

Sensitization and Allergic Response and Intervention Therapy in Animal Models

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A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with heat-killed *E. coli* preparations showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was provided, additional experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need additional study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

The incidence of allergy to peanuts appears to be increasing worldwide, accounting for the majority of fatal and near-fatal anaphylactic reactions to foods (1). Compared to traditional immunotherapy options of allergen desensitization, the only recommended strategy to manage peanut allergy is strict avoidance of foods containing peanuts. Because peanut or peanut products are often used in a number

of processed foods or provided in meals without the peanut-allergic individual's knowledge of its presence, accidental ingestions are common (2, 3). Sicherer et al. (4) reported that up to 55% of peanut-allergic children experienced some type of reaction following accidental ingestion in a 5-year period. We have investigated several options that could contribute to the reduction of an allergic response following accidental ingestion of small amounts of peanuts. A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options that could potentially be extrapolated for use in children and adults.

Experimental Designs

Model 1. Mutation of Linear IgE-Binding Epitopes of Ara h 1 for the Preparation of a Hypoallergenic Ara h 1 Transgenic Peanut Plant (5)

Single point mutations were made in epitopes 7–15 and 18–22, resulting in a cDNA where all IgE-binding epitopes were altered. Polymerase chain reaction (PCR) fragments of the wild-type and mutant gene, minus the signal sequence and post-translational sequence, were cloned into the pGEM-T plasmid. The inserts contained restriction enzyme sites that permitted removing the gene inserts and placing them into the pET24b expression plasmid. The expressed protein contained a 5' T7-tag and a 3' His-tag, which were used in Southern and Western blot analysis for identification and purification, respectively. Recombinant protein expressed in BL21(DE3) *E. coli* cells was purified by Ni²⁺ affinity chromatography. Western blot analysis of the expressed protein using serum containing IgE from 57 peanut-sensitive individual was used to determine IgE binding to the wild-type and modified Ara h 1 protein. Of the 57 peanut-sensitive patients, 2–42% retained some IgE binding to the modified Ara h 1.

The modified Ara h 1 protein was then tested for native conformation prior to transfer of the gene into the seed. In the synthesis and deposition of seed storage proteins, mRNA derived from genes encoding the storage protein is translated on rough endoplasmic reticulum (ER) and undergoes a series of post-translational modifications as it transits through the ER and Golgi apparatus before being deposited as a seed storage protein (6, 7). During synthesis, transport, and

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Table 1. Symptom scores for Ara h 1 sensitization/desensitization model

Test group	Desensitization	Symptom scores
1	Ara h 1	3.7
2	rAra h 1	3.9
3	mAra h 1	3.4
4	<i>E. coli</i>	1.9
5	<i>E. coli</i> /wt Ara h 1	1.1
6	<i>E. coli</i> /mod Ara h 1	0.5

deposition, the seed storage protein is exposed to various proteolytic and glycolytic enzymes, which could alter the structure and/or conformation of the protein (8, 9). Initial attempts to prepare transgenic tobacco plants expressing modified Ara h 1 failed. Investigations into structural characteristics of the modified protein were then pursued to determine correct processing.

For the modified Ara h 1 protein to be translated and processed in the plant, it must survive the ER and Golgi in the correct structure, be assembled into trimers, and deposited in seed storage vacuoles if it is to be used as a potential hypoallergenic peanut allergen source. The Ara h 1 protein is a member of the vicilin family of seed storage proteins with a sedimentation coefficient of 7–8S (10). Using *in vitro* transcription/translation (11, 12) and endopeptidase susceptibility (13) of wild-type and modified Ara h 1, products were tested for correct folding and oligomerization. Transcripts produced from the T7 promoter of the pET16b construct were translated into nascent protein by using the rabbit Retic Lysate IVTTM Kit (Ambion, Austin, TX). Assembly into trimers was analyzed by isopycnic sucrose density gradient centrifugation. The position of the Ara h 1 products was determined by cosedimentation with known sedimentation standards. The resulting gradient profile of the wild-type translation products revealed the presence of monomer, trimer, and hexamers compared to modified protein, which existed as primarily a monomer.

