy extracts. Membranes were immunostained with pooled soy flake processing worker (SPW) serum IgE (serum pool was derived from 13 SPWs with a similar pattern of immunoreactivity to WT and TG soy extracts; Fig. 2, Lanes 22–34); reactive proteins were then excised from corresponding Imperial protein stained 2D gels and identified using mass spectrometry. (a) Mass spectrometric protein characterization of soy occupational allergens derived from WT extract (UniProtKB/Swiss-Prot accession number) include (1) β-conglycinin, α chain precursor (P13916, P11827), (2) β-conglycinin, β chain precursor (P25974), (3) Glycinin precursor contains Glycinin A3 subunit (P04347), (4) Glycinin G1 precursor contains Glycinin A1A subunit (P04776), (5) Glycinin G3 precursor contains Glycinin A subunit (P11828), (6) Glycinin G4 precursor contains Glycinin A5 subunit (P02858), (7) Basic 7S globulin (P13917), (8) Sucrose binding protein (Q04672). (b) Mass spectrometric protein characterization of soy occupational allergens derived from TG extract (UniProtKB/Swiss-Prot accession number) include (1) β-conglycinin, α chain precursor (P13916), (2) β-conglycinin, β chain precursor (P25974), (4) Glycinin G1 precursor contains Glycinin A1A subunit (P04776), (7) Basic 7S globulin (P13917), (9) NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (Q43272). ND, not detectable; MW, molecular weight standard.

present study, we identified and characterized the allergens to which SPWs were exposed and sensitized in their occupational environment.

Community asthma epidemics due to aerosolized soy have been reported in the United States and Spain [7–10, 13]. The unloading of soy from container vessels docked in shipping ports aerosolizes significant quantities of soy allergen into the surrounding environment [7, 10, 11, 13]. Patients who presented on asthma epidemic days were more likely to be sensitized to SH than patients on non-epidemic days [7]. Although these studies did not bronchially challenge subjects with soy extracts, immunological analysis of the extracts demonstrated that patients were sensitized to low molecular weight allergens associated with the protective SH that encases the soybeans [12, 36, 37]. IgE binding was greatest to SH allergens in patients that had asthma exacerbations during the unloading of soy at seaports [12, 37]. These allergens were later designated Gly m 1 (7 kDa), a hydrophobic SH protein and Gly m 2 (8 kDa), a defensin [12, 37].

Allergens derived from soy are well characterized food and inhalation allergens [2–6, 38, 39]. Occupational sensitization to soy has also been reported [14–20, 24, 25]; however, the allergens associated with many of these working groups are less well characterized except for bakers' asthma [15, 19, 24–26]. Baker and confectioner IgE reactivity is to higher molecular weight soybean storage proteins [15, 19, 24–26] that have been associated with occupational asthma [15, 25]. Assessments of soy allergy in occupational environments are difficult as dust composition varies depending on the occupation and work environment. In the current study, immunoblotting demonstrated that the 20% of workers with soy-specific IgE were sensitized to higher molecular weight proteins derived from the cupin family of proteins [34], specifically β-conglycinin (Gly m 5) and glycinin (Gly m 6) but not allergens of SH (Gly m 1 and Gly m 2). These findings are consistent with the results previously reported in bakers' asthma studies; however, we were unable to bronchially challenge the workers with soy extracts to determine whether occupational respiratory morbidity was predominantly associated with soy exposures.

Transgenic modifications to incorporate CP4-EPSP derived from *Agrobacterium tumefaciens* have facilitated crop resistance to the herbicide, glyphosate (Roundup®). The health effects associated with ingestion of CP4-EPSP containing soy has been extensively studied [38, 40–43]. These previous studies used food sensitized patients and no IgE immunoblot reactivity to CP4-EPSP was observed [38, 40, 42]. In the present study, inhalational exposure to TG soy did not result in specific IgE to a 48 kDa band identified as CP4-EPSP. Given the potential for inhalational exposures to CP4-EPSP in SPWs, these results further suggest that exposure to CP4-EPSP did not produce sensitization. However, bronchial challenge with purified CP4-EPSP would be required to definitively demonstrate the lack of allergenicity of CP4-EPSP.

