Differences in the Anaphylactic Response between C3H/HeOuJ and BALB/c Mice

Guadalupe Marco-Martín^{a, c} Alejandro La Rotta Hernández^{a, f}
María Vázquez de la Torre^{a, d} Yoko Higaki^a José Manuel Zubeldia^{a, b, e}
María Luisa Baeza^{a, b, e}

^aAllergy Service and Experimental Medicine Unit and ^bInstitute for Health Research Gregorio Marañón (IIS-GM), Hospital General Universitario Gregorio Marañón, Madrid, ^cAllergy Department, Hospital Clínico San Carlos, Madrid, ^dAllergy Department, Hospital Infanta Leonor, Madrid, ^eBiomedical Research Network on Rare Diseases (U761 CIBERER), Instituto de Salud Carlos III, Madrid, and ^fAllergy Department, Policlínica Miramar, Palma de Mallorca, Spain

Keywords

Anaphylaxis · Mouse models · Allergy

Abstract

Background: Anaphylaxis is a severe and potentially lethal allergic reaction whose incidence is increasing. Murine models can elucidate the underlying mechanisms and pave the way for appropriate therapeutic options. However, differences in strains and protocols hamper comparisons of data between researchers. We performed a parallel study of clinical and immune responses with 2 strains of mice, BALB/c and C3H/HeOuJ, in an allergen-induced systemic anaphylaxis protocol. Both strains have been widely used in allergy models, although they have not been compared in an intraperitoneal systemic model. **Methods:** Groups of 5-week-old female BALB/c and C3H/HeOuJ mice were intraperitoneally sensitized with peanut in the presence of adjuvants. Specific immunoglobulin (Ig) G1, IgG2a, IgE, total IgE, histamine release, and specific stimulated splenocyte cytokines, interleukin (IL)-4, IL-5, IL-10, IL-12, IL-13, and interferon (IFN)- γ , were assessed. At week 6, mice were intraperitoneally chal-

lenged with peanut. Anaphylaxis was evaluated by recognition of clinical symptoms and changes in body temperature.

Results: All peanut-sensitized mice induced Ig and developed anaphylactic symptoms upon challenge. Nonetheless, the C3H/HeOuJ strain demonstrated earlier and persistently higher IgG1 and IgG2a production, elevated IgE, and more severe clinical symptoms and histamine release than the BALB/c strain. In contrast, BALB/c exhibited higher release of IL-4, IL-5, IL-10, IL-13, and IFN- γ . **Conclusions:** Both models are suitable for studying anaphylaxis. Consequently, they could be used in research on the pathogenesis and therapy of anaphylaxis. However, according to the type of study performed, differences in the specific clinical, humoral, and cellular responses to antigens have to be considered.

© 2017 S. Karger AG, Basel

Introduction

Anaphylaxis is an acute, severe, and potentially lethal allergic reaction [1] that was first described by Richet [2] in 1904. Today, it affects between 0.05 and 2% of the pop-

ulation, and its frequency has risen progressively during the last few years [3]. The main cause of anaphylaxis is an IgE-mediated reaction triggered by an allergen, although alternative pathways involving IgG antibodies have been proposed [4].

Given the potential mortality of this reaction, the underlying mechanisms must be analyzed, and novel effective therapies sought. Multiple studies are being carried out. Specifically, much research is aimed at the development of models in laboratory animals, mainly mice, since this approach provides considerable advantages not only in financial terms, but also in terms of environmental and biological standardization thanks to inbred and genetically modified strains. Murine models have enabled us to considerably advance our knowledge of anaphylaxis [5, 6].

The first murine studies were performed in 1941 [7]. Since then, robust conclusions on the pathogenesis of anaphylaxis have been reported in various strains: the allergic phenotype of BALB/c mice has led this strain to be widely used for characterizing classic (IgE-FcεRI-mast cell-histamine) and alternative dependent pathways (IgG-FcγRIII-macrophage-platelet-activating factor) [8, 9] and for establishing the immunoregulatory mechanism underlying tolerance, which suppresses both Th₁ and Th₂ responses [10, 11]. Furthermore, 129SvEvBrd mice are more susceptible to IgE-mast cell-mediated anaphylaxis than BALB/c mice through their increased sensitivity to histamine and an increase in mast cell counts in tissue, thus enabling a more in-depth study of IgE-mediated anaphylaxis and mast cell biology [12]. The essential nature of specific immunoglobulins and functional mast cells was defined for systemic anaphylaxis using C57BL/6 wild-type and knockout mice [13]. Jonsson et al. [14] proved the involvement of FcγRIV, neutrophils, and platelet-activating factor in active and passive systemic anaphylaxis using the same murine model. Li et al. [15] generated a murine model of peanut-induced anaphylaxis in C3H/HeJ mice with clinical symptoms that resembled those of food-mediated human anaphylaxis and where the contribution of peanut-specific IgE, histamine release, and mast cell degranulation was demonstrated. Moreover, this model has proven suitable for studying diverse treatments for anaphylaxis, such as a Chinese herbal medicine formula, which completely blocks anaphylactic reactions [16]. Protection of enteric helminth-infected C3H/HeJ mice against systemic anaphylaxis in a food allergy model has also been reported [17, 18]. Lastly, transgenic mice have proven useful in demonstrating inhibition of mast cells and basophil degranulation by

