



An oral Brown Norway rat model for food allergy: comparison of age, sex, dosing volume, and allergen preparation

Kirsten Pilegaard*, Charlotte Madsen

Institute of Food Safety and Nutrition, Danish Veterinary and Food Administration, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

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Abstract

The purpose of the presented experiments was to study the possibility of using the Brown Norway rat as a model for food allergy in our laboratory. Specific serum IgE against ovalbumin (OVA) was induced after dosing male and female Brown Norway rats daily by gavage for 35 days. The influence of various preparations of allergen: OVA grade II, OVA grade V, and fresh egg white, age (4 versus 8 weeks), dosing volumes, and animal suppliers was studied. A general finding was that females had statistically significantly higher specific IgE and IgG titres and number of responders than males. Egg white preparation, age, dosing volume, and animal supplier did not statistically significantly influence the median IgE and IgG titres and number of responders. The difference between immune responses in males and females could not be attributed to variations in daily intake of OVA or exposure via the lung. In our hands, the oral Brown rat food allergy model gives rise to a moderate number of IgE responders, 13–38 and 38–75% in males and females, respectively. For further experiments with this model in our laboratory, females seem the sex of choice. © 2003 Elsevier Ireland Ltd. All rights reserved.

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1. Introduction

Food allergy is an adverse reaction to an otherwise harmless food or food component that involves an abnormal response of the immune system to specific protein(s) in foods. The majority of food allergies are mediated by allergen-specific IgE antibodies that cause an immediate type of reactions with symptoms occurring from minutes to a few hours after ingestion of the offending food (FAO, 2001).

The prevalence of food allergy in the Western world is up to 8% in children and 2% in adults (Sampson and Burks, 1996). Therefore, one of the concerns of the regulatory authorities before accepting a genetically modified food or a novel food, i.e. a food not previously eaten by the population, is whether the food causes allergy (FAO, 2001).

At present, no validated animal model is available for testing whether a food or proteins derived from a food are allergenic. In recent years, efforts have been made for development of animal models for studying the immediate type of food allergies. An ideal animal model should be able to rank well-known strong, medium, and weak food allergens as well as proteins that are immunogenic but not allergenic, i.e.

* Corresponding author. Tel.: +45-33-95-60-00; fax: +45-33-95-60-01.

E-mail address: kpi@fdi.dk (K. Pilegaard).

proteins recognized by the immune system but not known as causes of food allergy. If such a ranking of well-known food proteins could be done satisfactory in an animal model, a validation of the model is subsequently needed to ensure that the results can be repeated in other laboratories and eventually the test may be incorporated in a guideline, e.g. OECD-guideline.

Guinea pigs, mice, and rats have been used in food allergy models. For years a guinea pig model in which the potential allergen is given orally has been used. After intravenous (i.v.) challenge, the measured end point is bronchospasm/anaphylaxis. However, the antibodies eliciting the allergic reactions in guinea pigs are not IgE but probably IgG1 (Pahud et al., 1988). A model with Balb/c mice given protein by intraperitoneal injection without the use of adjuvant is under development (Dearman et al., 2000, 2001, 2002; Dearman and Kimber, 2001). Also rats have been used for the development of food allergy models with and without adjuvants. Especially, the Brown Norway rat strain, a so-called high IgE responder strain, has been studied. Atkinson et al. (1996) used Brown Norway rats in a model where the production of specific IgE was studied after oral dosing with ovalbumin (OVA) and intraperitoneal administration of carrageenan as an adjuvant. The same model has been used for ranking of food proteins (Miller et al., 1998). A model without adjuvant seems preferable since an adjuvant may skew the IgE response making the ranking of strong and weak allergens difficult or may induce a false-positive IgE response with a nonallergenic food/protein. One of the advantages of using a rat model instead of a mouse model is that rats can easier be bled serially whereupon changes in individual immunoglobulin responses can be followed. Without adjuvant, OVA-specific IgE and IgG were induced in Brown Norway rats after gavage dosing with OVA whereas other tested rat strains: Wistar, Hooded Lister, and Piebold Virol Glaxo only developed specific IgG (Knippels et al., 1999a). When sensitized, the immune system of Brown Norway rats and humans recognizes similar allergens (Knippels et al., 2000). Specific IgE antibodies against OVA, ovomucoid and ovotransferrin were found in all sera from young patients with previously proven allergy to hen's egg white and in all sera from Brown Norway rats dosed by gavage with an extract of hen's egg white proteins (Knippels et al., 2000).

Hen's egg white is a common food allergen. OVA and ovomucoid have been identified as some of the major allergens from egg white (Sampson and Burks, 1996). OVA is one of the extensively studied proteins in animal models both in the Brown Norway rat models and in the murine model (Atkinson et al., 1996; Knippels et al., 1998a, 1999a,b; Dearman et al., 2000, 2001, 2002).

