

A Neonatal Swine Model of Allergy Induced by the Major Food Allergen Chicken Ovomucoid (Gal d 1)

Prithy Rupa Korinne Hamilton Melissa Cirinna Bruce N. Wilkie

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ont., Canada

Key Words

Allergy • Egg • Food hypersensitivity • Neonatal swine model • Ovomucoid

Abstract

Background: Food allergy is a serious health problem for which a validated outbred large animal model would be useful in comparative investigations of immunopathogenesis and treatment and in testing hypotheses relevant to complex host-environmental interactions in predisposition to and expression of food allergy. **Objective:** To establish a neonatal swine model of IgE-mediated allergy to the egg protein ovomucoid (Ovm) that may mimic human allergy. **Methods:** In order to induce Ovm sensitivity, piglets at days 14, 21 and 35 of age were sensitized by intraperitoneal injection of 100 µg of crude Ovm and cholera toxin (50, 25 or 10 µg). Controls received 50 µg of cholera toxin in phosphate-buffered saline. The animals were challenged orally on day 46 with a mixture of egg white and yoghurt. Outcomes were reported as direct skin tests, clinical signs, IgG-related antibody and passive cutaneous anaphylaxis. **Results:** Sensitized pigs developed immediate wheal and flare reactions, and after oral challenge, sensitized but not control animals displayed signs of allergic hypersensitivity. Serum IgG-related, Ovm-specific antibodies were detected only in the sensitized pigs and IgE-mediated antibody response to Ovm was confirmed by positive passive cutaneous anaphylaxis reactions induced by sera of sensitized but not by heat-treated

sera from Ovm-sensitized pigs or sera of unsensitized control pigs. **Conclusion:** The present results confirm induction of Ovm-specific allergy in pigs and provide opportunity to investigate allergic predisposition and immunopathogenesis of IgE-induced Ovm allergy using outbred neonatal swine. This may better simulate allergic disease in humans and allow investigation of candidate prophylactic and therapeutic approaches.

Copyright © 2007 S. Karger AG, Basel

Introduction

Food-induced allergy has increased in prevalence in recent years such that in North America 4% of adults and 6% of children have food allergies [1]. Food allergy involves an idiosyncratic reaction of the immune system to ingested foods and is responsible for substantial morbidity and mortality. Severe allergic reaction to food may lead to anaphylaxis and death. The most common food allergens, in decreasing rank order of frequency of related disease, are milk, eggs, peanut, soy, wheat, fish, shell fish and tree nuts. These account for more than 90% of severe adverse reactions to food [2]. Eggs are therefore the second most common allergen amongst small children [3].

Eggs are a source of dietary proteins as well as essential vitamins and minerals, and elimination of eggs from the diet presents a risk to the balance of nutritive intake. Also, in many cases eggs are 'hidden' ingredients in foods, the

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2007 S. Karger AG, Basel
1018–2438/08/1461–0011\$24.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Bruce Wilkie
Department of Pathobiology, Ontario Veterinary College
University of Guelph
Guelph, Ont. N1G2W1 (Canada)
Tel. +1 519 824 4120, ext. 54760, Fax +1 519 824 5930, E-Mail bwilkie@uoguelph.ca

presence of which is hard to detect, making accidental exposures common. Thus, prophylactic and therapeutic interventions for egg allergy are urgently needed. Most allergic children react to egg white protein, the major allergens of which are ovomucoid (Ovm), ovalbumin, ovomucoid, lysozyme [4]. Symptoms and signs of egg allergy include skin rashes or hives, gastrointestinal distress, dyspnoea and, in severe cases, anaphylaxis. Ovm is the dominant allergen causing most of the allergic reactions to hen's egg white [5]. In order to study immunopathogenic mechanisms of Ovm-induced allergy, an outbred animal model is needed that incorporates features of the target species, man. Preferably the animal model should be homologous, that is, signs and time course in the animal closely resemble those of naturally sensitized humans.

