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BALB/c and C3H mice are both suitable as peanut allergy models

To the editor,

Peanut allergy is a potentially life-threatening IgE-mediated disease with few therapeutic options available; subcutaneous (s.c.) immunotherapy, for example, showed a too high safety risk in the few trials performed.¹ Despite peanut oral immunotherapy being shown to increase tolerance to ingested peanuts in children and adolescents,² the development of effective and safe allergen-immunotherapies (AITs) is still an important goal in the field of peanut allergy research.

An integral part of the development of new peanut AITs is the establishment of reliable preclinical mouse models. We have recently attained preclinical proof-of-concept for a peanut AIT based on Ara h 2 displayed on cucumber mosaic virus-like particles (CuMV_{TT}/CuMV_{TT}-Ara h 2 named VLP Peanut).³⁻⁵ All experiments were performed in BALB/c mice sensitized with peanut extract formulated in aluminium hydroxide as an adjuvant. Interestingly, a recent publication by Paolucci et al. in *Clinical and Experimental Allergy* concluded that C3H, but not BALB/c mice could be rendered allergic to peanuts and may serve as a peanut allergy model.⁶ This observation is contradictory with our and other studies that have successfully used BALB/c mice as a peanut anaphylaxis model.^{3-5,7} To clarify matters, we contacted the authors of the aforementioned study and designed an experimental setup to directly compare BALB/c with C3H mice using our sensitization protocol. In addition, we tested if VLP-based peanut AIT also induced protection against anaphylaxis in the C3H strain, similar to what we observed in BALB/c mice. Accordingly, C3H and BALB/c mice (Envigo) were sensitized to peanut by two injections (D0, D7) of 5 µg peanut extract formulated in 200 µL Alhydrogel (InvivoGen) intraperitoneally (i.p.) as described earlier.⁴ Fourteen days after sensitization, mice were vaccinated s.c. either with 100 µg VLP Peanut or with 100 µg VLP control (CuMV_{TT}) three times with intervals of 21 days. As a further control, non-sensitized C3H mice were included in the study (Figure 1A). No non-sensitized BALB/c were included as we have previously demonstrated that they show no signs of anaphylaxis or specific IgE.^{3,4} Mice were challenged intravenously (i.v.) with 20 µg peanut extract and body core temperature was measured in intervals of 10 min for 1 h. Upon peanut challenge, systemic anaphylaxis was observed in both BALB/c and C3H mice treated with non-modified VLP as control, indicated

by a dramatic drop in body core temperature. VLP Peanut vaccinated BALB/c and C3H mice showed no or weak anaphylactic reactions upon allergen challenge. Thus, in both strains, VLP Peanut immunization resulted in significantly reduced anaphylaxis after challenge compared to control mice, in line with previous results in BALB/c mice^{3,4} (Figure 1B).

Next, we assessed the Ara h 2-specific IgG levels in serum obtained immediately before the challenge. Due to sensitization against peanut, VLP control mice developed Ara h 2-specific IgG in the absence of vaccination. In C3H and BALB/c mice, vaccination increased Ara h 2-specific IgG titers by a factor of 10. In sensitized VLP control mice as well as in sensitized VLP Peanut vaccinated mice, the level of Ara h 2-specific IgG was higher in C3H compared to BALB/c mice. Non-sensitized C3H mice formed Ara h 2-specific IgG only after vaccination with VLP Peanut (Figure 1C). Importantly, sensitization induced Ara h 2-specific serum IgE in both strains with a trend to higher titers in C3H mice (Figure 1D).

We have previously shown that Ara h 2-specific serum IgG mediates protection from allergic reactions by neutralizing the allergen as well as by engaging the FcγRIIB inhibitory receptor on mast cells and basophils.^{3,4} In line with Paolucci et al.,⁶ we observed slightly increased Ara h 2-specific serum IgG and IgE titers in C3H compared to BALB/c mice. A possible explanation for the different antibody responses between both strains might be the distinct MHC haplotypes, which is H-2^k in C3H and H-2^d in BALB/c mice, resulting in different antigen presentation. However, this is speculation, which would require more investigation.

