



Review

Current advances of murine models for food allergy

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ABSTRACT

Food allergy affects an increasing population in Western world but also developing countries. Researchers have been taking great efforts in identifying and characterising food allergens using molecular tools. However, there are still many mechanistic hypotheses that need to be tested using an appropriate *in vivo* experimental platform. To date, a number of mouse models for food allergy have been established and provided valuable insights into food allergenicity, development of therapies and allergic inflammation mechanisms. Nevertheless, a large diversity of protocols have been developed for the establishment of relevant mouse models. As a result, comparisons of outcomes between different models are very difficult to be conducted. The phenotypes of mouse models are greatly influenced by genetic background, gender, route of allergen exposure, the nature and concentration of food allergens, as well as the usage of adjuvants. This review focuses on IgE-mediated food allergy, compares the differential approaches in developing appropriate murine models for food allergy and details specific findings for three major food allergens, peanut, milk and shellfish.

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1. Introduction

1.1. Food allergy

Food allergy is an important public health issue in industrial countries due to the increasing prevalence and the potential life-threatening consequence, effecting up to 8% of children and 4% of adults (Nieuwenhuizen and Lopata, 2005; Platts-Mills, 2015; Ashley et al., 2015). Food allergies are also becoming increasingly important in developing countries (Lee et al., 2013), particular to more exotic food sources. Food allergy or hypersensitivity can be defined as adverse reactions by the immune system to food compounds that are allergens. Patients with food allergy often have itchy flushing skin, diarrhea and/or vomiting, as well as respiratory distress, and are under risks of fatal anaphylaxis after exposure to allergens (Wang and Sampson, 2011). We will focus on IgE-mediated food allergy in this review.

Allergic mechanisms consist of two phases: the sensitisation and the effector phases (see Fig. 1). Food allergens are captured by antigen-presenting cells (APCs), processed and presented to T

helper (Th) lymphocytes. In sensitised individuals, Th2 responses activate B cells to produce antigen-specific IgE antibody after the exposure to allergens. During the effector phase clinical symptoms occur because of the enhanced Th2-type pattern of immunological responses to the same allergens after subsequent exposures. So far, there are still many questions to be investigated about the mechanisms, multiple risk factors and events leading to sensitisation towards food compounds (Ashley et al., 2015; Lack, 2012). Unfortunately, currently all therapeutic approaches for food allergy are still in clinical phase I-III and patients can only protect themselves through strict avoidance of all allergen-containing food products (Burks et al., 2008). However, many important questions about food allergy cannot be answered by *in vitro* molecular experiments alone, and an appropriate *in vivo* experimental platform becomes an urgent need. Mechanistic research or modifications of experimental conditions using human studies cannot be easily performed in patients. Moreover, the amount and types of tissue samples that can be collected from patients with food allergy are very restricted. Therefore, animal models become very valuable research tools of choice. Without taking risks on human lives, animal models help us getting more insights into three major aspects of food allergy: (1) the evaluation of food allergens, (2) studies on immunological responses and mechanisms and (3) the development of preventive and therapeutic treatments (Oyoshi et al., 2014).

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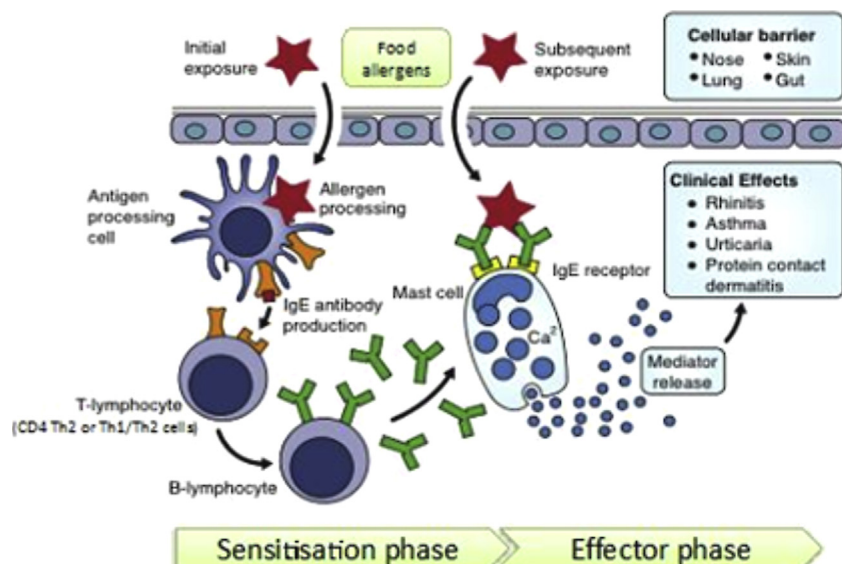


Fig. 1. Humoral and cellular mechanisms of allergic inflammation (Modified and adapted from Abdel Rahman et al. (2015)).

1.2. Mouse models for food allergies

Mice have some unique characteristics that contribute to its much wider use to study human biology than any other organisms. Importantly, the mouse has many physiological, genetic and immunological similarities to humans. The overall structure of the immune system of mice and humans are very similar (Haley, 2003), in spite of the necessary consideration of discrepancies between mouse and human innate and adaptive immune system (Mestas and Hughes, 2004). Mouse strains, which are well characterised provide an abundance of genetic resource due to its nature and a string of unique technological strengths. It allows intensive genetic manipulations, such as forward genetics, reverse genetics, transgenesis, targeted mutations and knock-ins/outs (Justice et al., 2011). With the application of these genetic engineering approaches on mice, a great number of humanized mouse strains with targeted modifications become available, hence, more hypotheses on human diseases can be tested. Moreover, as selective breeding is necessary in the production of disease models, the mouse, which has a short generation cycle, is an appropriate option.

The evaluation criteria of successfully establishing a mouse model for food allergy is the extent to which mice mimic human allergic reactions, including clinical symptoms as well as humoral and cellular responses to food allergens. To date, a large diversity of protocols to establish mouse models for food allergy have been published as illustrated in Table 1.

The comprehensive analysis of 26 established protocols highlight several important elements, including the mouse genders, genetic background of mouse strains, routes of sensitisation, nature of food allergens and usage of adjuvants that could impact the performance of a mouse model for food allergy.

In the following sections the detailed influence of each key impact factor is discussed on the levels and patterns of the induced allergy-like immune disorders in mice as well as the most important scientific highlighted.

2. Mouse strain

Genetic susceptibility seems to have a close association with the development of allergic diseases, however no particular genes have been identified predisposing humans to food allergy (Björkstén, 2005). Considering the unfeasibility of studying allergy risk fac-

tors directly on human subjects, mouse strains with defined genetic background become very useful in this field. Under the control of genetic variations and living environment, mice are essential for a better understanding of multi-mediator involved mechanisms underlying the allergic responses.

BALB/c mice are generally believed to be a Th2-biased strain, however reported to be resistant to the induction of hypersensitivity to cow's milk or peanut, in contrast to the C3H/HeJ strain (Morafo et al., 2003). In this study conducted by Morafo et al., three week-old mice of both strains were sensitised orally using a mixture of peanut or cow's milk with cholera toxin (CT) as a mucosal adjuvant. After one oral challenge, C3H/HeJ mice presented mild clinical reactions and a significantly increase of serum antigen-specific IgE and plasma histamine, while BALB/c mice only generated a high level of IgE antibody to peanut, but not cow's milk. No anaphylactic symptoms could be observed in BALB/c mice. Therefore, BALB/c mice, often used in modeling allergic diseases induced by systemic allergen sensitisation, might be not sensitive enough to mimic human allergic responses when orally sensitised. In addition, Morafo et al. found the higher expression level of IL-4 and IL-10 in C3H/HeJ only, but significantly increased Th1 cytokines in BALB/c, which suggests that C3H/HeJ and BALB/c mice may develop different immunologic response patterns to food allergens.