Piglets have been sensitized and tolerized to cow's milk and soy proteins with induced responses which resemble those of young children [6, 7]. Peanut allergy was induced in piglets as a model for comparative investigation of human gastrointestinal hypersensitivity to peanuts [8]. A similar animal model would be useful for investigation of allergic sensitization to eggs. Marked individual variation occurs in immune response of outbred pigs, as in humans, but in contrast to inbred mice, hence neonatal pigs may be better subjects for comparative studies of human allergy. Recently, hypersensitivity reactions to Ovm have been characterized in Balb/c mice and ablation of Ovm-induced allergic response by a modified mutant isoform of Ovm (hypoallergen) has been reported [9]. This is a high-IgE responder mouse strain and this system has obvious limitations. These include bias in allergic predisposition reflecting minimal genotypic diversity unlike that of humans or large outbred animals such as neonatal pigs. Experimental allergy in outbred pigs may provide opportunity for comparative studies of variables influencing immunological ontogeny hypothetically predisposing to the allergic phenotype. If validated, such an animal model may also be a realistic platform for discovery and testing of novel therapies and prophylactic/immunotherapeutic strategies for human allergic disease.

The present report describes methods to induce IgE-mediated antibody response and allergic hypersensitivity to Ovm in neonatal pigs. We hypothesized that systemic sensitization could be used to induce allergy to Ovm after oral challenge of neonatal swine and the frequency of induced allergic signs reflects occurrence of allergy-susceptible immune response phenotype. Immune response was monitored as direct skin test reactions to Ovm, Ovm-

specific serum antibody detected by enzyme-linked immunosorbent assay (ELISA) and passive cutaneous anaphylaxis (PCA) reactions. Clinical scores were assigned for orally induced hypersensitivity reactions. We report for the first time an outbred animal model (neonatal swine) to the major egg white allergen Ovm that mimics human egg allergy.

Materials and Methods

Experimental Design and Animals

The study was designed to induce allergy to Ovm in neonatal swine. The subjects were outbred piglets (Yorkshire; 10 days old) from 3 different litters, with a litter size of at least 12 piglets per litter, maintained under specific pathogen-free conditions (University of Guelph, Arkell Research Station), weaned at 21 days and housed with free access to food and water. All piglets within each litter were reared by the same mother. The sow's diet did not contain any egg protein. Equal numbers of animals within each litter were assigned randomly to 4 treatments in a split-litter design ($n = 3/\text{treatment group}$). The experimental design and treatments are illustrated in figure 1. Animal use was approved by the local Animal Care Committee under guidelines of the Canadian Council for Animal Care.

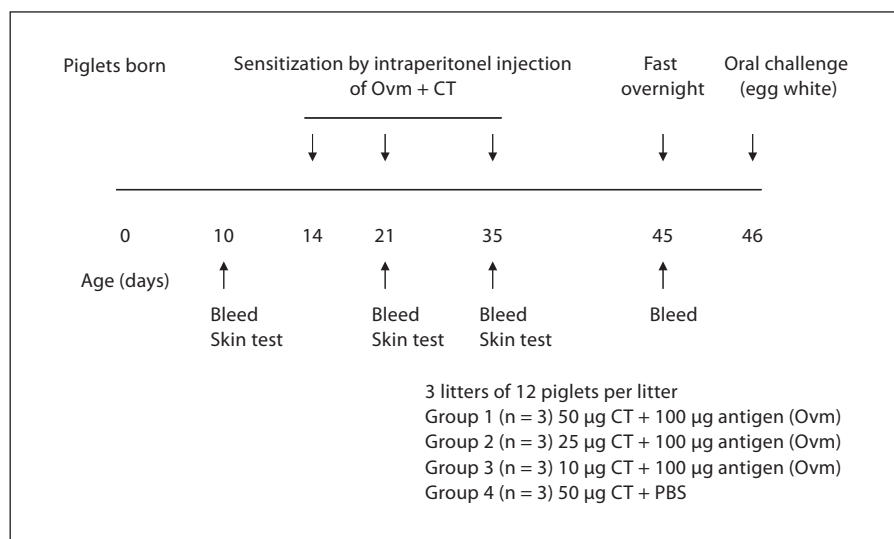
Purification of Ovm

Intact crude Ovm was isolated according to the procedure reported by Fredericq and Deutsch [10]. To 1 litre of egg white, an equal volume of 10% trichloroacetic acid was added and a homogeneous solution was made by constant stirring. The pH of the solution was adjusted to 3.5 with 1 N NaOH and the resulting precipitate was allowed to settle overnight at 4°C, followed by centrifugation at 5,000 g for 30 min at 4°C (Beckman J2-21M/E centrifuge; Beckman Instruments Inc., Palo Alto, Calif., USA). The supernatant was recovered, adjusted to pH 3.9 with 1 N NaOH and precipitated by adding twice the volume of absolute ethanol and incubating overnight at 4°C. The precipitate was collected by centrifugation (5,000 g for 15 min) and dissolved in 5 ml of Milli-Q water. The resulting solution was extensively dialyzed against Milli-Q water (6K-8K cut-off dialysis membrane; Fisher Scientific, Pittsburgh, Pa., USA) for 2 days at 4°C. The material thus obtained is a crude form of Ovm which is a dominant allergen in egg white matrix [5]. The sample was analyzed using SDS-PAGE (data not shown) and protein concentration was determined using a colorimetric assay (DC protein assay; Biorad Laboratories, Mississauga, Canada) with a bovine serum albumin standard.