A prerequisite for the full development of specific antibodies eliciting allergic reactions after oral administration of a protein, is that the guinea pigs, rats or mice are kept on a diet free from the studied protein for at least two generations (Pahud et al., 1988; Knippels et al., 1998a; Christensen et al., 2003).

We have developed a Brown Norway rat model dosing newborn rats with OVA in the mouth (Madsen and Pilegaard, 2003). It was expected that the animals would be primed resulting in a high number of rats developing OVA-specific IgE when after weaning the animals were dosed with OVA by gavage. However, we found that this administration schedule did not give rise to a higher number of IgE responders compared to animals dosed solely after weaning.

Since Knippels showed that gavage dosing for 42 days of young, adult Brown Norway rat without the use of adjuvant gave rise to a high number of IgE-positive responders when dosed with the strong allergen, OVA, we wanted to adapt the model to our laboratory conditions. In our experiments, the dosing period was reduced to 35 days as it was apparent from Knippels' results that the number of responders with OVA-specific IgE as well as the titres were similar Days 35 and 42 (Knippels et al., 1999a, 2000).

Male and female Brown Norway rats bred for at least three generations on an egg-free diet in our animal facilities were used. In all experiments, OVA-specific IgG and IgE titres in serum and number of responders were studied. Four experiments were performed. One compared Brown Norway rats from the supplier Charles River used by Knippels et al. (1998b, 1999a,b) with Brown Norway rats from our colony. A second experiment studied responses in Brown Norway rats dosed with OVA from 4 or 8 weeks of age. A third experiment compared various preparations of hen's egg: dried egg white, OVA and fresh egg white. In a fourth experiment, the gavage volume was decreased to 0.1 ml per rat and compared with the volume of 0.5 used in all our previous experiments.

2. Methods

2.1. Animals

In one experiment, the supplier experiment, rats were purchased from Charles River, Sulzfeld, Germany. They were bred and raised on a commercially available egg-free rodent diet (Special Diet RM3(E)SQC, SDS, Witham, Essex, UK). Animals in all other experiments were Brown Norway rats originating from Iffa Credo, France, bred in our own animal facilities. They had been kept on the above-mentioned egg-free diet for at least three generations. The rats were weaned at 21 days of age and afterwards housed in wire cages (two to three per cage) with a 12 h day:night cycle with light from fluorescent tubes from 9 a.m. to 9 p.m. The room temperature was $22 \pm 1^\circ\text{C}$ and the relative humidity $55 \pm 5\%$. Diet and acidified tap water were given ad libitum. The animals were observed twice a day and clinical signs were recorded. Body weight (b.w.) was recorded once a week. The guidelines formulated in Council of Europe's 'Convention for the protection of vertebrate animals used for experimental and other scientific purposes' were followed. Treatments of the rats followed procedures approved by the Animal Experimentation Inspectorate.

2.2. Ovalbumin

Animals were dosed with OVA grade II (A 5253, Lot 47H0966, crude, dried egg white, Sigma, St. Louis, USA), OVA grade V (A 5503 Sigma, Lot

78H7007, minimum purity 98%) or organic, pasteurized frozen egg white purchased in a local food store. Before the beginning of an experiment, solutions for dosing were made, divided in aliquots, and frozen at -20°C . Fresh aliquots were used every day. The protein content of egg white is approximately 10%. The OVA content of OVA grade II and of fresh egg white is approximately 50% (Yunginger, 1997). Positive control sera for OVA were produced by immunizing Brown Norway rats i.p. with OVA grade V (A 5503 Sigma, Lot 78H7007). The content of endotoxin was 192 EU/mg in the abovementioned batch of OVA grade II. The endotoxin assay was performed at Statens Serum Institute, Copenhagen.

2.3. Experimental design

In all experiments, test solutions were administered by gavage for 35 days using a blunt-ended stainless steel animal feeding needle. An overview of the experiments is shown in Table 1.

2.3.1. Supplier experiment

Fourteen Brown Norway rats, 5-week-old, were purchased from Charles River. From 6 weeks of age, eight animals, four males and four females, were dosed with 0.5 ml tap water containing 1 mg OVA (grade II). Six rats, three males and three females, served as control animals and were left untreated.