The influence of the production process on the IgE binding by the soy proteins was additionally evaluated. Extracts from post-processed soy were shown to have less allergen compared with pre-processed soy extracts. Although lot-to-lot variation was not examined, soy flakes undergo various preparative processes during the production phase, including heat, chemical, and washing treatments. Heat during transportation and storage has been shown to generate additional soy allergen determinants [44]; however, we found that other chemical and preparative processes used at the Tennessee processing plant appeared to reduce antigenicity. Further study would be required to understand the influence of the soy production process on high molecular weight soybean storage allergens.

In this study, the prevalence of sensitization to soy was higher by ImmunoCAP analysis than by SPT [17]. Discordance between immunodiagnostic tests is well known for other aeroallergen sources; however, the protein and allergen contents of commercially available soy SPT extracts have been previously shown to contain Gly m 5 and Gly m 6 [45]. In the present study, the sources of soy for the commercial SPT and ImmunoCAP extracts are not known and could have included SH proteins, soybean storage proteins, or a combination of both SH and soybean allergens. This may account for some of the reported variations between SPT and ImmunoCAP results. In addition, allergen cross-reactivity by cross-reactive carbohydrate determinants (CCD) could also account for the observed discordance [27, 46–48]. The *N*-linked glycan moieties of soy allergens, including β-conglycinin and Gly m Bd 28k have been previously reported to be IgE determinant sites [46–48]. Inhibition with glycan moieties or deglycosylation significantly reduced IgE binding to Gly m Bd 28k but it was not completely eliminated in some patients [47]. Although these studies demonstrate that glycan moieties may contribute to the over estimation of soy reactivity, the clinical significance of CCDs in soy sensitization remains the subject of continued clinical research. Furthermore, bronchial challenge, SPT, and inhibition immunoblot studies of SPWs to purified high molecular weight soybean storage allergens Gly m 5 and Gly m 6 may also provide further evidence as to whether respiratory morbidity is associated with occupational exposure to these allergens.

NIOSH recommended that the company take a number of steps to reduce worker exposures to dusts in the processing plant [17]. These included engineering controls, such as additional ventilation and the installation of process enclosures and changes in work practices, such as cleaning spills with HEPA filtered vacuums instead of shovelling, brushing, or sweeping. In addition, the use of personal protective equipment was included as measures to reduce personal exposure to soy at the processing facility [17]. Despite these steps, sensitized workers with work-related asthma may need to be relocated away from further exposure.

In conclusion, occupational exposure to soy flake was associated with sensitization to soy and asthma outcomes. Immunoblotting analysis demonstrated that SPWs with soy-specific IgE reacted to multiple soy antigens. The most relevant proteins that bound IgE included the high molecular weight soybean storage proteins belonging to the cupin family of proteins, specifically β-conglycinin (Gly m 5) and glycinin (Gly m 6); allergens known to be involved in baker's asthma. Occupational sensitization amongst SPWs was distinct to that observed in community asthma epidemics, in that, IgE reactivity was not observed to the low molecular weight SH allergens. SPWs are predominantly exposed to dehulled soy flake, thus reactivity to hull allergens was not expected or observed, but we cannot rule out that these proteins are not relevant occupational allergens in other soy processing facilities. Variations in IgE reactivity were not observed between WT and TG extracts further suggesting that CP4-EPSP is not an allergen. Overall, the results of this study demonstrate that workplace exposures to higher molecular weight soybean storage proteins, β-conglycinin (Gly m 5) and glycinin (Gly m 6), may contribute to occupational sensitization and respiratory morbidity.

Clinical relevance

High molecular weight soybean storage allergens, β-conglycinin (Gly m 5) and glycinin (Gly m 6), may contribute to occupational sensitization and respiratory morbidity among SPWs.

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