Received: 16 March 2023 | Revised: 23 August 2023 | Accepted: 11 September 2023

DOI: 10.1111/cea.14398

RESEARCH LETTER

WILEY

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).

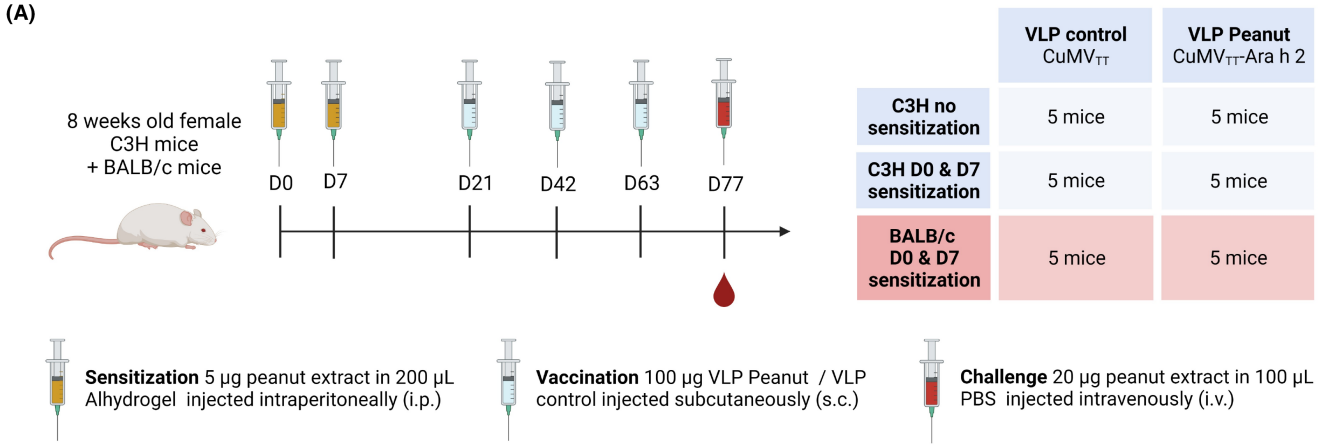
Next, we assessed the Ara h 2-specific IgG levels in serum obtained immediately before the challenge. Due to sensitization against peanut, VLP control mice developed Ara h 2-specific IgG in the absence of vaccination. In C3H and BALB/c mice, vaccination increased Ara h 2-specific IgG titers by a factor 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).

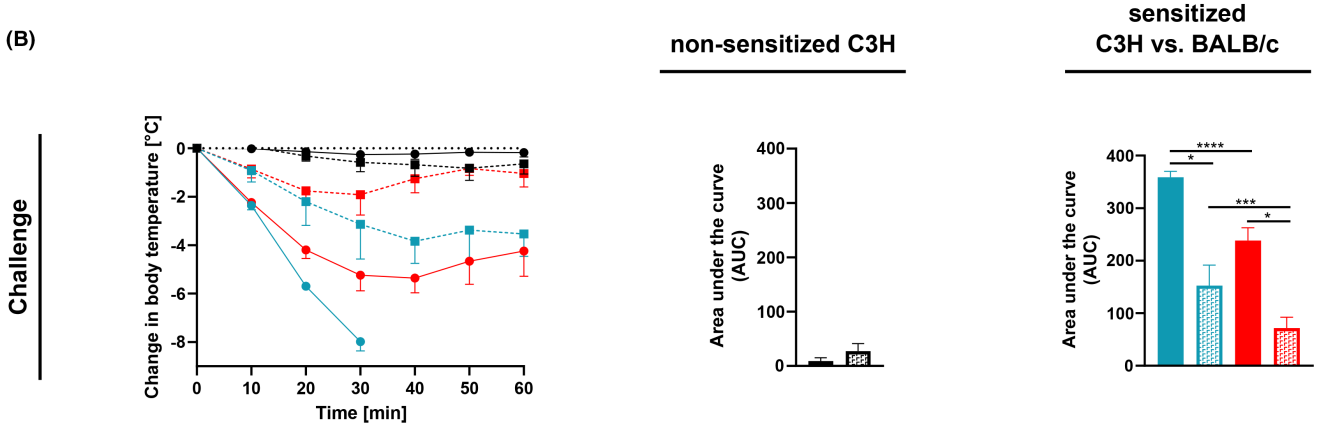
We have previously shown that Ara h 2-specific serum IgG mediates protection from allergic reactions by neutralizing the allergen as well as by engaging the FcγRIIB inhibitory receptor on mast cells and basophils.[3,4] In line with *Paolucci* et al.,[6] we observed slightly increased Ara h 2-specific 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 $H\text{-}2^k$ in C3H and $H\text{-}2^d$ in BALB/c mice, resulting in different antigen presentation. However, this is speculation, which would require more investigation.