These data demonstrate that both BALB/c and C3H mice may be used as valid models for peanut allergy. However, different sensitization regimens may lead to different outcomes, highlighting the importance of optimizing the induction protocol of peanut allergy for individual mouse strains. In the report by Paolucci et al.,⁶ sensitization comprised four weekly injections, while we used two injections in 7 days interval.⁴ Also, the dose of aluminium hydroxide used by Paolucci et al.⁶ was 0.15 mg, while we used 2 mg per mouse and injection. Finally, the source of peanut allergen extract for sensitization and challenge differed. While we use an in-house extract from roasted peanut kernels (Intersnack) as described by Koppelman et al.,⁸ Paolucci et al.⁶ used either a skin-prick-test solution

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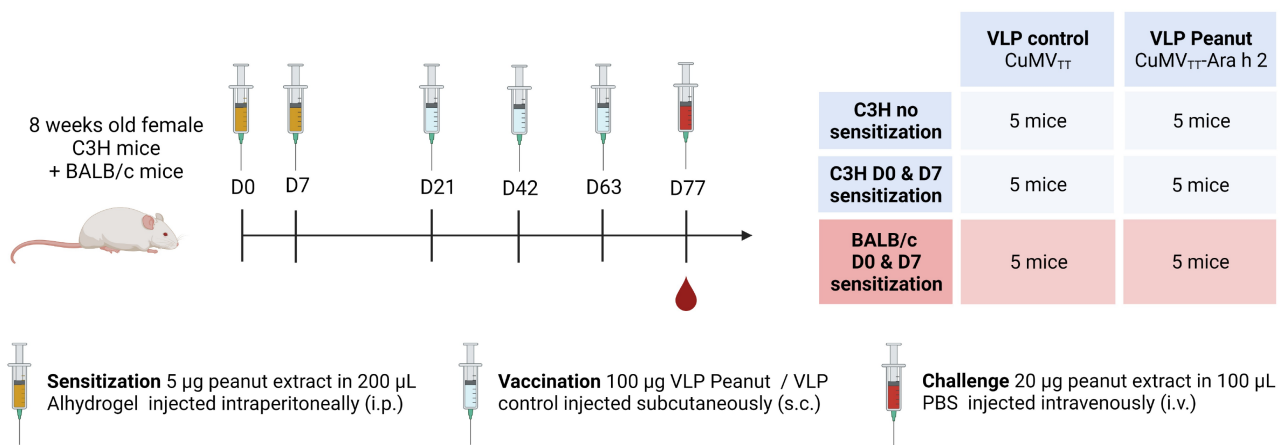
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(Allergopharma) or an in-house extract from partially defatted peanut flour containing 50% proteins from Golden Peanut (Alpharetta) for sensitization. When selecting a suitable model for peanut allergy, the route for sensitization should also be considered carefully. Other

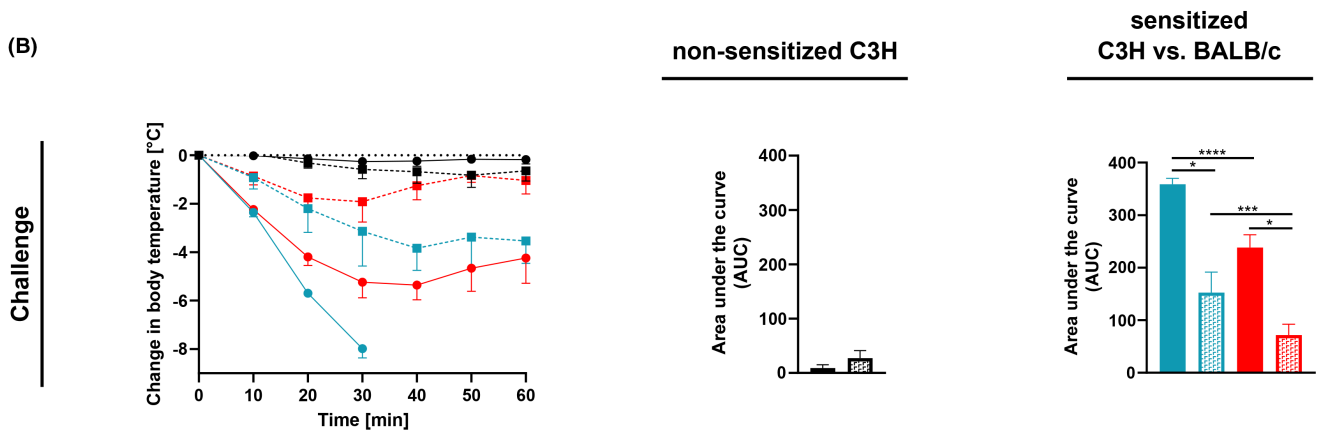
than i.p., mice have been effectively sensitized to peanut by percutaneous or oral application of the allergen.⁹

In conclusion, peanut allergy may be induced independently of the strain. However, optimized regimens and doses need to be

