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## Platelet-activating Factor does not Mediate UVB-induced Local Immune Suppression

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### Abstract

The lipid mediator Platelet-activating factor (PAF) and oxidized glycerophosphocholine PAF agonists produced by UVB have been demonstrated to play a pivotal role in UVB-mediated systemic immunosuppression. Importantly, employing the ability of distant UVB irradiation to inhibit contact hypersensitivity (CHS) responses to the chemical antigen dinitrofluorobenzene (DNFB) to an area of unirradiated murine skin, we and others have demonstrated that UVB-mediated systemic immunosuppression was only observed in PAF-R expressing wild type (WT) mice and not in PAF-R-knockout (*Pafr*<sup>-/-</sup>) mice. As it is not known if PAF is involved in UVB-mediated local immunosuppression, these studies compared local UVB on CHS responses in WT versus *Pafr*<sup>-/-</sup> mice. We demonstrate that the application of DNFB onto UVB exposed (locally) area of mouse skin resulted in a similar significant inhibition of subsequent CHS responses in both WT and *Pafr*<sup>-/-</sup> mice compared to sham-irradiated control mice. Furthermore, the expression of langerin, a marker for the presence of Langerhans cells was substantially reduced equally in the epidermal ears of UVB-irradiated WT and *Pafr*<sup>-/-</sup> mice compared to their respective sham control groups. These findings indicate that the PAF-R is not involved UVB-induced local immunosuppression.

### Introduction

Ultraviolet B (290–320nm; UVB) exposure found in sunlight mediates various biological processes ranging from vitamin D metabolism to the induction of inflammatory responses and skin cancer in humans (1,2). In addition to its ability to damage DNA, UVB is well known to exert an immunosuppressive effect via inhibiting cell-mediated immune responses (3,4). UVB-induced immunosuppression is divided into two distinct types, systemic and local immunosuppression (4). UVB-mediated systemic immunosuppression has been classically measured by the ability of distant UVB irradiation to inhibit contact hypersensitivity responses to chemical antigens such as 2, 4-dinitrofluorobenzene (DNFB)

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or delayed type hypersensitivity responses to antigens such as *Candida* (5–7). In contrast, local immunosuppression is observed when UVB irradiation to an area of skin results in the inability to sensitize with an antigen in the UVB-irradiated area. Experimental studies with mice indicate that systemic immunosuppression usually necessitates higher doses of UVB than local immunosuppression (4). Moreover, local immunosuppression involves dendritic cells and TNF- $\alpha$  (8–10), whereas UVB systemic immunosuppressive effects involve mast cells, cyclooxygenase-2, reactive oxygen species, and Platelet-activating factor (1-*O*-alkyl-2-acetyl glycerophosphocholine, PAF) (5–7, 11–12). However, both local and systemic UVB-mediated immunosuppressive responses involve regulatory T cells and IL-10 (5, 13). Therefore UVB-mediated local versus systemic immunosuppression appears to occur via different mechanisms yet share common pathways.

Studies have demonstrated that UVB exposure, through its ability to act as a pro-oxidative stressor, induces the generation of oxidized glycerophosphocholines (ox-GPC) that act upon the PAF receptor (PAF-R) (7, 14–15). Several lines of evidence indicate that these Ox-GPC PAF-R agonists mediate the UVB systemic immunosuppressive response. First, UVB-mediated systemic immunosuppression is blocked by PAF-R antagonists, mimicked by PAF-R agonists, and is not present in PAF-R-deficient mice (5–7). Second, UVB-mediated systemic immunosuppression is inhibited by systemic antioxidants, and by pretreatment with the PAF- and Ox-GPC metabolizing enzyme serum PAF-acetylhydrolase (7). Finally, treatment with UVB-irradiated GPC also mimicks UVB-mediated systemic immunosuppression (5).

Since both systemic and local UVB-mediated immunosuppression appear to be dependent upon cytokines IL-10 and TNF- $\alpha$ , and PAF-R activation in various cell types including keratinocytes also generates these cytokines (5, 16–17), the present studies sought to define whether PAF was involved in UVB-mediated local immunosuppression. Using *Pafr*<sup>-/-</sup> mice, the present studies demonstrate that UVB-induced local immunosuppression does not depend upon the PAF-R.

## Material and Methods

### Reagents and UVB irradiation source

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless indicated otherwise. As previously reported, our ultraviolet (UV) source was a Philips F20T12/UVB lamp (6, 7). The intensity of the UVB source was measured before each experiment using an IL1700 radiometer and a SED240 UVB detector (International Light) at a distance of 8 cm from the UVB source to the anesthetized mice. All chemicals used in the irradiation protocols were first tested to ensure there was no ability to absorb UVB (i.e., sunblock effect) by testing the intensity of UVB (as measured by detector) irradiation underneath a Kodacel membrane with/without application of the dose used in the in vitro or in vivo protocol.

### Mice

Female C57BL/6-wild type mice (PAF-R expressing; age 6–8 week) were purchased from The Charles River Laboratories. Age-matched female *Pafr*<sup>-/-</sup> mice on a C57BL/6 background, generated as described previously (18), were a kind gift of Professor Takao Shimizu (University of Tokyo Department of Biochemistry). These mice were housed under specific pathogen-free conditions at the Indiana University School of Medicine. All procedures were approved by the Animal Care and Use Committee of Indiana University School of Medicine.

