

A Mouse Model of Anaphylaxis and Atopic Dermatitis to Salt-Soluble Wheat Protein Extract

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Keywords

Wheat allergy · Systemic anaphylaxis · Mouse model · Immediate hypersensitivity · Atopic dermatitis · Cytokine · Chemokine

Abstract

Background: Wheat allergy and other immune-mediated disorders triggered by wheat proteins are growing at an alarming rate for reasons not well understood. A mouse model to study hypersensitivity responses to salt-soluble wheat protein (SSWP) extract is currently unavailable. Here we tested the hypothesis that SSWP extract from wheat will induce sensitization as well as allergic disease in mice. **Methods:** Female BALB/cJ mice were weaned onto a plant protein-free diet. The mice were injected a total of 4 times with an SSWP (0.01 mg/mouse) fraction extracted from durum wheat along with alum as an adjuvant. Blood was collected biweekly and SSWP-specific IgE (SIgE) and total IgE (TIgE) levels were measured using ELISA. Systemic anaphylaxis upon intraperitoneal injection with SSWP was quantified by hypothermia shock response (HSR). Mucosal mast cell degranulation was measured by the elevation of mMCP-1 in the blood. The mice were monitored for dermatitis. Skin tissues were used in histopathology and for measuring cyto-

kine/chemokine/adhesion molecule levels using a protein microarray system. **Results:** Injection with SSWP resulted in time-dependent SIgE antibody responses associated with the elevation of TIgE concentration. Challenge with SSWP elicited severe HSR that correlated with a significant elevation of plasma mMCP-1 levels. Sensitized mice developed facial dermatitis associated with mast cell degranulation. Lesions expressed significant elevation of Th2/Th17/Th1 cytokines and chemokines and E-selectin adhesion molecule. **Conclusion:** Here we report a mouse model of anaphylaxis and atopic dermatitis to SSWP extract that may be used for further basic and applied research on wheat allergy.

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Introduction

Hypersensitivity reactions to wheat proteins, including wheat allergy, are increasingly recognized as a growing public health problem of global significance adversely affecting both wheat consumers as well as the wheat industry [1, 2]. Recent estimates show that the combined prevalence of celiac disease, wheat allergy, and non-celiac gluten sensitivities in the USA is surprisingly up to 10% [1–3]. Wheat allergy, in particular, affects both children

and adults with an estimated overall prevalence of up to 1–3% in the USA [2, 3].

Wheat is the third most produced cereal (after corn and rice) globally [4, 5]. A wheat-free diet is the only recommended treatment for wheat allergy at present, thus necessitating millions of wheat-sensitive consumers around the world to avoid wheat for life [1, 2, 6]. This has resulted in major negative impacts on the wheat industry and on the quality of life of wheat-sensitive consumers and their families [1–3, 7]. As a result, the economic impact of the health issues related to wheat on the global wheat industry is expected to grow at an unprecedented rate [3–5]. Consequently, urgent intervention methods are needed to address this escalating global food safety issue. However, fundamental mechanisms about why and how wheat proteins trigger a variety of immune-mediated disorders are incompletely known, making it difficult to develop novel intervention strategies.

Wheat is implicated in multiple types of immune-mediated adverse reactions, as follows: (i) wheat causes typical food allergy (e.g., anaphylaxis, atopic dermatitis, etc.) mediated by IgE antibodies; (ii) it causes non-IgE or cell-mediated reactions (e.g., eosinophilic esophagitis, eosinophilic gastritis); (iii) it also triggers autoimmune diseases associated with autoantibody production and autoreactive T cell responses (celiac disease, dermatitis herpetiformis); (iv) it is linked to neuroimmune reactions (e.g., gluten ataxia), and (v) more recently, it is linked to a new disease known as non-celiac gluten sensitivity [1–3, 6–9].

It is noteworthy that wheat proteins are thought to initiate the adverse immune reactions reviewed above. Interestingly, wheat contains not only salt-soluble allergens but also alcohol-soluble and acid-soluble protein allergens that can trigger IgE-mediated reactions in humans [8, 1–3]. Previously, elegant mouse models of wheat hypersensitivity to alcohol-soluble wheat gluten proteins were developed [10–14]. One study evaluated the immune response to purified lipid transfer protein (LTP) in mice [11]. However, anaphylaxis and a dermatitis disease model has so far not been reported for LTP. Furthermore, a mouse model to study systemic anaphylaxis, mast cell degranulation responses, dermatitis, and cytokine/chemokine responses to the salt-soluble wheat protein (SSWP) extract is also unavailable at present.

Here we tested the hypothesis that SSWP extract from a durum wheat will not only sensitizes BALB/c mice, but will also elicit allergic disease. Our results demonstrate the development of immediate hypersensitivity to SSWP with robust readouts of sensitization as well as systemic anaphylaxis and mucosal mast cell degranulation. We also re-

port the development of facial dermatitis lesions in SSWP-sensitized mice and identify selectively elevated cytokines, chemokines, and adhesion molecules in the lesion.

Materials and Methods

Chemicals and Reagents

The following were purchased from the indicated sources in parenthesis. Biotin conjugated rat anti-mouse IgE paired antibodies and isotype standards (BD BioSciences, San Jose, CA, USA); p nitro phenyl phosphate (Sigma, St Louis, MO, USA); Streptavidin alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA, USA).

