Assessment of the Allergic Potential of Food Protein Extracts and Proteins on Oral Application Using the Brown Norway Rat Model

Léon M.J. Knippels and André H. Penninks

TNO Nutrition and Food Research, Department of Target Organ Toxicology, Working Group Experimental Immunology, Zeist, The Netherlands

The need for widely accepted and validated animal models to test the potential allergenicity and potency of novel (biotechnology-derived) proteins has become an important issue for their safety evaluation. In this article, we summarize the results of the development of an oral sensitization protocol for food proteins in the rat. Young Brown Norway rats were exposed to either various purified allergenic proteins (e.g., ovalbumin, partly purified), a whole food (cow's milk), or total protein extracts (hen's egg white, peanut) by daily gavage dosing during 42 days without the use of an adjuvant. The results showed that Brown Norway rats can be sensitized orally to the various allergenic food proteins tested, resulting in antigen-specific immunoglobulin (Ig) G and IgE responses, without the use of adjuvants. Animals orally exposed to cow's milk or total protein extracts of egg white also developed specific IgE and IgG antibodies that recognized the same proteins compared with antibodies from patients allergic to egg white or cow's milk. We also studied local and systemic immune-mediated effects. In ovalbumin-sensitized rats, some clinical symptoms of food allergy were studied upon an oral challenge with ovalbumin. The results demonstrated that gut permeability was increased and that in some animals breathing frequency and systolic blood pressure were temporarily decreased. The results obtained show that the Brown Norway rat provides a suitable animal model for food allergy research and for the study of relative allergenicity of existing and novel food proteins. *Key words:* allergic potential, Brown Norway rats, challenge effects, egg-white proteins, food allergy, food proteins, IgE, milk proteins, oral sensitization, ovalbumin, passive cutaneous anaphylaxis. *Environ Health Perspect* 111:233–238 (2003). [Online 21 January 2003]

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Food allergy is an area of growing interest for several reasons. First, although evidence is scientifically insufficient for proof, there are indications that the number of individuals suffering from food allergy is increasing. Second, it is anticipated that for rapid developments in biotechnology, which will result in the introduction of new proteins and new biologic varieties for application in food, the potential allergenicity will pose a major additional concern in their safety assessment. The first structured approach to assess the potential allergenicity of novel proteins was the decision tree jointly prepared by the International Food Biotechnology Council (IFBC) and the International Life Science Institute (ILSI) Allergy and Immunology Institute (Metcalfe et al. 1996). The various elements of this decision tree provide important and relevant information on suspected characteristics of the protein related to allergy. However, because no widely accepted animal models were available at that time, the IFBC/ILSI decision tree did not include animal testing procedures, although its importance was stressed (Metcalfe et al. 1996). Nevertheless, it is clear that only this kind of testing will allow direct determination of the (relative) sensitizing potential of existing and novel proteins. An Expert Consultancy on Allergenicity of Foods Derived from Biotechnology, formed under the auspices of the Food and Agriculture Organization (FAO) and World Health Organization (WHO), released a revised decision tree approach in 2001 (FAO/WHO 2001), which includes the introduction of animal models. As mentioned in the conclusions of that report, validation of these animal models is still an ongoing process. The Codex Open-Ended Working Group on Allergenicity, which convened in Vancouver, British Columbia, Canada, 10–12 September 2001, suggested therefore that animal models be again labeled as "Areas requiring further development" (Codex 2001); this document indicates that "the use of animal models, once developed and validated, could enhance the weight of evidence used" (p. 8). Along with these discussions, research is proceeding by various groups to make progress in the development of suitable animal models.

In this article we summarize information concerning the progress made in a new rat model for allergenicity testing. The results obtained up to now indicate that this Brown Norway (BN) rat model is likely to become a valuable tool, not only for the improvement of our knowledge on food allergy in general, but also for the assessment of the allergenic potential and potency of genetically engineered foods.

