

Utility of Animal Models for Evaluating Hypoallergenicity

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Introduction

Food allergy affects as many as 5-8 percent of young children and 3-4% of adults (Sampson, 2004). Cow's milk protein allergy (CMPA) is the most common in young infants, with a 2-3 percent incidence (Saarinen et al, 1999; Sampson, 2004). When breastfeeding is not possible, hypoallergenic (HA) cow's milk based formulas are usually given during the first months of life for prevention of CMPA. Depending on primary (sensitization) or secondary (triggering) prevention, the requested quality of HA formulas may be different. Besides in vitro methods, in vivo and ex vivo animal models are helpful in assessing residual allergenicity and the preventive effect of HA formulas. The oral sensitizing capacity of a formula can be examined by either the guinea pig (IgG1a mediated) or the mouse (IgE) models. The triggering IgE mediated allergenicity is tested by a parenteral rat model with oral gavage for intestinal mast cell protease (RMCPII) release. These animal models are also used for testing the oral tolerance inducing capacities of formulas. Together with cellular in vitro assays, animal models are very helpful in predicting allergenicity and the tolerogenic potential of hypoallergenic infant formulas.

For reducing the allergenicity of cow's milk proteins, IgE-binding and T cell epitopes have to be destroyed or inactivated. A number of processes are available to achieve this. For example heating cow's milk proteins above 80°C will destroy the globular structure of proteins, and consequently conformational epitopes but not sequential ones. Enzymatic hydrolysis is by far the most efficient process for disrupting sequential and conformational epitopes and therefore allergenicity reduction is best achieved by this method. Depending on the type of enzymes used and the conditions of hydrolysis, peptides of different length may be obtained carrying more or less allergenicity. Porcine trypsin/chymotrypsin are frequently used for producing hypoallergenic formulas but proteases extracted from bacteria or of fungal origin are increasingly also used.

Physico-chemical methods are frequently used for examining the amount of proteins/peptides obtained after heat and enzymatic hydrolysis processing. SDS-PAGE and peptide profiles give usually a good picture on allergenicity reduction. Immunological *in vitro* tests are often used for the evaluation of residual allergenicity. ELISA inhibition or uptake (Elisa sandwich) methods are very helpful for the determination of IgG and IgE *binding* epitopes, using respectively allergen specific polyclonal animal sera or human patient sera. On the other hand, the IgE mediated mast cell *triggering* capacity of allergenic epitopes can be measured with the help of a functional *in vitro* assay. We have set up such an assay in our laboratory, based on peritoneal rat mast cells passively sensitized with specific rat IgE and labelled with ³H-Serotonin (Figure 1). Cells are triggered for mediator release with standard dilutions of the allergen or test formula dilutions (Fritsché et al., 1990).