Mice

BALB/cJ female weanlings (The Jackson Laboratory, Bar Harbor, ME, USA) were maintained on a plant protein-free diet (AIN-93M) throughout the study. Adult-female mice (4–6 weeks old) were used in sensitization as described below. Adult female mice without sensitization were kept in the same room and used as the controls. All animal procedures used were in accordance with the Michigan State University policies.

Preparation of SSWP Extract from Durum Wheat Flour

Flour of the Carpio variety, a durum type of wheat, was used for the protein extraction. The salt-soluble protein fraction was prepared using a standard method previously published [15]. Ten grams of flour in 100 mL of 0.5 M NaCl were stirred continuously for 2 h at 20°C followed by centrifugation (5,000 g, 10 min) at 20°C. The supernatant was frozen overnight at –70°C and then freeze-dried. The protein content was measured by the Bio-Rad method. Freeze-dried samples were stored at –70°C until use.

Sensitization and Bleeding

Groups of mice ($n = 4$ –5/group) were injected by the intraperitoneal (IP) route 4 times (days 0, 10, 24, 40) with SSWP extract (0.01 mg/mouse/injection) plus alum (1 mg/mouse). Sham-sensitized mice received alum alone as an injection. Blood was collected from the saphenous vein on days 26 and 46 after the first injection.

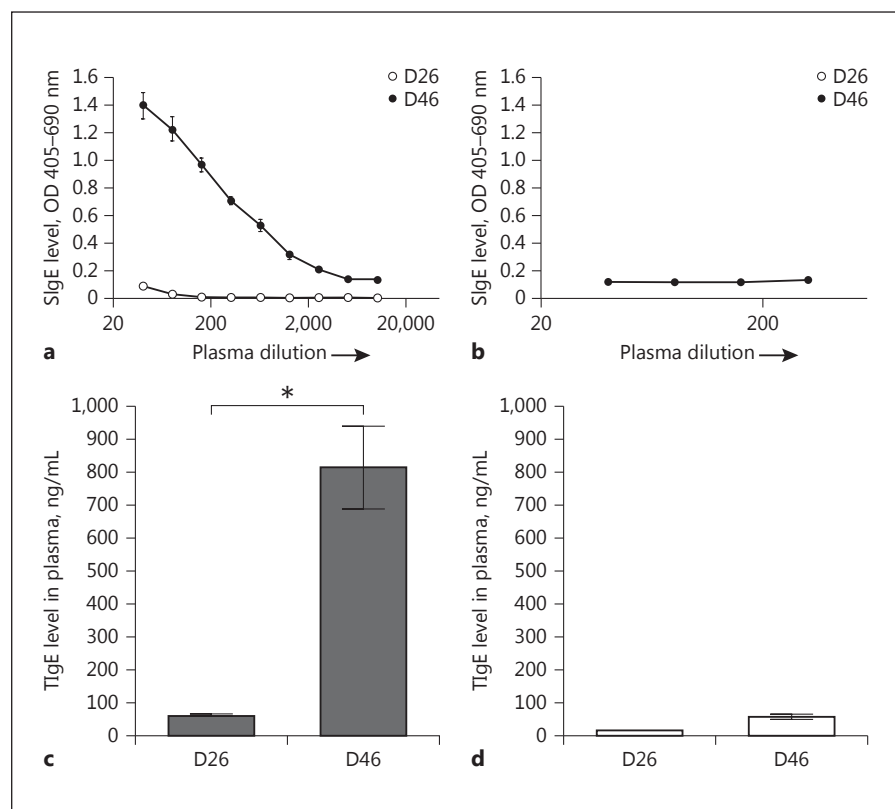
Measurement of SSWP-Specific IgE Antibody Levels and Total Plasma IgE Concentration

SSWP-specific IgE (SIgE) and total IgE (TIgE) were measured using optimized ELISA-based methods described by us previously [16–18]. Briefly, the TIgE was quantified by a sandwich ELISA using paired antibodies (i.e., a capture anti-mouse IgE antibody and a biotin-labelled anti-mouse IgE detection antibody) and an IgE isotype standard (BD Biosciences). The SIgE levels were measured by coating ELISA plates with SSWP (5 mg/mL) followed by blocking, sample additions, and then detection using a biotin-labelled anti-mouse IgE detection antibody (BD Biosciences) as described previously [16–18].

Systemic Challenge and Quantitation of Hypothermia Shock Response by Measurement of Rectal Temperature

The SSWP-sensitized mice and sham-sensitized mice were challenged by the IP route with SSWP (0.5 mg/mouse). Rectal tem-

Fig. 1. Development of specific IgE antibody response and elevation of plasma TIgE levels upon injection with SSWP fraction in BALB/cJ mice. Groups of mice ($n = 4/\text{group}$) were injected with SSWP fraction (0.01 mg/mouse) as described in Materials and Methods; blood was collected on the indicated days after injection and SIgE antibody levels and plasma TIgE concentrations were determined using ELISA-based methods. **a** SIgE antibody levels on day 26 (D26) and day 46 (D46) after first sensitization in allergic mice. **b** SIgE antibody levels on D26 and D46 after sham sensitization (i.e., alum alone-injected mice). **c** TIgE concentration (mean $\pm$ SE) on D26 and D46 after first sensitization in allergic mice. * $p < 0.05$. **d** TIgE antibody levels on D26 and D46 after sham sensitization (i.e., alum alone injected mice).



perature (RT) was measured before and at every 10 min up to 30 min after challenge, and the change in temperature was calculated and used in analyses.

Quantitation of Mucosal Mast Cell Protease-1 Levels in the Blood

Plasma samples were used in the measurement of mMCP-1 levels using an ELISA-based kit. The protocol supplied by the manufacturer was used in the analysis (eBioscience).

