Received: 12 October 2023 | Revised: 19 December 2023 | Accepted: 14 January 2024

DOI: 10.1111/all.16038

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

LETTERS

Tissue-specific contribution of mucosal-associated invariant T cells to allergic airway inflammation

To the Editor,

Mucosal-associated invariant T (MAIT) cells are major histocompatibility complex (MHC) class I-related protein 1 (MR1)-restricted T cells which exert anti-microbial as well as homeostatic functions, populate all mucosal tissues as well as the skin and have a microbiome-dependent development, making their contribution to allergic diseases likely.[1] Whether that contribution is protective or pathogenic remains unresolved. Indeed, we recently described the disease promoting activity of MAIT cells in atopic dermatitis (AD), while others made opposing observations for asthma[2,3]—the latter observation having since been validated and expanded using a transgenic mouse strain artificially enriched in MAIT cells.[4]

So far, the allergic potential of MAIT cells has only been studied in single tissues, each receiving distinct imprints from resident MAIT cells.[5] To determine which imprint prevails during allergic propagation from the skin to the lung, we compared $MR1^{-/-}$ and WT mice in an experimental model of the allergic march, comprised of epicutaneous sensitization and airway challenge to house dust mite (HDM; Figure 1A). In WT mice, this model induced a robust HDM-specific IgE response and eosinophilic lung infiltration, detectable in both the bronchoalveolar lavage fluid and lung tissue (Figure 1B–E). Remarkably, $MR1^{-/-}$ mice were virtually protected from disease, as reflected by all disease parameters (Figure 1B–E). To circumvent the potentially confounded phenotype of $MR1^{-/-}$, due to simultaneous removal of pathogenic and protective MAIT cells in the skin and lung, respectively, we next co-applied HDM and the MAIT cell antagonist Ac-6-FP (previously used as topical treatment against experimental AD[2]) to spatiotemporally block MAIT cells in the skin of WT mice. As shown in Figure 1B–E, Ac-6-FP-treated WT mice closely phenocopied the reduced eosinophilia and IgE production observed in $MR1^{-/-}$ mice, suggesting that the pathogenic contribution of MAIT cells is restricted to the skin and that MAIT cells may influence both innate and adaptive features of allergen sensitization. To start addressing the possibility that skin MAIT cells directly interact with dendritic cells,[6] we determined the proportion of activated (CD86+) type-2 dendritic cells (DC2) in the skin draining lymph node of WT mice epicutaneously sensitized with HDM and observed a significant reduction in the presence of Ac-6-FP (Figure S1A). Skin MAIT cells also influenced the innate sensitization of the pulmonary microenvironment, as reflected by the significant inhibition of eosinophilic (pre-)activation (CD69+) (not observed in the blood) and the increase of alveolar macrophages in $MR1^{-/-}$ mice, conferring additionally protective effects[7] (Figure S1B).

To confirm the responsiveness of lung-resident MAIT cells in this model, we examined their number and activation state (CD25 and CD69 expression), which we found to be significantly elevated at endpoint (Figure 1F–H). While Ye et al.[3] have previously shown that pulmonary MAIT cell numbers decrease upon repeated allergen administration, as early as Day 6 after the initial exposure, the pulmonary consequences of epicutaneous sensitization (Figure S1) may have qualitatively altered the response to HDM and avoided MAIT cell decline. We also observed that our baseline number of pulmonary MAIT cells was comparatively much lower, which may be related to different environmental conditions (i.e. lower environmental bioburden), as reported for cutaneous MAIT cells.[3,8] In such a setting, HDM, as a carrier of microbial metabolites, may provide sufficient antigenic stimulation to influence MAIT cell activation/expansion. Consistently, we found i.n. HDM to increase the percentage of Nur77-positive pulmonary MAIT cells—a surrogate for recent TCR signalling—albeit without reaching statistical significance, and modestly compared to the positive control 5-OP-RU (Figure 1I).