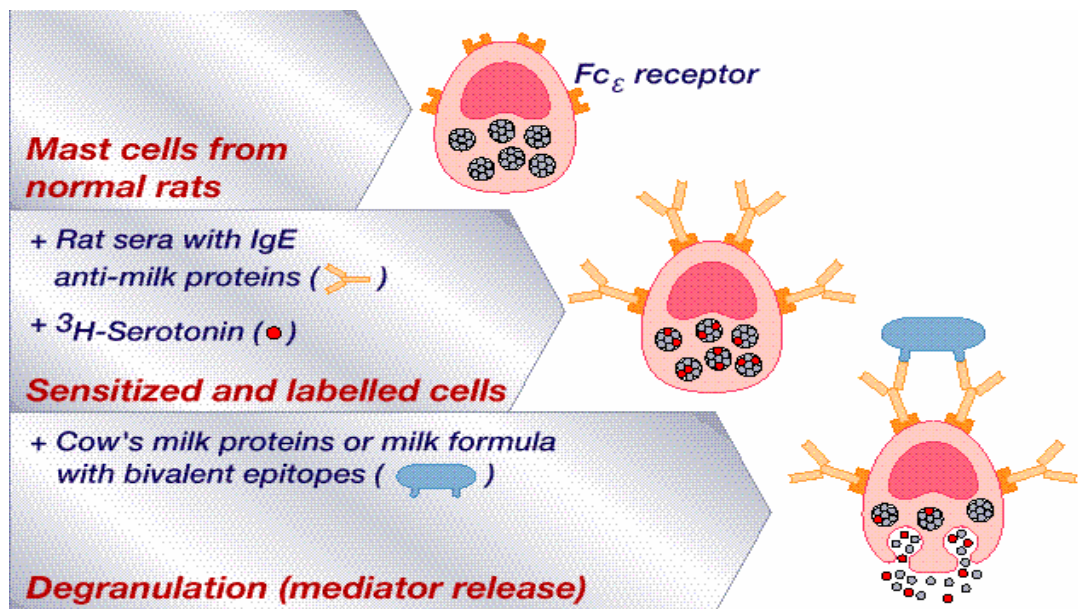


Figure 1: Determination of allergenicity by *in vitro* triggering of IgE sensitized and ³H-Serotonin labelled mast cells

For the determination of the immunological *in vivo* allergenicity, animal models are very helpful. The IgE dependent allergic reaction is composed of two phases: an inducing step, where the immune system of the host is sensitized by the allergen, ending up in specific IgE anti-allergen antibodies production which bind to specific cell surface receptors on mast cells in target organs; the second step is a triggering phase mediated by the allergen binding to these IgE and stimulating mediator (histamin) release from mast cells. For evaluating allergenicity of food antigens both phases should be examined by appropriate tests.

Historically, guinea pigs (Devey et al., 1976) and rats (Fritsché et al., 1990) have been used to investigate allergenicity of food proteins. The Brown Norway rat has been reported to be a useful model for the investigation of food allergy because intraperitoneal sensitization generates IgG and IgE antibodies to a range of milk proteins that are of similar specificity to those produced by humans (Atkinson et al., 1994). Recently, oral mouse models have also been reported which use cholera toxin as an adjuvant.

There is a significant obstacle to the development of oral murine models of food allergy, namely the strong innate tendency to develop oral tolerance to ingested antigens.

Rat parenteral model

The rat parenteral model appears to be adequate for measuring both the IgE specific sensitizing (production of IgE antibodies) and triggering (IgE-mediated RMCPII release) capacities of food allergens. It further allows the determination of the tolerizing capacity of an infant formula.

Sensitization/allergic triggering

We use the following protocols: Brown-Norway high IgE responder rats are injected subcutaneously with the allergen in presence of Al(OH)₃. Fourteen days later the primary IgE response is determined by ELISA in animal sera. For the evaluation of the in vivo triggering activity of a product containing the allergen, above sensitized rats are gavaged with the test product, bled after 2 hours and the level of rat mast cell protease II (RMCPII) determined in the serum. After a booster injection of the allergen, the secondary IgE response as well as spleen / lymph node lymphocyte proliferation and culture supernatant cytokine determinations are done. This parenteral rat model provides a good indication on the IgE inducing capacity of standard and hypopallergenic infant formulas: moderately and extensively hydrolysed cow's milk formulas induced respectively 100 to 10'000 times less IgE antibodies than a standard milk formula (Fritsché et al.,1990). Further, intestinal mast cells are primary targets of food allergens in IgE dependent hypersensitivity. In the rat model, the specific protease (RMCPII) is released into blood after intestinal mast cell triggering. This protease, determined by ELISA in serum, is a good indicator of the IgE mediated allergic triggering capacity of infant formulas at the intestinal level. Standard formulas stimulated the highest specific release in our model, whereas hypoallergenic formulas triggered low or no RMCPII release (Figure 2)