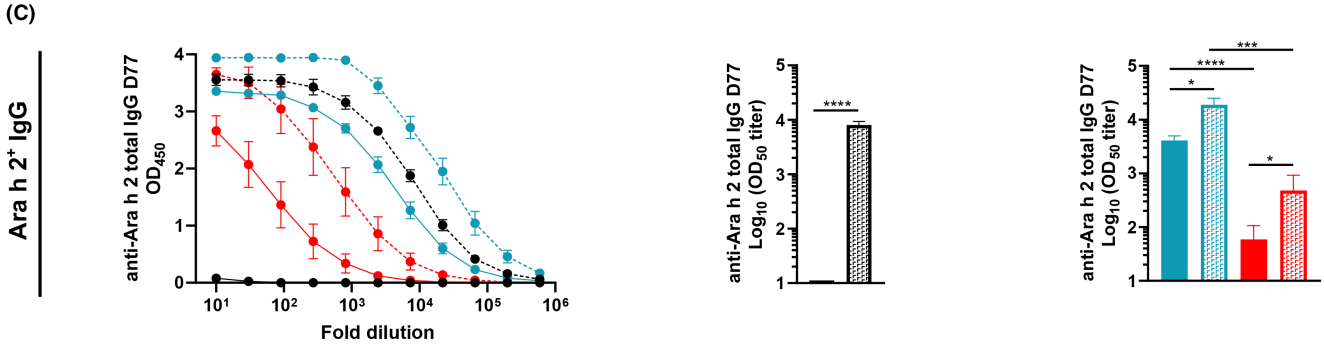
These data demonstrate that both BALB/c and C3H mice may be used as valid models for peanut allergy. However, different sensitization regimens may lead to different outcomes, highlighting the importance of optimizing the induction protocol of peanut allergy for individual mouse strains. In the report 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 *Koppelmann* et al.,[8] *Paolucci* et al.[6] used either a skin-prick-test solution

(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 pericutaneous 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







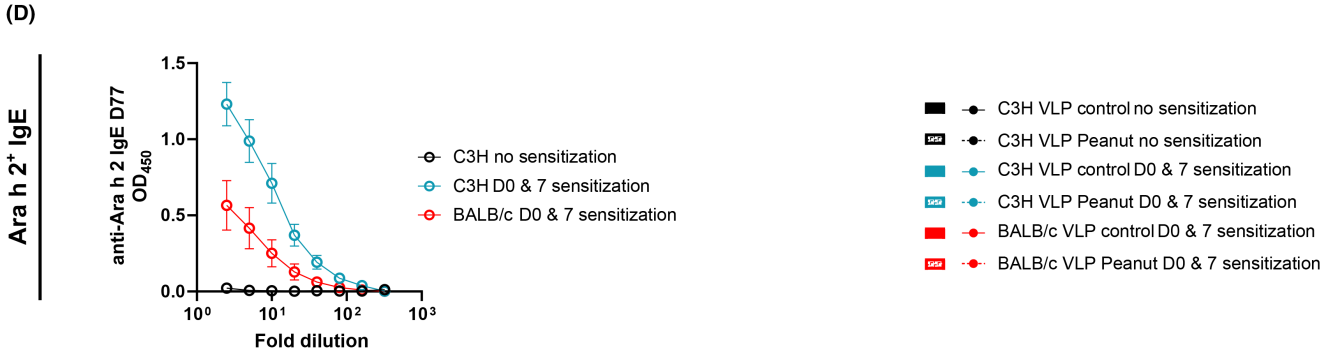


FIGURE 1 Comparison of the BALB/c and C3H mouse model for the assessment of VLP Peanut-mediated protection from anaphylaxis. (A) Schematic illustration of the mouse anaphylaxis model including sensitization and vaccination of BALB/c and C3H mice ($n = 5$ for all groups) for the assessment of systemic anaphylaxis and antibody responses. Scheme created with BioRender.com. (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, OD_{450} shown left, Log_{10} OD_{50} titre shown right. (D) Ara h 2-specific serum IgE pooled per mouse strain illustrated as reciprocal titration curves, OD_{450} shown. Data depicted as mean ± 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.

