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

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Background

UV-B radiation and plant pollen are two environmental factors 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.

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 is 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.

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 h. 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 addition of APE.

Results

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 (Figure 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 lactate dehydrogenase (LDH) release assay are provided as supplementary data (Figure S1). Furthermore, we could show by using protein

and non-protein containing APE fractions, that a protein factor is crucial for the induction of IL-1β and IL-18 release (Figure S2).

In order 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 figure 1B shows the caspase-1 p20 subunit was concentration depended enhanced after the stimulation with Amb APE.

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 figure 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 versus non atopic: 26.8 ± 2.7 pg/ml; $p < 0.001$). Analysis of IL-18 levels in cell supernatants showed similar results as observed for IL-1β (Figure 2C). Both Amb APE and UV-B induced significant IL-18 release in single use and exerted a strong additive 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 (Figure 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 LDH release assays did not show any difference in the calculated cell death rate between unstimulated and stimulated cells (Figure 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 combinated stimulation (Figure S3B).

Conclusions

In summary our results support the hypothesis that pollen influence 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 participate in drafting the article. SAO participate 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.

Conflict of interest

The authors declare that there are no conflicts of interests.

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.

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Figure legends

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 release was measured by ELISA. Results were normalized to control mean values; n = 3 – 7. **(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.

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 $^{\#}$ $p < 0.05$; ** and $^{\#\#}$ $p < 0.01$; *** and $^{\#\#\#}$ $p < 0.001$ for one-way ANOVA followed by Tukey post hoc analysis.