DOI: 10.1111/all.15673

Can birch pollen directly influence the IL-4/IL-4R interaction to modulate Th2 responses?

To the Editor,

Birch pollen (BP) is a clinically relevant aeroallergen source affecting up to 16% of the European population. In predisposed individuals, BP exposure triggers Th2 immune responses that orchestrate allergic sensitization.[1] The key cytokine for Th2 differentiation, interleukin-4 (IL-4), binds with high affinity to IL-4R alpha (IL-4Rα) to form the active type-I IL-4 receptor with the common gamma (γC) chain. Downstream, induced GATA3 mainly regulates the expression of type 2 cytokines. Although the initiation of Th2 differentiation by pollen sources is still unclear, the reported adjuvant function of

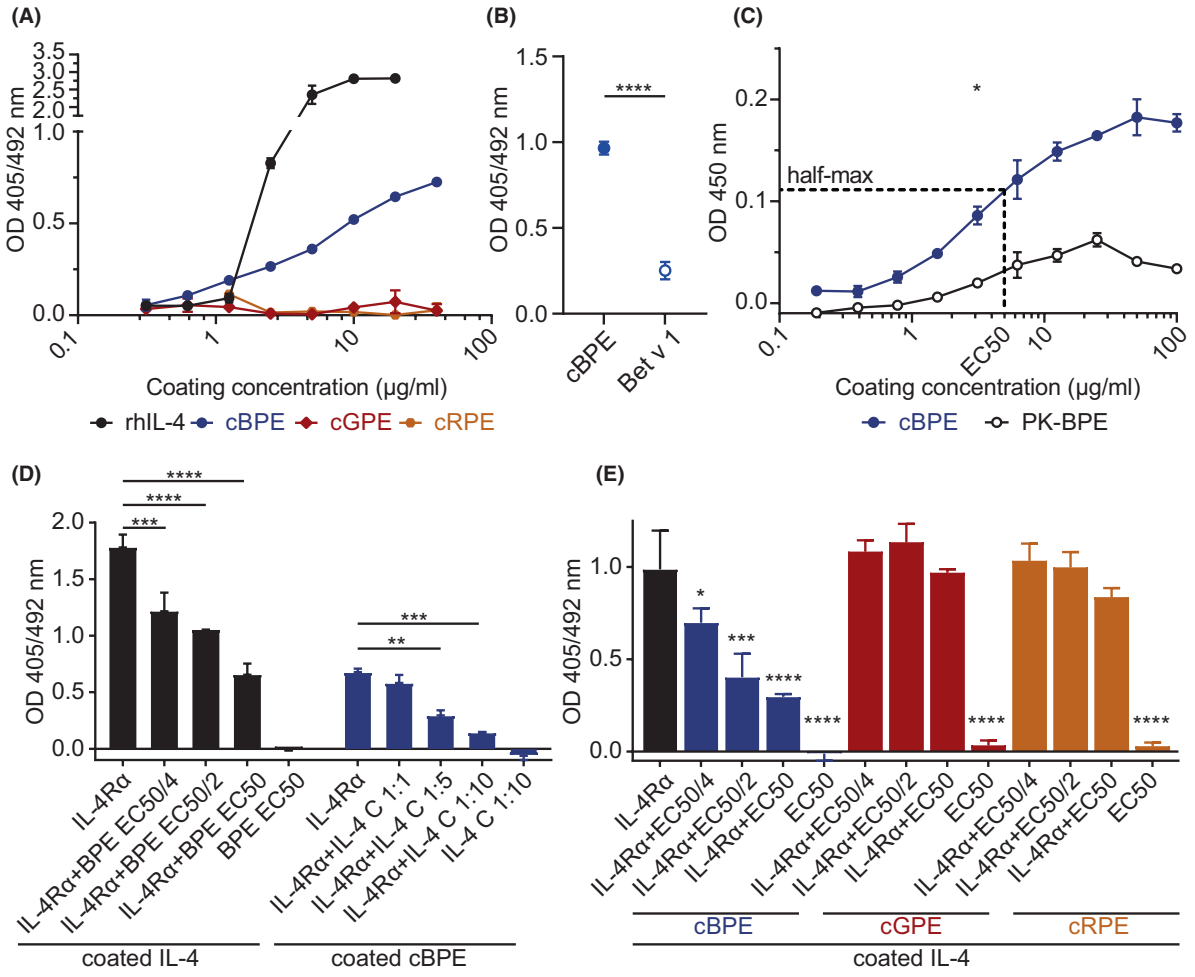

FIGURE 1 Binding activity of a birch pollen extract (BPE)-derived protein to IL-4Rα. IL-4Rα-binding ELISAs comparing BPE to recombinant human (rh)IL-4, commercial grass (cGPE) or ragweed (cRPE) pollen extracts (A), Bet v 1 (B) and to proteinase K-digested cBPE (PK-BPE, C). IL-4Rα-binding evaluated in competition and inhibition ELISAs regarding rhIL-4 (D, E). IL-4Rα plus cBPE (EC50 = 5 µg/mL) or rhIL-4 ($C_{1:1}$ = 8.25 µg/mL; IL-4:IL-4Rα mol-ratio) added simultaneously to coated rhIL-4 or cBPE, respectively (D). IL-4Rα pre-incubated with pollen extracts added to coated rhIL-4 (E). Statistical significance indicates $p < .05$ (*), $p < .01$(**), $p < .001$ (***), and $p < .0001$ (****).

specific BP-derived compounds other than the major allergen[2,3] suggests that Th2-inducing signals originate from the BP source itself.

We, therefore, aimed to study the role of the IL-4/IL-4R pathway in BP-induced Th2 responses by (i) investigating the ability of BP extracts (BPE) compared with other pollen species to bind IL-4Rα via ELISA, (ii) screening for latent IL-4Rα ligands by fractionating BPE via size exclusion chromatography (SEC), and (iii) assessing associated Th2 responses in vivo using IL-4 reporter mice. Finally, (iv) the functional downstream signaling elicited by the interaction with the IL-4R was examined in pilot in vitro assays.