2.3.2. Age experiment

Forty-five Brown Norway rats from our own colony were divided into three groups. Each group consisted

Table 1

An overview of the experiments: supplier of Brown Norway rats, test substance, dose of test substance, dosing volume, and age at which dosing commenced

Experiments	Supplier of rats	Test substance	Dose of test substance per rat (mg)	Dosing volume (ml)	Dosing start at age (weeks)
Supplier	Charles River	OVA (grade II)	1	0.5	6
Age	Own colony	OVA (grade II)	1	0.5	4
	Own colony	OVA (grade II)	1	0.5	8
Egg white	Own colony	OVA (grade II)	1	0.5	4–7
	Own colony	OVA (grade V)	0.5	0.5	4–7
	Own colony	Egg white		0.5	4–7
Dosing volume	Own colony	OVA (grade II)	1	0.1	5

of rats from 3 to 7 litters. The control group, eight males and five females, was dosed from 4 weeks of age with 0.5 ml tap water. A second group, eight males and eight females, was dosed from 4 weeks of age with 1 mg OVA (grade II) in 0.5 ml tap water. A third group, eight males and eight females, was 8 weeks of age when dosing began but otherwise the dosing schedule was identical to the second group.

2.3.3. Egg white experiment

The control group, five male and five female Brown Norway rats, was left untreated. A second group was dosed with 1 mg OVA (grade II) in 0.5 ml tap water, a third with 0.5 mg OVA (grade V) in 0.5 ml tap water, and a fourth received 0.5 ml tap water with fresh egg white corresponding to 0.5 mg OVA. All dosed groups consisted of eight males and eight females and the rats were from our own colony. Dosing began when the rats were 4–7 weeks old. Each group consisted of rats from 6 to 7 litters.

2.3.4. Dosing volume experiment

Sixteen, eight males and eight females, 5-week-old Brown Norway rats from our own colony received 1 mg OVA (grade II) in 0.1 ml tap water. The males originated from 3 litters and the females from 4 litters.

2.4. Sampling of serum

Blood was drawn on rats fasted overnight Days 28, 35, and 42 after commencement of dosing. Blood samples were taken from the orbital plexus in rats anaesthetized s.c. with 0.15 ml/100 g Hypnorm/Dormicum, consisting of one part of Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) (5 mg/ml), one part of Dormicum (Roche, Hvidovre, Denmark) (5 mg/ml) and two parts of sterile water. Individual serum samples were prepared and stored at -20°C until analysis.

2.5. Enzyme linked immunosorbent assay (ELISA)

Serum samples were analyzed for specific IgE (capture ELISA) and specific IgG (direct ELISA). In each ELISA plate, a positive and a negative control serum pool was included to check the reproducibility of test results. The positive sera were from animals immu-

nized i.p. with 100 μg OVA (grade V) in 3% $\text{Al}(\text{OH})_3$ Day 1, followed by 10 μg OVA i.p. Days 14, 28, and 48. The negative sera were from undosed animals. Analysis of individual test sera was done once. The test for specific IgE was modified after Knippels et al. (1998b) as described earlier (Madsen and Pilegaard, 2003) using a capture ELISA with monoclonal mouse anti-rat IgE as capture layer, serial dilutions of serum (starting at 1:4) and digoxigenin coupled OVA as third layer. Detection of OVA-specific IgG was done using a direct ELISA with OVA coated microtitre plates as described in Madsen and Pilegaard (2003).

2.6. Statistics

Differences between groups were considered statistically significant at $P < 0.05$. Number of IgG or IgE responders were compared using Fischer's exact test. Median IgG or IgE titres were compared for male and female data separately, and for all groups. The Mann–Whitney test was used for comparison of two groups. For comparison of more than two groups, Kruskal–Wallis test was used followed by Dunn's test if statistically significant. If no statistically significant effect was found within each sex, male data were pooled and compared to pooled female data. Data from experiments where rats were dosed with 1 mg OVA (grade II) in 0.5 ml tap water were pooled and compared with data from animals receiving 0.1 ml. Relationship between initial dosing volume, or mean intake of pure OVA and titres was tested by linear regression. Excluding animals not eliciting IgG titres, it was analyzed whether OVA-specific IgG titres giving rise to IgE responses differed from IgG responses not giving rise to IgE.

3. Results

There was no effect of dosing on the clinical appearance of the rats. In general, data from Day 42 after commencement of dosing are reported making comparison with data from studies performed by others possible. The number of OVA-specific IgE-responders Days 35 and 42 was very similar. In male rats 14/48 and 13/48 responded Days 35 and 42, respectively, and in females 31/48 and 30/48.