means of the interaction between FcRI and a human immunoglobulin Fcγ-Fcε bifunctional fusion protein, thus paving the way for allergen immunotherapy [19]. However, not all murine strains are suitable for investigation of allergy; in particular, AKR/J and CBA/J have proven unsuitable for the induction of food allergy [20, 21] despite being widely used in the evaluation of allergic respiratory diseases [22, 23].

Although all murine models have been validated, it has been observed that a suitable murine strain is essential in order to obtain the most satisfactory clinical and immunological response. However, few studies compare the specific features of the various strains. Consequently, it is hard to compare data between experiments. Li et al. [21] contrasted allergic intramuscular sensitization and anaphylactic symptoms in AKR, BALB/c, and C3H/HeSn mice and recorded an absence of anaphylactic reactions in AKR and BALB/c mice that was correlated with the absence of reaginic IgG1 or IgE in these groups. Thus, allergic sensitization was strain dependent in their model. In addition, Morafo et al. [20] and Smit et al. [24] contrasted susceptibility to induction of food allergy, respectively, in C3H/HeJ and BALB/c and C3H/HeOuJ, BALB/cByJ, and C57BL/6J mice. These authors showed that C3H/HeJ and C3H/HeOuJ mice had more severe anaphylactic symptoms than BALB/c and BALB/cByJ mice. Nevertheless, the oral sensitization rate was higher in BALB/cByJ than C3H/HeOuJ mice. As a consequence, various murine strains and protocols should be analyzed to find the most suitable model for specific questions.

Finally, the high variability in technical procedures between laboratories and investigators means that there is a need to define strain-specific features. BALB/c and C3H/HeOuJ have been widely used in food allergy models and specifically in anaphylaxis models. However, they have rarely been compared and have never been investigated in an intraperitoneally induced systemic anaphylaxis model. In order to define the best strain for further use in pathogenic and therapeutic studies and based on a previously validated murine model, we compared the immune and anaphylactic responses in C3H/HeOuJ and BALB/c strains in systemic, intraperitoneally induced, allergen-mediated anaphylaxis [25].

Materials and Methods

Mice and Extracts

Five-week-old female BALB/c and C3H/HeOuJ mice were purchased from Charles River Laboratories (Barcelona, Spain). All mice were maintained under a 12-h light/dark cycle with free ac-

cess to water and peanut-free chow in the animal unit of the Experimental Medicine Department of the Hospital General Universitario Gregorio Marañón (Madrid, Spain), which adheres to the European regulations for animal experimentation (Directive: 86/609/EEC). The experiments were approved by the local institutional animal care and use committee, which adheres to international procedures for animal care.

Peanuts were purchased from Productos Manzanares S.A., Spain. A total of 370 g of fresh peanuts was washed in distilled water, ground, and mixed with 500 mL of phosphate-buffered saline (PBS) (pH 7.4). After shaking overnight, the mix was centrifuged at 18,000 g for 30 min at 4°C; the aqueous fraction was collected and recentrifuged to remove residual traces of fat and insoluble particles. Thereafter, it was dialyzed to PBS with a 3-kDa Millipore® membrane, concentrated in a Hamilton® chamber, passed through a 0.2-µm pore filter, and frozen at -40°C until use. The protein concentration was calculated to be 3 mg/mL using the Bradford assay.

Pertussis toxin and aluminum (alum) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Concanavalin A Sepharose was obtained from Pharmacia (now Phadia, Uppsala, Sweden).

Sensitization and Challenge Protocols

Groups of 7 C3H/HeOuJ and BALB/c mice received 3 weekly intraperitoneal injections of peanut extract (500 µg) adsorbed in alum (2 mg) and pertussis toxin (300 ng) as adjuvants. The control groups of both strains received equal intraperitoneal doses of saline solution and adjuvants. Six weeks after the initial sensitization dose, all mice were intraperitoneally challenged with peanut extract and maintained under careful observation for 30 min (Fig. 1).

Assessment of Anaphylactic Responses

Anaphylactic symptoms were evaluated every 5 min for 30 min by a blinded observer using the clinical score system described by Xu et al. [26], as follows: 0, no symptoms; 1, mild shock including itching, ruffling of fur, dyspnea, and decrease in spontaneous activity; 2, moderate shock including prostration, sluggish gait, and slight activity after prodding; 3, severe shock including complete paresis and no activity following prodding with or without convulsions; 4, death within 30 min.