C3H/HeJ mice are very often used to develop models for peanut-allergy, because C3H/HeJ mice are anaphylaxis-susceptible. This characteristic is probably due to a Toll-like receptor (TLR)-4 mutation in C3H/HeJ mice that blocked intestinal LPS (lipopolysaccharide) signaling (Qureshi et al., 1999). Both C3H/HeJ and C57BL/10ScNHsd strain with TLR4-deficiency showed more Th2-biased reactivity to peanuts by means of oral sensitisation with peanuts and CT as adjuvant. In contrast, the same administration of peanuts in MHC-matched or congenic controls (C3HeB/FeJ, C3H/HeOuJ, CBA/J, C57BL/10SnJ and C57BL/6J) were not able to induce the increased level of antigen-specific IgE and histamine, or any anaphylactic symptoms (Bashir et al., 2004). In addition, TLR-4 deficiency was not sufficient to confer BALB/c mice with proper clinical reactions to peanut (Berin et al., 2006). Accordingly, more differences than only the TLR-4 genetic mutation between C3H/HeJ and BALB/c mice contribute to the different susceptibility to food allergies.

AKR/J mice were successfully used in a peanut allergen-induced murine anaphylactic model to monitor the new immunoprophylactic strategy (Roy et al., 1999). However, following the same

Table 1

A summary of 26 different mouse models for food allergy established within the past two decades, categorised by sensitisation and challenge routes (Table 2) (Hsieh et al., 2003; van Halterena et al., 1997; Bohnen et al., 2013; Yamaki and Yoshino, 2012; Golias et al., 2012; Adel-Patient et al., 2005; Untersmayr et al., 2003; Nieuwenhuizen et al., 2006; Baeza et al., 2005; van der Ventel et al., 2011; Kirstein et al., 2010; Liu et al., 2013; Man et al., 2004; Frossard et al., 2007; Li et al., 1999; Morafo et al., 2003; Li et al., 2001; Ganeshan et al., 2009; Noval Rivas et al., 2013; Song et al., 2010; Li et al., 2000; Schiavi et al., 2011; Rupa and Mine, 2012; Sun et al., 2007; Zhou et al., 2010; Rupa and Mine, 2006; Brunner et al., 2009; Parvataneni et al., 2009; Roy et al., 1999; Swoboda et al., 2007).

Mouse strain, gender and age	Sensitisation				Challenge			Evaluation	Reference
	Route	Time points (Day)	Food dose	Adjuvant or solvent	Route	Time points (Day)	Dose		
BALB/c 8–12 week Female	I.P.	0	10µg OVA	Alum	I.G.	26	50mg OVA	Anaphylaxis score (see Table 2) IgE, IgG2a Histamine Proliferation Cytokine Histology of jejunum In vivo IFN-γ neutralization	(Hsieh, Hsu et al. 2003)
		16	20µg OVA	PBS					
BALB/c 8–12 week Female		0	2µg OVA	AH		18	1mg OVA	IgE Histamine (whole gut tissue) Anti-cytokine treatment	(van Halterena, van der Cammena et al. 1997)
		14	1µg OVA	PBS					
BALB/c 6–10 week Female		0, 14	50µg OVA x2	1mg alum		21, 22, 23, 24, 25, 26	CEW x6 (dose was not mentioned)	Anaphylaxis score (fur, feces) IgE, IgG2a, IgG1 Cytokine (MLN) Histology of jejunum Weight change BAA	(Bohnen, Wangorsch et al. 2013)
BALB/c Male		0, 14	50µg OVA x2	4mg alum		28–(43)	50mg OVA in saline x6, every 3 days	Anaphylaxis score Diarrhea score IgE, IgG2a, IgG1 Cytokine (MLN) mMCP-1 BT Histology of intestine	(Yamaki and Yoshino 2012)
BALB/c (H-2b) 2 month Female		1, 14	60µg OVA OR heated OVA x2	1mg AH		28–49	15mg OVA x10 at 2–3 days intervals	IgE, IgG2a, IgG1, IgA (serum) Cytokine Histology of jejunum Histamine (fecal) Diarrhea mMCP-1 FACs (Treg in spleen & MLN) Activities of EBBE in jejunum	(Golias, Schwarzer et al. 2012)
BALB/c 6 week Female		I.P. 1, 25	100µg WPE x2	Alum		-	-	IgE, IgG2a, IgG1 Cytokine Histology of intestine Histamine (fecal)	(Adel - Patient, Bernard et al. 2005)
BALB/c 3 week Female		I.G. 1, 6, 12, 18, 24, 30	5mg homogenized WPE x6	0.6µg CT					
BALB/c 3 week Female		I.G. 1, 6, 12, 18, 24, 30	5mg CMP x6	0.6µg CT					
BALB/c 8 week Female	I.P.	I.P. 0, 56	50µL caviar extract (40mg/mL)	100µL aluminium hydroxide	I.V.		125µL caviar extract (40mg/mL)	Skin test IgE, IgG2a, IgG1 Proliferation Electron microscop (antrum mucosa)	(Untersmayr, Schöll et al. 2003)
		I.G. 0, 56	150µL caviar extracts (20mg/mL)	2 mg sucralfate			125µL caviar extract (40mg/mL)		
BALB/c 8–10 week Female IL-4 ^{-/-} and IL-4Rα ^{-/-}		Week 0 and Week 8	2 larvae x2	-		Week 11	5mg <i>Anisakis</i> protein extract	Anaphylaxis score IgE, IgG2a, IgG2b, IgG1 (serum) Cytokine Histology of lung mMCP-1 (lung)	(Nieuwenhuizen, Lopata et al. 2006)
C3H/HeJ 6 week Male		2, 3, 4 weekly injections	100µg A.s	PT (300ng) + Alum (1mg)		Week6	I.V. 200µg OR I.G. 2mg	Anaphylaxis score IgE, IgG2a, IgG1 Histamine Cytokine	(Baeza, Conejero et al. 2005)

Table 1 Continued.

