Pollen and UV-B radiation strongly affect the inflammasome response in human primary keratinocytes

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LETTER TO THE EDITOR

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1 | BACKGROUND

UV-B radiation and plant pollen are two environmental factors that human skin is exposed to during outdoor stays and which are known to provoke inflammatory responses like sunburn or allergic reactions.[1,2] UV-B irradiation is confirmed to activate the NLRP3 inflammasome in human keratinocytes and to induce an inflammatory reaction via the cytokines IL-1β and IL-18.[3] However, for pollen, it is unknown if they can induce inflammasome-associated mechanisms in human keratinocytes and if simultaneous exposure to UV-B has any additional effects.

2 | QUESTIONS ADDRESSED

The current study investigates the impact of pollen alone or in combination with UV-B irradiation on human primary keratinocytes. Central question and aim of this study was to test the potential of pollen substances to activate inflammasome mechanisms in human keratinocytes and to compare it with effects triggered by UV-B alone or by the combination of the two factors.

3 | EXPERIMENTAL DESIGN

Human primary keratinocytes from healthy and atopic donors were stimulated with aqueous pollen extract (APE) in different concentration ranges for 3 or 4 hours. Supernatants and cells were harvested for ELISA cytokine quantification or Western blot analysis. For UV-B treatment, keratinocytes were irradiated with 90 mJ/cm^2 broadband UV-B using an UV therapy device (UV 800K; Waldmann, Villingen-Schwenningen, Germany) before the addition of APE.

4 | RESULTS

4.1 | Aqueous pollen extract from different plant species induces inflammasome-associated cytokine release and caspase-1 activation

To investigate the potential of pollen substances to induce IL-1β and IL-18 release, human primary keratinocytes were stimulated with different aqueous pollen extracts (APEs). ELISA analysis showed that all tested APEs induced the secretion of both cytokines (Fig. 1a). Comparing the different pollen extracts, the most prominent effect for IL-1β was achieved using A. *artemisiifolia* (Amb) extract. Furthermore, extracts of *B. pendula* (Bet), *P. pratense* (Phl) or *P. sylvestris* (Pin) induced a twofold increase in IL-1β level. In contrast to IL-1β, the release of IL-18 was most prominent in reaction to Phl APE. However, also all other tested extracts led to enhanced IL-18 levels in the keratinocyte supernatant with Amb APE being the second best inducer. In addition, Amb and Bet APE supported enhanced IL-1α release, which was, however, not as prominent as seen for IL-1β and IL-18. Effects on mRNA levels as well as the independence of cell death are provided as supplementary data (Fig. S1). Furthermore, we could show using protein and non-protein containing APE fractions that a protein factor is crucial for the induction of IL-1β and IL-18 releases (Fig. S2).

To be activated, IL-1β and IL-18 have to be cleaved by caspase-1. Therefore, Western blotting of cell lysates was used detecting the active caspase-1 subunit p20 as a measure for inflammasome activation. As Fig. 1b shows, the caspase-1 subunit p20 was concentration depended enhanced after the stimulation with Amb APE.

4.2 | APE and UV-B enhance the secretion of inflammasome-associated cytokines in an additive manner and dependent on the donor's atopy status

To test the strength of APE as an inflammasome activator and to compare its effects with UV-B irradiation, experiments with both factors alone but also in combination were conducted. Figure 2a illustrates that IL-1β release provoked by Amb APE-reached levels comparable to those resulting after UV-B irradiation. Astonishingly, a combination of the two factors had a more than additive effect as compared to stimulation with either factor. In addition, the difference between cells from atopic and non-atopic donors was studied. As demonstrated in Fig. 2b, both groups showed significantly enhanced levels of IL-1β in the cell supernatant after APE stimulation as compared to controls. Furthermore, the additive effect with UV-B was maintained in cells of non-atopic and atopic donors. However, in most cases, cells from atopic donors showed higher IL-1β levels in the supernatant. Besides this, the combination of APE and UV-B irradiation resulted in a nearly three times higher IL-1β release in atopic donors compared to non-atopic donors (atopic: 98.4±22.4 pg/mL vs non-atopic: 26.8±2.7 pg/mL; P<.001).