Sensitization of Pigs with Ovm

Three outbred litters of naïve Yorkshire piglets (University of Guelph, Arkell Research Station) were used in a split-litter design for induction of Ovm hypersensitivity. Pigs were bled at 10 days of age via the retro-orbital sinus to obtain pre-sensitization sera and were randomly assigned to 4 treatment groups ($n = 3/\text{group}$). Three groups received intraperitoneal injection of 100 µg Ovm with various doses (10, 25 or 50 µg) of cholera toxin (CT; List Biological Laboratories Inc., USA) in 200 µl of phosphate-buffered saline (PBS; 0.1 M, pH 7.4) administered on days 14, 21 and

Fig. 1. Experimental design and time lines. Three individual litters were used in this study. Each treatment group within each litter consisted of 3 piglets that were 10 days old at the start of the treatment regimen. Piglets received 100 µg of Ovm with various doses of CT (50, 25 and 10 µg) in a final volume of 200 µl phosphate-buffered saline solution. Control piglets received only CT (50 µg).



35 (fig. 1). The fourth group (control) received 50 µg of CT alone. Blood was collected for serum on days 21, 35 and 45 to detect progression in immune response. After coagulation for 1 h at room temperature, individual sera were collected and stored at -20°C for detection of IgG (whole molecule)-related antibodies to Ovm. Equal amounts of individual sera of littermates were pooled (sensitized and controls pooled separately) for PCA reaction. On day 45, the animals were fasted overnight and challenged orally on day 46 by feeding 40 ml of egg white mixed in yoghurt at a ratio of 2:3 (v/v) administered using a 60-ml Monoject syringe (BD Bioscience) with attached polypropylene tube. Pigs were monitored for clinical signs of allergy after the challenge (0–60 min).

Serum Antibody Response to Ovm

Sera were analyzed for anti-Ovm antibodies by ELISA. Briefly, polystyrene, high-binding, 96-well flat-bottomed plates (Immulon 2HB; VWR International, Mississauga, Canada) were coated with 100 µl/well of Ovm (100 µg/ml) in 0.05 M carbonate bicarbonate buffer (pH 9.6) by incubating overnight at 4°C . The plates were washed 3 times (Automatic plate washer, ELX405; BioTek Instruments Inc., Winooski, Vt., USA) with 200 µl/well of PBS containing 0.5% Tween 20 (PBST), prior to blocking the wells with 200 µl/well of 3% Tween in PBS for 1.5 h at room temperature. Plates were washed 3 times with PBST and sera diluted to 1:100 in PBST were added in triplicates at 100 µl/well and incubated at room temperature for 2 h. Following washing 3 times with PBST, 100 µl of alkaline phosphatase-conjugated rabbit anti-pig IgG (whole molecule; Sigma, Oakville, Canada) diluted to 1:8,000 was added to the plates and incubated for 1 h at room temperature. Plates were washed and a solution of polynitrophenol-phosphatase substrate (Sigma; 1 mg/ml; 100 µl/well) in diethanolamine buffer, pH 9.8, was added and incubated in the dark at room temperature. Controls included wells without serum and with positive (pooled day 35 sera that had high antibody) and negative sera (pooled sera from day 10). Optical densities (OD) of wells were measured at 405 nm using an ELISA plate reader (EL808; BioTek Instruments Inc.) when the OD of the positive control reached 1. The net absorbance value was calculated for all

samples by the instrument software to correct for nonspecific binding effects by subtracting the blank absorbance (without serum) value from the absorbance value for the well receiving Ovm antigen. The mean OD of each triplicate test serum was expressed as a percentage (percentage positivity) of the positive control as follows: % change OD = [sample OD/(positive control OD – negative control OD)] $\times 100$.