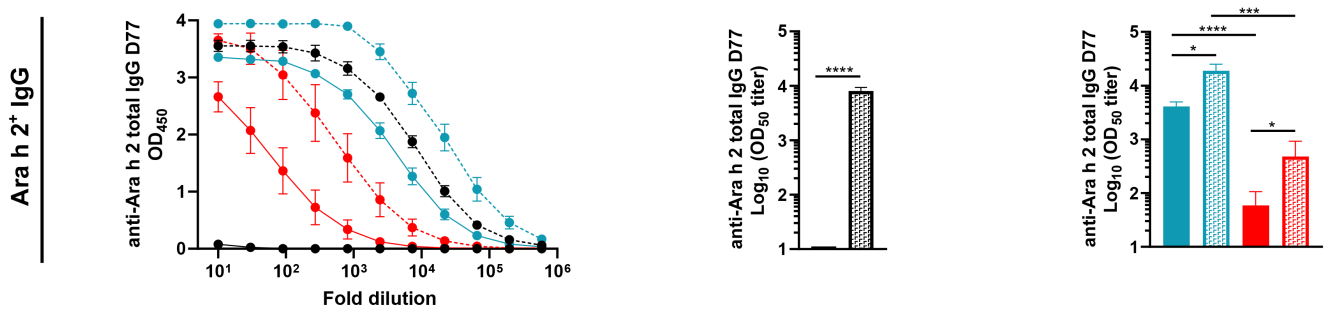
(A)



(B)



(C)



(D)

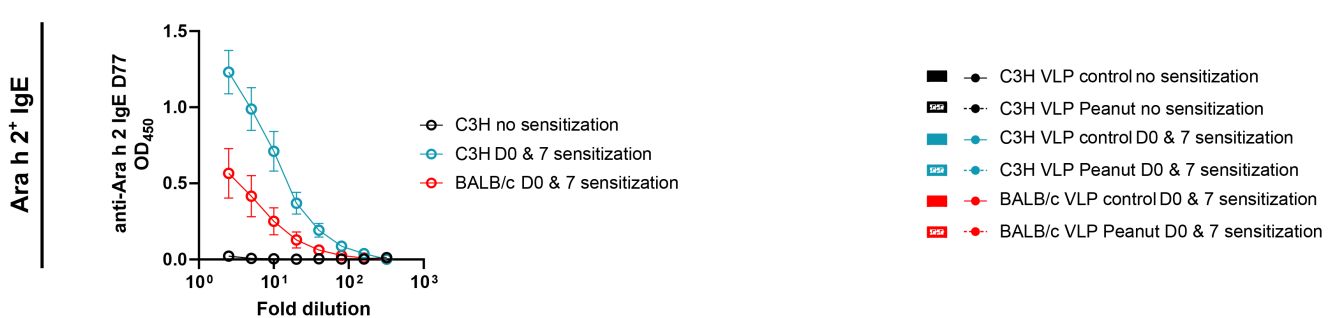


FIGURE 1 Comparison of the BALB/c and C3H mouse model for the assessment of VLP Peanut-mediated protection from anaphylaxis. (A) Schematic illustration of the mouse anaphylaxis model including sensitization and vaccination of BALB/c and C3H mice ($n = 5$ for all groups) for the assessment of systemic anaphylaxis and antibody responses. Scheme created with [BioRender.com](https://www.biorender.com). (B) Left: Change in body core temperature of vaccinated mice after challenge with 20 μg whole peanut extract given i.v. Last observation carried forward for mice that fulfilled the termination criterion of 6°C body core temperature reduction. Right: Area under the curve (AUC) relative to baseline at time point zero (0°C) of body core temperature–time diagram. (C) Ara h 2-specific serum IgG illustrated as reciprocal titration curves, OD₄₅₀ shown left, Log₁₀ OD₅₀ titre shown right. (D) Ara h 2-specific serum IgE pooled per mouse strain illustrated as reciprocal titration curves, OD₄₅₀ shown. Data depicted as mean \pm SEM. Statistical analysis using unpaired *t*-test for comparison of two and ordinary one-way ANOVA with Tukey correction for comparisons of multiple groups. $p < .05$ (*), $p < .01$ (**), $p < .001$ (***), $p < .0001$ (****).

Key messages

- BALB/c and C3H mice are both suitable as preclinical peanut allergy models.
- VLP Peanut immunization protects sensitized mice from anaphylaxis upon peanut allergen challenge.

established for each individual strain to obtain a reliable preclinical model for peanut allergy.

KEYWORDS

allergy immunotherapy, mouse model, peanut allergy

AUTHOR CONTRIBUTIONS

PK and MB were involved in the design of experiments. PK, JS, and MB were involved in the methodology. PK, JS, and MB were involved in the acquisition of data, interpretation, and analysis of data. PK, JS, MP, MV, TK, PJ, and MB were involved in writing, revising, and editing the manuscript. MB was involved in study supervision. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

Thomas M. Kündig is a scientific advisor to Mabyon AG. Martin F. Bachmann is a board member of Saiba AG. All other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT


The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Animal procedures were performed in accordance with the Swiss Animal Act (455.109.1–September 2008, 5th) at the DBMR of the


University of Bern. All animal experiments were conducted by protocols approved by the Cantonal Veterinary Office Bern, Switzerland (Licence BE79/2021).

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