The trimer fraction of wild-type was tested for enzyme stability by an endopeptidase assay (13). Proteins isolated from sodium dodecyl sulfate–gel electrophoresis (SDS–PAGE) gels were sequenced by W.M. Keck Foundation Biotechnology Resource Laboratory, New Haven, CT. Results indicated that the 65 kilodalton (kDa) protein had an N-terminal sequence that began at residue 85 as numbered from the start codon of the cDNA sequence, while that of a 59 and 6 kDa protein had N-terminal sequences beginning at residue 141 and 85, respectively. N-terminal sequencing of native Ara h 1 protein, not subjected to enzyme digestion, confirmed that the N-terminal sequence began at residue 85.

Confirmation of the wild-type processing to oligomeric structures suggested that the mutations for the modified Ara h 1 protein were not being properly processed in pET16b. The failure to express the modified Ara h 1 in transgenic tobacco plants and the failure of the monomer form to oligomers may be the result of improper folding in the primary mutated Ara h 1 sequence. Future studies will need to address structure of the modified Ara h 1 protein to determine whether if post-translational processing prevents expression in the tobacco plant and correct folding for oligomerization.

Model 2. Mutation of Linear IgE-Binding Epitopes of Ara h 1 for the Preparation of an Immunotherapeutic Agent to Alleviate Peanut-Allergic Response in a Murine Animal Model (5)

Although it was not possible to prepare an Ara h 1 hypoallergenic peanut plant by using transgenic technology, the modified Ara h 1 protein was used to investigate its ability to down-regulate an ongoing peanut allergy. Historically, immunotherapy with allergen extracts has been predominantly applied to inhalant and vespid venom allergies. Immunotherapy with food allergen was successful in a child treated for fish allergy (14), and pollen-specific immunotherapy was successful for individuals with oral allergy syndrome (15, 16). However, a high rate of adverse systemic side effects was noted in peanut-sensitive individuals treated with peanut extracts, and immunotherapy was largely abandoned in the treatment of peanut-allergic individuals (17, 18).

Table 2. Crude peanut and soybean desensitization study design: Experiment 1

Procedure	Time point	Group 1	Group 2	Group 3	Group 4	Group 5
Sensitization	Days 0, 7, 21	Ara h 1	Ara h 1	Ara h 1	PBS	Ara h 1 ^a
Desensitization	Days 35, 37, 39	0.1 mg CPE ^b	0.1 mg SPE ^b	PBS	PBS	
	Days 42, 44, 46	0.25 mg CPE	0.25 mg SPE	PBS	PBS	
	Days 49, 51, 53	0.5 mg CPE	0.5 mg SPE	PBS	PBS	
	Days 56, 58, 60	0.5 mg CPE	0.5 mg SPE	PBS	PBS	
Challenge	Day 76	CPE	CPE	CPE	CPE	

^a Challenged/sacrificed at Day 35 to determine symptom score sensitization.

^b CPE = Crude peanut extract; SPE = soy protein extract.