Cutaneous Hypersensitivity

Intradermal skin tests were performed in all animals, prior to sensitization on day 10 and after sensitization on days 21 and 35 to test for immediate active hypersensitivity reactions. Injection sites on the inner thighs were marked with an indelible ink pen. For each skin test, 100 µl (100 µg) of Ovm in PBS was injected intradermally using a tuberculin syringe and 25-gauge needle. The negative control was PBS. All intradermal tests (serum and PBS) were performed and read at the same time. One investigator performed all injections. Each site was examined individually by at least 2 observers for wheal and flare response 15 min after injection and consensus was reached regarding positivity or negativity.

Clinical Scores

Hypersensitivity responses were evaluated using a scoring system after induction by oral challenge with egg white on day 46. The response was scored visually by at least 3 observers in a blinded fashion 0–60 min after oral challenge. Each pig was individually assessed and scored as follows: 0 = no signs; 1 = immobility, lethargy, malaise, scratching, rash; 2 = diarrhea, emesis; 3 = increased respiratory rate, neck extension; 4 = forced expiration; 5 = other signs (various). Total scores were obtained by adding individual scores assigned to each animal.

Passive Cutaneous Anaphylaxis

Sera were pooled from treatment groups (CT-treated controls and sensitized) and aliquots were treated by heating at 56°C for 4 h to inactivate putative IgE-anti-Ovm PCA-mediating antibodies. Sera were injected intradermally in duplicate 100 µl vol-

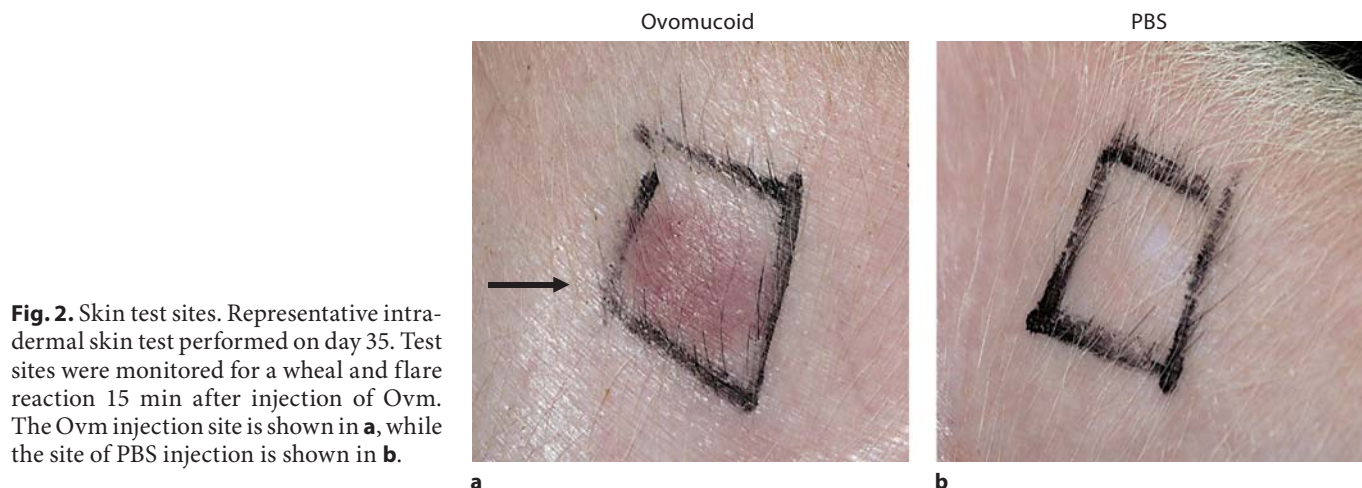


Fig. 2. Skin test sites. Representative intra-dermal skin test performed on day 35. Test sites were monitored for a wheal and flare reaction 15 min after injection of Ovm. The Ovm injection site is shown in **a**, while the site of PBS injection is shown in **b**.

umes into marked sites on the inner thighs of naïve pigs. After 24 h, 5 mg of Ovm in 1.0 ml of sterile PBS was injected via a dorsal ear vein using a 23-gauge needle. Cutaneous injection sites were examined by at least 3 observers for expression of a wheal and flare reaction. Sera obtained from sensitized pigs in each of the 3 litters were also tested individually for PCA response and each site was graded (+, ++, +++, +++) on the basis of relative severity.