Clinical Scoring of Dermatitis Lesions

Mice were monitored for the development of any symptoms of dermatitis. The progression of the lesions was monitored and images were captured using digital photography. The severity of dermatitis was determined using the following scoring method based on the literature. The clinical severity of the lesion was determined based on symptoms of redness, swelling, oozing/crusting, skin thickening, and the surface area affected. The intensity was assessed as none (0), mild (1), moderate (2), or severe (3). The dermatitis scores were expressed as the sum of the individual score graded from 0 to 3 for each of the criteria (maximum combined score 15, minimum 0).

Skin Tissue Collection, Protein Extraction, and Analysis of Cytokines, Chemokines, and Adhesion Molecules

Mice were euthanized within 1 h after IP challenge with SSWP (0.5 mg/mouse). Dermatitis-affected skin tissues from allergic mice and from sham-sensitized mice were collected, snap-frozen

in liquid nitrogen, and stored at -70°C . The protein extract was prepared using the method described by us previously [18]. Briefly, skin tissue was weighed and immersed in T-PER extraction buffer (Thermo Scientific 78510) containing protease inhibitor (Sigma P8340; 10 μL inhibitor per 1 mL of T-PER buffer). The ratio of tissue to extraction buffer (1 mL per 100 mg of tissue) was kept constant. The tissue was homogenized by ultrasonication for 30 s, twice, at an interval of 5 min. After 15 min of resting, the mixture was centrifuged (13,500 g, 10 min) at 4°C . Supernatant was collected and the protein concentration determined (Bio-Rad kit). Protein supernatants were frozen in aliquots at -70°C until they were sent on dry ice for the analyses of immune markers. The Ray Biotech's Quantibody array (Mouse Cytokine Array 4000) service was used for the quantitation of cytokines, chemokines, and adhesion molecule levels. Samples were run in quadruplicate using standards as detailed by the service provider (<http://www.raybiotech.com/mouse-cytokine-array-q4000.html>).

Histopathology and Mast Cell Degranulation Analysis

Formalin-fixed skin tissues were processed by the Michigan State University Histopathology and Cytology Service Laboratory using standard methods described previously [18]. Paraffin sections (Reichert Jung 2030 rotary microtome) were stained with toluidine blue. The numbers of mast cells (intact and degranulated) in 20 high-power fields were counted and the percent degranulation was calculated as described earlier [18].

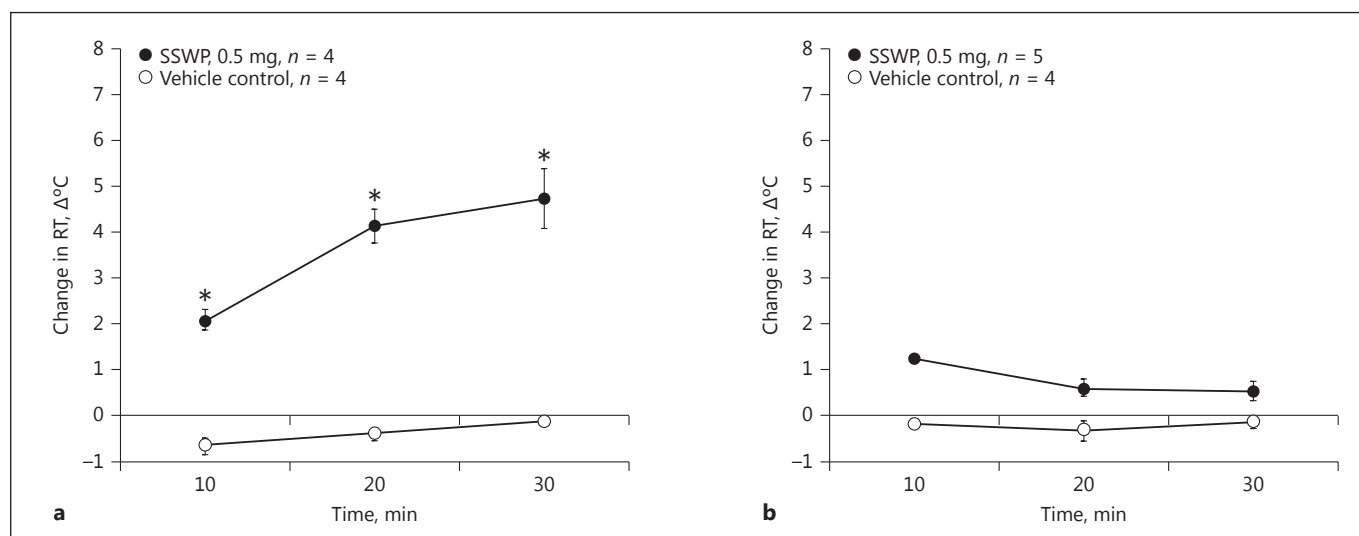


Fig. 2. Elicitation and quantitation of systemic anaphylaxis by HSR upon challenge with SSWP fraction in sensitized versus sham-sensitized mice. **a** After confirming sensitization, mice were challenged by IP injection with SSWP (0.5 mg/mouse) or vehicle alone. RT was measured just prior to challenge and after challenge at 10-min intervals for 30 min to quantify the HSR. Changes in RT at 10,

20, and 30 min from baseline prechallenge RT ($\Delta^{\circ}\text{C}$) are shown. * Student *t* test, $p < 0.05$. **b** Sham-sensitized mice were challenged by IP injection with SSWP (0.5 mg/mouse) or vehicle. Changes in RT at 10, 20, and 30 min from baseline prechallenge RT ($\Delta^{\circ}\text{C}$) are shown.