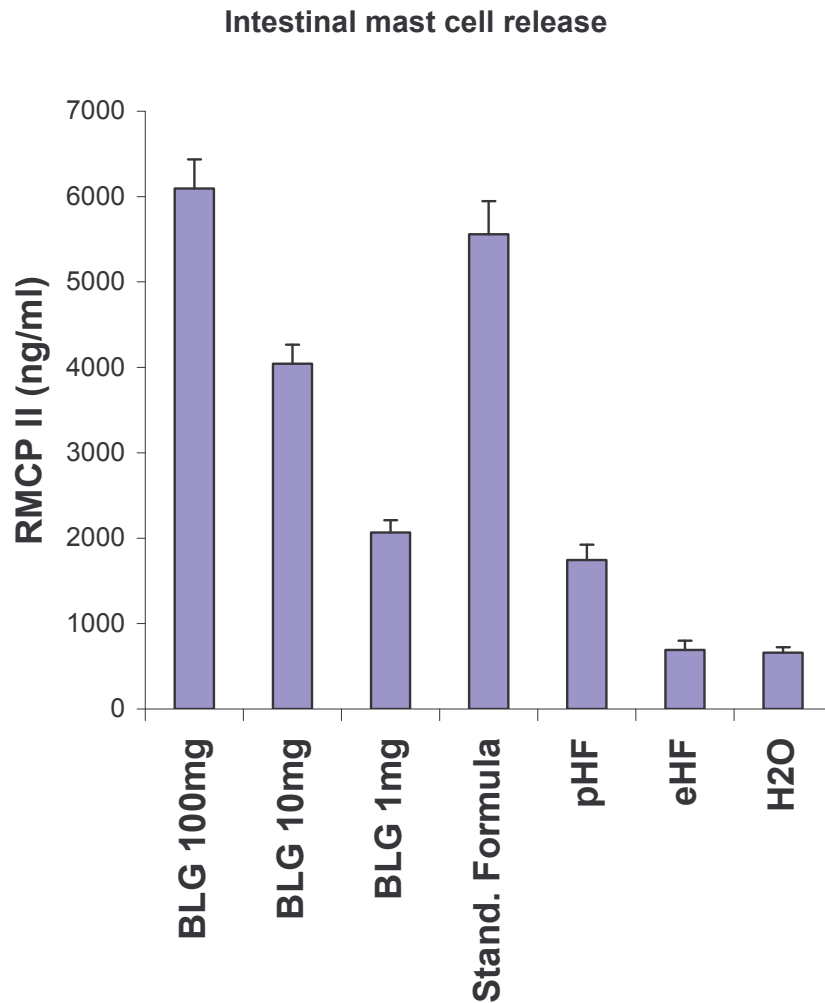


Figure 2: RMCP II release from BLG sensitized rats challenged orally with BLG or infant formulas

Induction of oral tolerance

Oral administration of protein antigens induces specific immunologic hyporesponsiveness (tolerance) to these antigens. Induction of oral tolerance with intact proteins has been well documented with a number of antigens in several animal models. Oral tolerance to bovine serum albumin for example has been shown in a Black Norwegian rat model (Thomas et al, 1974). Immune regulation by the induction of oral tolerance is thought to prevent food allergy (Mowat, 1987). It has been shown that induction of oral tolerance is dependent on the age of the host, the dose of antigen administered (Strobel, 1984) and the nature of the antigen. Although oral tolerance to dietary proteins has been extensively investigated with intact antigens, few studies with antigen fragments or digests have been done.

The protocol we use is the following: For induction of oral tolerance to cow's milk proteins, Sprague-Dawley rats are given different experimental liquid milk formulas ad libitum in their drinking bottles and a solid "milk-free" pellet diet from days 1 to 19 of the experiment. For challenge, rats are immunized on day 5 by subcutaneous injection of a selected cow's milk protein (e.g. β LG) in the presence of aluminium hydroxide. Animals are sacrificed on day 20 and sera analysed for specific IgE antibodies (Figure 3).