The reduced IgE-binding of the modified Ara h 1 suggested a plausible alternative to treatment of peanut allergy. In order to determine the effect of treatment with the modified Ara h 1, we used the C3H/HeJ peanut allergy model. Five-week-old female C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) were sensitized intraperitoneally with 0.5 mg native Ara h 1 in the presence of 2 mg alum on Day 0 and boosted on Days 7 and 21. Desensitization consisted of 3 weekly intraperitoneal injections beginning on Day 45 with the following groups of animals: (1) 50 µg native Ara h 1; (2) 50 µg recombinant Ara h 1; (3) 50 µg modified Ara h 1; (4) 2 OD₆₀₀ units of heat-killed *E. coli* cells; (5) 2 OD₆₀₀ units heat-killed *E. coli* containing pET24b plasmid wild-type Ara h 1 insert; (6) 2 OD₆₀₀ units of heat-killed *E. coli* with the plasmid pET24b modified Ara h 1; and (7) phosphate-buffered saline (PBS) carrier. For *E. coli* at 2 OD₆₀₀ units, *E. coli* BL21 freezer stocks were grown up in 100 mL Luria broth at 37°C in the presence of 30 mg/mL Kanamycin to a density of OD₆₀₀ of 0.6–0.9. The cultures were induced with 1mM isopropyl- β -D-thiogalactopyranoside (IPTG), and continued incubations were maintained until they reached a density of 1.5–2.0 OD₆₀₀. The culture was heat-killed at 65°C for 30 min, and the cells were pelleted at 3000 rpm for 20 min. Appropriate dilutions were made so that 30 µL stock solution containing 66.66 OD/mL was obtained and used to immunize mice. IgE immunoblots, anti-His (carboxy terminal), and anti-T7 (amino terminal) of SDS-PAGE blots of the pET24b plasmid vector were used to confirm the presence of Ara h 1.

On Day 73, all mice were intraperitoneally challenged with 1 mg native Ara h 1 and observed for clinical symptoms at 15 and 30 min post-challenge by the following scoring system: 0—no reaction; 1—scratching; 2—lethargy, easily prodded to action; 3—lethargy, cyanotic; 4—lethargy, not prodded to activity; 5—convulsion, death (Table 1). After physical symptom analysis, surviving animals were euthanized and the spleens were obtained for proliferation and cytokine analysis. To determine the relative effects of the desensitization, serum levels of allergen-specific IgG1, IgG2a, and total IgE were measured by enzyme-linked immunosorbent assay (ELISA) prior to sensitization and again on Day 42, representing pre- and post-desensitization collections, respectively. The proliferation assay consisted of 4×10^6 /mL splenocytes

Table 3. Symptom scores and core body temperatures for Experiment 1

Group	Symptom scores ^a	Core body temperatures, °C
1	±1.0	±35
2	±2.0	±33
3	±3.4	±31
4	0	±37
5	±3.5	±30

^a The use of ± indicates that the scores were rounded to the nearest whole value. Insufficient animal numbers prevented statistical numbers.

challenged with 10 µg native Ara h 1, rice protein, or *E. coli* protein. Cells were incubated for 3 days, after which 1 µCi/well of tritiated thymidine was added overnight. Cells were then harvested onto filter paper by using the Packard Filtermate 96 and analyzed with the Packard Matrix 9600 Direct Beta Counter (Downers Grove, IL). A blastogenic index was calculated by averaging triplicate samples and dividing the radiolabeled incorporation of antigen-treated cells by media alone with tritiated thymidine. Cytokine analysis of stimulated splenocytes was determined from separate incubations of 4×10^6 /mL treated with the antigens for 24 h with 10 µg native Ara h 1. Supernatants were harvested and assayed by ELISA for INF-gamma, IL-4, and IL-5 using commercial kits (BioSource International, Camarillo, CA). In light of the symptom scores and variable immunoglobulin, proliferation scores, and cytokine levels, it was difficult to assign specific desensitizing effects of Ara h 1 proteins in the presence of *E. coli*, which offered protection. Experiments determining the role of endotoxins and *E. coli* need to be assessed.

In summary, although some benefit was observed with the mutated Ara h 1 desensitization schedule, the results suggested that mice desensitized with heat-killed *E. coli* preparations showed increased protection on challenge, suggesting that bacterial protein was acting as an adjuvant to modify the immune system. Symptom score responses to mice desensitized with either recombinant protein were similar to

Table 4. Crude peanut and soybean desensitization study design: Experiment 2

Procedure	Time point, day	Group 1	Group 2	Group 3	Group 4	Group 5 ^a
Sensitization ^b	0, 7, 21	CPE	CPE	CPE	PBS	CPE
Desensitization	35, 37, 39	0.1 mg CPE	0.1 mg SPE	PBS	PBS	
	42, 44, 46	0.25 mg CPE	0.25 mg SPE	PBS	PBS	
	49, 51, 53	0.5 mg CPE	0.5 mg SPE	PBS	PBS	
	56, 58, 60	0.5 mg CPE	0.5 mg SPE	PBS	PBS	
Challenge	76	CPE	CPE	CPE	CPE	

^a Sacrificed at Day 35 to verify sensitization.