Western Blot Analysis

The wheat protein was subjected to electrophoresis, followed by the identification of IgE binding proteins as described previously [17]. Briefly, plasma from wheat-allergic mice was added for the binding of IgE antibodies to wheat proteins followed by horseradish peroxidase-conjugated anti-mouse IgE antibody for detection using a chemiluminescence method (RayBiotech, Norcross, GA, USA).

Statistical Analysis

The comparison of groups for significance was performed using the Student *t* test. Pearson correlation analysis was used to test the relationship between mMCP-1 levels and the drop in RT and the severity of dermatitis. An online software service was used in these analyses (<http://www.socscistatistics.com/tests/pearson/>). The statistical significance level was set at 0.05.

Results

The SSWP Extract Induces a Specific IgE Antibody Response and Elevates TIgE Levels in BALB/c Mice

Blood samples collected after sensitization were used in the measurement of SIgE antibodies using the published method [16]. As evident, low levels of SIgE antibody levels were detectable by day 26 after first sensitization, but reached significantly high levels by day 46 (Fig. 1a). To confirm SIgE elevation, the plasma TIgE

concentration was measured in these samples. As shown (Fig. 1c), a time-dependent increase in TIgE was observed. Sham-sensitized mice did not show elevation of SIgE or TIgE (Fig. 1b, d).

SSWP-Sensitized Mice Exhibit Acute Hypothermia Shock Responses upon Challenge

After IP challenge with SSWP, systemic anaphylaxis was quantified by hypothermia shock response (HSR). As evident (Fig. 2a), SSWP-sensitized mice exhibited significant HSR during the next 30 min postchallenge. Vehicle alone did not elicit a significant HSR (Fig. 2a). Sham-sensitized mice did not show HSR (Fig. 2b).

SSWP-Sensitized Mice Exhibit Robust Mucosal Mast Cell Degranulation That Correlates with HSR

As evident (Fig. 3a), IP challenge with SSWP elicited a robust elevation of mMCP-1 compared to the levels found in the prechallenge plasma sample. Sham sensitized mice did not show elevation of mMCP-1 upon SSWP challenge (Fig. 3b).

Furthermore, we performed a Pearson correlation analysis of data obtained from each mouse. As evident (Fig. 3c), a significant positive correlation ($r = 0.87$) between the mMCP-1 concentration and the drop in RT at 10 min postchallenge was observed. Modest positive cor-