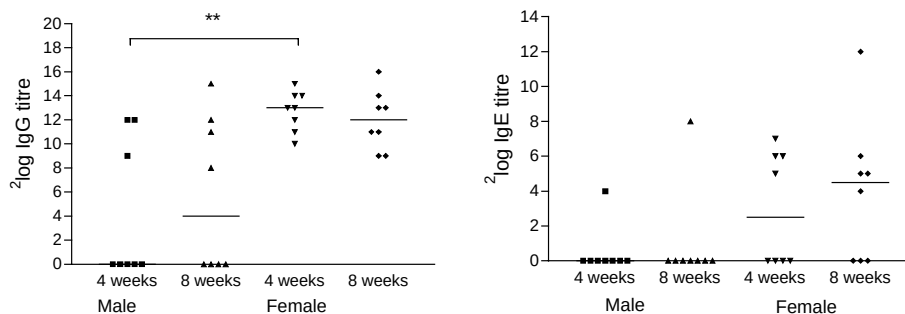


Fig. 1. Individual specific IgG (left) and IgE titres (right) to ovalbumin (OVA) Day 42 in male and female Brown Norway rats dosed daily by gavage for 35 days with 1 mg OVA (grade II) in 0.5 ml tap water. The rats were dosed from 4 or 8 weeks of age ($n = 8$). Horizontal lines indicate medians. ** $P < 0.01$, comparison between median IgG-titres.

3.1. Supplier experiment

Fifty percent, 2/4 males and 2/4 females, of the Brown Norway rats purchased from Charles River, developed OVA-specific IgE Day 42 after dosing by gavage with OVA grade II (1 mg in 0.5 ml tap water per rat) for 35 days. The $^2\log$ titres ranged from 4 to 5. For both male and female rats 75% was IgG-positive Day 42.

3.2. Age experiment

No effects on OVA-specific IgG or IgE titres or the number of IgG or IgE responders were observed between groups dosed from 4 or 8 weeks of age when comparing males and females separately (Fig. 1). A

statistically significantly higher median OVA-specific IgG titre was found in female rats compared to male rats dosed from 4 weeks of age.

3.3. Egg white experiment

Neither in male nor female rats did gavage dosing with equal amount of OVA in various egg white preparations: 1 mg OVA (grade II), 0.5 mg OVA (grade V), or 0.5 mg OVA from egg white for 35 days result in statistically significant differences of median IgG titres or IgE titres or number of responders when tested Day 42 (Fig. 2). No difference was observed in median OVA-specific IgG titres between male and female rats dosed with 1 mg OVA (grade II) whereas median IgG titres were statistically significantly higher in females

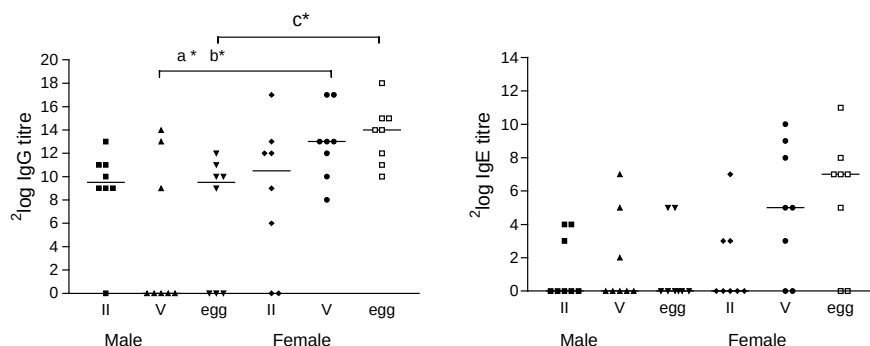


Fig. 2. Individual specific IgG (left) and IgE titres (right) to ovalbumin (OVA) Day 42 in male and female Brown Norway rats dosed daily by gavage for 35 days with 1 mg OVA grade II (II) in 0.5 ml tap water, 0.5 mg OVA grade V (V) in 0.5 ml tap water, or 0.5 ml tap water with fresh egg white (egg) ($n = 8$). All doses correspond to 0.5 mg OVA per rat per day. Horizontal lines indicate medians. * $P < 0.05$. (a, c): Comparison between median IgG titres; (b): comparison between number of OVA-specific IgG responders.

compared to males both after dosing with OVA (grade V) and egg white. Additionally, a statistically significantly higher number of females than males elicited an IgG response after dosing with OVA grade V.

3.4. Dosing volume experiment

Using 1 mg OVA (grade II) but reducing the dosing volume from 0.5 to 0.1 ml had no statistically significant influence on median titres or number of OVA-specific IgG or IgE responders neither in male nor female rats. Data on number of responders are shown in Table 2. When using 0.5 ml tap water, the intake volumes at the commencement of the dosing period ranged from 2.5–8.9 ml/kg (b.w.) (in males) to 3.7–10.0 ml/kg b.w. (in females). A dosing volume of 0.1 ml tap water gave rise to an initial volume ranging from 0.7–1.0 ml/kg b.w. (in males) to 1.0–1.2 ml/kg b.w. (in females). The initial volumes and the corresponding IgG and IgE titres are shown in Fig. 3. No linear relationship between initial volumes and OVA-specific IgG titres was seen in male and female rats or in male rats concerning IgE. In female rats a statistically significant inverse relationship ($r^2 = 0.14$, $P = 0.04$) between dosing volume per kg b.w. and OVA-specific IgE titres was seen.