Commercial (c)BPE showed a dose-dependent binding activity to IL-4Rα (Figure 1A), which was not attributable to the major allergen, Bet v 1 (Figure 1B). The activity of the natural ligand IL-4 was higher than for cBPE. By contrast, neither timothy grass (cGPE) nor short ragweed (cRPE) pollen extracts exhibited this IL-4Rα-binding activity (Figure 1A; Figure S1-S3A), indicating a BP-specific effect. Proteinase K-mediated degradation of cBPE (PK-BPE) abolished the binding signal (Figure 1C; Figure S1-S3B), inferring a protein ligand. In inhibition and competition approaches, cBPE was able to block the binding of IL-4Rα to IL-4 dose-dependently and, conversely, IL-4 hindered IL-4Rα to interact with cBPE (Figure 1D,E; Figure S1-S3C), implying similar binding interaction. Interestingly, BPE from self-collected pollen (sBPE) displayed a stronger binding activity than cBPE (Figure 2A). Via interpolation from an IL-4 standard curve, an IL-4Rα-binding activity of 37% for sBPE versus 20% for cBPE was measured. SEC-generated fractions of sBPE (sF1-7) and cBPE (cF1-5) both showed an increased binding activity from low (LMW) to high (HMW) molecular weight fractions. The augmented binding activity observed for sF4-sF1 (86, 65, 55, 39%) and cF1 (47%) suggests the presence of a HMW ligand (Figure 2A; Figure S1-S3). Consistently, compared with original cBPE, only the HMW fraction significantly induced a Th2 response in vivo, measured by the induction of IL-4/eGFP expression in CD4-positive (+) lymphocytes (Figure 2B). Immunization with sBPE similarly induced a significant increase in 13% IL-4/eGFP$^+$CD4$^+$ cells (Figure 2C). Blocking of IL-4Rα on sBPE-stimulated human naïve CD4$^+$T cells increased anti-inflammatory IL-10 secretion, and in HEK reporter cells cBPE stimulation interfered with IL-4-induced STAT6 phosphorylation (Figure S1-S3A,B), preliminarily suggesting the activation of a non-canonical pathway downstream of IL-4R. BPE was demonstrated earlier to act on T cells favoring IL-5 and IL-13 expression compared with Th1-associated TNFα and IL-2.[3] IL-4R signaling was also shown to regulate IL-10 secretion to establish Th2 dominance[4] and IL-4Rα-deficiency in FoxP3$^+$Tregs exacerbated airway inflammation in a house dust mite sensitization model.[5]