Analysis

Data were analyzed using InStat Package 4.0 (GraphPad Software Inc., San Diego, Calif., USA). The confidence interval was set to be at least 95% ($p \leq 0.05$) in assessing significance. Comparisons were made between day 10 versus day 45 serum IgG-anti-Ovm and control (CT) versus sensitized (Ovm) antibody. The primary data for comparisons of antibodies detected were analyzed with a one-way ANOVA test using animal as the experimental unit followed by Tukey's multiple comparison test for significant differences between treatment groups. The relationship between PCA reaction and clinical score was evaluated by linear regression and Pearson correlation analysis (GraphPad Prism, version 4.0).

Results

Direct Skin Test

Most test animals that were sensitized to Ovm showed strong skin reactivity (7/8, 8/8 and 7/9 test animals in the first, second and third litters, respectively) to intradermal Ovm injections on day 35. The test sites developed a central wheal and flare at about 15 min after injection with diameter usually >1 cm. The control pigs (CT treated) and the PBS-injected site, however, did not respond. Skin tests were negative on days 10 and 21. A representative positive skin test is illustrated in figure 2.

Oral Challenge with Egg White

Following oral challenge with egg white, clinical signs occurred within 1–45 min and ranged from minor skin reactions, including periocular and focal erythema, facial edema and wheals, to diarrhea and vomiting. Usual locations for skin erythema reactions were the flank, vulva, face, particularly around the eyes, mouth and ears, dorsal surface of the back, as well as areas around the base of the tail. Reactions were initially localized to one part and later spread over the entire body surface causing redness. One of the 3 litters (litter 2) did not exhibit allergic signs and therefore the scores were not determined (except for 1 pig that was scored 1). Seven of eight sensitized pigs in the first litter, 1/8 pigs in the second litter and 5/9 pigs in the third litter expressed clinical signs. Unsensitized control animals did not develop clinical signs. Clinical signs for individual pigs are given in table 1. Both individual and litter differences in clinical sign response were obvious. There was no apparent effect of variable dose of CT on response to oral challenge. Representative images of control pigs (CT treated) versus allergic pigs (Ovm treated) and skin erythema reactions are illustrated in figure 3.

Serum Antibody Response to Ovm

Sera obtained on day 10 before treatment and after sensitization on day 45 were analyzed by ELISA to detect Ovm-specific antibody activity. ANOVA confirmed significant increase in Ovm-specific serum antibodies in antigen-sensitized pigs from day 10 to 45 ($p \leq 0.05$) in all 3 litters. High individual variation was observed between pigs in detection of Ovm-specific antibodies, even within the same litter. Sera of CT-treated negative control pigs

Table 1. Treatment, gender and measurement of Ovm-induced serum antibody activity (IgG – whole molecule), skin test response, PCA and clinical signs in individual pigs

A Treatment	Sex	Ovm-specific IgG, day 45	PCA score day 45	Skin test day 35	Clinical score day 46	Clinical signs day 46
50 µg CT (control)	F	15.479	–	–	0	no response
50 µg CT (control)	M	10.6533	–	–	0	no response
50 µg CT (control)	M	8.01782	–	–	0	no response
10 µg CT + 100 g Ovm	F	245.694*	++	+	5	emesis, rash, scratching, diarrhea
10 µg CT + 100 µg Ovm	M	208.797*	++	+	0	no response
10 µg CT + 100 µg Ovm	M	200.074*	++	+	5	malaise, rash, scratching, emesis
25 µg CT + 100 µg Ovm	F	239.644*	+++	+	1	scratching
25 µg CT + 100 µg Ovm	F	245.026*	++	+	2	emesis
25 µg CT + 100 µg Ovm	F	148.924*	++++	+	9	malaise, scratching, rash, diarrhea, forced expiration
50 µg CT + 100 µg Ovm	F	235.078*	++	+	2	scratching, rash
50 µg CT + 100 µg Ovm	M	134.037*	++	+	3	malaise, rash, scratching
B Treatment	Sex	Ovm-specific IgG	PCA score	Skin test	Clinical score	Signs
50 µg CT (control)	F	10.942	–	–	ND	ND
50 µg CT (control)	F	14.8037	–	–	ND	ND
50 µg CT (control)	M	14.332	–	–	ND	ND
10 µg CT + 100 µg Ovm	F	320.661*	++++	+	ND	ND
10 µg CT + 100 µg Ovm	F	327.398*	++	+	ND	ND
25 µg CT + 100 µg Ovm	M	333.748*	++++	+	ND	ND
25 µg CT + 100 µg Ovm	M	332.761*	+++	+	ND	ND
25 µg CT + 100 µg Ovm	F	332.032*	++	+	ND	ND
50 µg CT + 100 µg Ovm	F	326.754*	+++	+	1	scratching
50 µg CT + 100 µg Ovm	M	313.495*	++++	+	ND	ND
50 µg CT + 100 µg Ovm	M	338.554*	++	+	ND	ND
C Treatment	Sex	Ovm-specific IgG	PCA score	Skin test	Clinical score	Signs
50 µg CT (control)	F	4.950	–	–	0	no response
50 µg CT (control)	F	5.1752	–	–	0	no response
50 µg CT (control)	M	10.318	–	–	0	no response
10 µg CT + 100 µg Ovm	F	100.723*	++	+	7	scratching, malaise, immobility, rash, difficulty in breathing, neck extension
10 µg CT + 100 µg Ovm	F	40.823*	–	+	5	scratching, immobility, rash, erythema
10 µg CT + 100 µg Ovm	M	62.038*	–	–	0	no response
25 µg CT + 100 µg Ovm	F	89.907*	–	–	0	no response
25 µg CT + 100 µg Ovm	M	69.785*	–	+	0	no response
25 µg CT + 100 µg Ovm	M	33.655*	–	+	5	scratching, immobility, rash, erythema
50 µg CT + 100 µg Ovm	F	44.970*	–	+	0	no response
50 µg CT + 100 µg Ovm	M	56.766*	–	+	6	scratching, immobility, rash, forced expiration
50 µg CT + 100 µg Ovm	M	80.296*	++	+	5	scratching, immobility, rash, erythema