3.5. Dose response

The calculated daily intake of pure OVA was 3.4 ± 0.7 mg/kg b.w. (mean $\pm$ S.D.) in male rats and 4.7 ± 0.6 mg/kg b.w. in female rats. No relationship between mean intake and OVA-specific titres was found (Fig. 4).

3.6. Responses in male and female rats

Females had statistically significantly higher responses than males when data from the four groups dosed with 1 mg OVA (grade II) were compared ($P < 0.001$ for OVA-specific IgG titres, $P < 0.01$ for number of IgG responders, $P < 0.05$ for OVA-specific IgE titres and number of IgE responders). The higher the OVA-specific IgG titre the more likely that the animal also developed an OVA-specific IgE response. In males $^2\log$ IgG titres were 9.6 ± 1.7 (mean $\pm$ S.D.) in IgE-negative animals versus 12.4 ± 1.9 in IgE-positive ($P < 0.01$). In female rats $^2\log$ IgG titres were 10.4 ± 2.0 in IgE-negative versus 14.0 ± 1.8 in IgE-positive rats ($P < 0.001$), excluding rats not eliciting an OVA-specific IgG response. It was found that a rat eliciting a specific IgE response always simultaneously had a specific IgG response.

Table 2

Mean ovalbumin (OVA)-specific IgG and IgE titres in positive responders, and the number of OVA-specific IgG and IgE responders Day 42 in male (M) and female (F) Brown Norway rats

Experiment	Sex	IgG responders	$^2\log$ IgG titres (mean $\pm$ S.D.)	IgE responders	$^2\log$ IgE titres (mean $\pm$ S.D.)
Age (weeks)					
4	M	3/8	11.0 ± 1.7	1/8	4
8	M	4/8	11.5 ± 2.9	1/8	8
4	F	8/8	12.8 ± 1.7	4/8	6.0 ± 0.8
8	F	8/8	12.4 ± 2.3	5/8	6.4 ± 3.2
Egg white					
OVA (grade II)	M	7/8	10.3 ± 1.5	3/8	3.7 ± 0.6
OVA (grade V)	M	3/8	12.0 ± 2.6	3/8	4.7 ± 2.5
Fresh egg white	M	5/8	10.4 ± 1.1	2/8	5.0 ± 0.0
OVA (grade II)	F	6/8	11.5 ± 3.7	3/8	4.3 ± 2.3
OVA (grade V)	F	8/8	12.9 ± 3.1	6/8	6.7 ± 2.7
Fresh egg white	F	8/8	13.6 ± 2.6	6/8	7.5 ± 2.0
Dosing volume (ml)					
0.1	M	4/8	11.8 ± 4.2	3/8	7.3 ± 1.5
0.1	F	7/8	13.0 ± 1.6	6/8	6.5 ± 0.5

The animals were dosed by gavage with 1 mg OVA (grade II) in 0.5 ml tap water from 4 or 8 weeks of age, with 1 mg OVA (grade II), 0.5 mg OVA (grade V), or with fresh egg white in 0.5 ml tap water, or with 1 mg OVA (grade II) in 0.1 ml tap water. The dosing period was 35 days.