Food Allergy Model in BN Rats

To study the sensitizing potential of new proteins, animal models need to satisfy several important criteria (Penninks and Knippels 2001; Taylor and Lehrer 1996), which are not easy to achieve in one single model. Important criteria to consider are selection of species and strain, production of a Th2 response [resulting in immunoglobulin (Ig) E production], tolerance to most food proteins, route of exposure for sensitization and challenge, and the use of adjuvants. Because preliminary studies at our institute showed promising results in the rat, we started to develop an oral feeding protocol to sensitize rats to food proteins. Comparative sensitization studies with Wistar, Piebald Virol Glaxo (PVG), Hooded Lister, and BN rats, using ovalbumin (OVA), a well-defined egg-white allergen, as a first model allergen, revealed that only the BN rats developed OVA-specific IgE antibodies (Knippels et al. 1999b). This is in line with the knowledge that the BN rat is a high-immunoglobulin (particularly IgE) responder strain and thus, to a certain degree, resembles atopic humans in their genetic predisposition to react more readily with an IgE production to antigens. Because of this preferential response and the results obtained in the comparative study, the BN rat was chosen for the further development of an oral sensitization model (Knippels et al. 1998b). In subsequent studies (Knippels et al. Unpublished data), a whole food [cow's milk (CM)], whole protein extracts of hen's egg white (HEW) and peanut, and purified strong-allergenic (Arah1 from peanut and Pen a1 from shrimp), weak-allergenic (Sol t1 from potato tuber), and nonallergenic (beef tropomyosin) proteins were also used.

A critical factor that heavily affects the result of oral sensitization studies is the unscheduled dietary pre-exposure of test animals and their parental generation to the antigen under investigation. When oral sensitization studies with proteins are performed, one must ensure that at least two generations of animals are bred on a diet free of the antigen under investigation for the animals to be immunologically naive. It has

Address correspondence to A.H. Penninks, TNO Nutrition and Food Research, Department of Target Organ Toxicology, PO Box 360, 3700 AJ Zeist, The Netherlands. Telephone: 31-30-6944564. Fax: 31-30-6960264. E-mail: Penninks@voeding.tno.nl

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been shown that exposure of the parental generation to the antigen under investigation can influence the outcome of sensitization studies with the offspring (Knippels et al. 1998a).

In the sensitized BN rats, we also studied whether, upon renewed oral antigen exposure challenge, clinical reactions could be observed as are those in food-allergic patients upon exposure to the offending food. The many different clinical signs or physiologic reactions observed upon renewed oral antigen exposure in food-allergic patients frequently include effects on gastrointestinal physiology, whereas only in some patients are effects on the respiratory and/or cardiovascular system noted. Therefore, in OVA-sensitized rats, oral challenges were performed by intragastric intubation 10 days after the oral sensitization period of 42 days, with doses of OVA varying between 10 and 100 mg. As part of the oral challenge, effects on blood pressure, gastrointestinal permeability, and breathing frequency were investigated (Knippels et al. 1999a).

Materials and Methods

Proteins. The various different antigen sources used in the oral sensitization studies were either prepared or obtained from different suppliers. The total HEW protein extract was prepared essentially according to the method of Bernhisel-Broadbent et al. (1994). A more detailed description of the preparation of this protein extract is given by Knippels et al. (2000). Other antigen sources used were OVA (Fluka Chemie, Buchs, Switzerland; purity, 70%) and skimmed CM (Albert Heijn, Zaandam, The Netherlands; 4 g protein/100 mL milk). The purified proteins used—Arah1, Sol t1, Pen a1, and beef tropomyosin—were purified from their native foods (peanut, potato tuber, raw brown shrimp, and raw beef, respectively). Verification of purity and identity were followed by SDS-PAGE and N-terminal sequencing or MALDI TOF, respectively. Ara h1 (molecular weight, 60–67 kDa; purity, 88.0%), Sol t1 (molecular weight 40–43 kDa; purity, 92.6%), Pen a1 (molecular weight, 37–38 kDa; purity, 94.6%), and beef tropomyosin (molecular weight, 35–39 kDa; purity, 99.3%) were prepared by Gary Bannon et al. (Unpublished data).

Rats. For the sensitization studies, young male BN rats, 4–6 weeks old at study initiation, were obtained from Charles River (Sulzfeld, Germany). The rats were housed in an animal room maintained at 23 ± 3°C, with a light/dark cycle of 12 hr each and a relative humidity of 30–70% during the experiment and for at least 10 days before study initiation. The animals were housed in stainless-steel wire cages in groups of four or five and had free access to food and tap water. The rats were bred and raised on a commercially available rodent diet that was HEW-protein and CM-protein free (SDS Special Diet Service, Witham, England). Prestudy blood samples were always tested for HEW-, CM-, and peanut-protein–specific antibodies to ensure the use of immunologically naive animals with respect to the antigens under investigation. All animal studies were approved by an independent ethical committee.