BALB/c 5–6 week Female	I.P.	0, 7, 14	50µg raw or cooked PC	1.5% alum	I.N.	21, 22, 23	100µg raw PC, cooked PC, purified PC parvalbumin, or rCyp c1.01	IgG1, IgG2b, IgE eosinophil peroxidase (lung) Cytokine Histology of lung BAL	(van der Ventel, Nieuwenhuize n et al. 2011)
BALB/c 6–8 week Female Wide type and IL-4Rα ^{-/-}		0, 21	2 larvae x2	-		42, 43, 44	0.1mg <i>Anisakis</i> protein extract	IgE, IgG2a, IgG2b, IgG1 (serum) Cytokine Histology of lung BAL Airway hyperresponsiveness	(Kirstein, Horsnell et al. 2010)
BALB/c 6–8 week Male	I.G.	Once a week for 5 weeks	OVA 0.2mg in 0.3ml saline x5	20µg CT	I.G.	Week6	OVA (No dose mentioned)	Diarrhea BT IgE Cytokine mMCP-1 Histology of jejunum	(Liu, Wu et al. 2013)
C3H/HeJ 3 week Female		Once a week for 5 weeks	1.0 mg/g BW homogenized CM	0.3µg / BW (g) CT		Week6	1x CM (No dose mentioned)	Anaphylaxis score	(Man, Bertelli et al. 2004)
C3H/HeOuJ 4–5 week Female		0, 7, 14, 21	200mg BLG x4	10µg CT 0.2mol/L NaHCO ₃		28	100mg BLG	Anaphylaxis score Vascular leakage IgE, IgG1, IgG2a, and/or IgA BT(ear) Cytokine (IL-10) Proliferation	(Frossard, Steidler et al. 2007)
C3H/HeJ 3 week Female		Once a week for 6 weeks	0.01mg, 0.1mg, 1.0mg, or 2 mg/ BW (g) CM	0.3µg / BW (g) CT		Week7	2 doses of CM (30mg/mouse) given 30 minutes apart	Anaphylaxis score Vascular leakage IgE Histamine PAC Cytokine Histology of intestine & lung & ear Skin test	(Li, Schofield et al. 1999)
C3H/HeJ 3 week Female and BALB/c		Once a week for 5 weeks	Homoge-- nized CM 1mg/g BW x5	0.3µg / BW (g) CT		Week6	33mg	Cytokine Anaphylaxis score IgE Histamine	(Morafo, Srivastava et al. 2003)
C3H/HeJ 5 week Female and BALB/c		Once a week for 5 weeks + Two boosts at 2- week intervals	10mg PNx5 + 50mg of protein-CT x2	20µg CT 1.5% NaHCO ₃		Week14	50mg	Cytokine Anaphylaxis score IgE Histamine	(Morafo, Srivastava et al. 2003)
C3H/HeJ 5 week Female		0, 7, 21	5mgx3 PE	10µg x3 CT		Week11 (Week4- week11, herbal formula or water)	10mg CPE	Anaphylaxis score IgE, IgG, IgA Histamine Proliferation Cytokine BT Histology of ear	(Li, Zhang et al. 2001)
BALB/c 4- to 8-week Female Or C57Bl/6 mice		Once a week for 8 weeks	100 µgx8 OVA OR 100µgx8 WPE	10µg CT or various SEB (better in eosinophil and score) 10µg SEB		Week9	5mg	Blood eosinophil quantification Cytokine Anaphylaxis score IgE, IgG2a, IgG1 Histamine Real time PCR Histology of jejunum BT	(Ganeshan, Neilsen et al. 2009) However, (Noval Rivas, Burton et al. 2013)
C3H/HeJ 5 week Female		Weekly for 5 weeks; boosted at week6 and week8	Ground peanut (10 mg x5; 50 mg x2)	20µg CT		Week14, week18 (after i.g. treatment of sham)	200mg	Anaphylaxis score FACs (blood basophil numbers and peritoneal mast cell numbers and FcεRI expression) BT	(Song, Qu et al. 2010)

Table 1 Continued

C3H/HeJ 5 week Female		0, 7	5mgx2 (higher score& IgE) or 25mgx2 GWP	10µg x2 CT		21 (week3) Week 5	10 mg CPE divided into two doses at 30- to 40-minute intervals; Mice surviving – re-challenged	Anaphylaxis score Vascular leakage IgE Histamine PCA Proliferation Histology of ear	(Li, Serebrisky et al. 2000)
C3H/HeJ 8 week Female		Once a week for 4 weeks	100µg pST x4	10µg CT		Week5	600µg pST	IgE, IgA (fecal) Real Time (cytokine and TF expression of intestinal tissue) Anaphylaxis score Histamine (fecal) IgE, IgG2a, IgG1 FACs (T cell subpopulations)	(Schiavi, Barletta et al. 2011)
BALB/c mice 6–8 week Female	I.G.	Twice per week for 4 weeks	1mg of Ovm x2x4	10 µg CT	I.P.	Week11 (After 4wk- PBS oral treatment)	20mg Ovm	Serum histamine Cytokine Anaphylaxis score IgE, IgG2a, IgG1 IgA (fecal) FACs (Treg cells in blood)	(Rupa and Mine 2012)
C57BL/6 6–8 week Female		Once a week for 1, 2, 3, or 4 weeks	1mg PN (from PN butter)	10µg CT		2 weeks after the last sensitisation	5mg CPN	Anaphylaxis score Vascular leakage IgE, IgG2a, IgG1 Histamine and leukotriene levels BT	(Sun, Arias et al. 2007)
								Late-phase peritoneal &cutaneous responses to CPE Cytokine	
C3H/HeJ, C57BL/6 genetic modification		Once a week for 7 weeks	200µgx7 BSA	10µg x7 CT		56	1mg BSA	Anaphylaxis score Vascular leakage IgE, IgG2a, IgG1 Histamine Spleen cell transfer FACs Cytokine	(Zhou, Kawasaki et al. 2010)
BALB/c mice 6–8 week Female		I.P. 0, 7, 21, 28, 35	50µg DIII x5	2.0 mg AH	I.P.	42	1mg DIII +alum	Cytokine Anaphylaxis score IgE, IgG2a, IgG1 Histamine	(Rupa and Mine 2006)
BALB/c 6–8 week Female		I.G. 0, 7, 14, 21, 28, 35, 42 and 49,	0.2mg OVA x8 Or Alum	SUC - -	-	-	-	skin test IgE, IgG2a Histamine Cytokine	(Brunner, Wallmann et al. 2009)
BALB/c 6–8 week Female	Other	Cutaneous exposure to allergen with a 4- day interval for 4 weeks	0.05, 0.5 or 1mg CNP	-	I.G.	Day6 following the 4th exposure	15mg	Cytokine Anaphylaxis score IgE, IgG2a, IgG1 Histamine Histology of intestinal tissues BT	(Parvataneni, Gonipeta et al. 2009)
AKR/J 4–5 week Male		0, 8, 16-i.g. 1, 9, 17-i.p.	1mg x3 0.5µgx3 CPE	CT AH	I.P.	24	200µg of r-Arah2 protein	Anaphylaxis score Vascular leakage IgE, IgG2a Histamine	(Roy, Mao et al. 1999)
BALB/c 8 week		S.C. 4-wk intervals	10µg parvalbumin mutant X3	AH		-	-	IgG1 and IgE	(Swoboda, Bugajska- Schretter et al. 2007)

The different colors mark the allergen categories: purple = egg allergy, blue = milk allergy, green = peanut & tree nut allergies, grey = fish allergy, and orange = shellfish allergy. **Administration:** I.P. = intraperitoneal, I.G. = intragastric, I.V. = intravenous, S.C. = subcutaneous; Adjuvant: CT = cholera toxin, SEB = staphylococcal-enterotoxin B, AH = aluminum hydroxide, SUC = sucralfate, PT = pertussis toxin; **Allergen:** PN = peanut, GWP = ground whole peanut, CPE = crude peanut extracts, WPE = whole peanut extracts, r = recombinant, A.s. = Anisakis simplex (A.s) proteins, PC = pilchard extract, BLG = β-lactoglobulin, CNP = cashew nut protein extract, CEW = chicken egg white, pST = purified shrimp tropomyosin, OVA = ovalbumin, Ovm = egg-allergen ovomucoid, CMP = cow's milk protein, DIII = ovomucoid third domain; **Evaluation:** IFN = interferon, IL = interleukin, BT = body temperatures, BW = body weight, MLN = mesenteric lymph node, BAA = basophil activation assay, TF = transcription factor, EBBE = enterocyte brush-border enzymes, mMCP = mouse mast cell protease, Treg = T regulator cell. (For interpretation of the references to color in this table, the reader is referred to the web version of this article.)

protocol of the well-established anaphylaxis model for milk allergy using C3H/HeJ by Li et al. (1999), this research group failed to generate an appropriate allergic phenotype using AKR/J or CBA/J mice. The different results in AKR/J mice indicate the great diver-

sity of genetic susceptibility to different food allergies in mice, similar to the fact that only some human individuals getting sensitised to food allergens (Morafo et al., 2003). The same conclusion was reached when comparing C3H/HeOuJ mice and C3H/HeJ mice.