Ovm-specific IgG (whole molecule) serum antibody activity was measured by ELISA using alkaline phosphatase-conjugated rabbit anti-pig IgG (whole molecule) and antibody activity is reported as percentage of positive control serum pool optical density. ND = Not determined; M = male; F = female. * Significant difference ($p \leq 0.05$) from negative controls. A, B and C represent 3 different litters.



Fig. 3. Skin erythema. **a** Representative images of control pigs versus allergic pigs after oral challenge. **b** Representative image of skin erythema reaction in the abdominal region of an allergic pig.

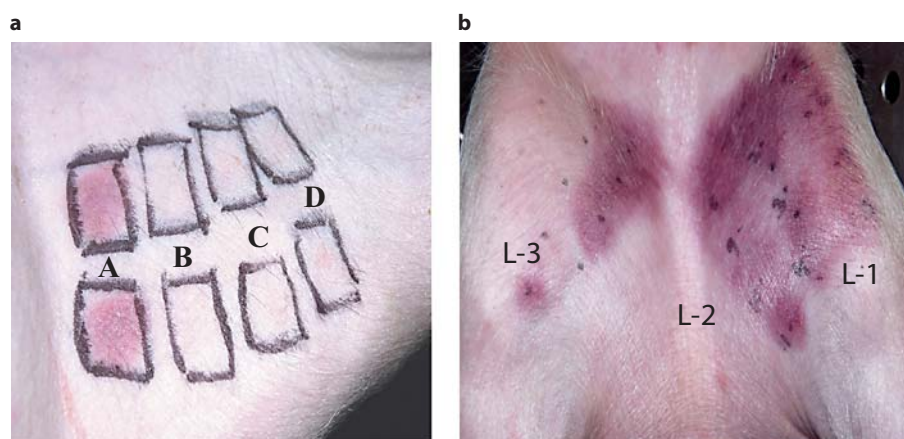


Fig. 4. Ovm-induced, IgE-mediated PCA. PCA reaction in a naïve pig was determined using either pooled or individual sera. **a** PCA response mediated by pooled sera. Pooled anti-Ovm serum (0.1 ml) from sensitized pigs was injected intradermally into a naïve pig and after a latent period of 24 h, Ovm (5 mg) was injected intravenously to induce PCA. A = Reaction mediated by Ovm-sen-

sitized pig serum samples; B = sites that received heat-inactivated Ovm-sensitized pig serum; C = sites that received sera of pigs that were treated with CT only; D = sites that received heat-inactivated sera from CT-treated pigs. **b** PCA reaction of individual pigs. Sera of individual sensitized pigs from each of 3 litters (L-1, L-2 and L-3) were tested as described in Methods.

did not change in ELISA values from day 10 to 45 and were significantly lower ($p \leq 0.05$) than values of Ovm-sensitized pigs. There was no apparent effect of variable dose of CT on antibody response. The antibody activity of individual animals' sera was expressed in relation to the activity of the positive control (table 1).