Oral sensitization protocol. In the ultimate standard protocol used for most studies, the BN rats were exposed to the various proteins or protein extracts by daily gavage dosing, using a 18-gauge stainless steel animal feeding needle (standard dose is 1 mg protein/mL tap water, 1 mL/animal) during 6 weeks, without the use of an adjuvant. Blood samples were obtained from the orbital plexus under light CO_2 anesthesia at weekly intervals or by exsanguination from the abdominal aorta at sacrifice. After coagulation for 1 hr at room temperature, the blood samples were centrifuged (Heraeus Minifuge T, Osterode, Germany) for 20 min at 2,000 *g* and 4°C to obtain sera. The sera were stored at –20°C until analyses for antigen-specific IgG titers by enzyme-linked immunosorbent assay (ELISA) and antigen IgE by ELISA and passive cutaneous anaphylaxis (PCA) test in naive rats.

Assays for antigen-specific IgG and IgE antibodies. Serum antibodies (IgG, IgE) specific for the various allergenic proteins (extracts) tested were measured by ELISA. The procedures used to detect specific IgG and IgE antibodies to the purified proteins or protein extracts are described in detail in publications by Knippels et al. (1998a, 1998b, 1999a, 1999b, 2000). In summary, for the detection of antigen-specific IgG, 96-well microtiter plates (flat-bottomed; Maxisorp, NUNC, Roskilde, Denmark) were coated overnight at 4°C with 100 µL/well of the antigen in carbonate buffer, pH 9.6. After three washing steps with tap water containing 0.4% Tween 20 (Merck, Hohenbrunn, Germany), 100 µL/well phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; Sigma Chemicals Co., St. Louis, MO, USA) and 0.02% Tween 20 (PBS/BSA-Tween 20) was added. After 1 hr incubation at 37°C, the plates were washed, and serial dilutions of rat serum in PBS/BSA-Tween 20 were added to the wells and incubated for 1 hr at 37°C. After washing, 100 µL/well peroxidase-conjugated goat anti-rat IgG (H+L) (Zymed, San Francisco, CA, USA; diluted 1:500) in PBS/BSA-Tween 20 was added. After incubation for 1 hr at 37°C, the plates were washed again, and an enzyme substrate solution of 3,3′,5,5′-tetramethylbenzidine (TMB; Sigma Chemicals; 100 µL/well; 6 mg/mL dimethyl sulfoxide) was added. The plates were developed at room temperature for 5–15 min. Finally, 100 µL/well of 2N H_2SO_4 was added. Optical densities were read spectrophotometrically at 450 nm with an ELISA plate reader (Microplate Reader, Bio-Rad Laboratories, Richmond, VA, USA). Positive and negative control samples were incorporated for each 96-well plate. A preserum pool was used as negative control. The pooled preserum was measured at a 1:4 dilution. The average extinction in negative control wells, to which three times the standard deviation was added, provided the reference value taken to determine the titer in the test sera. Each test serum was titrated starting at a 1:4 dilution, and the reciprocal of the greatest serum dilution giving an extinction higher than the reference value was read as the titer. All analyses were performed in duplicate.

For the detection of antigen-specific IgE, 96-well microtiter plates were coated overnight at 4°C with 100 µL/well mouse anti-rat IgE (MARE-1, Zymed) at a concentration of 1.5 µg/mL in carbonate buffer, pH 9.6. The plates were washed, and 100 µL/well of PBS/BSA-Tween 20 was added. After incubation for 1 hr at 37°C, the plates were washed and diluted rat serum samples were added and incubated for 2 hr at 37°C. The plates were washed, and then 100 µL/well of a solution of an antigen-digoxigenin (Boehringer, Mannheim, Germany) conjugate was added as prepared according to the manufacturer's instructions. Incubation with antigen-digoxigenin was performed for 1 hr at 37°C, and after washing, 100 µL/well peroxidase-conjugated sheep anti-digoxigenin Fab fragments (Boehringer) diluted 1:3,000 in PBS/BSA-Tween 20 was added. After incubation for 1 hr at 37°C, the plates were washed again, and an enzyme substrate solution of TMB was added. Plate development, measurement, and titer elaboration were as described for the OVA-specific IgG ELISA.