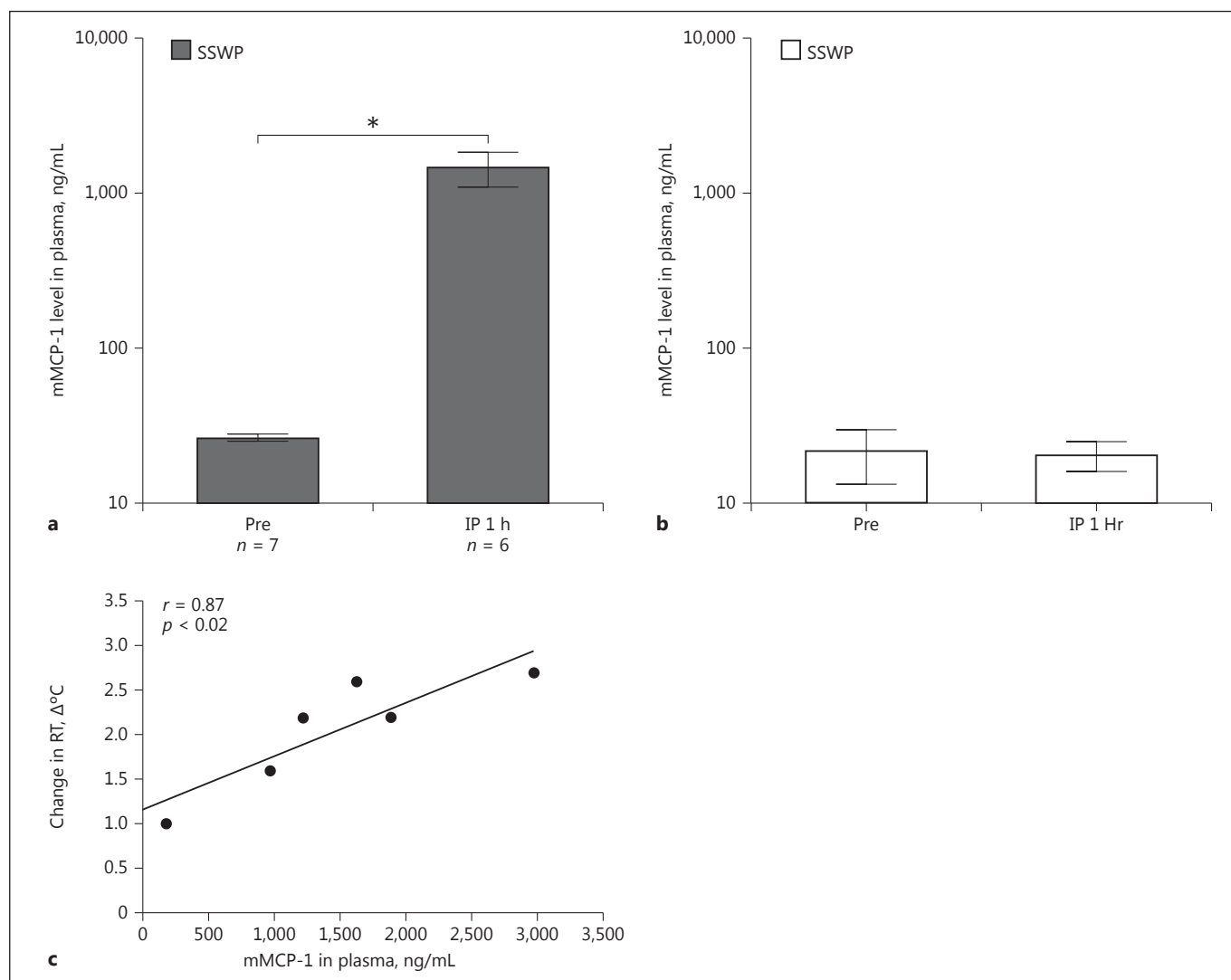


Fig. 3. Mucosal mast cell degranulation after challenge with the SSWP fraction and its correlation with HSR in sensitized mice. **a** Groups of SSWP-sensitized mice were challenged by the IP route with an SSWP fraction (0.5 mg/mouse). Plasma collected at the indicated times from sensitized mice were used in the measurement of mMCP-1 levels by the ELISA method. * $p < 0.05$. **b** Groups of sham-sensitized mice were challenged by the IP route with

the SSWP fraction (0.5 mg/mouse). Plasma collected at the indicated times was used in the measurement of mMCP-1 levels by the ELISA method. **c** The mMCP-1 concentration determined from each mouse sample was used to correlate with a drop in RT; data show the Pearson correlation coefficient obtained for mMCP-1 levels and the drop in RT at 10 min postchallenge.

relations were observed at 20 ($r = 0.52$) and 30 min ($r = 0.28$) that were not statistically significant.

SSWP-Sensitized Mice Develop Dermatitis Lesions Associated with Mast Cell Degranulation

The SSWP-sensitized mice (5/8), but neither the non-sensitized mice (0/4) kept in the same facility nor the sham-sensitized mice (0/5) developed marked dermatitis lesions and alopecia (Fig. 4a). Mice were also clinically

scored for severity of dermatitis using the method described in the Materials and Methods section (Fig. 4b). Furthermore, histopathology studies demonstrated significant degranulation of mast cells in the lesions (Fig. 4c, d). We conducted a Pearson correlation analysis to determine the relationship between plasma mMCP-1 levels and skin lesion development and severity. There was a significant positive correlation between the 2 readouts ($r = 0.85$, $p < 0.01$).