Table 2

The most common anaphylaxis scoring system used in published studies of food allergy mouse models (Roy et al., 1999; Song et al., 2010; Rupa and Mine, 2012).

Score	Anaphylactic symptoms
0	No symptoms
1	Scratching and rubbing around the nose and head
2	Puffiness around the eyes and mouth, pilar erecti, reduced activity and/or decreased activity with increased respiratory rate
3	Wheezing, labored respiration, and cyanosis around the mouth and tail
4	Slight or no activity after prodding, or tremor and convulsion
5	Death

C3H/HeOuj mice are a negative control strain when induced with peanut (Bashir et al., 2004), however are good allergy responder as demonstrated for the establishment of a β -lactoglobulin-induced anaphylaxis model (Frossard et al., 2007). Peanut-treated C3H/HeJ mice were observed to have more severe clinical symptoms than β -lactoglobulin-treated C3H/HeJ mice (Berin et al., 2006). It is worth noting that in the studies of comparing murine susceptibilities to different foods, mice should be sensitised and/or challenged with the same amount of respective food allergens. Thus crude extracts of foods containing multiple allergenic proteins with various allergic efficacies are not well suited for these studies.

C57BL/6 mice are generally regarded as a less Th2-prone strain than BALB/c due to a relatively weak allergic response to allergens. However, with an advantage of the enormous genetic variants available, C57BL/6 mice could become a more convenient platform targeting essential elements of the immune system involved in the pathogenic mechanisms. Moreover, a recently established asthma model might provide a more faceted comparison between C57BL/6 and BALB/c mice (Gueders et al., 2009). In this study, BALB/c mice showed a greater level of inflammation in lung tissue, including the increased number of mast cells and concentration of IL-4, IL-5 and IL-13, which validate the bias of BALB/c to Th2. While, surprisingly, C57BL/6 exhibited much more pro-inflammatory cytokines and eosinophilic inflammation in bronchoalveolar lavage fluid (BALF) than BALB/c mice, which may reflect a mixed pattern of Th1/Th2.

In conclusion, the genetic background of the chosen mouse strain can affect hyperresponsiveness and allergic inflammation in the murine model. The selection of experimental mouse strains is very important for the characterization of allergic diseases, and greatly dependent on the allergen types and the target phase or features of particular allergic diseases.

3. Mouse gender

Gender has been widely believed as one of the potential risk factors in the development of food allergy. Gender preference in food allergy could also possibly be affected by age. A typical pattern is found in some investigations on the basis of a large number of subjects in peanut allergy or tree nut allergy. In a telephone survey of 13,493 participants in the United States (Sicherer et al., 2003), generally no significant difference of peanut or tree nut allergy sensitisation rates were found among different age groups. However, among children's, the prevalence of peanut or tree nut allergy was much higher among males than females, while the female/male ratio of adult patients with peanut or tree nut allergy reached almost 2. The same trend was also observed in asthmatic patients (Lack, 2012). Overall, the male predominance of allergic diseases occurred in children but the situation was reversed in female predominance among adults. Accordingly, the gender effect on allergy prevalence is possibility associated with some of the physiological and immunological changes during different growth stages. In contrast the Multiple Logistic Regression Models constructed on the basis of 38,480 children in the United State could not identify gender as a significantly related characteristic with food allergy (Gupta et al., 2011). Furthermore very similar prevalence rates of peanut

allergy or cashew allergy were reported among males and females in 141 English children (Clark et al., 2007).

Some studies on animal models have also compared the influence of different mouse genders on allergic responses. In a model of Brown Norway rats (Pilegaard and Madsen, 2004), repeated oral dosing with OVA provoked a significantly higher level of OVA-specific IgE and IgG antibodies and a much higher incidence rate among female rats compared to males. Two studies on BALB/c mouse models of airway hyperreactivity supporting this gender preference (Hayashi et al., 2003; Melgert et al., 2005). In these studies, a more severe lung inflammation in female or castrated males as compared to uncastrated males was detected, which included a higher level of antigen-specific IgE antibody and IL-4 cytokines, the upregulation of CD4+ cells and a remarkable lung peribronchial infiltration of lymphocytes and eosinophil. Compared to males, less regulatory T cells were detected in female lungs, which suggested a relatively weak protection in female lungs against allergen challenges, hence worsening the inflammatory responses. In addition to the deficient regulatory T cells, a lower level of testosterone in females and castrated males were speculated to contribute to a stronger airway inflammation. Moreover, estrogen is another sex hormone that has been well studied for its effects on gender difference in allergic diseases (Chen et al., 2008). Recent experimental evidence was derived from a passive systemic anaphylaxis mouse model. This study revealed the regulatory function of estrogen in the production of endothelial nitric oxide synthase and nitric oxide without impacting mast cells, contributing to an increased anaphylaxis severity in female mice (Hox et al., 2015).

Although the majority of mouse models of food allergy have been conducted on female mice, so far, some male mouse models have also succeeded in providing novel insights into food allergy, particularly the development of more specific remedies for a better immunological regulation of allergic diseases. For example, in a CT-dependent ingestion model using male adult BALB/c mice (Liu et al., 2013), a unique subpopulation of tolerogenic B cells in intestine tissues was characterised. More importantly, in the effector phase of food allergy, this cell strain was found to be able to control the activation of murine effector T-cells through the expression of transforming growth factor (TGF)- β and to inhibit Th2 inflammation of murine intestine tissues greatly after adoptive transfer. Another recent male mouse model highlighted rapamycin as a potential preventive and therapeutic drug in food allergy (Yamaki and Yoshino, 2012). In the preventive part of this study, male mice with the administration of rapamycin prior to oral challenge of OVA showed that mast cell responses were locally (intestine) and systemically (serum mouse mast cell protease-1) reduced and consequently less severe allergy-related symptoms were observed. In addition the therapeutic treatment with, rapamycin dramatically decreased the levels of antigen-specific IgE antibody as well as several cytokines including IFN- γ , IL-4, IL-13, IL-9, IL-10, and IL-17.

To summarise, regardless of possible gender preference in human food allergy, female mice may be a better choice for the model establishment of severe airway allergic conditions, while male mice can be well employed in studies of food allergy prevention and therapies. The few published investigations did not explain the reasons of mouse gender preference, and male mice should not

automatically excluded to develop a mouse model for food allergy. Further research to link the physiological and immunological characteristics of the two genders with different applications in food allergy will be necessary.

4. Routes of sensitisation

The first phase of the IgE-mediated allergy cascade responses is sensitisation, which is represented by the development of antigen-specific IgE antibodies after exposure to an allergen. People with food allergy actually have often difficulty recognising their routes of sensitisation because no symptoms are experienced at this early stage. Tree-nut specific IgE antibody was detected in more than 50% of the children patients with tree nut allergy who did not realize their history of exposure (Sicherer et al., 1998). In the same study, a similar situation was seen among patients with peanut allergy. The first acute reactions to peanut occurred at a median age of 24 months, which suggested one or multiple exposure(s) that sensitised most individuals at an earlier stage. However, 72% of young patients thought the event that led to initial allergic reactions was their first exposure to peanuts. In fact, allergens potentially obtain access to lymphoid organs via three main routes: ingestion, inhalation and skin contact. Subsequent allergen exposure is boosting the production of antigen-specific IgE antibody. Different sensitisation routes have been established in animal models of food allergy, including intragastric (I.G.), intraperitoneal (I.P.), epicutaneous (E.C.), intranasal (I.N.) and subcutaneous (S.C.) administration. Because the clinical allergenic potency of proteins and immunological responses can vary greatly depending on the exposure route and the fate of the allergen, selecting a reasonable sensitisation route is very critical in developing the best possible mouse model for food allergy.