Passive Cutaneous Anaphylaxis

Sensitization to Ovm was confirmed by PCA. To test the hypothesis that Ovm had induced IgE-mediated allergic reactions, ability of sera to mediate PCA was tested

using pooled sera from sensitized and control pigs in all 3 litters, as well as sera from Ovm-sensitized individual pigs. Only sera obtained from Ovm-sensitized pigs in all 3 litters induced positive PCA response. A representative PCA response for pooled sera is illustrated in figure 4a. The PCA reactivity was abolished by heat inactivation, confirming the role of IgE-anti-Ovm antibodies in the hypersensitivity reactions. Control (CT) sera (native and heat inactivated) failed to elicit a positive PCA response. Linear regression analysis showed a strong correlation between PCA reactions and clinical scores in both the lit-

ters that exhibited allergic signs (litter 1: $p < 0.0124$, $r^2 = 0.5192$; litter 3: $p < 0.0463$, $r^2 = 0.3408$; GraphPad Prism). Considerable individual differences were observed in the PCA response as represented in figure 4b and table 1.

Discussion

A neonatal swine model of food allergy to Ovm is described. The approach was intended to provide an outbred large animal experimental system more closely mimicking humans to allow investigation of potential interventions in the neonatal period that might either enhance or diminish immunological predisposition to food allergy. The prominent individual variation that exists between and within the litters is seen to provide opportunity to investigate genetic and environmental effects upon expression of allergy.

The results indicate that systemic sensitization of pigs with Ovm induced IgE-mediated allergic reactions after oral challenge. Earlier attempts to sensitize pigs using aluminium hydroxide adjuvant rather than CT failed. Piglets sensitized with Ovm and CT and orally challenged with egg white had clinical signs similar to those of allergic human patients including gastrointestinal, dermatologic and respiratory signs. The failure of one litter to express allergic signs after oral challenge reflects litter variation of unknown etiology. Others have described experimental induction of allergy to peanuts [8], soy [6] and milk proteins [7] in pigs. However, eggs are the second most frequent cause of allergic reaction in the USA [1] and a report of egg allergy recurrence, demonstrating that egg allergy may have a fluctuating course, has recently been reported [11], which stresses the need to validate a model for intervention studies on egg allergies. In addition to the present report of successful allergization and oral elicitation in neonatal pigs of sensitization to Ovm, our unpublished results confirm similar results using peanut proteins.

The use of pigs for comparative studies of human allergy offers advantages over the commonly utilized inbred mice. These include the outbred genetic background of pigs similar to the target human population, which may realistically mimic the complexity of individual variation in response to environmental factors influencing predisposition to and expression of allergic diseases. Young piglets can be housed and fed under the same conditions, together in a specific pathogen-free facility and the litter size (12–14) is large enough to allow controlled experiments differentiating genetic and environmental

effects on individual variation in allergic manifestations.

Most animal models proposed or used to characterize the sensitizing and desensitizing activity of allergens are based on measurement of serological responses, especially of IgE-associated antibodies and the mediators of type 1 allergic signs. Due to the unavailability of anti-swine IgE, in the present study serum antibodies to Ovm were measured using anti-swine IgG (whole molecule) to detect essentially all antibody activity without isotype bias. The sensitized animals, but not the adjuvant-treated controls, developed a significant antibody response after systemic injection of Ovm with CT, which confirms that the adjuvant (CT) alone does not elicit response but, upon co-administration with Ovm, enhances the immunogenic and allergenic response. The presence of IgE-related, Ovm-specific antibodies was confirmed by heat-labile PCA reactivity of sensitized pig serum.

The porcine immune system and intestine are continuously changing as the young pig matures [12] and litter as well as individual variation in immune response is pronounced [13]. Pigs have high individual variation in cytokine production and in the type 1/type 2 bias of cytokines produced [14]. This variation relates to litter, age and gender of the pig. Heritability of immune response in pigs is in the order of 20% [15] and pigs can be selected for high and low immune response, confirming significant genetic, litter, dam and sire contributions to variable immune response phenotype [16]. Nevertheless, variation between littermates suggests important epigenetic and environmental effects on variation in immune response [17]. In the present study there was significant individual variation within and between litters, in serum antibody to Ovm and in manifestation of allergic signs after oral challenge. The model is important in that it will allow investigation of hypotheses dealing with early neonatal influences on ontogeny of immune response leading to allergic predisposition and to control of predisposition to allergic diseases. Its power derives from the inherent similarity of the experimental subjects and the target species with respect to simulation of genetic variation and ability to control interactions between genome and controlled variations in environment. Candidate variables include the effect of orally administered probiotic bacteria, modified Ovm (hypoallergen) and immunoregulatory cytokines to alter allergic immune response phenotype. The experimental allergy model would also allow evaluation of these interventions in the context of potentially altered frequency and severity of systemically induced food allergy to a common allergen, Ovm. Sensiti-

zation and challenge for oral induction of Ovm-induced hypersensitivity are in progress, and will further validate the model for future intervention studies to treat and prevent food allergy to eggs.