At the same time, body core temperature was registered every 2 min using a rectal thermometer (Measurement Specialties, Temperature Products Group, Dayton, OH, USA).

Antibody Assays

Mice were anesthetized weekly with inhaled isoflurane and bled in order to measure specific IgG1 (sIgG1), IgG2a (sIgG2a), IgE (sIgE) against peanut, and serum polyclonal IgE levels.

Measurement of sIgG1 and sIgG2a Levels

First, 96-well plates were coated with 50 µL of 0.001 µg/µL peanut extract per well, diluted in carbonate buffer, and left overnight at 4°C. Plates were then blocked with 1% bovine serum albumin in PBS at room temperature for 2 h, washed with borate-buffered saline/0.05% Tween 20 (Sigma), and incubated with blood samples or standards overnight at 4°C. A standard curve was created using serial dilutions of hyperimmune mouse serum in each plate. Alkaline phosphatase-linked anti-IgG1 or anti-IgG2a (Southern Biotechnology Associates, Birmingham, AL, USA) and streptavidin (Zymed, San Francisco, CA, USA) was added, then washed, and

Fig. 1. Study protocol. Groups of C3H/HeOuJ and BALB/c mice received 3 weekly intraperitoneal (i.p.) injections of peanut adsorbed in alum and pertussis toxin. Control groups of both strains received equal i.p. doses of saline solution and adjuvants. All mice were i.p. challenged at week 6.

incubated with 2.63 mg/mL of *p*-nitrophenyl phosphate (Boehringer Mannheim, GmbH, Germany). Absorbance was read at 405–650 nm within 1 h. Arbitrary units (AU) were determined by extrapolation of the absorbance value of the standard curve built with hyperimmune serum.

Measurement of sIgE Levels

Peanut sIgE was measured using ELISA based on a similar method. In this case, samples were previously incubated with protein-G Sepharose beads (Pharmacia, Uppsala, Sweden) to remove antigen-specific IgG. Protein-G-absorbed sera were added to the peanut-coated plates and incubated overnight at 4°C. Plates were incubated with goat biotinylated anti-IgE at 6 µg/mL (Becton Dickinson, Pharmingen, San Diego, CA, USA) before being washed and incubated with horseradish peroxidase-linked streptavidin at 0.62 µg/mL. After washing, TMB peroxidase was added (3,3',5,5'-tetramethyl benzidine; Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA). The reaction was stopped with phosphoric acid. Absorbance was read at 450–650 nm. AU were determined by extrapolation of the absorbance value of the standard curve constructed with hyperimmune serum.

Measurement of Serum Polyclonal IgE Levels

Serum polyclonal IgE levels were determined using ELISA based on a similar method. In this case, anti-mouse IgE and purified mouse IgE (Pharmingen) were used for coating plates and creating the standard curve, respectively.

Measurement of Histamine Release

In a parallel experiment, 6 sensitized mice of each strain were bled 15 min after the intraperitoneal challenge. Blood was collected in chilled tubes containing 7.5% potassium-EDTA. After centrifugation (900 g) for 10 min at 4°C, plasma aliquots were frozen at -80°C until use. Histamine levels were determined using an enzyme immunoassay kit (ImmunoTECH Inc., Marseille, France) according to the manufacturer's recommendations. For baseline histamine measurements, blood was collected 24 h before the challenge. The mice used for this determination were not included in the final analyses of anaphylactic symptoms.

Fig. 2. a Measurement of serum levels of specific IgG1 (**a**) and specific IgG2a (**b**) in sensitized and control groups for 6 weeks. Means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

Fig. 3. Serum levels of total IgE (**a**) and peanut-specific IgE (**b**) in peanut-sensitized groups were measured at weeks (W) 0 and 6. Means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$; ns, non-significant.

Splenocyte Cytokine Profiles

After the peanut challenge, survivors were sacrificed with isoflurane, and mouse spleens were harvested, teased to prepare single-cell suspensions, and resuspended in RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mmol/L glutamine, 0.05 mM M2-mercaptoethanol, and 1% penicillin-streptomycin (complete medium). Splenocytes were incubated at 5×10^5 cells per well in 96-well plates in a final volume of 250 μ L of complete medium with peanut (0.1 mg/mL) added at 37°C in a 5% CO₂ atmosphere. Culture supernatants were collected at 72 h, and samples were frozen at -80°C until use. Samples were tested for the presence of IL-4, IL-5, IFN- γ , IL-10, IL-13, and IL-12 p40 using ELISA, as recommended by the manufacturer (BD Biosciences, San Diego, CA, USA) or as described by Baeza et al. [25].