^b Sensitization was performed with 0.5 mg/mouse on Days 0, 7, and 21.

Table 5. Symptom scores and core body temperatures: Experiment 2

Group	Symptom scores ^a	Core body temperatures, °C
1	±1.0	±37
2	±1.2	±37
3	±4.0	±33
4	0	±37
5	±3.0	±32

^a The use of ± indicates that the scores were rounded to the nearest whole value. Insufficient animal numbers prevented statistical numbers.

sham-desensitized mice, while those treated with native Ara h 1 had reduced symptom scores. This suggests a prominent role for the glycosylated form of Ara h 1. In light of these results, continued experiments on dose effects and the treatment with heat-killed *E. coli* may be beneficial for alleviating peanut-allergic responses in the mouse peanut allergy model. Additional studies are being designed to determine the relative role of *E. coli* in the assessments of immunoglobulins and cytokines.

In continuing studies and in collaboration with Hugh Sampson, Mount Sinai School of Medicine, engineered recombinant protein and heat-killed *Listeria monocytogenes* co-administration showed protection in the peanut-induced murine model (19). Conclusions from both studies clearly suggest that the protective effect is associated with an up-regulation of Th1 and a down-regulation of Th2 responses. It remains to be seen if the results of using modified peanut allergens in association with bacteria can be extrapolated to potential treatment of peanut allergy to reduce symptoms from hidden peanut anaphylaxis events.

Model 3. Allergen Homologs as Desensitizing Reagents in a Murine Food Allergy Model (20)

Based on a novel idea that seed storage proteins in the legume family share significant amino acid homology and the clinical data suggesting that individuals with peanut allergy can tolerate other legumes (21), a soybean desensitization protocol could potentially be used for immunotherapy. The

specific aim under investigation is to determine if soybean extract can provide protection from peanut allergic anaphylactic responses in the peanut allergic murine model. Preliminary results in the murine food allergy model suggest that soybean immunotherapy could potentially provide benefit to peanut-allergic patients by reducing anaphylaxis following ingestion of hidden peanut food sources.

Five groups of 5-week-old C3H/HeJ female mice were sensitized with native Ara h 1 and desensitized (Table 2). In this preliminary protocol, animals were sensitized with enriched Ara h 1 (Day 0 = 0.25 mg; Day 7 = 0.125 mg; Day 14 = 0.05 mg), followed by desensitization with either crude peanut extract (CPE) or soybean protein extract (SPE) and challenged with crude peanut extract. To determine Ara h 1 sensitization, Group 5 was challenged/sacrificed at Day 35. Symptom scores following sensitization alone were in the range of 3–4, clearly indicating anaphylactic response to peanut challenge. At Day 76, all animals were challenged, and symptom scores and core body temperatures were recorded. The results are shown in Table 3.

The results suggested that sensitizing with Ara h 1 and desensitization with crude peanut extract (Group 2) had the most profound effect, whereas the soy protein extract (Group 2) showed a more modest effect than did animals receiving a sham PBS desensitization (Group 3). The controls (Group 4) clearly were healthy animals with no symptom scores or decrease in body temperature following the crude peanut extract challenge. The results suggested that both peanut and soybean could be used as desensitizers in animals sensitized to the major allergen, Ara h 1. However, following natural exposure to peanuts, sensitization to a single allergen is unlikely. A second experiment was designed to determine the effect of CPE sensitization followed by CPE and increased concentrations of soybean extract to determine the relative effects of these 2 desensitization protocols (Table 4).