The gastrointestinal tract is believed to be the most common exposure sites to food allergens. As a result, oral delivery of allergens into the gastrointestinal tract of animals is the most widely used route of sensitisation in food allergy models. However, similar as humans, animal models have an innate tendency to develop oral tolerance to a myriad of food proteins (Helm, 2002). Consequently, some adjuvants are often required to overcome oral tolerance, as for example CT which is a well-known mucosal adjuvant. However, the involvement of CT does not reflect usual human pathogenic mechanism of food allergy. CT is an endotoxin produced by *Vibrio cholera*. The non-toxic subunit B of CT links to the allergen of interests and may have the capacity to bind to the receptor on intestinal epithelial cells, which increases allergen uptake (Brunner et al., 2010; Snider et al., 1994). It has been reported that CT is able to enhance Th2-biased responses and suppress Th1-mediated responses in some autoimmune diseases (Su et al., 2004) and delayed-type hypersensitivity (Rask et al., 2000). In addition to the modification of T-cell responses, the imbalance of intestinal fluid balance has been suggested to contribute to CT actions as well (Cox et al., 2006). However, to date, the exact mechanisms through which CT strengthens the murine Th2 immune response still remains unclear. Staphylococcal enterotoxin B (SEB) is another utilised adjuvant that could bypass the state of tolerance by oral administration together with food allergens in mice. SEB is known as causing food poisoning, but could act as a super-antigen to virtually stimulate Th2 lymphocyte activities in food allergy models. In particular, unlike CT/allergen-sensitised BALB/c mice, intense eosinophilia is presented in the blood and intestinal tissues of SEB/allergen sensitised BALB/c mice, which is also a hallmark of pathological characteristics seen in patients with food allergy (Ganeshan et al., 2009). Furthermore, some studies suggested an association of SEB with the induction of other allergic diseases, such as atopic dermatitis and chronic rhino sinusitis. Therefore, SEB might be a better option of adju-

vants to enhance the effectiveness of murine sensitisation with food allergens. However, there is still no evidence to show the necessary involvement of SEB as an environmental risk factor in the development of human food allergy.

Instead of breaking the oral tolerance, Bowman evaluated the allergenic potency of different foods by the failure to induce tolerance in adult C3H/HeJ mice (Bowman and Selgrade, 2008). In this study, mice orally exposed to food extracts without adjuvants received subsequently I.P. treatment of food extracts. The tolerance indicator was the decreased production of antigen-specific serum IgE in “tolerant” mice compared to those without feeding of extracts.

The experiment results showed that foods containing non-allergens (turkey, spinach) were more tolerising than foods containing strong allergens (peanut, Brazil nut), while foods containing resolving allergy (egg white) were most likely tolerant. From this perspective, murine immunologic tolerance to foods can mimic human oral tolerance in many ways.

In addition, an adjuvant-free mouse model study of rice allergy demonstrated the effectiveness of repeated I.G. gavage to elicit hypersensitivity in BALB/c mice (Chen et al., 2011). The typical allergic responses of food allergy include mild clinical symptoms, antigen-specific IgE antibody response and small intestine inflammation were all detectable in this mouse model of rice allergy, which placed this model the first successful gastrointestinal mouse model in the absence of adjuvants.

The intraperitoneal route (I.P.) of sensitisation has a relatively high efficiency to systemically trigger the production of mouse circulating IgE and IgG antibody. Alum is frequently used to increase the sensitivity to allergens through establishing allergen sites of high concentration to stimulate macrophages. The clinical utility of alum in human has been approved (Lambrecht et al., 2009). However, the usage of alum in food allergy model is still in doubt because of its non-physiological nature. Therefore, in the absence of alum, systemic (I.P.) administration of proteins in mice has been applied, but only for the purpose of screening novel allergens (Dearman et al., 2003; Dearman and Kimber, 2007; Verma et al., 2012). Different inherent allergenic potentials of proteins could be identified by the measurement of IgE antibody responses in the high IgE responder, the BALB/c mouse.

The epicutaneous route (E.C.) of sensitisation has been applied to establish adjuvant-free BALB/c models. Allergens were applied to an area of the mouse back or belly skin with hair removed, and then this area was covered with a non-latex, non-occlusive bandage for a short period, followed by a longer resting period. The sensitisation was conferred by repeated transdermal exposure. Mouse models of hazelnut (Birmingham et al., 2006), cashew nut (Parvataneni et al., 2009), Anisakis (Nieuwenhuizen et al., 2009) and milk whey protein allergy (Gonipeta et al., 2009) have been established using this approach. These studies published the successful induction of antigen-specific IgE antibody, Th2-biased cytokines and clinical responses in dose-dependent manners. In another example of an adjuvant-free mouse model for hazelnut allergy, similar to persistent human hazelnut allergy, BALB/c mice could respond to transdermal challenges of hazelnuts after a longer resting period after transdermal sensitisation. This study discovered mechanisms underlying the long-term hypersensitivity with long-lasting memory response of murine antigen-specific IgE antibody and splenocyte IL-4 responses (Gonipeta et al., 2010).

The intranasal route (I.N.) of sensitisation is often applied for aerosolized allergens to elicit food allergy. An investigation in 2007 showed 1/4 of all allergic reactions were due to hidden allergens, with 35% of fish-allergic patients and 22% of egg-allergic reactions caused by allergens hidden in other foods or food vapours (Anibarro et al., 2007). Subsequently it was demonstrated by Kirstein et al. (2010) that also food borne parasite allergens can

generate allergic sensitisation through allergen inhalation. Acute allergic airway hyperreactivity was also reported in peanut-allergic patients who inhaled airborne peanut at school or on commercial airlines. Moreover, IgE reactivity to multiple spice allergens was found in spice mill workers exposed to high concentration ($>10\text{ mg/m}^3$) of inhalable spice dust (van der Walt et al., 2010). Therefore, the immune reaction to aerosolized food allergens has been an essential aspect of food allergies in both domestic and working environments (Jeebhay et al., 2001). Another related issue for attention is the immunological cross-reactivity and consequently is responsible for sensitising over 30% of the world-population. Therefore inhalation of environmental allergens can play a considerable role in either sensitisation or effector phases to contribute to food allergies.

However, airway exposures to allergens are usually overlooked in the establishment of food allergy models. Only a few intranasally food-challenged mouse models have been published and even less models with airway sensitisation. In a recent study of fish allergy, a mouse model was established for a better understanding of work related inhalant allergy during food-processing (van der Ventel et al., 2011). After I.P. sensitisation, pilchard-sensitised mice received intranasal challenges of the same allergens. Increased levels of antigen-specific IgE antibody, Th2 biased cytokines of IL-4, IL-5 and IL-13, as well as the lung inflammation and cells infiltration, but no allergy-related clinical symptoms were reported in this model. A comparative study of respiratory sensitisation and oral sensitisation in peanut allergy provided one of the very few published nasally sensitised model using C57BL/6 mice (Fischer et al., 2005). However this study used CT conjunct with peanut extracts to orally initiate the immune responses. In Table 3 the main difference of two models are highlighted, that were sensitised by two mucosal routes and nasally challenged. Additionally, many mouse models of allergic diseases, such as allergic rhinitis (Hellings et al., 2001) and asthma (Brusselle et al., 1995), are often developed with aerosolized OVA as allergen using a similar protocol, which could be good references for the establishment and analysis of novel inhalant models of food allergy.