Animals: 4 week old Sprague-Dawley rats, 8 rats /group

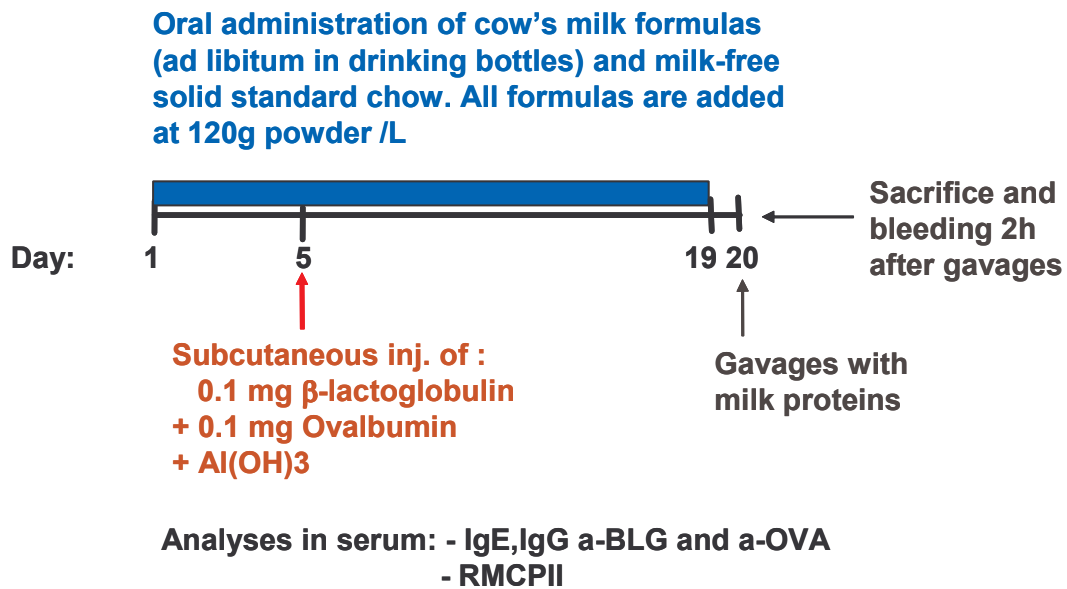


Figure 3: Rat parenteral model for oral tolerance induction

We have shown with the help of this model that partially hydrolysed whey proteins (pHF) are able to induce oral tolerance to intact whey proteins whereas extensively hydrolysed whey proteins (eHF) are unable to achieve this (Fritsché et al., 1997). This was demonstrated at the levels of the IgE response and intestinal mast cell secretion (Figure 4). Similarly, moderately hydrolysed soy proteins (with pancreatic enzymes) could be shown with this model to induce oral tolerance to intact soy proteins whereas strongly hydrolysed soy proteins are unable to achieve this.

Oral tolerance induction with HA formulas

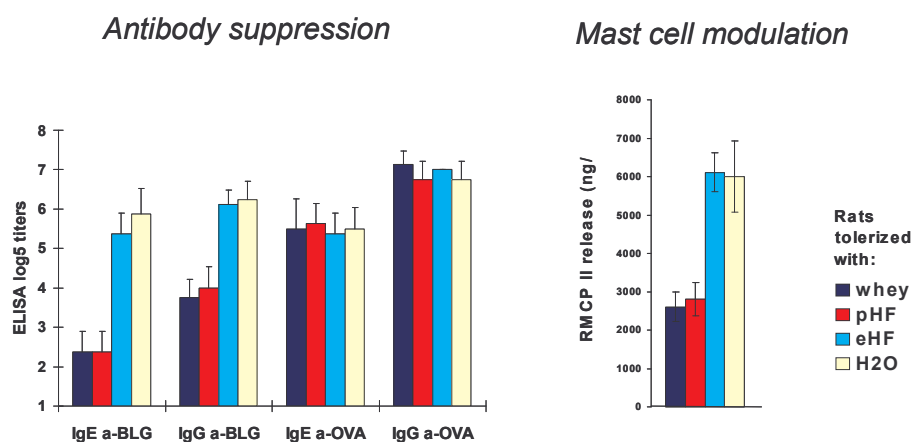


Figure 4: IgE and RMCP II suppression following oral tolerance induction to \square LG with intact or moderately hydrolysed whey based infant formulas