The results of symptom scores and corresponding core body temperatures are shown in Table 4. Evidence from the Group 5 animals challenged and sacrificed on Day 35 was taken as a measure of sensitization to CPE as demonstrated by the symptom score and core body temperature. Group 4, the nonsensitized/CPE challenged animals at Day 76 did not show any evidence of symptoms and the core body temperature was normal compared to Group 3, the peanut-sensitized/CPE challenged animals at day 76, which mirrors the response seen

Table 6. Comparison of food sensitization in the swine model: Experimental design (timeline)

Sensitization ^a	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Days 7, 8, 9, 17, 25	Day 25	Day 32	Day 39	Day 46	Day 53	Day 60
			Skin	Oral	Skin	Oral
			Test	Challenge	Test	Challenge
	Bleed	Bleed	Bleed	Bleed	Bleed	Bleed

^a Sensitization group: (1): egg 500 µg; (2): egg 1000 µg; (3): soy 500 µg; (4): soy 1000 µg; (5): peanut 500 µg; (6): PBS/CT.

Table 7. Swine symptomatic evaluation of allergen challenge

Grade	Symptoms
0	No evidence of any reaction or signs
1	Coughing, gagging, evidence of stomach contractions; reduced activity, tendency to lie down, easily activated to movement; minor rashes
2	Vomiting; lethargy/malaise; tremors; convulsions; reduced activity that could not be activated by prodding; major areas of edematous rashes
3	Respiratory distress and symptoms requiring epinephrine; evidence of diarrhea 18–24 h after challenge

at Day 35 with Group 5 animals. In both the peanut- and soybean-desensitized groups (1 and 2, respectively), symptom scores were reduced by 2 scoring grades, suggesting that the desensitization schedules were effective in reducing the allergic response. The core body temperatures confirmed that temperatures were normal (Table 5).

Peanut-specific IgG1 was reduced approximately 5–7-fold in the soy-desensitized animals (Group 2; >0.2 mg/mL) compared to peanut desensitization (Group 1; ± 3.5 mg/mL). No statistical evaluations for IgG1 levels were made as these were regarded to be preliminary studies designed to direct future studies. The post-sensitization blastogenic response to peanut and soybean extract was significantly reduced ($P < 0.001$) in both Groups 1 and 2 compared to Group 3. Cytokine analysis revealed a Th1 pattern in the peanut and soy desensitization schemes compared to Group 3, i.e., IFN-gamma levels were prominent ($P > 0.001$), 100 pg/mL for peanut and >1750 pg/mL for soybean.

Combined, these 2 preliminary studies suggest that soy immunotherapy can be used to down-regulate an ongoing peanut-specific response in the C3H/HeJ peanut-allergic mouse model. The symptom scores and core body temperatures were decreased in both experimental protocols. However, mechanistic studies are still necessary to determine the role of peanut or soy desensitization in this model and

whether the data can be applied to treatment of individuals with peanut allergy. Significant is the route of administration (intraperitoneal versus oral) and lack of prior exposure. The mice are unlikely to have had exposure to soy products as compared to peanut-allergic individuals. Future studies are being designed to determine the optimal sensitizing/desensitizing doses and to delineate the molecular mechanisms leading to decreased clinical symptoms of peanut/soybean immunotherapy.

Model 4.: Comparison of Allergenic Sensitization/Response to Soybean and Egg in the Neonatal Swine Food Allergy Model (22)

A natural disease model that can be used to analyze gastrointestinal (GI) food allergy has been developed in the Yorkshire/Landrace outbred strain of swine (23). The model is being used to determine the immunoregulatory mechanisms of clinically relevant GI immediate hypersensitivity to develop a model for threshold food immune sensitization/allergic response levels for prediction of novel proteins potential allergenicity, and to offer therapeutic options for treatment of food allergy.

Skin tests and oral challenge by gastric intubation performed with the respective foods established an allergen-specific symptomatic response. Orally challenged piglets responded with no symptoms (controls), mild symptoms (egg and soybean—body rashes, lethargy), and more severe symptoms (peanut—vomiting, diarrhea, respiratory distress), comparable to human food allergic reactions. Skin tests confirmed sensitization to the respective food with little or no response to nonsensitizing foods. Therapeutic options for peanut allergy are preliminary and suggest that probiotic therapy may alter an ongoing peanut allergy. The neonatal swine model reflects the pathological features in food-allergic humans and can therefore be useful in investigations of mucosal-associated lymphoid tissues of the GI and respiratory tract.