Next, we compared WT and $MR1^{-/-}$ mice in allergen sensitization/challenge models which bypass the skin, including HDM and *Alternaria alternata* (*Alternaria*) lung-only models, and ovalbumin (OVA) lung challenge after intraperitoneal sensitization. In strong contrast to epicutaneously sensitized mice, WT and $MR1^{-/-}$ showed similar levels of pulmonary eosinophilia, in terms of both frequency and absolute numbers, when initially and repeatedly challenged with HDM or *Alternaria* via the airways, notwithstanding a significant decrease in the number of eosinophils in $MR1^{-/-}$ mice challenged with *Alternaria* (Figure 2A,B). To reconcile these observations with the reported protective function of MAIT cells in this setting, we crossed $MR1^{-/-}$ with IL-4/IL-13 dual reporter mice (4C13R) and indeed observed significantly higher $IL\text{-}4^{+}$ and $IL4^{+}IL\text{-}13^{+}$ $CD4^{+}$ T-cell frequencies in the lung-draining lymph node of 4C13R × $MR1^{-/-}$ mice after two successive i.n. HDM

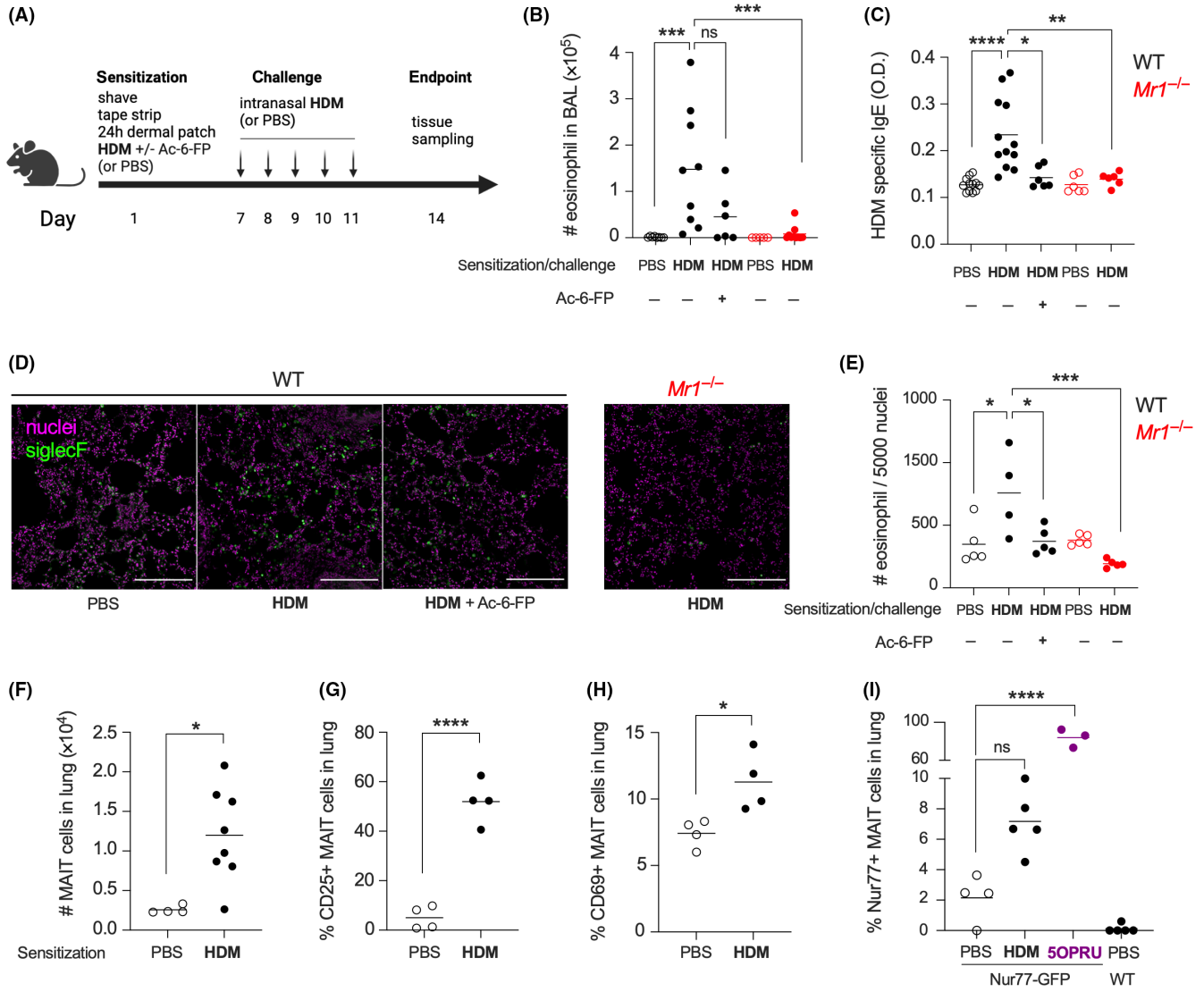

FIGURE 1 MAIT cell dependence of allergic propagation from the skin to the lung. (A) Protocol. (B) Eosinophil counts in the BAL, (C) HDM-specific IgE and (D–E) eosinophil quantification by confocal microscopy in lung tissue. Assessment of MAIT cell (F) numbers and expression of (G) CD25 and (H) CD69 in lungs. (I) Expression of Nur77 by pulmonary MAIT cells 24 h after i.n. HDM or 5-OP-RU. Schema of treatment regimen created with BioRender.com. Significance by one-way ANOVA or Mann–Whitney test (F–H): $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$, ns, non-significant.