Oral mouse model

Several tests in mice, using adjuvants like cholera toxin, have been published which succeeded in inducing oral IgE mediated sensitisation to cow's milk proteins (Li et al., 1999). Briefly, three week old C3H/HeJ mice are sensitized orally with the allergen in the presence of cholera toxin (CT), a mucosal adjuvant, once a week for 5 successive weeks, followed by an oral challenge with a large dose of the allergen one week later. The model allows, beside clinical symptom scoring, the determination of IgE, T cell cytokine measurement and determination of mouse mast cell protease 1 (MMCP-1) as markers of allergic sensitization. Another oral mouse model was recently published (Adel-Patient et al, 2005) where Balb/c mice were orally sensitized with peanut proteins in the presence of CT.

There appears to be further a strain selectivity for optimal oral sensitization to different allergens (Morafo et al, 2003). Depending on the protocole used for sensitization, these mouse models allow to mimick either gastrointestinal, dermatologic or respiratory symptoms, which may be helpful for preclinical studies of defined allergen modulation.

We have further used this oral mouse model for the evaluation of the tolerizing capacity of an antigen. We have shown that orally sensitized mice mounted a \square LG specific IgE response

when gavaged with β LG in the presence of cholera toxin. A single gavage of whey proteins given prior to the onset of oral sensitization resulted in the suppression of both specific and bystander IgE (Von-der-Weid et al., 2001).

Guinea pig oral ad lib model

Guinea pigs can be sensitized by the oral route without adjuvants making it a good model, close to the human situation. Difficulties associated with PCA (passive cutaneous anaphylaxis) testing and the fact that the reagenic antibody response is of the IgG1a subtype limit the use of guinea pigs as a suitable model with which to study CMPA (Poulsen et al., 1987). It is nevertheless a helpful model for the determination of the natural “spontaneous” capacity of a product to induce sensitization or oral tolerance. We have for example shown that oral ad libitum administration of moderately hydrolysed whey proteins to Dunkin-Hartley guinea pigs during 5 weeks before an oral challenge during 2 weeks with intact whey proteins prevents the induction of specific anti-cow milk protein reagenic IgG1a antibodies. This demonstrated that oral tolerance could be induced by prefeeding with a tolerogenic non-sensitizing formula, in a similar approach as the one occurring in infant feeding.

Other models

Besides above small laboratory animals, dogs and swine have been reported as useful models for investigations involving food allergy. The advantage of both models lies in their propensity to develop clinical symptoms of food allergy, primarily gastrointestinal and dermatological reactions.

The atopic dog model is based on an inbred colony of high IgE-producing dogs; to elicit sensitization, dogs are immunized with a live virus vaccine, followed by subcutaneous injections with food antigens over a course of weeks. Skin test titrations were used to assess the allergenicity of processed foods (Buchanan et al, 1997).

The neonatal swine model has been used mainly because these animals resemble humans in gastrointestinal physiology and in the development of mucosal immunity.. Young piglets have been used as models for sensitization/tolerance to cow’s milk and soy proteins (Bailey et al, 1994).

Discussion

While in vitro and cellular assays are very helpful tools for evaluating residual allergenicity in food products (infant formulas, processed foods), their sensitizing capacity as well as their tolerogenic potential can however be evaluated only with in vivo animal models. There exists up to now no ideal model of food allergy. The parenteral route leads to stronger but less physiologic sensitizations than oral administration with adjuvants. Therefore, for preclinical studies, the use of several selected ones may be recommended.

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