Passive cutaneous anaphylaxis. To confirm the presence or absence of anaphylactic antibodies, rat sera were also tested by PCA essentially as described previously by Ovary (1964). In short, naive (untreated) BN rats were shaven on the back and flanks and injected intradermally with 0.1 mL of the test serum in serial dilutions, followed 64 hr later with an intravenous injection of 1 mL of a 1:1 mixture of a solution of the protein or protein extract studied (5 mg/mL sterile saline) and a solution of Evans blue (2% in sterile saline). After 20–30 min, the diameter of dye extravasation at the site of the serum injection was measured. The reaginic titer was read as the reciprocal of the greatest dilution giving a colored spot of at least 5 mm in diameter. Positive and negative control sera as used in the ELISAs were assayed simultaneously with the test sera on each animal used for the PCA tests.

Determination of challenge reactions. The procedures used for the detection of the respiratory function, blood pressure, and gut permeability on an oral challenge of sensitized rats are described in detail elsewhere (Knippels et al. 1999a). Main characteristics are presented briefly here.

Respiratory function. Both control and OVA-sensitized animals were orally challenged with 2 mL of increasing doses of an OVA solution in tap water. Respiratory frequency was assessed using a plethysmograph with a separate head and body chamber and matched pressure transducers. Rats were restrained in tubes placed in the body chamber with the open end of the tube fitting into the front chamber. Breathing frequencies were determined by recording the pressure signal in the volume-calibrated body chamber. Before challenge, the respiratory function was measured constantly for 15 min and immediately upon an oral challenge with OVA constantly during the first 10 min and thereafter for periods of 30 sec once every 5 min for a total period of 6 hr.

Blood pressure. During the sensitization period, the rats were trained to get used to the measurement equipment (an inflatable pressure cuff around the tail and a distal taped sensor) to avoid stress during the experiments. The control and the OVA-sensitized animals were orally challenged with 2 mL of a 5 mg/mL OVA solution in tap water or 2 mL of tap water. Subsequently, the systolic blood pressures were recorded at intervals during a period of 7 hr.

Gut permeability. To determine possible changes in gut permeability, the uptake of a bystander protein was determined in time after the challenge. Control and OVA-sensitized animals were orally challenged with 2 mL tap water or 2 mL of an OVA solution in tap water. The animals received an additional intragastric dose of β-lactoglobulin (β-LG; 1 mL of a 100 mg/mL solution in tap water) 30 min after the oral OVA challenge. Blood samples were collected from the orbital plexus under light CO_2 anesthesia at various time points after the β-LG administration. Sera were used for the quantification of β-LG by ELISA.