Statistical Procedures

The results were shown as the mean of 3 different experiments. Data were analyzed using GraphPad Prism software (v4; GraphPad Software Inc, La Jolla, CA, USA). Serum IgE, sIgE, histamine, cytokines, and anaphylactic responses were analyzed using a 2-tailed t test with the Welch correction, while ANOVA was used for the sIgG1 and sIgG2a analyses. Data are expressed as means $\pm$ SEM, and p values < 0.05 were considered statistically significant.

Fig. 4. Changes in body temperature and clinical score (CS) in sensitized and control groups after the peanut challenge (0 min). ** $p < 0.01$, *** $p < 0.001$.

Results

Humoral Immune Responses

Mice were bled weekly for the determination of sIgG1 and sIgG2a, sIgE, and serum IgE levels. Upon sensitization, both strains produced sIgG1 and sIgG2a, which increased progressively during the following weeks. However, C3H/HeOuJ IgG levels increased earlier and remained persistently higher than in BALB/c mice (mean $\pm$ SEM sIgG1 and sIgG2a levels in the C3H/HeOuJ group at week 5 were $203,998 \pm 42,281$ and $81,774 \pm 17,403$ vs. $16,057 \pm 6,840$ and $6,221 \pm 1,380$ AU in the BALB/c group, respectively). No specific immunoglobulin production was detected in the control groups (Fig. 2).

Both strains produced peanut sIgE. As previously observed with sIgG, C3H/HeOuJ mice produced higher levels (29.7 ± 4.7 AU/mL) than BALB/c mice (5.5 ± 1.3 AU/mL; $p = 0.007$; Fig. 3). Figure 3 also shows total IgE levels of both strains at the beginning (week 0) and at the end (week 6) of the experiment. There were no statistical differences between the strains for total IgE at week 6. However, both produced IgE during the sensitization period (week 0 vs. 6; $p = 0.0064$ for C3H/HeOuJ and $p = 0.0137$ for BALB/c mice). No sIgE or total IgE was detected in the control groups.

Systemic Anaphylaxis after Peanut Challenge

At week 6, all BALB/c and C3H/HeOuJ mice were intraperitoneally challenged with 1 mg of peanut. Rectal temperature and symptom score were evaluated for 30 min.

Fig. 5. Histamine release in BALB/c- and C3H/HeOuJ-sensitized mice 10 min after peanut challenge. Means $\pm$ SEM. ** $p < 0.01$.

All sensitized mice developed anaphylactic symptoms and experienced a steep decrease in body temperature. Marked differences were found between the sensitized and nonsensitized groups (Fig. 4). Significant differences following the decrease in body temperature were recorded after 10 min between the sensitized and control C3H/HeOuJ groups (0.94 vs. 0°C drop; $p < 0.01$); this delay was 8 min in the BALB/c mice (1.86°C drop in the sensitized group vs. 0°C drop in the control group, $p < 0.01$).

Compared with BALB/c mice, C3H/HeOuJ mice experienced a more severe reaction: the clinical score was higher (3–4 in C3H/HeOuJ vs. 2 in BALB/c mice), and the rectal temperature dropped more markedly than in BALB/c mice from 14 min (1.9°C drop in C3H/HeOuJ vs. 1.34°C drop in BALB/c mice; $p < 0.01$), reaching the greatest differences at 30 min (4.42°C drop in C3H/

Fig. 6. Measurement of splenocyte cytokines (IL-4, IL-5, IL-13, IFN- γ , IL-10, and IL-12) in the sensitized groups of both mouse strains. Means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

HeOuJ vs. 2.7°C drop in BALB/c; $p < 0.001$). Moreover, sensitized C3H/HeOuJ mice developed an even faster and more severe reaction. No variations were observed between the control groups of either strain.

Histamine Release

In a parallel group of mice, plasma histamine levels were determined in both strains 15 min after peanut challenge. Consistent with the clinical symptoms, histamine levels in C3H/HeOuJ mice ($19,608 \pm 4,005$ nM) were found to be higher than in the BALB/c group ($2,895 \pm 562$ nM; $p = 0.0043$; Fig. 5). Histamine release was not detected in the control groups.

Cellular Immune Responses

Mice were sacrificed after challenge, and cytokines from peanut-stimulated splenocytes were analyzed. Th₂ (IL-4, IL-5, and IL-13), Th₁ (IFN- γ and IL-12), and a regulatory cytokine (IL-10) were measured in both groups. In general, and in contrast to anaphylactic symptoms, BALB/c mice produced higher levels of all cytokines than C3H/HeOuJ mice (Fig. 6), although significant differences were found in IL-4 (134 ± 54 pg/mL in BALB/c vs. 37 ± 12 pg/mL in C3H/HeOuJ; $p = 0.0381$) and IL-5 ($1,901 \pm 656$ pg/mL in BALB/c vs. 554 ± 87 pg/mL in C3H/HeOuJ; $p = 0.008$). Likewise, Th₁ cytokine (IFN- γ) levels were higher in BALB/c than in C3H/HeOuJ mice

(677 ± 224 and 171 ± 18 pg/mL, respectively; $p = 0.048$). No IL-12 was released upon stimulation in C3H/HeOuJ or BALB/c mice.