By exploring the existence of intrinsic Th2 inducers within BPE, our results revealed the peculiar feature of BP-derived compound(s) contained in the HMW fraction and affected by protein degradation to interact with IL-4Rα, associated with the induction of Th2 response in vivo. Several microbial as well as plant-derived cytokine mimics with immunomodulating functions have already been described,[6] yet this is the first study to present a natural IL-4Rα ligand other than IL-4. Considering IL-4R expression on various immune cells, concrete downstream effects mediated by BP via the IL-4R pathway to prime Th2 response in vivo remains to be investigated. The identification of BP-intrinsic immunostimulators is relevant to better understand the mechanisms initiating BP allergy and will provide novel therapeutic targets.

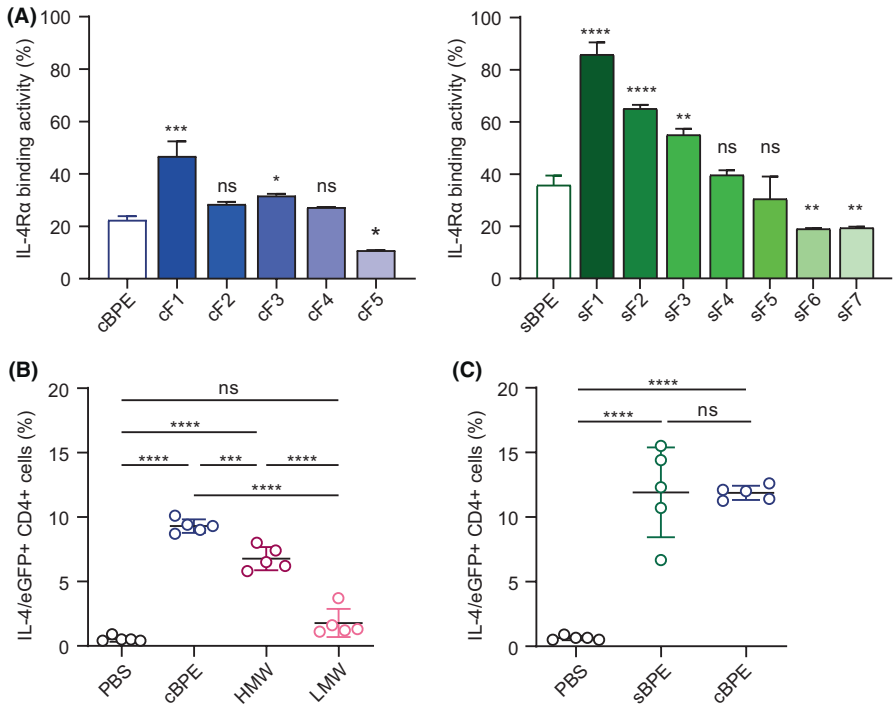

FIGURE 2 IL-4Rα-binding activity and in vivo Th2 response induced by a HMW fraction of BPE. IL-4Rα-binding ELISA with size exclusion chromatography fractions (F) of self-collected (s)BPE, sF1-7, or commercial (c)BPE, cF1-cF5 (coating adjusted to protein concentration, A). IL-4Rα-binding capacity quantified using the rhIL-4 standard curve. Immunization of 4Get mice ($n = 5$ per group) with PBS, 3kDa-filter-generated high (HMW) and low (LMW) molecular weight fractions (B) or sBPE and cBPE (C). Frequency of IL-4/eGFP-positive (+) CD4$^+$T cells detected in inguinal skin-draining lymph nodes 5 days post-injection. eGFP: green fluorescent protein. Statistics indicate $p < .05$ (*), $p < .01$ (**), $p < .001$ (***), $p < .0001$ (****), and not significant (ns).