A pregnant Yorkshire/Landrace outbred sow at Day 108 of gestation was maintained on a peanut-soybean-free diet and allowed to farrow under normal conditions. A litter born to the sow was allowed to nurse until Day 21, at which time they were weaned onto peanut-soybean-free nursery diets. All animals were

Table 8. Swine skin test to egg and peanut 25 μ g id^a

Group	Egg	Peanut	Soybean	PBS	Histamine
1 (egg 500 μ g)	9 $\times$ 7 ^b	0	0	0	15 $\times$ 16
2 (egg 1000 μ g)	6 $\times$ 5	0	3 $\times$ 4	0	15 $\times$ 13
3 (soy 500 μ g)	0	0	12 $\times$ 12	0	11 $\times$ 11
4 (soy 1000 μ g)	0	2 $\times$ 3	6 $\times$ 6	0	8 $\times$ 10
5 (peanut 500 μ g)	0	10 $\times$ 9	5 $\times$ 5	0	9 $\times$ 9
6 (PBS/CT)	0	0	0	0	5 $\times$ 8

^a Average of 2 animals/group; id = intradermal.

^b Numbers represent wheal responses measured at right angles.

Table 9. Swine oral challenge symptom scores to egg, soybean, and peanut, 10 g ig^a

Group	Piglet	6 Week	8 Week	Group	Piglet	6 Week	8 Week
1 (egg 500 µg)	1	NR ^b	2	4 (soy 1000 µg)	7	NR	NR
	2	3	NR		8	NR	NR
2 (egg 1000 µg)	3	NR	NR	5 (peanut 500 µg)	9	NR	NR
	4	NR	NR		10	NR	NR
3 (soy 500 µg)	5	3	3	6 (PBS)	11	NR	NR
	6	3	3		12	NR	NR

^a Symptom score for each animal is represented; ig = intragastric.

^b NR = No response.

maintained according to National Research Council regulations and the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences and the Arkansas Children's Hospital Research Institute. The litter of 12 piglets was divided into 6 groups (2 piglets/group) and underwent intraperitoneal sensitization with crude defatted peanut or soybean extracts or lyophilized whole egg extract with 100 µL cholera toxin (CT) as adjuvant according to the timeline shown in Table 6.

Beginning at Day 25 and weekly thereafter, blood was drawn by cardiac puncture and analyzed for allergen-specific IgG and lymphocyte proliferation. At Weeks 5 and 7, piglets were intradermally skin tested for allergen-specific responses. At Weeks 6 and 8, piglets were intragastrically challenged with 10 g ground peanut or soybean meal or lyophilized whole egg and with the respective sensitizing food in PBS after an overnight fast. Physical symptoms were evaluated by 3 independent investigators by the symptom evaluation scoring system (Table 7).

Skin test results averaged over the 2 time periods showed wheal and flare responses to the respective sensitizing food (Table 8). Egg- and soy-sensitized animals sensitized with 1000 µg crude protein extracts showed some cross-reactivity to

soybean and peanut, respectively. A similar skin test response of the 500 µg peanut-sensitized animals to soy at the 1000 µg sensitized concentrations suggests a possible dose-dependent sensitization of soy that will cause a soy skin test positive result. Experiments with dosing schedules for these 3 foods need to be evaluated to confirm sensitizing dose and using skin testing as a measure of allergic response.