Results

Oral sensitization effects. Our studies have shown that BN rats, bred and raised on a diet free of the antigen to be tested, can be sensitized by daily dosing with the antigen via the enteral route without the use of adjuvants. The standard protocol used is based on the results of various studies with mainly OVA as allergen. In these studies, not only the mode of application (gavage vs. *ad libitum* in drinking water) but also the dose (0.002, 0.02, 0.2, 2, or 20 mg OVA/dose) and the frequency of application (daily, twice a week, once a week, once every 2 weeks) were studied (Knippels et al. 1998b). These studies revealed that the best results were obtained from daily intragastric dosing with 1 mg OVA during 42 days. Besides OVA-specific IgG, OVA-specific IgE antibody responses were found in these animals as measured by ELISA and PCA. Optimal OVA-specific IgE antibody responses were observed around days 28–35. The percentage of IgE responders in our OVA studies in general exceeded 80%. However, occasionally, no OVA-specific IgE responses were induced upon daily gavage dosing with OVA in our studies. Less frequent administration of 1 mg OVA by gavage, once a week or once every 2 weeks, did not induce specific IgG or specific IgE antibody responses. Upon gavage dosing twice a week for 6 weeks with 1 mg OVA, only one of four animals developed an OVA-specific IgG and IgE response. *Ad libitum* exposure to OVA in the drinking water resulted in only OVA-specific IgG, and no OVA-specific IgE was detected. Recent studies by Akiyama et al. (2001) demonstrated, however, that their OVA-dosing regime in the drinking water produced OVA-specific IgE titers, although they confirmed that daily gavage dosing of BN rats showed higher OVA-specific IgE titers. That the BN rat was indeed the most suitable strain for oral sensitization studies was confirmed in comparative studies using different strains of rats (Knippels et al. 1999b). Upon oral exposure of Wistar, PVG, Hooded Lister, and BN rats to OVA, it was apparent that only BN rats developed OVA-specific IgE antibodies. Subsequent studies (Knippels et al. 2000), using daily gavage dosing of the animals for up to 9 weeks to various concentrations (0.5, 1, 2.5, 5, 10, and 15 mg protein/mL/day) of OVA, HEW-protein extract, or CM, showed the best results in specific IgG antibodies at a dose of 1 mg OVA/mL/day versus 10 mg for HEW and CM protein/mL/day (Figure 1A–C). Clear OVA-specific IgE titers were also observed (Figure 1D). Because it is technically not possible to measure CM and HEW protein-specific IgE responses by ELISA, reaginic antibody responses were detected by PCA. However, despite observation that by immunoblotting the sera of HEW- and CM-sensitized rats showed IgE antibodies to the main allergens in HEW and CM, only a low number of IgE responders were observed as measured by PCA. An explanation for this apparent discrepancy may be that the HEW and CM IgE titers were not high enough to be measured by PCA, because it was shown in previous studies that the PCA was less sensitive than ELISA (Knippels et al. 1998b).

An important observation for the BN rat model was that the profile of allergens recognized by the immune system of the BN rat appeared comparable with the profile of allergens recognized by allergic patients. Specific IgG antibodies in sera from both HEW-allergic patients and rats orally sensitized to HEW proteins recognized a whole repertoire of proteins, the profile of which appeared to be the same. Although HEW is a complex mixture of more than 20 proteins, the specific IgE antibodies in sera from both HEW-allergic patients and rats orally sensitized to HEW proteins recognized the same proteins (mainly ovotransferrin, OVA, ovomucoid, and to a lesser extent lysozyme), all claimed to be the major allergens for HEW-allergic humans (Ebbehoj et al. 1995), and no reaction was observed against any other protein present in the HEW-protein extract. The same phenomenon was observed when the patterns of protein recognition by antibodies in sera from rats orally sensitized to CM proteins and antibodies present in sera from CM-allergic patients were compared. The induced antibodies were directed mainly against β-LG and, to a lesser extent, against the caseins. Although CM contains more than 30 proteins, no reaction was observed against any other protein present in CM. These results indicate that upon daily intragastric dosing with HEW proteins or CM proteins, the specific protein recognition of induced antibodies in the BN rat is comparable with that observed in sera from allergic patients. The same phenomenon was described in BN rats intraperitoneally sensitized with CM, which produced a profile of IgE antibodies to milk proteins similar to that observed in humans (Atkinson et al. 1996).

Although the induced antibodies in the BN rat apparently react to relevant proteins comparable with the human situation, it remains to be elucidated whether the induced specific antibodies in the rat react to the same epitopes as the antibodies in the sera from patients.

More recently, several oral sensitization studies were performed in BN rats with crude raw- or roasted-peanut protein extracts and with some purified allergenic proteins and one nonallergenic protein (Knippels et al. Unpublished data). Between crude raw or roasted peanut extracts, no clear differences were observed in the sensitizing potency as measured by the Th2-mediated IgG2a production at dose levels ranging from 0.01 to 10 mg of peanut protein/day. From day 7 onward, the number of positive responders and the magnitude of the IgG2a response increased in time. However, at each time point the magnitude of the IgG2a response was almost similar in the different dose groups, indicating the sensitizing potency of even very low doses of peanut protein. As measured by PCA, only a limited number of

animals were IgE positive. Using the purified three major peanut allergens, Ara h1, Ara h2, and Ara h3, the IgG2a responses were determined in sera of rats sensitized orally or intraperitoneally with peanut proteins. After intraperitoneal sensitization, the IgG2a antibodies were directed mainly toward Ara h2 and, to a lesser extent, to Ara h1; after oral sensitization, they were directed against all three major peanut allergens. This observation may indicate that upon oral or intraperitoneal sensitization, a different sensitization profile may be induced.