Regulatory cytokine (IL-10) levels were measured in both groups and were slightly higher in BALB/c mice (615 ± 198 vs. 351 ± 64 pg/mL; $p = 0.23$).

Cytokine release was not detected in the control groups.

Discussion

Anaphylaxis is a systemic, life-threatening hypersensitivity reaction whose incidence is increasing in industrialized countries [27] and whose analysis in humans is complex [28]. We recommend our patients to avoid allergens and provide them with emergency treatment in case an anaphylactic reaction occurs. Currently, there is no way to prevent sensitization or any specific treatment for patients who are already sensitized [29]. Therefore, novel therapies should be investigated. Murine models have been found to be a good approach for studying anaphylaxis and its underlying immunological pathways [5, 6]. BALB/c mice have traditionally been considered an appropriate strain for developing allergy mouse models [8, 9]. Nevertheless, other strains, such as C3H/HeOuJ mice, have been shown to be a good alternative for studying

anaphylaxis and investigating novel therapies [16]. While multiple anaphylaxis studies have been conducted in BALB/c and C3H/HeOuJ strains, they have rarely been compared and have never been investigated in an intraperitoneal systemic anaphylaxis model.

We present an anaphylaxis model with a potent allergen. Peanut was selected because it is a strong allergen for humans and not to mimic a food allergen: our objective was not to develop a food allergy model. We aimed to develop a robust model of anaphylaxis to evaluate clearly the changes in anaphylactic signs and symptoms that appear in mice and thus minimize any subjective perceptions that may arise. Although murine peanut allergy models have been reported to accurately resemble human anaphylaxis [20], the clinical symptom scores were lower in BALB/c [20] and C3H/HeOuJ mice [30] receiving peanut orally than systemically. As shown elsewhere, intraperitoneal sensitization has proven successful [25, 31], food allergens have been widely used in models of systemic anaphylaxis [14, 32, 33]; both classic and alternative pathways were activated in a mouse model of systemic sensitization [8]. As our primary aim was to study systemic anaphylaxis (activating IgE and IgG pathways) expressed by sharp clinical and immunological responses in both strains, we developed a systemic sensitization protocol. It has been reported that the number of boosts, rather than the total amount of antigen received, is associated with the increase in allergen-specific IgE, IgG1, and IgG2a levels [21], and with the severity of the anaphylactic reactions [25, 32]. In a previously developed systemic anaphylaxis model, 3 weekly intraperitoneal doses were more suitable for the study than 2 or 4 doses [25]. Therefore, we opted for 3 weekly intraperitoneal doses in our sensitization protocol to be able to induce and unequivocally quantify the anaphylactic response.

Furthermore, since purified antigens are poorly immunogenic, adjuvants have been widely added to sensitization protocols in order to intensify antigen sensitization [34]. Alum is widely recognized as a pro-Th₂ adjuvant [35], and this effect is associated with its ability to induce augmented antigen capture by dendritic cells, thus increasing antigen presentation and activation of T cells [34]. Similarly, pertussis toxin promotes secretion of IL-2, IFN- γ , IL-5, IL-4, and antigen-specific IgG1, IgG2a [36], and IgE [37]. Interestingly, the use of adjuvants in our sensitization protocol produced different immune responses in each strain. Thus, it is highly likely that there may be some genetic features in each strain that generated these varied immune responses.

In this study, clinical and immune responses to intraperitoneally induced systemic anaphylaxis were compared between C3H/HeOuJ and BALB/c mice. Sensitization to peanut was demonstrated in both strains by an increase in antigen-specific immunoglobulins (sIgE and sIgG). Moreover, an anaphylactic response was verified in each strain by their clinical score and a marked drop in body temperature upon challenge that correlated with plasma histamine release. Our results indicate that both strains are highly suitable as a murine model of systemic anaphylaxis. Nevertheless, remarkable differences in response were observed. Although an increase in antigen-specific IgE and IgG levels was observed in BALB/c mice, the increase was much lower than that generated by the C3H/HeOuJ strain. Likewise, BALB/c mice developed milder anaphylactic symptoms, which corresponded to lower plasma histamine levels. In contrast, antigen-stimulated splenocytes from BALB/c mice led to much higher cytokine levels in Th₁, Th₂, and T_{reg} patterns than the C3H/HeOuJ strain. The study of spleen cytokine production by C3H/HeOuJ mice revealed minimal cytokine release. Thus, C3H/HeOuJ mice exhibited a vigorous humoral response while, curiously, BALB/c mice showed more pronounced lymphocyte activation.