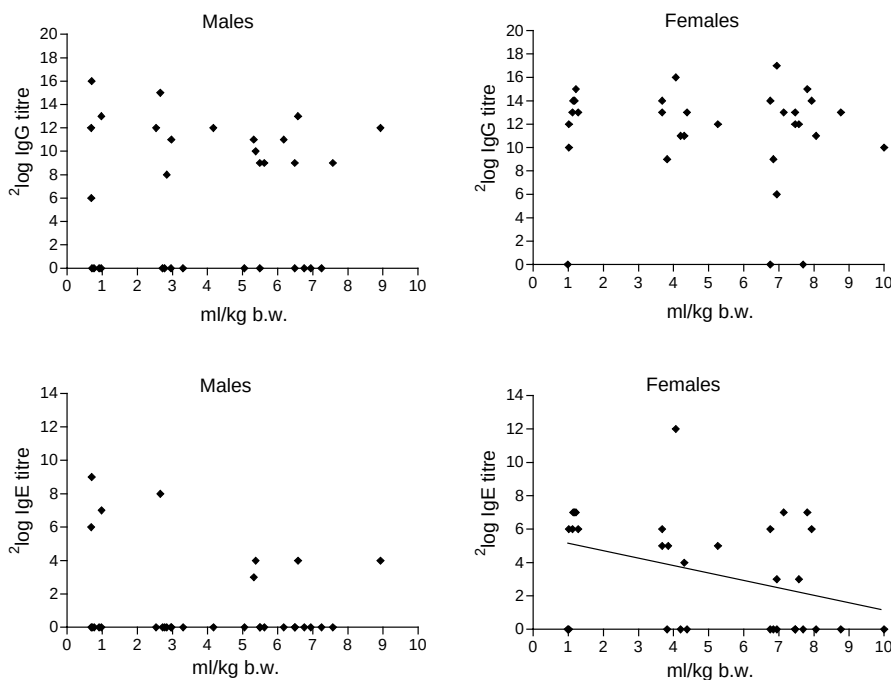


Fig. 3. Relationship between initial gavage dosing volume per kg body weight (b.w.) and the specific IgG (upper panel) or IgE titres (lower panel) to ovalbumin (OVA) Day 42 in male (left) or female (right) Brown Norway rats dosed daily by gavage for 35 days with 1 mg OVA (grade II) in either 0.1 or 0.5 ml tap water ($n = 32$). Regression lines with slopes varying statistically significantly ($P < 0.05$) from 0 are shown.

4. Discussion

At Day 42, OVA-specific IgE was detected in 50% of the Brown Norway rats from Charles River dosed by gavage with 1 mg OVA for 35 days. A lower number of positive responders than reported by Knippels and co-workers in experiments on rats from the same supplier (Table 3). The Brown Norway rat strain is an inbred strain, i.e. brother and sister are mated for generations. Variation in characteristics may therefore exist between Brown Norway colonies. However, a variation in genetic makeup cannot explain the difference in number of responders observed between the two laboratories since Brown Norway rats from Charles River were in our hands no better in developing specific IgE than rats originating from Iffa Credo and bred and raised in our animal facilities for generations. The number of OVA-specific IgE responders was 13–38% in males and 38–75% in females (Table 2).

Whether dosing commenced when the rats were 4 or 8 weeks of age had no influence on the IgG or IgE titres

or number of responders. Thus, age had no influence on the response and subsequently in other experiments age within groups varied from 4 to 7 weeks of age.

Dosing with OVA from various sources and purity: OVA grade II, OVA grade V, or OVA from fresh egg white did not result in statistically significant differences in OVA-specific IgG or IgE titres or number of responders in male or female rats. In our experiments, the possible outcome of dosing with preparations of egg white is: no OVA-specific IgG or IgE, OVA-specific IgG and no IgE, or both IgG and IgE. The phenomenon that the rats did not raise an IgG response against OVA has not been reported in the studies by Knippels or Dearman when dosing with 1 mg OVA but was also observed in the study by Zhang and Crevel (1999) where 6/10 Brown Norway rats did not elicit an IgG response (Table 3). Knippels et al. (2000) found that increasing the dose to 10 mg OVA gave rise to a lower number of responders, 4/6 developing an OVA-specific IgG response and 5/6 developing an IgE-response, compared to 6/6 responders with

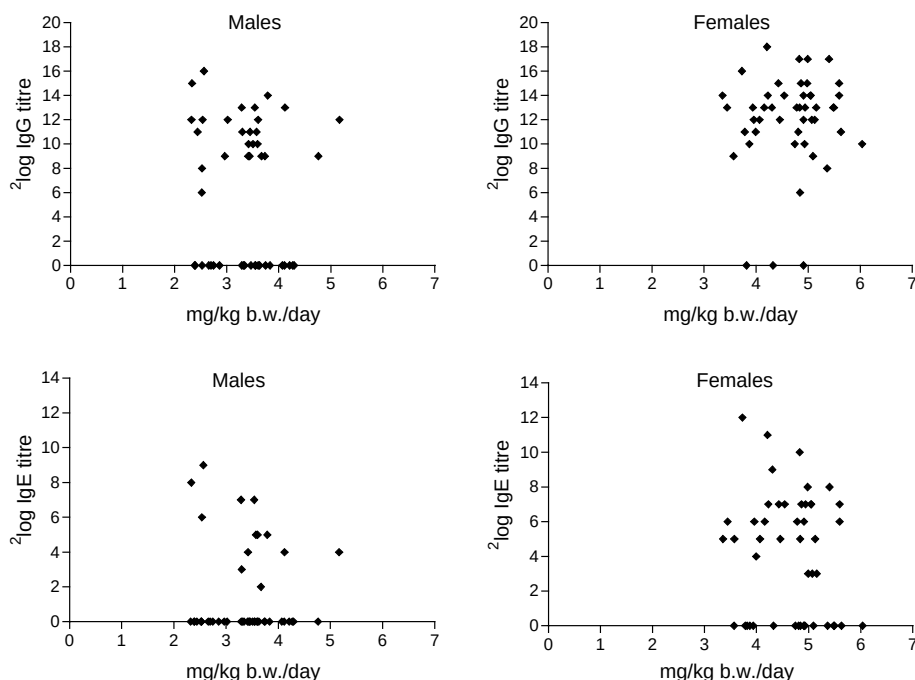


Fig. 4. Relationship between mean intake of pure ovalbumin (OVA) and specific IgG (upper panel) or IgE titres (lower panel) to OVA Day 42 in male (left) or female (right) Brown Norway rats dosed daily for 35 days with 1 mg OVA (grade II) in either 0.1 or 0.5 ml tap water, with 0.5 mg OVA (grade V) or with fresh egg white in 0.5 ml tap water ($n = 48$). All doses were equivalent to 0.5 mg pure OVA. Slopes of regression lines were not statistically significantly different from 0 and are not shown.