ACKNOWLEDGEMENTS

We acknowledge Aleksandra Nonic for the technical assistance. Open access funding provided by Inselspital Universitatsspital Bern.

FUNDING INFORMATION

This publication was funded by the Swiss National Science Foundation (SNF grant 310030_185114).

CONFLICT OF INTEREST STATEMENT

Thomas M. Kündig is a scientific advisor to Mabylon 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).

Pascal S. Krenger[1,2,3]
Jan Sobczak[1,2,3]
Marta Paolucci[4]
Thomas M. Kündig[4,5]
Pål Johansen[4,5]
Monique Vogel[1,2]
Martin F. Bachmann[1,2,6]

[1]*Department of Rheumatology and Immunology, University Hospital of Bern, Bern, Switzerland*
[2]*Department of BioMedical Research, University of Bern, Bern, Switzerland*
[3]*Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland*
[4]*Department of Dermatology, University of Zurich, Zurich, Switzerland*
[5]*Department of Dermatology, University Hospital Zurich, Zurich, Switzerland*
[6]*Nuffield Department of Medicine, Centre for Cellular and Molecular Physiology (CCMP), The Jenner Institute, University of Oxford, Oxford, UK*

Correspondence
Martin F. Bachmann, Department of Rheumatology and Immunology, University Hospital of Bern, Bern, Switzerland.
Email: martin.bachmann@me.com

ORCID

Pascal S. Krenger https://orcid.org/0009-0008-7074-3842
Jan Sobczak https://orcid.org/0000-0002-5271-7826
Marta Paolucci https://orcid.org/0000-0001-5371-0514
Thomas M. Kündig https://orcid.org/0000-0003-3863-8766
Pål Johansen https://orcid.org/0000-0002-5055-6299
Monique Vogel https://orcid.org/0000-0002-5219-4033
Martin F. Bachmann https://orcid.org/0000-0003-4370-2099

REFERENCES

1. Anagnostou K, Clark A. Peanut immunotherapy. *Clin Transl Allergy*. 2014;4(1):4-9.

2. AR101 oral immunotherapy for peanut allergy. *N Engl J Med*. 2018;379(21):1991-2001.
3. Sobczak JM, Krenger P, Storni F, et al. The next generation virus-like particle platform for the treatment of peanut allergy. *Allergy Eur J Allergy Clin Immunol*. 2023;78:1980-1996. doi:10.1111/all.15704
4. Storni F, Zeltins A, Balke I, et al. Vaccine against peanut allergy based on engineered virus-like particles displaying single major peanut allergens. *J Allergy Clin Immunol*. 2020;145(4):1240-1253.e3.
5. Storni F, Cabral-Miranda G, Roesti E, et al. A single monoclonal antibody against the Peanut allergen Ara h 2 protects against systemic and local Peanut allergy. *Int Arch Allergy Immunol*. 2020;181(5):334-341.
6. Paolucci M, Homère V, Waeckerle-Men Y, et al. Strain matters in mouse models of peanut-allergic anaphylaxis: systemic IgE-dependent and Ara h 2-dominant sensitization in C3H mice. *Clin Exp Allergy*. 2023;53(5):550-560.
7. Manzano-Szalai K, Pali-Schöll I, Krishnamurthy D, Stremnitzer C, Flaschberger I, Jensen-Jarolim E. Anaphylaxis imaging: non-invasive measurement of surface body temperature and physical activity in small animals. *PloS One*. 2016;11(3):1-17.
8. Koppelman SJ, Jayasena S, Luykx D, et al. Allergenicity attributes of different peanut market types. *Food Chem Toxicol*. 2016;91:82-90. doi:10.1016/j.fct.2016.02.016
9. Smeekens JM, Kulis MD. Mouse models of food allergy in the pursuit of novel treatment modalities. *Front Allergy*. 2021;2:810067.