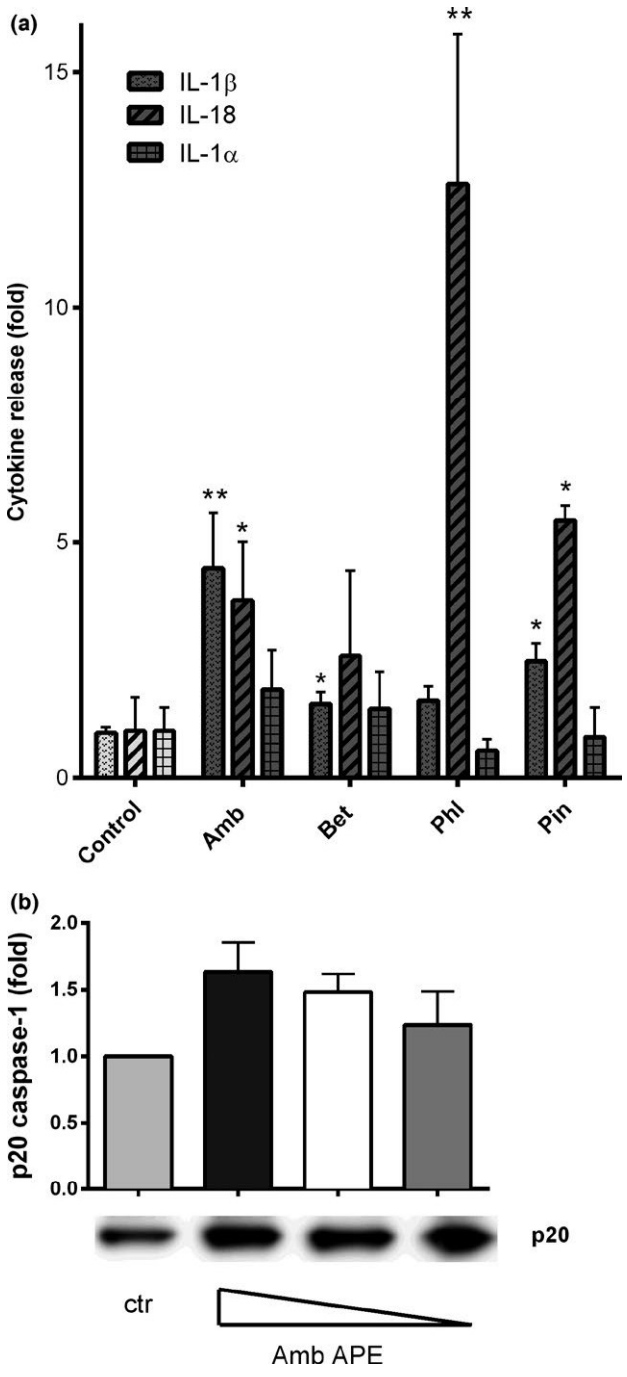

FIGURE 1 Aqueous pollen extract (APE) from different plant species induces inflammasome-associated cytokine release and caspase-1 activation in keratinocytes. Human primary keratinocytes were treated for 3 h with Amb, Bet, Phl or Pin APE (10 mg/mL). (a) IL-1β, IL-18 and IL-1α releases were measured by ELISA. Results were normalized to control mean values; n=3–7. Data are shown as mean +SEM with $^{*}P<0.05$; ** $^{**}P<0.01$; Mann Whitney test. (b) Western blot detecting caspase-1 p20 unit after 3 h of Amb APE stimulation (10, 2.5 and 1.25 mg/mL), n=2. Data are shown as mean +SEM

Analysis of IL-18 levels in cell supernatants showed similar results as observed for IL-1β (Fig. 2c). Both Amb APE and UV-B induced significant IL-18 release in single use and exerted a strong additive