For predictive screening of new proteins, the assessment of relative allergenicity in animal models will be a very important aspect in relation to validation and acceptance. Therefore, a collaborative study with two industrial partners (Monsanto in the United States and Syngenta in the United Kingdom) was begun recently using some selected and purified allergenic and nonallergenic proteins (Knippels et al. Unpublished data). We exposed BN rats by gavage at different dose levels in the presence and absence of an oral adjuvant. BN rats were also sensitized intraperitoneally at one dose level of each protein. Ara h1 (purified from peanut), Pen a1 (purified from shrimp), and Sol t1 (purified from potato tuber) were used as strong to weak allergens, respectively, and tropomyosin (purified from raw beef) as a nonallergenic protein based on human experience. Two identical studies were performed with these purified proteins, with animals of different suppliers in the two studies, and the preliminary results indicated marked differences between these studies. Despite assurances of the animal suppliers, there are indications that the different findings regarding the relative allergenicity of the tested proteins in the two studies performed were caused by the unexpected pre-exposure of the animals of the first study to one of the allergens used and to a cross-reacting allergen. In the second study, the oral sensitizing potential decreased in the order Ara h1 > Pen a1 > Sol t1, with no sensitization to beef tropomyosin in both studies.

The probable cause of the different results obtained in the above-mentioned identical sensitization studies stresses again that the unscheduled dietary pre-exposure of the test animals to the test protein will affect the results of the sensitization studies. Previous studies have demonstrated that the exposure of the parental generation to the antigen under investigation influenced the outcome of the sensitization study with the offspring (Knippels et al. 1998a). BN rats bred and raised on a soy-protein–containing diet for several generations were found to have soy-specific IgG antibodies. These antibodies remained detectable in serum of the parental animals after feeding for 6 months on a soy-free diet. Even in serum of their F_1 generation of offspring, raised further on a soy-free diet for periods up to 6–12 month, soy-specific IgG was still detectable. In the second, third, and fourth generation of offspring, bred and raised on a soy-free diet, no soy-specific IgG was detected, and oral sensitization could be achieved again in these rats. To get immunologically naive and responsive rats for oral sensitization studies with proteins, it will therefore be of importance to ensure that the rats have been bred and raised for at least two generations on a diet free of the protein to be tested.

In summary, the results described support that the BN rat may provide a suitable animal model for food allergy research and to study the relative allergenicity of (novel) food proteins.

Challenge effects. To characterize the developed BN rat model in more detail, additional studies were performed to investigate local and systemic immune-mediated effects upon enteral challenge and to study mechanisms involved in sensitization (Knippels et al. 1999a). The possible occurrence of local and systemic effects upon an oral challenge was investigated in OVA-sensitized animals by monitoring gut permeability, and respiratory functions and blood pressure, respectively. On an oral challenge with OVA, gut permeability was increased as evidenced by an increased uptake of a bystander protein (β-LG). One hour after an OVA challenge followed by an oral dose of β-LG 30 min later, the amount of β-LG in the sera of previously sensitized rats was significantly higher compared with nonsensitized animals (Figure 2). Several models of intestinal hypersensitivity to food proteins have shown that antigen challenge of the sensitized intestine causes alterations in ion transport, permeability, and motility (Berin et al. 1997; Crowe and Perdue 1992) and in the release of mediators in anaphylactic reactions such as histamine, platelet-activating factor, prostaglandins, and leukotrienes and in the release of some newly formed cytokines that have been shown to alter mucosal function in experimental models (Heyman et al. 1994; Kanwar et al. 1994). However, up to now it has not been fully revealed whether the increased macromolecular passage is mainly due to transcellular or paracellular transport. Despite this, our finding that a significant amount of intact β-LG is present in sera of sensitized animals—together with the findings of Scudamore et al. (1995) who showed that the release of rat mast cell protease II, a known rat mucosal mast cell mediator, increases epithelial permeability via a paracellular route—suggests an increased epithelial permeability in our animals via a paracellular route, although an increased permeability via the transcellular route cannot be excluded.