We showed the induction of anaphylactic reactions to be strain dependent, as corroborated by other authors. In a model of intramuscular peanut-induced anaphylaxis, Li et al. [21] reported more severe symptoms and higher levels of antigen-specific IgG1 and IgG2a in C3H/HeSn mice than in BALB/c mice. In contrast with our data, these authors observed higher cytokine release in C3H/HeSn mice than in BALB/c mice and no anaphylactic symptoms in BALB/c mice. Interestingly, no antigen-specific IgE was observed in C3H/HeSn, thus contrasting with our results. Nevertheless, an oral peanut sensitization study showed heterogeneous outcomes in the anaphylactic responses in C3H/HeOuJ mice [24] and C3H/HeJ mice [20] compared with BALB/c mice. In both cases, C3H/HeOuJ and C3H/HeJ always had a higher clinical score upon challenge than the BALB/c group, as demonstrated in our data. However, in their model, C3H/HeOuJ mice were shown to generate increased amounts of antigen-specific IgA and IgG2a, while C3H/HeJ mice [20] and BALB/c mice [24] showed an increase in antigen-specific IgE levels. Another difference with our model is the cytokine release pattern. C3H/HeJ mice released increased amounts of IL-4 and IL-10, whereas BALB/c mice demonstrated enhanced levels of IFN- γ [20]. Additionally, Pablos-Tanarro et al. [38] recently compared C3H/HeOuJ and BALB/c mice in an ovalbumin-based oral

sensitization protocol. In contrast with our results, both strains showed similar sIgG1 and sIgE levels. Although BALB/c mice released mouse mast cell protease 1 and exhibited anaphylactic symptoms, no changes in body temperature were recorded, thus indicating a discrepancy between both techniques in this model. Consistent with our results, the C3H/HeOuJ group exhibited a more severe anaphylactic response, and BALB/c mice released higher levels of IL-13, IL-5, and IFN- γ than the C3H/HeOuJ mice. These discrepant findings can be accounted for by differences in the route of sensitization (oral vs. systemic), the antigen challenge (oral vs. subcutaneous), and the adjuvant used (cholera toxin vs. alum).

It has been suggested that the variations in allergic responses observed between BALB/c and C3H/HeOuJ mice may be attributable to immune variations, as is the case with the Toll-like receptor (TLR). TLR4, specifically, is activated by LPS [39], and some authors suggest that it is required for the development of Th₂ responses [40]. However, in other studies, it was shown that LPS might inhibit allergic sensitization [30, 38, 41]. Likewise, in a systemic peanut-induced anaphylaxis model, severe anaphylactic shock was induced independently of contamination with LPS and TLR signaling pathways [42]. Some authors have suggested that the different antigens used and strain backgrounds, rather than TLR4 activation, account for these dissimilar results [41].

The strains we used might also be subject to differences in cellular activation, as reflected in Th₁, Th₂, and T_{reg} cytokine release patterns. Both in our study and elsewhere [20, 21, 25, 38, 43], Th₁ and Th₂ cytokine patterns are present in murine anaphylaxis models. Interestingly, this finding may not correlate with variations in immunoglobulin secretion, since BALB/c mice produced augmented Th₂ cytokine levels and reduced sIgE, sIgG1, and sIgG2a levels, and a less pronounced anaphylactic response. Further research is needed to investigate cell activation in our model.

IgE-mediated anaphylaxis is well established in humans. Nevertheless, some anaphylactic events occur in the presence of relatively large amounts of antigen-specific IgG antibodies [44], and it has been suggested that an alternative IgG anaphylactic pathway may also be found in humans [45]. In our model, increased antigen-specific immunoglobulin levels indicate that the classic (IgE-Fc ϵ RI-mast cell-histamine-dependent) anaphylaxis pathway and the alternative (IgG-Fc γ RIII-macrophage-platelet-activating factor-dependent) anaphylaxis pathway are activated in both strains [9]. The higher levels of both sIgE and sIgG1 pathways in C3H/HeOuJ probably led them to experience more severe clinical anaphylaxis than the BALB/c mice. The activation of both pathways in our model may facilitate the development of novel therapies for anaphylaxis in humans regardless of the pathway activated. However, these mouse models are subject to limitations, such as the timeline of the study and the fact that murine data must be confirmed in humans.

In conclusion, both lineages of mice are suitable for the evaluation of systemic anaphylaxis and for future research on treatment in this model. However, the sensitization and challenge protocols should be selected meticulously in order to closely mimic the features of the anaphylactic reaction studied. It is highly likely that anaphylaxis pathways will depend on the strain studied. As a consequence, strain selection is a key issue when evaluating anaphylaxis: BALB/c mice will be the more appropriate strain if the cellular response is to be assessed, whereas the C3H/HeOuJ strain will be the more appropriate one if clinical response is the object of evaluation.

Disclosure Statement

The authors declare that they have no conflicts of interest.