Table 3

OVA-specific IgG and IgE titres and the number of OVA-specific IgG and IgE responders after daily oral gavage dosing of 1 mg OVA in 1 ml tap water per rat without the use of adjuvant for 42 days as reported in the literature

OVA		Brown Norway rats						
Purity (%)	Supplier	Sex	Supplier	Dosing began at age (weeks)	IgG responders	$^2\log$ IgG titres (mean $\pm$ S.D.)	IgE responders	$^2\log$ IgE titres ^a (mean $\pm$ S.D.)
70	Fluka	M	Charles River, Sulzfeld, Germany	4–6	8/8	12.8 ± 3.2	7/8	6.5 ± 2.8 Knippels et al. (1998b)
70	Fluka	M	Charles River, Kent, UK	4–6	6/6	9.3 ± 2.7	6/6	6.3 ± 2.9 Knippels et al. (1999a)
70	Fluka	M	Charles River, Sulzfeld, Germany	4–6	n.s.	n.s.	20/28 ≥ 5	n.s. Knippels et al. (1999b)
70	Fluka	M	Charles River, Sulzfeld, Germany	4–6	6/6	n.s.	6/6	6.8 ± 3.0 Knippels et al. (2000)
n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4/6	n.s. Knippels et al. (2002)
98	Sigma	F	Harlan Seralab, Oxfordshire, UK	8–10	6/6	n.s.	0/6	n.s. Dearman et al. (2001)
n.s.	n.s.	n.s.	n.s.	n.s.	4/10	n.s.	n.s.	n.s. Zhang and Crevel (1999)

The rats in references Knippels et al. 1998b, 1999a,b, 2000 had been bred and raised on an OVA-free diet, no information was given on the diet in Knippels et al. (2002), Zhang and Crevel (1999), and the diet was comprised primarily of cereals (Dearman et al. (2001)). In Knippels et al. 1998b, 1999a,b, 2000, IgE was measured with a capture ELISA, in Knippels et al. (2002), Dearman et al. (2001) by passive cutaneous anaphylaxis test; n.s.: not specified.

^a $^2\log$ IgE titres are given for responders.

1 mg. As all rats in our experiment were dosed with a fixed dose, the mean OVA intake varied from 2.3 to 5.2 mg/kg b.w. per day in males to 3.4–6.0 mg/kg b.w. per day in females. No relationship between mean intake of OVA and IgG or IgE titres was found (Fig. 4). Since daily intake of OVA in male and female rats are overlapping, the higher titres found in female rats compared to males seem to be a sex difference and not to be caused by variation in daily intake.

Exposure via inhalation to very low doses of OVA elicits an IgE response in rodents. Fifty percent (3/6) male Brown Norway rats exposed to less than 1 mg/m³ of respirable OVA aerosols for 30 min weekly for 6 weeks had developed OVA-specific IgE after the last day of exposure (Siegel et al., 2000). Administration of OVA by inhalation (25 mg/l) to BALB/cJ female mice for 20 min per day for 10 consecutive days followed by weekly inhalation for 6 weeks, resulting in a cumulated dose of 1.0 µg OVA in the respiratory system, elicited an IgE response in 4/9 animals (Clausen et al., 2003). Elvig-Jørgensen et al. (2002) have demonstrated that a test substance may unintentionally end up in the lung of some of the rats when high volumes of test solutions (10 or 20 ml/kg b.w.) are used. The mechanism is thought to be inhalation of the test substance deposited in the pharyngeal cavity after reflux from the stomach. This effect was not seen when using doses of 1 ml/kg b.w. twice daily. Whether pulmonary exposure takes place between the studied 1 or 10 ml/kg b.w. per day, where most of our data from rats dosed with 0.5 ml are found, has to our knowledge not been studied. However, reduction of the volume from 0.5 to 0.1 ml did not change the OVA-specific IgG or IgE titres or the number of responders. Neither was a linear relationship between initial volume/kg b.w. and IgG titres in males or females, or IgE titres in males observed. An inverse linear relationship between initial volume/kg b.w. and female IgE titre was found indicating that IgE titres are lower at higher volumes but only based on few data close to 10 ml/kg b.w. per day and no statistical significance is found if the single observation at 10 ml/kg b.w. is excluded.