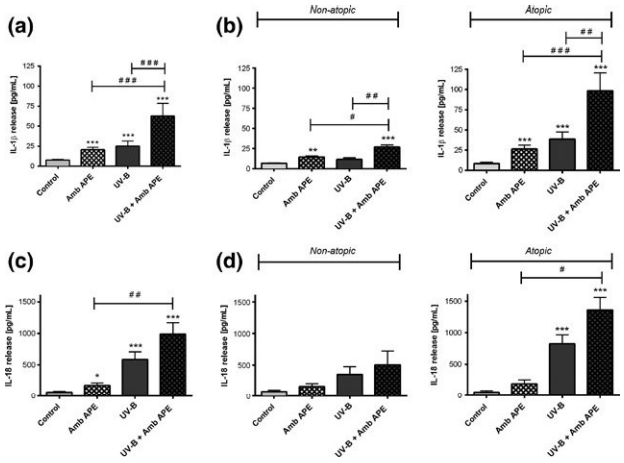

FIGURE 2 APE and UV-B enhance the secretion of inflammasome-associated cytokines in an additive manner and dependent on the donor's atopy status. Human primary keratinocytes were treated for 4 h either with Amb APE (2.5 mg/mL) alone or in combination with UV-B exposure (90 mJ/cm^2). (a) IL-1β release provoked by Amb APE and UV-B in all donors. (b) IL-1β release separated according to keratinocyte donor's atopy status: non-atopic (left) and atopic (right). (c) IL-18 release provoked by APE and UV-B in all donors. (d) IL-18 release separated according to keratinocyte donor's atopy status: non-atopic (left) and atopic (right). Data are shown as mean+SEM. (a–d) n=10 (n=5 non-atopic, n=5 atopic). * and $^{\#}P<.05$; ** and $^{\#\#}P<.01$; *** and $^{\#\#\#}P<.001$ for one-way ANOVA followed by Tukey's post hoc analysis

effect in combination. However, in contrast to the results for IL-1β, UV-B was more effective in inducing IL-18 release than Amb APE. This effect was also observed when considering the results of atopic and non-atopic donor's cells separately (Fig. 2d). In addition, the separate analysis illustrates that cells from atopic donors released higher IL-18 levels in response to Amb APE or UV-B and showed a significantly stronger additive effect when compared to non-atopic cells. Corresponding Lactate dehydrogenase (LDH) release assays did not show any difference in the calculated cell death rate between unstimulated and stimulated cells (Fig. S3a). Regarding the activation of caspase-1, Amb APE and UV-B showed inducing effects for caspase-1 p20 unit in single as well as in combined stimulations (Fig. S3b).

5 | CONCLUSIONS

In summary, our results support the hypothesis that pollen influences the immunological barrier of the skin by triggering the inflammasome system of keratinocytes on its own as well as by supporting the effects of UV-B irradiation. In detail, the current study showed that pollen substances, especially from A. *artemisiifolia* can induce IL-1β/IL-18 cytokine release and the activation of caspase-1 in comparable levels as the known inflammasome activator UV-B radiation. Thus, pollen themselves can provide a danger signal but also exert additive effects which can be important for

the initiation and persistence of inflammatory allergic skin reactions.

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AUTHOR CONTRIBUTIONS

DCD designed the research study, performed the research, analysed the data and wrote the manuscript. SGS designed the research study and participated in drafting the article. SAO participated in drafting the article and contributed to data interpretation. IB, JH, JD, DE and UF contributed essential reagents and provided intellectual input. OGr designed the research study and provided intellectual input. CTH obtained primary funding, and designed and oversaw the project.

ETHICS APPROVAL

The study was performed in adherence to the Declaration of Helsinki Guidelines. Patients were enrolled in the study after written informed consent, and the study was approved by the medical ethical committee of the Technical University Munich.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

Keywords
allergy, environmental factor, inflammatory mechanisms, interleukin1, Skin

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Data S1 Supplementary and methods
Data S2 Supplementary references
Figure S1 APEs induce inflammasome associated cytokine release independently of cell death and provoke an increase in mRNA levels of IL-1β and IL-18 pro-form
Figure S2 Protein components of APE are responsible for the induction of IL-1β and IL-18 release in keratinocytes
Figure S3 APE and UV-B enhance the secretion of inflammasome associated independently of cell death and induce Caspase 1 cleavage