administrations (Figure 2C), consistent with the higher concentration of IL-13 in lung tissue observed by Ye et al.[3] It is important to note that the extent of eosinophilic infiltration upon i.n. allergen administration, and its reproducibility or lack thereof across studies, may be related to qualitative batch differences between allergen extracts. Indeed, the dose of *Alternaria* that could be safely administered to our mice was 5 μg, while a single i.n. dose of 50 μg, as used by Ye et al. (sourced from the same manufacturer), proved lethal in our hands. Similarly, the varying levels of HDM-associated endotoxins have been shown to influence the transcriptomic signature of HDM-challenged airways.[9]

Lastly, intraperitoneal sensitization with OVA/alum and subsequent OVA challenge led again to almost identical pulmonary eosinophilia in WT and MR1$^{-/-}$ mice (Figure 2D), contrasting the moderately exacerbated phenotype observed in MR1$^{-/-}$ mice by Sasano et al.[10]

In summary, our observations indicate that the contribution of MAIT cells to allergic airway inflammation is dependent upon the route of sensitization. If sensitization occurs via the skin—of particular relevance for the allergic march—MAIT cells' contribution is pathogenic, including mechanistic aspects of both innate and adaptive nature. On the contrary, if allergen is first encountered via the airways, MAIT cells exert a protective function. It remains to be determined to what extent this dichotomy is related to intrinsic functional differences between skin- and lung-resident MAIT cells, tissue-specific combinations of T-cell receptor (TCR)-dependent and -independent activation and/or MAIT cells' local interaction partners (e.g. DC and epithelial cells).