Knippels and co-workers used a dosing volume of 1 ml in male Brown Norway rats and found the number of IgE responders varying from 67 to 100% (Table 3). If the body weight of these male rats is similar to the one seen in our 4-week-old rats, the initial dosing volume was 12.9 ± 3.0 ml/kg b.w. (mean $\pm$ S.D.), a

volume where some of the dosing material could end up in the lung (Elvig-Jørgensen et al., 2002). In the study by Dearman et al. (2001), no IgE responders were observed when 1 ml OVA solution was used for gavage dosing of female Brown Norway rats beginning at 8–10 weeks of age. Based on data from our female 8-week-old rats, the estimated initial dosing volume was 8.0 ± 0.6 ml/kg b.w. Our data show that dosing female rats with a volume of up to 10 ml/kg b.w. and male rats with a volume up to 9 ml/kg b.w. do not increase the likelihood of developing specific IgE compared to volumes around 1 ml/kg b.w. making it unlikely that volumes under 10 ml/kg b.w. end up in the lung initiating an immune response. The passive cutaneous anaphylaxis test as used by Dearman and co-workers is less sensitive than ELISA (Knippels et al., 1998b, 2000). This may also contribute to explaining the lack of IgE responders in the Dearman study. Whether a gavage dosing volume higher than 10 ml/kg b.w. leads to pulmonary exposure initiating an IgE response via the respiratory system and not the gastrointestinal tract should be further investigated.

A general finding in our experiments was higher mean or median titres in females compared to males and a higher number of females eliciting OVA-specific IgG and IgE antibodies than male rats. The finding was unexpected and not observed by others since Knippels and co-workers have studied male rats only and Dearman et al. (2001) female rats in a single study. This difference between males and females seems to be caused by sex and not by differences in dosing volume per weight or daily intake.

A relationship between the IgG and IgE titres was found. It is not possible to guess whether a rat is an IgE responder only by observing the IgG titre but the higher the IgG titre the more likely that the rat also elicits an IgE response.

The batch of OVA grade II used was contaminated with endotoxin (192 EU/mg). It seems not to be uncommon that commercial protein preparations contain endotoxin, e.g. endotoxin contents varied from 17 to 157 EU/mg in eight batches of hen egg lysozyme from Sigma, and the concentration of endotoxin was 0.5 or 50 EU/mg in bovine serum albumin from two suppliers (Reis e Sousa and Germain, 1999). Endotoxins are normally found in food and drinking water. As long as they get into contact with the digestion system they are apparently handled without problem (Petsch and

Ansprach, 2000). However, intravenous administration of endotoxin has a strong biological effect on the immune system and a threshold level of pharmaceuticals for i.v. administration is set to 5 EU/kg b.w. (Reis e Sousa and Germain, 1999; Petsch and Ansprach, 2000).

For the purpose of developing a Brown Norway rat model for food allergy, a high proportion of animals eliciting an IgE response when dosed with a strong allergen is of utmost importance. In comparison with the number of IgE responders found by Knippels and co-workers disappointingly few animals developed specific IgE in our experiments (Tables 2 and 3). We do not think that the differences in number of IgE responders are due to a difference in sensitivity between methods used. Both laboratories use a capture ELISA and a lower number of male IgE responders are found irrespectively of whether the mean IgE titres are lower or higher than the levels reported by Knippels. Additionally, the lower number of IgE responders is reflected in a simultaneous lower number of OVA-specific IgG responders. The low number of responders seen in male rats dosed with a strong allergen like OVA would not make identification of weaker allergens or ranking of allergens with various potencies possible. The number of responders seen in the female rats was higher but varied between experiments. If we should use the oral model for food allergy for examination of the potential allergenicity of a protein or a novel food of unknown allergenicity, female rats should be chosen and group sizes should be increased. Models like the murine model developed by Dearman and co-workers where proteins are administered by intraperitoneal injection could give an indication of the allergenic potential of a protein for hazard identification but whether the protein in question would also cause allergy when eaten is unresolved. The Brown Norway rat model without the use of adjuvant is a very attractive model when succeeding but too few responders were seen in our laboratory. These interlaboratory differences in number of responders, that were seen repeatedly in our experiments, cannot be attributed to different suppliers of Brown Norway rats or induction of oral tolerance due to feed contaminated with egg, and were seen both when using a daily gavage dosing volume per rat of 0.5 and 0.1 ml, and when studying various preparations of eggs. In conclusion, due to higher number of female than male

responders, it is in our laboratory most promising to use female Brown Norway rats for further studies with the oral Brown Norway rat model for food allergy. In the future, other dosing regimes will also be studied.

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