In addition to studies on local effects, the possible occurrence of systemic effects upon an oral challenge were studied by measuring the breathing frequency and systolic blood pressure. These studies revealed that an oral challenge with OVA did not induce a clear effect on the respiratory system or blood pres-

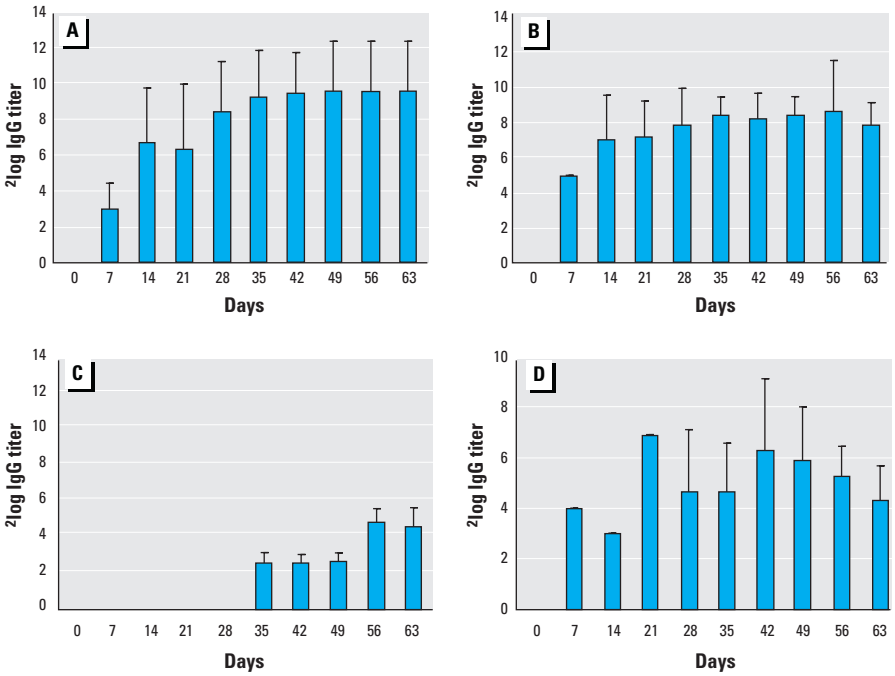

Figure 1. Time dependency of IgG responses specific to (*A*) OVA, (*B*) HEW protein, (*C*) CM protein, and (*D*) the OVA-specific IgE responses in young BN rats upon daily intragastric dosing with 1 mg OVA, 10 mg HEW protein, or 10 mg CM protein per rat each day for 63 days. Immunoglobulin titers were determined in blood samples obtained at weekly intervals. The data are presented as mean ^{2}log IgG/IgE titer ± SD of six rats/group. The numbers of responders at the respective time points are indicated in the bars.

sure in most animals. However, some animals demonstrated a temporary decrease in breathing frequency or systolic blood pressure. These observations indicate that systemic effects can be induced in orally sensitized animals on an oral challenge. In literature, a drop in breathing frequency below 70% of the normal breathing frequency is considered an indication of severe respiratory effects (Botham et al. 1989). Although we observed severe respiratory effects in only a few animals (~10–15% of the animals), this low incidence agrees with observations from food allergic patients, of whom only about 10% react with respiratory problems (Monteleone and Sherman 1997). In several animals (~40% of the animals), a decrease in systolic blood pressure was observed, but no dramatic drop in blood pressure, resulting in circulatory collapse, was observed. Again, the rather low incidence of cardiovascular effects upon oral challenge of the rats is in accordance with the human clinical practice.

Conclusion

It is evident that for assessment of the potential oral allergenicity of "novel proteins," new appropriate approaches are necessary. In this context, the development of predictive animal models is often indicated as urgently needed. In such predictive and widely accepted animal models, products with an unknown history of allergenicity or those that show one or more physicochemical characteristics of known allergens can be studied to demonstrate the ultimate proof for the presence or absence of sensitizing activity of the novel protein. In the past few years, several groups have studied animal models related to food allergy research. Studies in mice have shown that upon repeated enteral protein administration in combination with adjuvants, immune priming or sensitization can be achieved (Ito et al. 1997; Li et al. 1999, 2000). For assessment of potential allergenicity, the intraperitoneal route of exposure is also studied in mice (Hilton et al. 1997; Dearman et al. 2000 ; Kimber et al. 2000). Appropriate domestic models that are considered to be useful models to predict the potential allergenicity of novel proteins or that can provide comprehensive understanding of IgE-mediated disease mechanisms are the atopic dog model (Ermel et al. 1997) and the swine model (Helm et al. 2002). For the rat, studies on sensitization to food proteins in the presence of an adjuvant have also been described (Atkinson et al. 1996; Atkinson and Miller 1994).