ACKNOWLEDGEMENTS

The authors thank Univ. Prof. Dr. Fatima Ferreira of the Department of Biosciences and Medical Biology for her great support endorsed by productive discussions and providing lab equipment.

FUNDING INFORMATION

The research was supported by Austrian/German Joint Science Funds (FWF/DFG Project I5312), by the University of Salzburg priority program Allergy-Cancer-BioNano Research Centre, by the doctoral program Immunity in Cancer and Allergy—ICA funded by the Austrian Science Fund (FWF W1213) and by the Doctoral School Program Biomolecules of the University of Salzburg.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Lisa Pointner[1]
Vanda Adamkova[1]
Athanasios Bethanis[1]
Swetlana Gerkhardt[2,3]
Leopold Moelter[2,3]
Claudia Traidl-Hoffmann[2,3,4]
Stefanie Gilles[2,3]
Lorenz Aglas[1]

[1]*Department of Biosciences and Medical Biology, University of Salzburg, Salzburg, Austria*
[2]*Environmental Medicine, Faculty of Medicine, University of Augsburg, Augsburg, Germany*
[3]*Institute of Environmental Medicine, Helmholtz Zentrum München, Neuherberg, Germany*
[4]*Christine-Kühne-Center for Allergy Research and Education (CK-Care), Davos, Switzerland*

Correspondence
Lorenz Aglas, Department of Biosciences and Medical Biology, University of Salzburg, Hellbrunner Strasse 34, 5020 Salzburg, Austria.
Email: lorenz.aglas@plus.ac.at

ORCID

Lisa Pointner https://orcid.org/0000-0002-9591-968X
Vanda Adamkova https://orcid.org/0000-0002-2651-9527
Athanasios Bethanis https://orcid.org/0000-0002-9242-4593
Swetlana Gerkhardt https://orcid.org/0000-0003-1918-659X
Claudia Traidl-Hoffmann https://orcid.org/0000-0001-5085-5179
Stefanie Gilles https://orcid.org/0000-0002-5159-2558
Lorenz Aglas https://orcid.org/0000-0002-1236-5934

REFERENCES

1. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. *Allergy*. 2019;74(7):1237-1248.
2. Pointner L, Bethanis A, Thaler M, et al. Initiating pollen sensitization - complex source, complex mechanisms. *Clin Transl Allergy*. 2020;10:36.
3. Aglas L, Gilles S, Bauer R, et al. Context matters: TH2 polarization resulting from pollen composition and not from protein-intrinsic allergenicity. *J Allergy Clin Immunol*. 2018;142(3):984 e986-987 e986.
4. Balic A, Harcus YM, Taylor MD, Brombacher F, Maizels RM. IL-4R signaling is required to induce IL-10 for the establishment of T(h)2 dominance. *Int Immunol*. 2006;18(10):1421-1431.
5. Khumalo J, Kirstein F, Hadebe S, Brombacher F. IL-4Ralpha signaling in CD4+CD25+FoxP3+ T regulatory cells restrains airway inflammation via limiting local tissue IL-33. *JCI Insight*. 2020;5(20):e136206.
6. White MPJ, Smyth DJ, Cook L, Ziegler SF, Levings MK, Maizels RM. The parasite cytokine mimic Hp-TGM potently replicates the regulatory effects of TGF-beta on murine CD4(+) T cells. *Immunol Cell Biol*. 2021;99(8):848-864.

SUPPORTING INFORMATION

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

DOI: 10.1111/all.15677

Ara h 1 but not Ara h 2 strongly adheres to oral epithelium and remains in the oral area upon peanut release

To the Editor,

Major allergens are characterized by a rapid availability from their biological sources and high sensitization potential through the epithelial barrier.[1] The release of peanut allergens from their natural source by human mastication, their persistence, and interaction with oral epithelial cells as a previous step of allergic sensitization