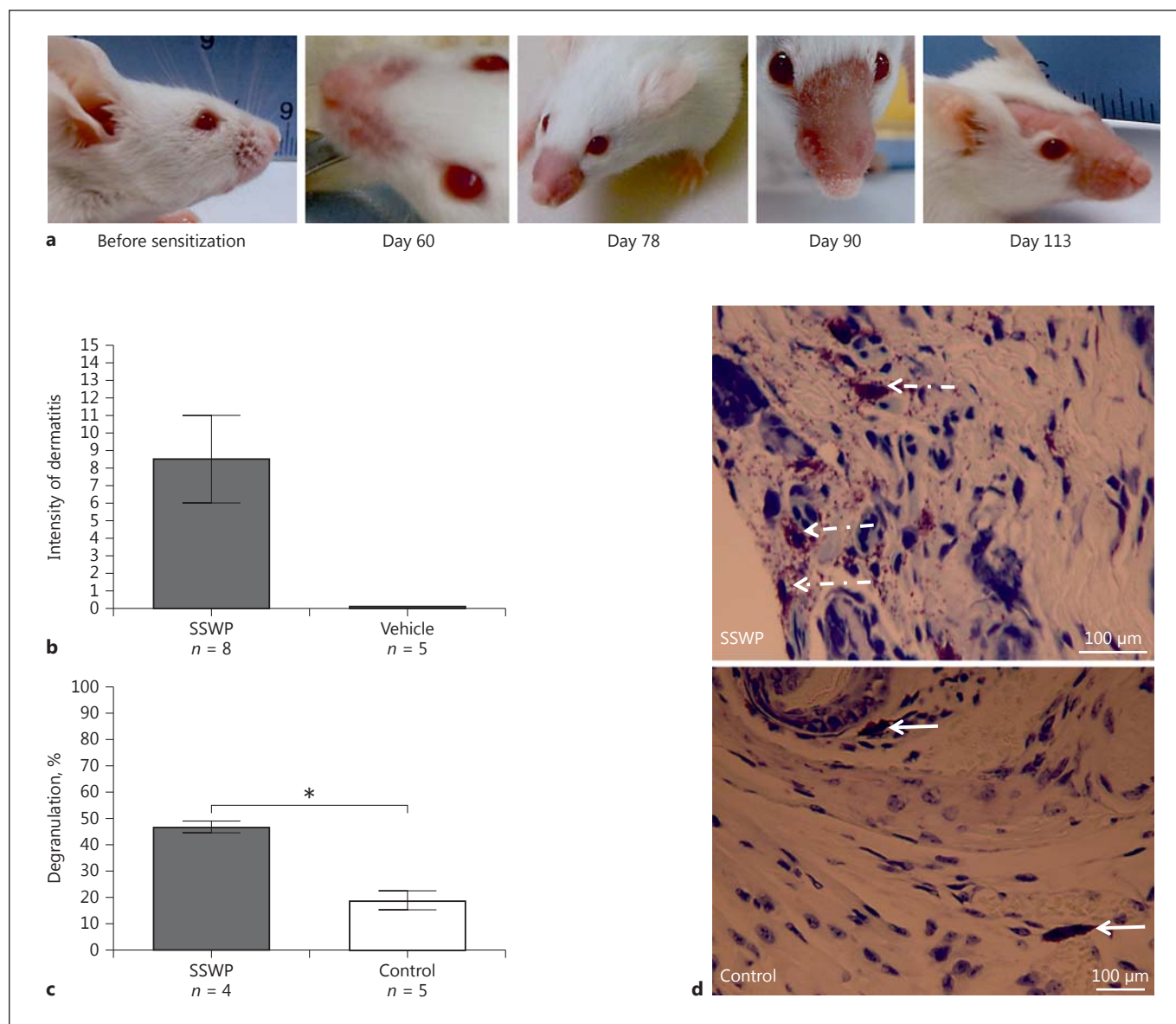


Fig. 4. Clinical scoring and histopathology of facial dermatitis lesions in SSWP-sensitized versus sham-sensitized mice. **a** Initiation of a facial lesion (day 60 after the first sensitization) and extensive progression of that lesion (up to day 113 after the first sensitization) in an allergic mouse. **b** The intensity of dermatitis in SSWP-sensitized mice versus sham-sensitized vehicle control mice determined using the clinical scoring method described in Materials

and Methods. **c** The percent degranulation of mast cells in skin lesions in allergic versus face skin from sham-sensitized control mice as determined by toluidine blue staining. * $p < 0.05$. **d** Extensive degranulation of mast cells (dotted arrows show toluidine blue-positive cells) in SSWP-sensitized mice versus intact mast cells (arrows) in skin tissue from sham-sensitized control mice.

Dermatitis Lesions Show Elevation of Th2/Th17/Th1-Associated Cytokines and Chemokines and E-Selectin Adhesion Molecule

We measured the protein levels of a large panel of cytokines, chemokines, and adhesion molecules in the pooled skin tissues collected from lesions and from

sham-sensitized healthy face skin. As evident, selective and significant elevations of cytokines (Table 1) and chemokines (Table 2), associated with Th2 and Th17 responses, were noted in the lesions. Notably, IL-5, IL-9, IL-23, and IL-33 were not significantly altered. In addition, proinflammatory/Th1-associated IFN- γ , TNF- α ,

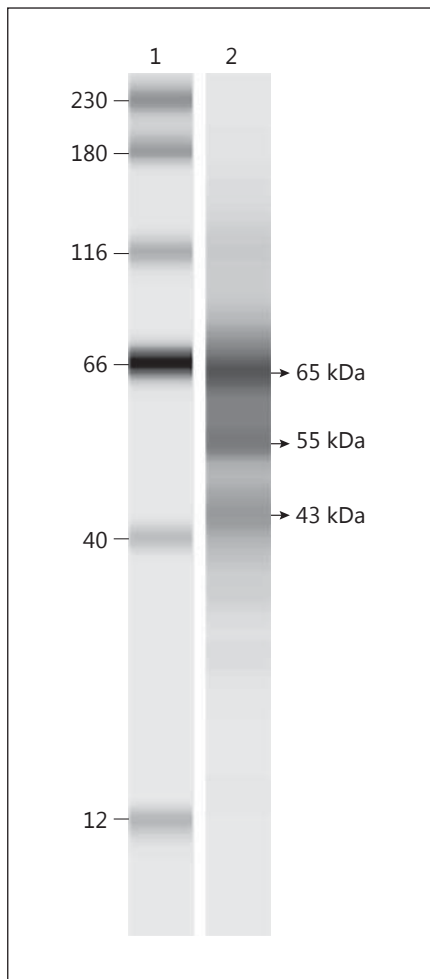


Fig. 5. Identification of 1 major and 2 minor wheat allergens in this model by Western blot analysis. The SSWP was subjected to SDS PAGE followed by the detection of IgE antibody-binding proteins by Western blot analysis as detailed in the text. Lane 1, molecular weight marker; lane 2, SSWP. The 3 IgE binding protein allergens are shown with arrows.

IL-1 β , and CXCL9 (MIG, a CXCR3 ligand) were also elevated. A significant elevation of only E-selectin was noted (Table 2).

Western Blot Analysis Identifies 1 Major Allergen and 2 Minor Allergens

We conducted Western blot analysis to identify the wheat allergens in this model. The analysis of wheat proteins for IgE antibody binding identified 3 protein allergens – 1 major allergen at 65 kDa and 2 minor allergens at 55 and 43 kDa (Fig. 5).