References

- 1 Sampson HA, Mendelson L, Rosen JP: Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380–384.
- 2 Richet C: De l'anaphylaxie ou sensibilité croissante des organismes à des doses successives de poison. *Arch Fisiol* 1904;1:129–142.
- 3 Lieberman P: Epidemiology of anaphylaxis. *Curr Opin Allergy Clin Immunol* 2008;8:316–320.
- 4 Berin MC, Sampson HA: Food allergy: an enigmatic epidemic. *Trends Immunol* 2013;34:390–397.
- 5 Oyoshi MK, Oettgen HC, Chatila TA, Geha RS, Bryce PJ: Food allergy: insights into etiology, prevention, and treatment provided by murine models. *J Allergy Clin Immunol* 2014;133:309–317.
- 6 Gonipeta B, Kim E, Gangur V: Mouse models of food allergy: how well do they simulate the human disorder? *Crit Rev Food Sci Nutr* 2015;55:437–452.
- 7 Weiser R: Studies on anaphylaxis in the mouse. *J Infect Dis* 1941;68:97–112.
- 8 Strait RT, Morris SC, Yang M, Qu XW, Finkelstein FD: Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* 2002;109:658–668.

- 9 Finkelman FD: Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol* 2007; 120:506–515; quiz 516–517.
- 10 Strid J, Thomson M, Hourihane J, Kimber I, Strobel S: A novel model of sensitization and oral tolerance to peanut protein. *Immunology* 2004;113:293–303.
- 11 Waeckerle-Men Y, Liang Y, von Moos S, Kundig TM, Johansen P: Multivalent paediatric allergy vaccines protect against allergic anaphylaxis in mice. *Clin Exp Allergy* 2014; 44:429–437.
- 12 Arumugam M, Ahrens R, Osterfeld H, Kottyan LC, Shang X, MacLennan JA, Zimmermann N, Zheng Y, Finkelman FD, Hogan SP: Increased susceptibility of 129SvEvBrd mice to IgE-mast cell mediated anaphylaxis. *BMC Immunol* 2011;12:14.
- 13 Sun J, Arias K, Alvarez D, Fattouh R, Walker T, Goncharova S, Kim B, Wasserman S, Reed J, Coyle AJ, Jordana M: Impact of CD40 ligand, B cells, and mast cells in peanut-induced anaphylactic responses. *J Immunol* 2007;179:6696–6703.
- 14 Jonsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, Shimizu T, Daeron M, Bruhns P: Mouse and human neutrophils induce anaphylaxis. *J Clin Invest* 2011;121:1484–1496.
- 15 Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, Stanley JS, Burks AW, Bannion GA, Sampson HA: A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* 2000;106: 150–158.
- 16 Srivastava KD, Kattan JD, Zou ZM, Li JH, Zhang L, Wallenstein S, Goldfarb J, Sampson HA, Li XM: The Chinese herbal medicine formula FAHF-2 completely blocks anaphylactic reactions in a murine model of peanut allergy. *J Allergy Clin Immunol* 2005;115:171–178.
- 17 Nagler-Anderson C: Helminth-induced immunoregulation of an allergic response to food; in Capron M, Trottein F (eds): *Parasites and Allergy. Chem Immunol Allergy. Basel, Karger, 2006, vol 90, pp 1–13.*
- 18 Bashir ME, Andersen P, Fuss IJ, Shi HN, Nagler-Anderson C: An enteric helminth infection protects against an allergic response to dietary antigen. *J Immunol* 2002;169:3284–3292.
- 19 Zhu D, Kopley CL, Zhang M, Zhang K, Saxon A: A novel human immunoglobulin Fcγ-Fcε bifunctional fusion protein inhibits Fcε RI-mediated degranulation. *Nat Med* 2002;8: 518–521.
- 20 Morafo V, Srivastava K, Huang CK, Kleiner G, Lee SY, Sampson HA, Li AM: Genetic susceptibility to food allergy is linked to differential TH2-TH1 responses in C3H/HeJ and BALB/c mice. *J Allergy Clin Immunol* 2003; 111:1122–1128.
- 21 Li X, Huang CK, Schofield BH, Burks AW, Bannion GA, Kim KH, Huang SK, Sampson HA: Strain-dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. *J Immunol* 1999;162: 3045–3052.
- 22 Li XM, Schofield BH, Wang QF, Kim KH, Huang SK: Induction of pulmonary allergic responses by antigen-specific Th2 cells. *J Immunol* 1998;160:1378–1384.
- 23 Blease K, Kunkel SL, Hogaboam CM: Acute inhibition of nitric oxide exacerbates airway hyperresponsiveness, eosinophilia and C-C chemokine generation in a murine model of fungal asthma. *Inflamm Res* 2000;49:297–304.