Here we describe an oral sensitization protocol in BN rats without the use of an adjuvant (Knippels and Penninks 2002; Knippels et al. 1998b). These results suggest that oral exposure to food proteins may produce a significant IgE response to the purified proteins. In addition, exposure to a whole food (CM) or a mixture of proteins (HEW and peanut-protein extracts) resulted in specific antibody responses toward several proteins, whereas most proteins present in CM and HEW extract did not elicit a specific IgE response in the BN rat. The profile of allergens recognized by the immune system of the BN rat appeared comparable with the profile of allergens recognized by allergic humans as measured by immunoblotting (Knippels et al. 2000). Along with oral sensitization, we also showed clinical effects after an oral challenge in the sensitized animals. We showed limited systemic effects on respiratory and circulatory functions and more clear local effects on gastrointestinal permeability upon oral OVA challenge of OVA-sensitized BN rats (Knippels et al. 1999a).

Although additional studies are needed with more purified strong and weak allergens, nonallergens, and allergenic whole foods or their protein extracts, to further validate the developed BN rat model, the results obtained up to now support the BN rat model as a useful animal model for studying oral sensitization to food proteins. This model is also useful for studying immune-mediated effects upon oral challenges with food proteins and to assess thresholds for challenge reactions in sensitized rats using rat mast cell protease II, based on the results of preliminary studies in peanut-protein–sensitized rats (Knippels et al. Unpublished data). In addition, the model seems promising for studying mechanistic aspects of food allergy as well as new prophylactic or therapeutic interventions for food allergy.

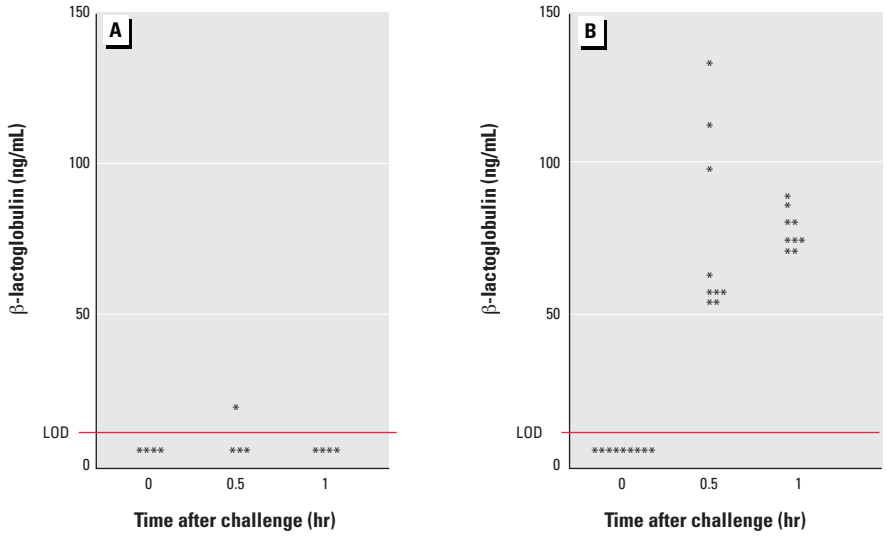

Figure 2. β-LG levels in sera of (*A*) nonsensitized control rats and (*B*) orally OVA-sensitized rats upon an oral challenge with OVA. Control animals were daily gavaged with 1 mL of tap water, and the OVA-sensitized rats with 1 mL of a solution of 1 mg OVA/mL, for 6 weeks. At day 49 all rats received an oral OVA challenge (100 mg/rat). A subsequent gavage dose with β-LG was given 30 min later, and the concentrations of β-LG in serum samples were followed in time as a measure for gastrointestinal tract permeability. The OVA-sensitized animals had anti-OVA IgE titers of ≥ 5. Limit of detection is 10 ng/mL.

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