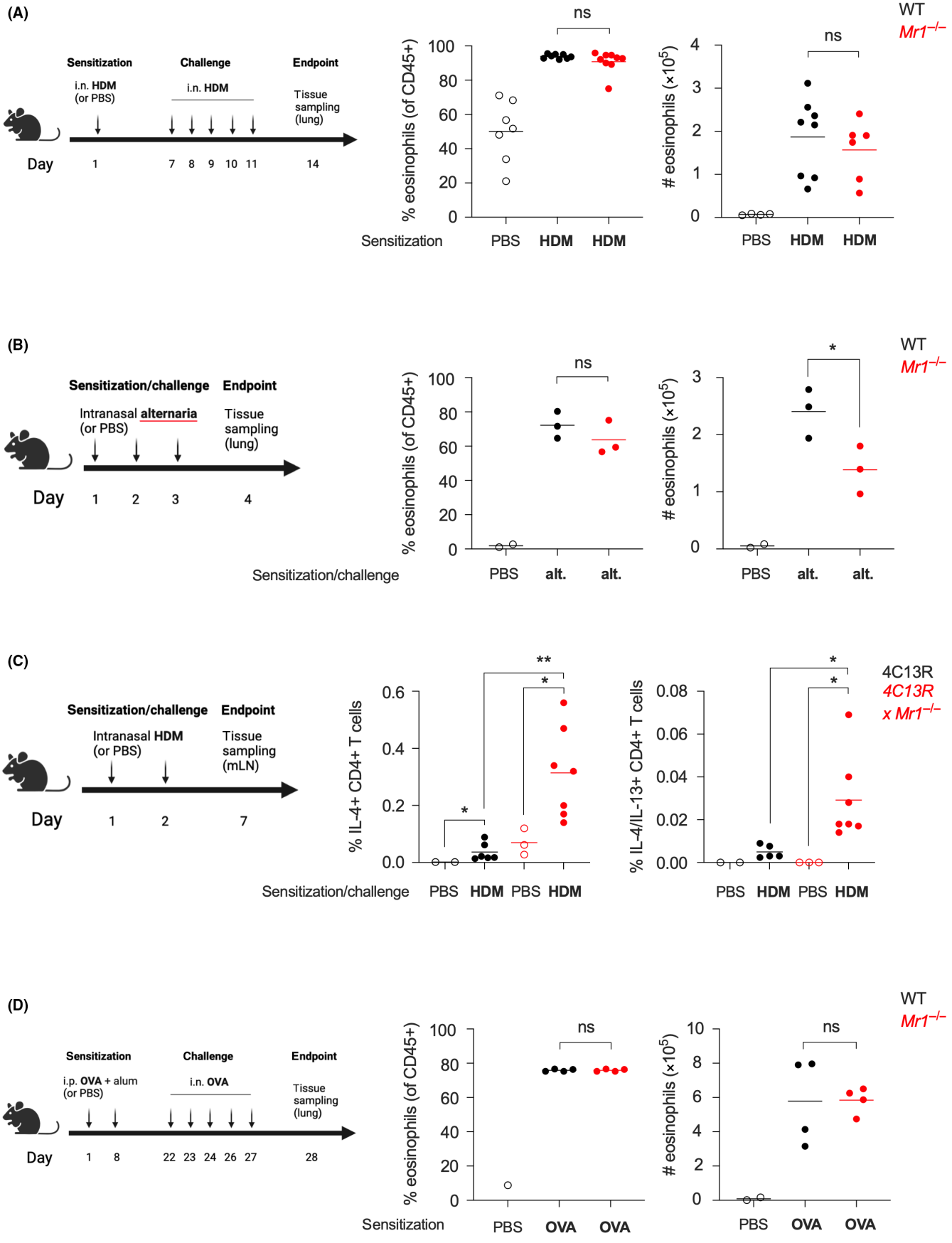

FIGURE 2 MAIT cell dependence of allergic lung inflammation models without skin involvement. (A) HDM lung-only model; protocol (left) and tissue eosinophilia (right). (B) *Alternaria* (alt.) lung-only model; protocol (left) and tissue eosinophilia (right). (C) Acute HDM challenge model; protocol (left) and IL-4- and IL-4/IL-13-producing $CD4^+$ T-cell frequencies (right). (D) OVA model; protocol (left) and tissue eosinophilia (right). Schemas of treatment regimen created with BioRender.com. Significance by one-way ANOVA: $^*p < .05$, $^{**}p < .01$, ns, non-significant.

FUNDING INFORMATION

This work was supported by the New Zealand Health Research Council Independent Organization Fund (14/1003) and the High-Value Nutrition National Science Challenge, New Zealand.