- 24 Smit JJ, Willemsen K, Hassing I, Fiechter D, Storm G, van Bloois L, Leusen JH, Pennings M, Zaiss D, Pieters RH: Contribution of classic and alternative effector pathways in peanut-induced anaphylactic responses. *PLoS One* 2011;6:e28917.
- 25 Baeza ML, Conejero L, Higaki Y, Martin E, Perez C, Infante S, Rubio M, Zubeldia JM: Anisakis simplex allergy: a murine model of anaphylaxis induced by parasitic proteins displays a mixed Th1/Th2 pattern. *Clin Exp Immunol* 2005;142:433–440.
- 26 Xu W, Tamura T, Takatsu K: CpG ODN mediated prevention from ovalbumin-induced anaphylaxis in mouse through B cell pathway. *Int Immunopharmacol* 2008;8:351–361.
- 27 Muraro A, Roberts G, Worm M, Bilo MB, Brockow K, Fernandez Rivas M, Santos AF, Zolkipli ZQ, Bellou A, Beyer K, Bindslev-Jensen C, Cardona V, Clark AT, Demoly P, Dubois AE, DunnGalvin A, Eigenmann P, Halken S, Harada L, Lack G, Jutel M, Niggemann B, Rueff F, Timmermans F, Vlieg-Boerstra BJ, Werfel T, Dhimi S, Panesar S, Akdis CA, Sheikh A: Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. *Allergy* 2014;69:1026–1045.
- 28 Brown SG, Stone SF, Fatovich DM, Burrows SA, Holdgate A, Celenza A, Coulson A, Hartnett L, Nagree Y, Cotterell C, Isbister GK: Anaphylaxis: clinical patterns, mediator release, and severity. *J Allergy Clin Immunol* 2013;132:1141–1149.e5.
- 29 Sicherer SH, Sampson HA: Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014;133: 291–307; quiz 308.
- 30 Bashir ME, Louie S, Shi HN, Nagler-Anderson C: Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. *J Immunol* 2004;172:6978–6987.
- 31 Rupa P, Mine Y: Engineered recombinant ovomucoid third domain can desensitize Balb/c mice of egg allergy. *Allergy* 2006;61: 836–842.
- 32 Nelde A, Teufel M, Hahn C, Duschl A, Sebald W, Brocker EB, Grunewald SM: The impact of the route and frequency of antigen exposure on the IgE response in allergy. *Int Arch Allergy Immunol* 2001;124:461–469.
- 33 Strait RT, Mahler A, Hogan S, Khodoun M, Shibuya A, Finkelman FD: Ingested allergens must be absorbed systemically to induce systemic anaphylaxis. *J Allergy Clin Immunol* 2011;127:982–989.e1.
- 34 Hogenesch H: Mechanism of immunopotential and safety of aluminum adjuvants. *Front Immunol* 2012;3:406.
- 35 Eisenbarth SC: Use and limitations of alum-based models of allergy. *Clin Exp Allergy* 2008;38:1572–1575.
- 36 Ryan M, McCarthy L, Rappuoli R, Mahon BP, Mills KH: Pertussis toxin potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD28. *Int Immunol* 1998;10:651–662.
- 37 Mu HH, Sewell WA: Regulation of DTH and IgE responses by IL-4 and IFN-γ in immunized mice given pertussis toxin. *Immunology* 1994;83:639–645.
- 38 Pablos-Tanarro A, Lopez-Exposito I, Lozano-Ojalvo D, Lopez-Fandino R, Molina E: Antibody production, anaphylactic signs, and T-cell responses induced by oral sensitization with ovalbumin in BALB/c and C3H/HeOuJ mice. *Allergy Asthma Immunol Res* 2016;8: 239–245.
- 39 Muzio M, Mantovani A: The Toll receptor family. *Allergy* 2001;56:103–108.
- 40 Dabbagh K, Dahl ME, Stepick-Biek P, Lewis DB: Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* 2002;168: 4524–4530.
- 41 Berin MC, Zheng Y, Domaradzki M, Li XM, Sampson HA: Role of TLR4 in allergic sensitization to food proteins in mice. *Allergy* 2006;61:64–71.
- 42 Khodoun M, Strait R, Orekov T, Hogan S, Karasuyama H, Herbert DR, Kohl J, Finkelman FD: Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol* 2009;123:342–351.
- 43 van Wijk F, Hartgring S, Koppelman SJ, Pieters R, Knippels LM: Mixed antibody and T cell responses to peanut and the peanut allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 6 in an oral sensitization model. *Clin Exp Allergy* 2004;34:1422–1428.
- 44 Umeda Y, Fukumoto Y, Miyauchi T, Imaizumi M, Shimabukuro K, Mori Y, Takemura H: Anaphylactic shock related to aprotinin induced by anti-aprotinin immunoglobulin G antibody alone; report of a case (in Japanese). *Kyobu Geka* 2007;60:69–71.
- 45 Castells MC: *Anaphylaxis and Hypersensitivity Reactions. Totowa, Humana, 2011.*