In the effector phase of food allergy, the airway exposure to food allergen seems less common than via the I.G. route. Also, the airway sensitisation has been less much studied. Therefore, a mouse model that receives nasal sensitisation but oral challenges might align better with some of the diagnosed food allergy patients with no clear exposure histology.

The subcutaneous route (S.C.) of allergens has been also applied in the establishment of some animal model, which are not discussed in this review due to causing skin inflammation and are unlikely to be acceptable for human applications (Bublin and Breiteneder, 2014).

5. The food allergen

In food allergy, there are eight groups of foods people are more commonly allergic to, which are egg, milk, peanut, tree nut, soy, wheat, fish and shellfish. Fig. 2 is based on a national survey of self-reported food allergy in 20, 686 individuals in the United States between 2007 and 2010 (McGowan and Keet, 2013). There seem to be three leading causes for food allergy, namely milk, peanut and

shellfish in both children and adults. In contrast to mouse studies of food allergy, the most intensively investigated food allergen sources are egg (mainly due to the wide usage of OVA as allergen), milk and peanut. As we are advancing the identification and characterisation of more allergens, mapping the IgE binding epitopes and the generation of recombinant proteins, more food allergy complications could be explored based on more specific investigations. An increasing number of mouse models have been developed with the introduction of different specific allergenic proteins (Table 4). In addition, the allergenicity of processed foods has been discussed in some mouse model studies. Here, the most recent findings for milk, shellfish and peanut allergies are summarised.

5.1. Milk allergens in mouse studies

Milk allergy is the most common food allergy in human's early life. 2%–3% of children younger than two years of age are suffering from cow's milk allergy and 60% of them showed IgE mediated (type I) allergic responses. A recent study from Australia reported up to 8% of infants with IgE confirmed allergy to milk (Osborne et al., 2011). Although up to 87% of infants could outgrow IgE-mediated milk allergy at three years of age (Høst et al., 1995), some individuals could not outgrow their allergy due to the deficient development of CD4⁺CD25⁺ Treg cells in their childhood (Karlsson et al., 2004). Moreover, IgE- and IgG-binding epitopes on α s1-, β - and κ -casein have been mapped with a recognition difference between patients with a persistent and transient histories of cow's milk allergy (Chatchatee et al., 2001). There are a variety of allergenic proteins contained in milk with varying structural and functional differences. However, the majority of mouse models of milk allergy have been developed with the use of whole milk or β -lactoglobulin, and the involvement of other milk proteins in the induction of allergic responses have been poorly studied.

Cow's milk consists of more than 25 different proteins, which can be divided into two major categories, whey and whole casein, accounting for approximately 18% and 82% of milk proteins respectively. BALB/c mice sensitised with modified milk of a casein/whey protein ratio of 40:60 showed a significantly lower diarrhea incidence and plasma histamine levels (Lara-Villoslada et al., 2005). Therefore the casein fraction might be more allergenic than the whey fraction of milk. The whole casein fraction is comprised of four proteins, α s1-, α s2-, β -, and κ -casein with little primary structure homology. The α s1-casein is the most abundant milk proteins and its allergenicity is frequently observed, whereas not a single protein could be identified as the dominant allergen in milk. Different binding specificity and intensity of sera IgE antibodies to multiple milk proteins, regardless of the amount of these proteins in milk, were recognised in 20 documented paediatric patients with milk allergy (Natale et al., 2004).

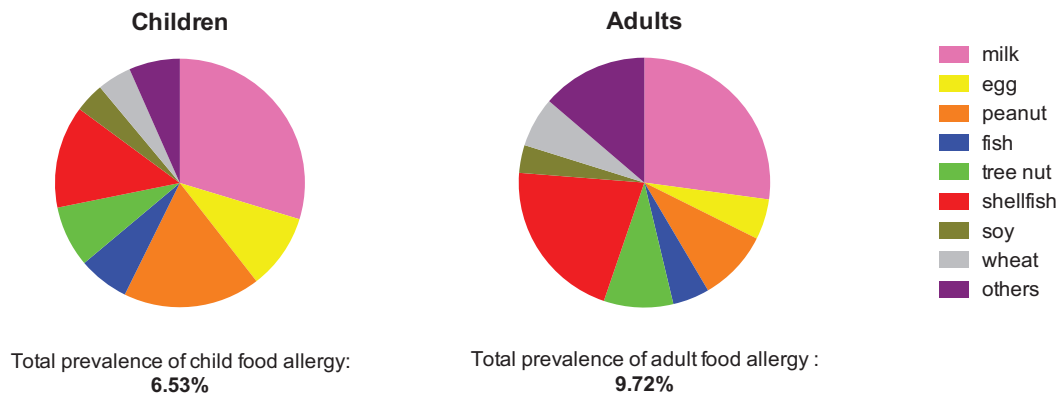
Although some mouse models have been successfully established using whole milk (Adel-Patient et al., 2005; Man et al., 2004; Li et al., 1999), very limited published models were established using single milk casein protein. There is one systemic induced BALB/c model with α s1-casein from goat's or sheep's milk (GSM), which is highly homologous to cow's milk. The severity of murine allergic reactions, including clinical symptoms and plasma levels of IgE antibody and mouse mast cell protease-I, was greatly dependent on the dose of α s1-casein (Hodgkinson et al., 2012). This dose-dependent manner suggests the important responsibility of α s1-casein in milk allergy.

In the whey fraction of milk, the two main allergenic proteins are β -lactoglobulin and α -lactalbumin. β -lactoglobulin has been recently studied using several different mouse models and some interesting studies may contribute to our knowledge of milk allergy therapeutics and mechanisms. By contrast, α -lactalbumin has been rarely studied *in vivo*. In 2011, Morin successfully estab-

Table 3

Comparison of the immune responses of mouse models for peanut sensitisation via the oral or nasal route (Fischer et al., 2005).

Immune responses	Oral sensitisation	Nasal sensitisation
Plasma levels of peanut-specific antibody	IgE: higher	IgG: higher
Lung inflammation	Significantly increased lung eosinophilia and higher Th2 type cytokines	Higher inflammatory reactions; rare eosinophils but massive lung neutrophilia
Response to nasal challenges of un-related food (OVA) or environmental allergen (<i>Dermathophagoides farina</i>)	Low level of lung inflammation	High level of lung inflammation
Airway hyperreactivity (enhanced pause)	Yes	Yes

**Fig. 2.** The prevalence proportions of self-reported food allergen sources of children and adults in the United States (McGowan and Keet, 2013).

lished an adjuvant free mouse model of milk allergy by I.P. sensitisation of β -lactoglobulin in germ-free BALB/c mice (Morin et al., 2011). In contrast, I.P. administration of whole casein or heated β -lactoglobulin could not induce antigen-specific IgE antibody responses in either germ-free mice or conventional mice, which indicates a lower allergenicity of whole casein or heated β -lactoglobulin compared to untreated β -lactoglobulin. Regarding to the heat effects on the allergenicity of β -lactoglobulin, an *in vitro* evaluation by indirect competitive ELISA reported that heat treatment could increase the allergenicity of β -lactoglobulin and also α -lactalbumin, with a peak at 90 °C, but dramatically decreased binding reaction at higher temperature (up to 120 °C) (But et al., 2009). Another important conclusion from Morin's adjuvant free mouse model of β -lactoglobulin allergy was that germ-free mice might have advantages over conventional mice when used in experimental models for food allergy. Because mice raised and maintained under *axenic* (germ-free) conditions lack normal intestinal microbiota, commensal organisms in particular, leading to a defective immune system (Smith et al., 2007). The complete development of commensal organisms in gastrointestinal tract after birth may reduce the susceptibility of individuals to milk allergy. This theory is supported by another mouse model. The pretreatment of transfected *Lactococcus lactis* with the capacity of producing IL-10 was demonstrated to have a protective effect on C3H/HeOJ mice before oral sensitisation with a mixture of β -lactoglobulin and CT (Frossard et al., 2007). Other milk proteins such as serum albumin and lactoferrin are also known milk allergens, however require more animal models for a better comparison of their allergenic potentials and further understanding the mechanisms of milk allergy.