Conclusions

In conclusion, we have described a method to induce Ovm-specific allergy by systemic sensitization of outbred pigs. The resulting allergy is IgE mediated and resembles human food allergy. The approach may have utility in

comparative investigations addressing variables, genetic and/or environmental, affecting induction, prophylaxis and therapy of allergies with relevance to allergy in the similarly outbred human population.

Acknowledgements

We gratefully acknowledge the skilled assistance of the University of Guelph Arkel Swine Research Station staff. This study was supported by grants to B.N.W. from the Natural Sciences and Engineering Research Council of Canada, Ontario Pork and the Ontario Ministry of Agriculture and Food.

References

- 1 Sampson HA: Update on food allergy. *J Allergy Clin Immunol* 2004;113:805–819.
- 2 Sampson HA, Mendelson T, Rosen JP: Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1993;327:380–384.
- 3 Hefle S, Nordlee J, Taylor S: Allergenic foods. *Crit Rev Food Sci Nutr* 1996;36:S69–S89.
- 4 Holen E, Elsayed S: Characterization of four major allergens of hen egg-white by IEF/SDS-PAGE combined with electrophoretic transfer and IgE-immunoautoradiography. *Int Arch Allergy Appl Immunol* 1990;91:136–141.
- 5 Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA: Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994;93:1047–1059.
- 6 Hankins CC, Noland PR, Burks AW Jr, Connaughton C, Cockrell G, Metz CL: Effect of soya protein ingestion on total and specific immunoglobulin G concentrations in neonatal porcine serum measured by enzyme-linked immunosorbent assay. *J Anim Sci* 1992;70:3096–3101.
- 7 Bailey M, Miller BG, Telemo E, Stokes CR, Bourne FJ: Altered immune response to proteins fed after neonatal exposure of piglets to the antigen. *Int Arch Allergy Immunol* 1994;103:183–187.
- 8 Helm RM, Furuta GT, Stanley JS, Ye J, Cockrell G, Connaughton C, et al: A neonatal swine model for peanut allergy. *J Allergy Clin Immunol* 2002;109:136–142.
- 9 Rupa P, Mine Y: Ablation of ovomucoid-induced allergic response by desensitization with recombinant ovomucoid third domain in a murine model. *Clin Exp Immunol* 2006;145:493–501.
- 10 Fredericq E, Deutsch HF: Studies on ovomucoid. *J Biol Chem* 1949;181:499–510.
- 11 Hu W, Katelaris CH, Kemp AS: Recurrent egg allergy in adulthood. *Allergy* 2007;62:709.
- 12 Brown DC, Maxwell CV, Erf GF, Davis ME, Singh S, Johnson ZB: Ontogeny of T lymphocytes and intestinal morphological characteristics in neonatal pigs at different ages in the postnatal period. *J Anim Sci* 2006;84:567–578.
- 13 Crawley AM, Mallard B, Wilkie BN: Genetic selection for high and low immune response in pigs: effects on immunoglobulin isotype expression. *Vet Immunol Immunopathol* 2005;108:71–76.
- 14 De Groot J, Kruijt L, Scholten JW, Boersma JAW, Buist GW, Engel B, et al: Age, gender and litter-related variation in T-lymphocyte cytokine production in young pigs. *Immunology* 2005;115:495–505.
- 15 Wilkie BN, Mallard BA: Selection for high immune response: an alternative approach to animal health maintenance? *Vet Immunol Immunopathol* 1999;72:231–235.
- 16 Mallard BA, Wilkie BN, Kennedy BW, Gibson J, Quinton M: Use of estimated breeding values in a selection index to breed Yorkshire pigs for high and low immune and innate resistance factors. *Anim Biotech* 1992;3:257–280.
- 17 Petry DB, Holl JW, Weber JS, Doster AR, Osorio FA, Johnson RK: Biological responses to porcine respiratory and reproductive syndrome virus in pigs of two genetic populations. *J Anim Sci* 2005;83:1494–1502.