Table 9 shows the results of oral challenges with the respective sensitizing food. In the 500 µg egg-sensitized animals, a 10 g egg challenge of the 2 piglet revealed different symptoms at the 2 challenging periods. Results from Group 1 (500 µg egg-sensitized) were different: Piglet 1 results suggest that the challenge at 6 weeks could have led to further sensitization similar to experiences with previous peanut challenges. Piglet 2, in comparison, showed what appeared to be a transient sensitization/allergic response, as no repeat challenge at 8 weeks caused any symptoms. No symptomatic responses were observed in Group 2 (1000 µg sensitized/10 g egg challenged) animals. In the soy-sensitized animals, Group 3 (500 µg soy-sensitized) piglets showed a consistent response at both 6 and 8 weeks; no response was detected in Group 4 (the 1000 µg soy-sensitized) group. In Group 5, the 500 µg peanut-sensitized animals, no clinical symptoms were observed; this is in contrast to previous sensitizing experiments where 90% of the animals responded with this sensitizing/oral challenge dose and may be the result of selecting 2 animals that would not normally respond to peanut, or the sensitization dose was not appropriate.

Figure 1 shows the food-specific IgG response to the different foods. During the course of the experiment, the 2 concentrations of egg did not provide IgG production above 1500 ng/mL, suggesting that either the egg is a weak antigen or the dose is not sufficient to stimulate immunoglobulin production. The 2 soy concentrations show an apparent dose-dependent effect, albeit a very transient effect peaking at Week 6 and falling off by Week 7. The peanut IgG concentration continued to rise during the course of 6 weeks, plateauing at Weeks 7 and 8.

Peripheral blood mononuclear cells (PBMCs) were isolated from each piglet, incubated with the respective protein extract to which the piglet was sensitized, PBS or PBS/CT to determine the cytokine profile (Table 10). The supernatant from the

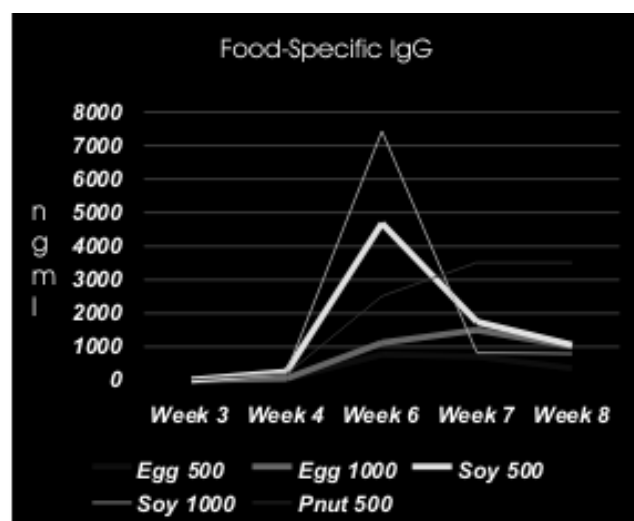


Figure 1. Food-specific IgG. Average values of 2 piglets/group to the respective sensitizing food.

Table 10. Peripheral blood cytokine profile in swine, pg/mL sensitizing agent

Cytokine	Egg	Soy	Peanut	PBS/CT ^a
IFN-gamma				
Week 5	18	291	753	<10
Week 6	29	188	574	<10
Week 7	<10	35	<10	<10
Week 8	<10	<10	<10	<10
IL-4				
Week 5	<10	25	84	<10
Week 6	<10	14	16	<10
Week 7	<10	<10	<10	<10
Week 8	<10	<10	<10	<10

^a CT = Cholera toxin.

challenged PBMC were collected, and the 2 cytokines IFN-gamma and IL-4 were measured by ELISA. The respective Th1/Th2 (IFN-gamma/IL-4) profile suggests that PBMC cytokine production was rapidly declining during the time frame under investigation. The combined results, although suggestive of positive responses, clearly suggest that further experimentations with different sensitization schedules and doses with greater numbers of animals in each group will be necessary for statistical significance.

Conclusions

In summary, both the murine animal models and the neonatal swine food allergy model suggest that the different experimental protocols are promising and that additional studies are needed. An allergic response profile in the neonatal swine food allergy model is similar to human food allergy with respect to sensitization/allergic response for peanut, egg, and soybean. Evidence presented suggests that optimization, with respect to dose and timing, for sensitization/allergic responses will be necessary to draw definitive conclusions from these preliminary experiments.

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