5.2. Peanut allergens in mouse studies

In recent decades there has been an increasing number of reported life-threatening anaphylactic shock caused through exposure to peanuts and most patients don't outgrow peanut allergy (Nwaru et al., 2014). As a result, peanut allergy is regarded as one of the most dangerous food allergy. So far, eleven Ara h-type allergenic

proteins have been identified with the capacity to bind specific IgE antibody from sera of peanut allergy patients, and a majority of them are peanut storage proteins (Schmidt et al., 2009). Ara h 1, Ara h 2 and Ara h 3 have been widely considered as the main peanut allergens with strong allergenic potency with more than 50% of patients reacting to these three allergens. Ara h 5 and Ara h 8 might be the main cross-reactive allergens with a variety of different pollen allergens (Mittag et al., 2004; Kleber-Janke et al., 2001; Kleber-Janke et al., 1999).

However, the results from *in vivo* assessments of the allergenicity of peanut allergens seem to conflict. Orally or systemically induced peanut-sensitised C3H/HeJ mice developed a stronger IgE, IgG1 and IgG2a antibodies response to purified Ara h 1 and Ara h 3 as compared to Ara h 2 and Ara h 6 (Van Wijk et al., 2004). Nevertheless, another orally sensitised C3H/HeJ model indicated the predominant allergenicity of Ara h 2 and Ara h 6 from roasted peanuts (Kulis et al., 2012). Ara h 2 and Ara h 6 are 2S albumin and seem to have more digestion-resistant properties than other proteins in peanuts. In this study, the depletion of Ara h 2 and Ara h 6 in peanut remarkably reduced the severity of murine allergy-like anaphylactic reactions after I.P. challenges. Moreover, Ara h 2 and Ara h 6 had similar desensitisation effects as the whole peanut extracts in an immunotherapy evaluated in mice. In addition, the presence of Ara h 1 and Ara h 2 specific IgE antibodies was also observed in an orally induced C3H/HeJ model using crude peanut extracts (Li et al., 2000). Moreover, both Ara h 1 and Ara h 2 were able to stimulate proliferations of spleen cells from sensitised mice, although not as effective as whole peanut extracts. Unfortunately, other possible peanut allergens were not assessed in the same study as comparisons.

Non-allergenic components in foods may contribute to the phenomenon that whole peanut extracts could be used to sensitise mice most effectively, in contrast to any of the individual peanut allergen. Fats, carbohydrate and non-allergenic proteins are very common ingredients of foods. The role of these components in the development peanut allergy has been rarely studied using murine immune system. The influence of peanut fat on the murine immune activation has been recently highlighted (Van Wijk et al., 2005).

Table 4

Characteristics of the main identified allergenic proteins derived from milk, peanut and shellfish, and their usages in the establishment of food allergy mouse models (<http://allergenonline.com>, <http://www.ncbi.nlm.nih.gov/>) (Mittag et al., 2004; Martins et al., 2005; Schmidt et al., 2009; Lopata et al., 2010; Hochwallner et al., 2014; Koeberl et al., 2014a).

Food source	Allergen name and nature			Molecular weight (kDa)	Representative mouse models implicated
Milk	Milk			NA	Hodgkinson et al. (2014), Poulsen et al. (1990)
	Processed milk			NA	and see more in Table 1
	Main allergenic proteins	α -Lactalbumin		14.2	ND
		β -Lactoglobulin		18.3	Morin et al. (2011)
		Bovine serum albumin		66.3	Dearman et al. (2000)
		Immunoglobulin		160	ND
		Lactoferrin		80	Debbabi et al. (1998)
		α s1-Casein		23.6	Hodgkinson et al. (2012)
		α s2-Casein		25.2	ND
		β -Casein		24	ND
Peanut	Peanut extracts			19	ND
				NA	Fischer et al. (2005), Berin et al. (2006) and see more in Table 1
	Processed peanut extracts			NA	Li et al. (2003), Adel-Patient et al. (2005)
	Main allergenic proteins	Lipid transfer protein	Ara h 9	9.8	ND
		Oleosin	Ara h 10	16	ND
			Ara h 11	14	
		Profiling	Ara h 5	15	ND
		α -amylase	NA	16	ND
		Conglutin (2S albumin)	Ara h 2	16.6	Li et al. (2000), Van Wijk et al. (2004), Kulis et al. (2012) reference for Ara h 7 is ND
			Ara h 6	18.0	
			Ara h 7	15	
		PR-10, Bet v 1 family member	Ara h 8	16.9	ND
		Agglutinin	NA	31	ND
		Cupin (vicilin-type, 7S globulin)	Ara h 1	63.5	Van Wijk et al. (2004), Li et al. (2000), Bashir et al. (2004)
					Van Wijk et al. (2004)
		Cupin (11S globulin, glycinin)	Ara h 3	60	reference for Ara h 4 is ND.
			Ara h 4	61	
		Phospholipase D	NA	91	ND
		Conarachin I	NA	142–295	ND
		α -arachin	NA	120–600	ND
Shellfish	Shellfish extracts			NA	ND
	Processed shellfish extracts			NA	ND
	Main allergenic proteins	Tropomyosin		34–39	Sand shrimp (<i>Metapenaeus ensis</i>): Capobianco et al. (2008)
		Arginine kinase		40	Mud crab (<i>Scylla paramamosain</i>): Shi et al. (2015)
		Myosin light chain		20	ND
		Sarcoplasmic calcium-binding protein		20	ND
		Troponin C		16.8	ND
		Myosin heavy chain		208–400	ND
		α -Actine		41	ND
		Triosphosphate isomerase		28	ND
		SERCA/Ca ²⁺ ATPase		122.2	ND

Abbreviation: NA = not applicable, ND = not done.

Peanut extracts or purified peanut proteins were S.C. injected into the hind footpad of BALB/c mice and one week later, popliteal lymph nodes (PLN) collected for analysis. Peanuts extracts, but not purified proteins including Ara h 1, 2 and 6 were able to induce PLN cell proliferation, cytokine production (IL-4, IL-10 and IFN- γ) in restimulation of PLN cell culture and activation of APCs. Compared with the peanut extracts containing 0.2% fat, the peanut-oil emulsion with 3.5% fat appeared to induce even stronger immune responses, which suggested peanut fat had a capacity to modify the allergenicity of peanut. In spite of the limited pathogenic relevance of PLN assay to food allergy, this study highlights that the factors affecting food allergenicity are not confined to antigenic proteins. The potential influence of different non-allergen food components or food-processing methods on food allergenicity can be analysed in detail using mouse models.