Table 1. Cytokine levels in the dermatitis skin tissue from SSWP-sensitized mice versus skin tissue from sham (alum alone)-sensitized healthy mice

Cytokine	Protein concentration, pg/mg skin tissue extract		
	dermatitis skin (n = 4 mice)	control mice skin (n = 5 mice)	p value ¹
IFN- γ	20.7 $\pm$ 6.8	1.5 $\pm$ 0.7	<0.05
IL-1 β	17.3 $\pm$ 4.5	6.7 $\pm$ 0	<0.01
IL-4	1.8 $\pm$ 0.4	0.2 $\pm$ 0.15	<0.01
IL-5	3.0 $\pm$ 1.6	5.7 $\pm$ 0	ns
IL-9	711.7 $\pm$ 188.8	139.8 $\pm$ 31.1	ns
IL-10	167.7 $\pm$ 23.8	33.7 $\pm$ 24.1	<0.01
IL-13	18.8 $\pm$ 2.1	9.2 $\pm$ 0	<0.001
IL-17	6.5 $\pm$ 1.8	2.8 $\pm$ 0	<0.01
IL-17E	252.2 $\pm$ 53.4	31.8 $\pm$ 13.6	<0.01
IL-23	5,991.7 $\pm$ 2,599	1,576.7 $\pm$ 506.05	ns
IL-33	661.9 $\pm$ 49.7	765.8 $\pm$ 16.95	ns
TNF- α	12.3 $\pm$ 3.5	2.9 $\pm$ 1.65	<0.05

Values are the mean $\pm$ SE. Mice were euthanized 1 h after intraperitoneal challenge with SSWP; skin tissues from dermatitis lesions (n = 4) and from face skin of sham-sensitized mice (n = 5) were collected and used in protein extract preparation as detailed in the text; cytokine levels in pooled samples were measured in quadruplicate using the RayBiotech system. ns, not significant. ¹ Student unpaired t test.

Table 2. Chemokine and adhesion molecule expression in the dermatitis skin tissue from SSWP-sensitized mice versus skin tissue from sham (alum alone)-sensitized healthy mice

Immune marker	Protein concentration, pg/mg skin tissue extract		
	dermatitis skin (n = 4 mice)	healthy skin (n = 5 mice)	p value ¹
CCL1 (TCA-3)	10.1 $\pm$ 1.3	4.9 $\pm$ 2.2	<0.05
CCL5 (RANTES)	1.7 $\pm$ 0.39	0.2 $\pm$ 0.05	<0.01
CCL11 (eotaxin)	5.8 $\pm$ 0.9	0.7 $\pm$ 0.1	<0.001
CCL12 (MCP-5)	5.0 $\pm$ 1.2	1.3 $\pm$ 0	<0.05
CCL22 (MDC)	5.6 $\pm$ 0.8	0.8 $\pm$ 0	<0.001
CCL24 (eotaxin-2)	6.6 $\pm$ 1.1	1.25 $\pm$ 0	<0.001
CXCL4 (PF4)	522.5 $\pm$ 61.6	388.9 $\pm$ 4.8	ns
CXCL9 (MIG)	20 $\pm$ 6.9	2 $\pm$ 1.1	0.05
CXCL16	33.9 $\pm$ 2.1	10.8 $\pm$ 0.7	<0.00001
CX3CL1 (fractalkine)	3,766.3 $\pm$ 945.7	2,569.1 $\pm$ 904.9	ns
MAAdCAM-1	303.5 $\pm$ 90.8	92.7 $\pm$ 22.2	ns
E-selectin	45.3 $\pm$ 4.9	20.5 $\pm$ 4.3	<0.01
L-selectin	954.7 $\pm$ 82	2,045.5 $\pm$ 207.7	ns
P-selectin	5,604.4 $\pm$ 331.8	6,657.8 $\pm$ 216.9	ns
VCAM-1	1,039 $\pm$ 26.1	2,087.9 $\pm$ 159.5	ns

Values are the mean $\pm$ SE. For methods, see Table 1 footnote. ns, not significant. ¹ Student unpaired t test.

Discussion

In this study, we tested the hypothesis that SSWP extract from a durum wheat flour will not only sensitize BALB/c mice but will also elicit clinical allergic disease. Our data collectively support this hypothesis. There are 6 novel and significant findings from this study: (i) systemic administration of SSWP along with alum elicited robust sensitization as evidenced by the time-dependent enhancement of circulating SIgE antibodies as well as elevation of TIgE levels; (ii) systemically sensitized mice developed severe HSR upon IP challenge; (iii) systemic challenge caused robust degranulation of mucosal mast cells as evidenced by elevation of mMCP-1 in the plasma; a significant positive correlation between mMCP-1 levels and the HSR was noted; (iv) most, but not all, of the SSWP-sensitized mice developed facial dermatitis associated with mast cell degranulation that correlated positively with mMCP-1 responses; (v) the dermatitis lesions expressed significantly elevated levels of selected cytokines and chemokines associated with Th2, Th17, and proinflammatory Th1 responses, and (vi) the dermatitis lesions also expressed high levels of only E-selectin adhesion molecule.

Human wheat allergic subjects can be sensitive to a number of proteins present in the alcohol-soluble, acid-soluble, and salt-soluble protein fractions from wheat [1–3, 8, 19, 20]. Although mouse models for wheat allergy have been developed for gluten proteins (gliadins), a mouse model for SSWPs – another major group of wheat allergens in both the USA and Europe – was not available. We therefore developed a mouse model and studied the IgE responses, the clinical responses, mast cell degranulation response, and cytokine/chemokine responses.

There are 5 useful mouse models that have previously been developed and characterized using gluten proteins (gliadins). Here we briefly compare and contrast these models with our mouse model. Bodinier et al. [10] developed a mouse model using gliadin/alum IP sensitization and intranasal challenge in BALB/c, B10.A/C3H/HeJ mice [10]. They studied IgE antibody responses and eosinophil influx into airways as disease markers and found BALB/c to be the best responder. Denery-Papini et al. [11] developed a BALB/c model using gliadins, and LTP1 plus alum by IP sensitization as in the above study and evaluated IgE responses [11]. They did not study anaphylaxis, mast cell responses, dermatitis, or cytokine/chemokine responses. Gourbeyre et al. [12] used native and deamidated gliadins plus alum and used IP sensitization

and IP challenge methods in BALB/c mice [12]. They studied IgE responses and symptom scores and histamine release. Abe et al. [13] also used deamidated gliadins plus alum and BALB/c mice, but used IP sensitization followed by intragastric challenges [13]. They studied IgE responses, intestinal permeability, intestinal anaphylaxis, and gut histamine release. Adachi et al. [14] reported a transdermal sensitization model using acid-hydrolyzed gluten [14]. They studied IgE responses and anaphylaxis upon IP challenge.