AUTHOR CONTRIBUTIONS

Conceptualization: AC, KW, KN, DOS, OG; Methodology and analysis: AC, KW, KN, KG, AS, RJ, AG; Writing—draft and editing: AC, KW, KN, DOS, OG; supervision, administration and funding: OG.

ACKNOWLEDGEMENTS

The manufacture of 5-(2-oxopropylideneamino)-6-D-ribitylamin ouracil (5-OP-RU) was carried out by Prof. Painter's laboratory financially supported by a programme grant from the New Zealand Ministry of Business Innovation and Employment (Award number RTVU1603). We are grateful to the Hugh Green Cytometry Centre, Malaghan Institute of Medical Research, for expert support with flow cytometry and confocal microscopy, staff of the Biomedical Research Unit, Malaghan Institute of Medical Research, for animal husbandry, and all colleagues at the Malaghan Institute for insightful discussion and advice.

CONFLICT OF INTEREST STATEMENT

The 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.

Alissa Cait[1,2]
Katherine Woods[1]
Karmella Naidoo[1]
Katie Gell[1,2]
Alfonso Schmidt[1]
Rosemary Jackson[1]
Alix Grooby[1]
David O'Sullivan[1,2]
Olivier Gasser[1,2]

[1]*Malaghan Institute of Medical Research, Wellington, New Zealand*
[2]*High-Value Nutrition National Science Challenge, Auckland, New Zealand*

Correspondence
Olivier Gasser, Malaghan Institute of Medical Research, Kelburn Parade, PO Box 7060, Wellington 6242, New Zealand.
Email: ogasser@malaghan.org.nz

Alissa Cait and Katherine Woods are joint first authors.

Karmella Naidoo and Katie Gell contributed equally to this work.

ORCID

Olivier Gasser https://orcid.org/0000-0002-8235-2274

REFERENCES

1. Godfrey DI, Koay HF, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. *Nat Immunol*. 2019;20(9):1110-1128.
2. Naidoo K, Woods K, Pellefigues C, et al. MR1-dependent immune surveillance of the skin contributes to pathogenesis and is a photobiological target of UV light therapy in a mouse model of atopic dermatitis. *Allergy*. 2021;76(10):3155-3170.
3. Ye L, Pan J, Pasha MA, et al. Mucosal-associated invariant T cells restrict allergic airway inflammation. *J Allergy Clin Immunol*. 2020;145(5):1469-1473.e1464.
4. Shimizu Y, Horigane-Konakai Y, Ishii Y, Sugimoto C, Wakao H. Mucosal-associated invariant T cells repress group 2 innate lymphoid cells in *Alternaria alternata*-induced model of allergic airway inflammation. *Front Immunol*. 2022;13:1005226.
5. Ataide MA, Knöpper K, Cruz de Casas P, et al. Lymphatic migration of unconventional T cells promotes site-specific immunity in distinct lymph nodes. *Immunity*. 2022;55(10):1813-1828.e1819.
6. Salio M, Gasser O, Gonzalez-Lopez C, et al. Activation of human mucosal-associated invariant T cells induces CD40L-dependent maturation of monocyte-derived and primary dendritic cells. *J Immunol*. 2017;199(8):2631-2638.
7. Miki H, Pei H, Gracias DT, Linden J, Croft M. Clearance of apoptotic cells by lung alveolar macrophages prevents development of house dust mite-induced asthmatic lung inflammation. *J Allergy Clin Immunol*. 2021;147(3):1087-1092.e1083.
8. Constantinides MG, Link VM, Tamoutounour S, et al. MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science*. 2019;366(6464):eaax6624.
9. Pascoe CD, Jha A, Basu S, et al. The importance of reporting house dust mite endotoxin abundance: impact on the lung transcriptome. *Am J Physiol Lung Cell Mol Physiol*. 2020;318(6):L1229-l1236.
10. Sasano H, Harada N, Harada S, et al. Pretreatment circulating MAIT cells, neutrophils, and periostin predicted the real-world response after 1-year mepolizumab treatment in asthmatics. *Allergol Int*. 2023;73:94-106.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.