Novel immunotherapies, mainly through the desensitisation effects of engineered peanut allergens, have been also analysed using murine immunity models. With the modification of the main IgE binding sites, recombinant peanut allergens rAra h 1, 2 and 3 had the potential to generate a much lower IgE antibody production than wild-type Ara h 1, 2 and 3. Thus, a frequent administration of rAra h 1–3 mixture in peanut/CT-sensitised mice could reduce the level of anaphylactic responses to oral

challenges. Interestingly, the S.C. co-administration of heat-killed *Listeria monocytogenes* (HKLM) (Li et al., 2003b) or the rectal co-administration of *Escherichia coli* (Li et al., 2003a) with rAra h 1–3 could dramatically enhance the effectiveness of desensitisation in the C3H/HeJ mouse model. Antigen-specific Th2 responses appeared to be shifted to a more Th1 responses. Unfortunately, the immune mechanisms in which HKLM and *E. coli* itself may play as potential bacterial adjuvants are largely unknown.

5.3. Shellfish allergens in mouse studies

With the increasing consumption of shellfish, the prevalence of shellfish allergies has been increasing in many industrialized as well as developing countries over recent years (Lopata et al., 2010; Sicherer et al., 2004; Chiang et al., 2007). Similar to peanut allergy, shellfish allergy often persists throughout adulthood and seems to be the most common food allergy in adults. A number of proteins have been identified as shellfish allergens (see Table 3). They are species-specific with different degrees of sequence homology considering the big number of shellfish species. Consequently, although various techniques have been used to characterize and quantify shellfish allergens (Abdel Rahman et al., 2015; Kamath et al., 2014), many of the shellfish allergens are still poorly studied at

the molecular and immunological level (Lopata and Lehrer, 2009). Mouse models have not been frequently applied in the clinical evaluation of allergenic shellfish components and the development of therapeutic interventions for shellfish allergy.

In the few model systems published, the major allergen tropomyosin (TM) has been considered as a good representative for sensitisation to shellfish, because the muscle protein tropomyosin is the most prominent pan-allergen responsible for allergic reactions to shellfish. For example, TM of the Brown shrimp (*Penaeus aztecus*) Pen a 1 is responsible for more than 75% of the shrimp-specific IgE antibody reactions (Daul et al., 1994) and TM has been the main allergenic protein identified in a variety of mollusks species (Chu et al., 2000; Asturias et al., 2002; Kamath et al., 2013). Thus, TM becomes a key allergen to better understand the cross-reactivity between a wider range of invertebrate species and also a suitable candidate allergen to be used in the induction of *in vivo* models for shellfish allergy. Particularly, shrimp tropomyosin accounts for approximately 20% of all shrimp proteins and the amino acid sequence identity among various edible shrimp species is 95–100% (Kamath et al., 2014; Koeberl et al., 2014b). In 2008, shrimp tropomyosin was initially used in oral sensitisation with CT as mucosal adjuvant in C3H/HeJ mice and elicited significantly increased titers of serum TM-specific IgE and IgG1 antibody (Capobianco et al., 2008). Oral challenge with high doses of purified TM triggered an appropriate level of anaphylactic reactions. The TM re-stimulated splenocytes from sensitised donor mice released significantly higher levels of IL-4, IL-13 and IFN- γ than control mice, which confirmed the Th1/Th2 pattern of immune reactions in shrimp allergy. In the same year, oral sensitisation with the recombinant TM rMet e 1 plus CT also successfully induced hypersensitivity in BALB/c mice (Leung et al., 2008). Unfortunately, no cell-mediated immune responses and local inflammations were assessed in these two studies. Recently, Shi established a mouse model of mud crab (*Scylla paramamosain*) TM allergy in BALB/c mice by systematic sensitisation (Shi et al., 2015). Oedema and lymphocytic infiltration of the jejunum was observed in TM/alum treated mice. On the basis of this mud crab TM model, the potential immunoregulatory effect of isolate sulfated polysaccharide from *Porphyra haitanensis* (PHPS) was investigated. Repeated systematic treatments of PHPS either before or after tropomyosin sensitisation could skew a mixed Th1/Th2 immune pattern to more Th1 responses to TM, suggesting a potential medical application of PHPS in crustacean allergy.

Different processing methods may impact on shellfish allergenicity (Kamath et al., 2013), however few mouse model studies have explained this topic. A very recent study of a I.G. mouse model revealed that high hydrostatic pressure, in combination with thermal treatment could significantly reduce the allergenicity of shrimp TM (Long et al., 2015). Such studies provide more perspectives for food processing and storage technology targeting people allergic to shellfish.

However, except for these animal models using TM, there are no animal models developed for the other shellfish allergens. Despite the importance of TM, particularly as a highly cross-reactive protein in I.G. and inhalant allergies, there are no published *in vivo* studies, warranting a detailed analysis of the mechanisms underlying cross-reactivity to TM derived from various allergen sources. The development of mouse models for shellfish allergy and cross-reactivity could greatly improve the specificity and accuracy of clinical diagnosis and medical advises on allergen avoidance.

6. Advances of food allergy mechanisms in mouse studies

The detailed immunological analysis of a mouse model not only plays a very necessary role to evaluate the level of murine responses

to allergens, but also reveals more knowledge of the molecular mechanism underlying allergic reactions to food. For example, a recent study found that insulin-like growth factor (IGF) 2 could stimulate Treg cell proliferation and improve Treg cell function *in vivo*. The IP administration of IGF2 and/or the adoptive transferring of Antigen (OVA)-specific Treg cells in an oral OVA/CT-sensitised mouse model significantly reduced the serum IgE responses and the inflammation in intestine (Yang et al., 2014). More molecular components that may play a regulatory role in the allergic pathway can be discovered using mice with target gene modified. The genetic depletion of sphingosine-kinase (SphK) 1 and 2 in mice could alter the production of Sphingosine-1-phosphate (S1P), thus suppressing the murine antibody responses to OVA in oral sensitisation with acid-suppression and subsequently the accumulation of CD4+ effector cells (Diesner et al., 2012). However, the OVA specific IgE and IgG1 level through IP sensitisation of OVA mixed with Al(OH)₃ was not affected by the absence of SphK 1 and 2. Therefore, SphK1 and 2 seems to play an important role in the regulation of CD4+ T cells leading to intestinal allergies.

Furthermore, in a study of mouse model for peanut allergy, the provirus integration site for Moloney murine leukemia virus (Pim) 1 kinase and downregulated expression of Runt-related transcription factor (*Runx*) were found to contribute to the development of intestine allergic responses via promoting Th2 and Th17 cell differentiation and expansion (Wang et al., 2012). The same research team also reported that the activation of Cyp11a1 may lead to peanut induced intestine anaphylaxis (Wang et al., 2013).

7. Conclusion

The availability of different mouse models greatly improves our knowledge on food allergies. The selection of different establishment parameters can contribute to the sensitisation and effector mechanisms of murine immunity. The majority of published mouse studies could reflect some of the essential features of human food allergy, such as boosted levels of antigen-specific IgE antibody in serum and Th2 cytokines in tissue and re-stimulated spleenocytes. However, the variability of mouse models increases the difficulty to compare the different *in vivo* studies on food allergens and food allergy. Such mouse studies might more easily conclude controversial views. Moreover, the consistency of mouse models between laboratories has to be validated.

There are still many food allergens and hypotheses of therapeutic or preventive strategy to be analysed using *in vivo* platforms. Further research on the role of non-allergen components of food and environmental factors in the induction of allergic responses will help to develop a more comprehensive understanding of food allergy.

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