Since BALB/c mice have been shown to respond to wheat gliadin proteins similarly to humans, we chose to use this strain of mice in our studies on SSWP [10, 11]. In contrast to the previous models, our model differs significantly in a number of ways. Firstly, we used SSWP extract for the first time; previous models used the alcohol-soluble proteins or purified LTP [10–14]. Secondly, we quantified the HSRs; among the previous models, only Adachi et al. [14] reported the utility of HSR for gluten sensitization [14]. Thirdly, we studied mucosal mast cell degranulation by quantifying the elevation of plasma levels of mMCP-1 postchallenge; notably this readout is a specific biomarker of IgE- (but not IgG1-) mediated anaphylaxis in mice [21]. This readout was not used in the existing wheat (gliadins or LTP1) allergy models discussed above. We also reported a significant positive correlation between mMCP-1 levels and HSR and with dermatitis for the first time. Fourthly, we report, for the first time, the development of facial dermatitis lesions in a mouse model; previous models did not report this common and very important human clinical phenotype associated with wheat allergy. Furthermore, we also provide data to explain the mechanism of dermatitis by identifying the elevation of selective Th2/Th17/Th1-associated cytokines and chemokines and E-selectin adhesion molecule in the dermatitis skin lesions.

Although we used the SSWP extract for the first time in this study, Denery-Papini et al. [11] previously studied mouse IgE responses (but not anaphylaxis, mast cell responses, or dermatitis, cytokine, or chemokine responses) to purified LTP1. The SSWP extract that we used in this study did not contain detectable LTP, a low-molecular-weight allergen (9 kDa), in SDS PAGE analysis, and Western blot also revealed no IgE binding proteins in the lower molecular weight range. Analysis of wheat proteins for IgE antibody binding identified 3 protein allergens – 1 major allergen at 65 kDa and 2 minor allergens at 55 and 43 kDa.

In order to elucidate the mechanism of atopic dermatitis in this model, we studied cytokine, chemokine, and adhesion molecule expression in the lesion. Elevation of the Th2/Th1-associated cytokines and chemokines in

atopic dermatitis lesions is well appreciated [22], although it had not previously been reported in a wheat allergy-associated dermatitis mouse model [23]. Here we report elevation of these Th2/Th1-associated cytokines and chemokines in the dermatitis lesion. In addition, elevation of CXCL16 was noted for the first time in atopic dermatitis.

Elevation of IL-1 β , TNF α , IFN- γ (all proinflammatory cytokines) is consistent with marked inflammation of the lesion. The elevation of selected Th17-associated cytokines (IL-17, IL-17E) in the lesions in this model of wheat allergy is highly significant. Although extensive evidence exists for the Th17 pathway in autoimmunity (e.g., psoriasis, rheumatoid arthritis, etc.) and airways allergy/asthma [24–26], its role in food allergy in general and in wheat allergy in particular, is not well understood. There are variable reports on the role of IL-17 in food allergies, implicating a pathogenic role as well as a marker of food tolerance [27, 28]. Nakajima et al. [29] proposed a key role for the Th17 pathway in a hapten-induced contact dermatitis mouse model. Our findings of selective elevation of Th17-associated cytokines in wheat protein-induced atopic dermatitis further support the hypothesis of a pathogenic role of elevated Th17 cytokines in food allergy-associated atopic dermatitis. Our data also show that among the adhesion molecules examined, E-selectin plays an important role in atopic dermatitis in this model. Taken together, these data provide molecular clues to explain the mechanism of SSWP-induced atopic dermatitis in this model.

Although we observed alopecia and dermatitis together in these mice, we do not know if initial itching/scratching led to alopecia in this model. Future behavioral studies will be necessary to address the relationship among dermatitis, alopecia, and itching/scratching behavior. Furthermore, not all mice sensitized with SSWP developed the lesions. The positive correlation between mMCP-1 response and dermatitis lesion severity suggests that stronger mast cell responses are associated with the development of dermatitis lesions.

The potential utility of mouse models in basic and applied biomedical research is well appreciated [30–32].

Along with previous mouse models of gliadin sensitivity, our new model of SSWP hypersensitivity with additional clinical (i.e., dermatitis) and mediator (i.e., mMCP-1) phenotypes should provide a novel opportunity for further research and development. Furthermore, identification of a selective set of cytokines/chemokines and one adhesion molecule in dermatitis lesions should provide clues for developing potential therapeutic targets for wheat-induced atopic dermatitis for the first time.

In conclusion, we report a mouse model of wheat allergy using SSWP with robust sensitization as well allergic disease elicitation readouts including HSR, mucosal mast cell degranulation response, and atopic dermatitis lesions for the first time. We also identified the selective set of cytokines, chemokines, and one adhesion molecule elevated in the dermatitis lesions. This model may be used for further basic and applied biomedical research on wheat allergy.

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Statement of Ethics

An institutional review board approved the animal procedures used in this study.

Disclosure Statement

The authors do not have any conflict of interest to declare.

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