## Contact hypersensitivity (CHS) reactions

CHS to DNFB was conducted as previously described (6,7) with modifications. In these studies we used a lower dose of UVB (1,200J/m<sup>2</sup>) and DNFB was painted onto UVB-exposed area of skin (model for local immunosuppression). In brief, to evaluate the effect of UVB on sensitization reactions, a 2.5 × 2.5 cm area of distal back skin was shaved in anesthetized WT and *Pafr*<sup>-/-</sup> mice to allow direct exposure to a single dose of UVB irradiation (1200 J/m<sup>2</sup>), with other areas shielded. One day later, this irradiated area of back skin was sensitized with the application 25 µl of 0.5% DNFB in acetone: olive oil (4:1, v/v). Seven days later, one of the dorsal sides of ears was challenged with painting of 10 µl 0.5% DNFB and the other ear painted with vehicle. After 24 hours, 5 mm punch biopsies were obtained from the ears and weighed. Our previous studies have demonstrated that measurement of weights of punch biopsies from ears correlated with measurement of ear thickness with calipers (7), and our laboratory prefers this methodology due to greater reproducibility in our hands.

## Immunohistochemistry analysis

The expression of langerin in the epidermal ear sheets of sham and UVB-irradiated WT and *Pafr*<sup>-/-</sup> mice was analyzed by immunohistochemistry. In brief, the dorsal halves of mouse ears were split mechanically from their ventral halves and incubated in 10 mM EDTA/PBS at 37°C for 60 minutes. Epidermal sheets were then removed from the dermis using a fine forceps, and mounted onto slides upside down. The specimens were then air dried, fixed with acetone, rehydrated with PBS, blocked with 1% BSA and stained with anti-mouse langerin/CD207 MoAb 929F3 (DENDRITICS, Rockefeller, Lyon), as per standard protocol (19). The numbers of Langerin-stained cells were quantified by counting four random high-power fields for each sample in blinded fashion.

## Statistical analysis

In the present study, at least five mice/group was used in all murine experiments. Differences between experimental and control groups were examined by a two-tailed Student's *t* test. Statistical significance was defined as a *p* value <0.05.

## Results and Discussion

While the role of the PAF system in mediating UVB-induced systemic immunosuppression has been extensively studied (5–7), and this process is involved in photocarcinogenesis (20), it is not known whether PAF mediates UVB-induced local immunosuppression. Given the fact that local UVB exposure can generate PAF-R agonists, which can augment the secretion of cytokines IL-10 and TNFα from the murine skin (5,17), the present study sought to determine the role of the PAF-R in UVB-induced local immunosuppression. As shown in figure 1A, in these studies, an approximately 2.5 × 2.5 cm area of shaved back skin was treated with 1,200J/m<sup>2</sup> UVB radiation. Identically treated mice without UVB served as controls (sham). One day following mock or UVB irradiation, irradiated back skin was sensitized with DNFB. Seven days after DNFB sensitization, the dorsal sides of ears were treated either with DNFB or vehicle control. Punch biopsies were taken from ears 24h later and inflammation assessed by weighing and comparing DNFB-treated with vehicle-treated ear skin. We observed that local UVB irradiation inhibited CHS to DNFB significantly in both WT and *Pafr*<sup>-/-</sup> mice (Figure 1B). These findings, particularly with *Pafr*<sup>-/-</sup> mice are in contrast to previous studies demonstrating that UVB-irradiation systemically inhibited CHS responses to DNFB (6,7) and DTH responses to Candida antigen (21) only in WT mice and not in *Pafr*<sup>-/-</sup> mice. The ability of UVB-induced local immunosuppression to affect both WT and *Pafr*<sup>-/-</sup> mice suggests that the local immunosuppression is not dependent upon the PAF-R.

Local immunosuppression induced by UVB has been shown to involve Langerhans cells (LCs), which are major antigen-presenting cells that trap antigens in the skin and trigger a cascade of immunologic events (8,22). Moreover, studies suggest that UVB-irradiation converts the immunogenic phenotype of LCs to a tolerogenic phenotype (23). The expression of LCs in the epidermis of skin has been shown to be measured by the presence of langerin/CD207, a highly useful and reliable marker for LCs (24). Therefore, we next analyzed the expression of langerin as a representative of UVB-induced local immunosuppression. In these studies, the dorsal side of the ears of WT and *Pafr*<sup>-/-</sup> were either sham- or UVB-irradiated with a dose of 1,200J/m<sup>2</sup>. After 24 hours, mice were sacrificed and the epidermal ear sheets from these mice were processed for the immunohistochemistry staining for langerin. As shown in Figure 2, the expression of langerin was substantially reduced in the epidermal ears of UVB-irradiated WT and *Pafr*<sup>-/-</sup> mice compared to their respective sham control mice. These findings fit with a previous report (25), and suggest that UVB exposure depletes LCs in a generalized way regardless the PAF-R status of the mice. Moreover, the idea that UVB-mediated LC depletion is PAF-R-independent is compatible with the findings detailed in Figure 1, that local UVB-radiation inhibits CHS to DNFB both in WT and *Pafr*<sup>-/-</sup> mice. These data are also consistent with the previous published reports demonstrating the involvement of epidermal LCs as a crucial event in mediating UVB-induced local immunosuppression (20,22,23). Yet, the role of epidermal LC in mediating UVB-induced local immunosuppression has been called into question by the recent report by Wang and colleagues, which provides evidence that dermal langerin-positive (non-LC) cells appear to be the key mediators (26).

In summary, the present studies indicate that PAF-R activation is not an important event in mediating UVB-induced local immunosuppression as has been defined for systemic immunosuppression. These data also indicate that UVB-mediated LC depletion does not involve the PAF-R.

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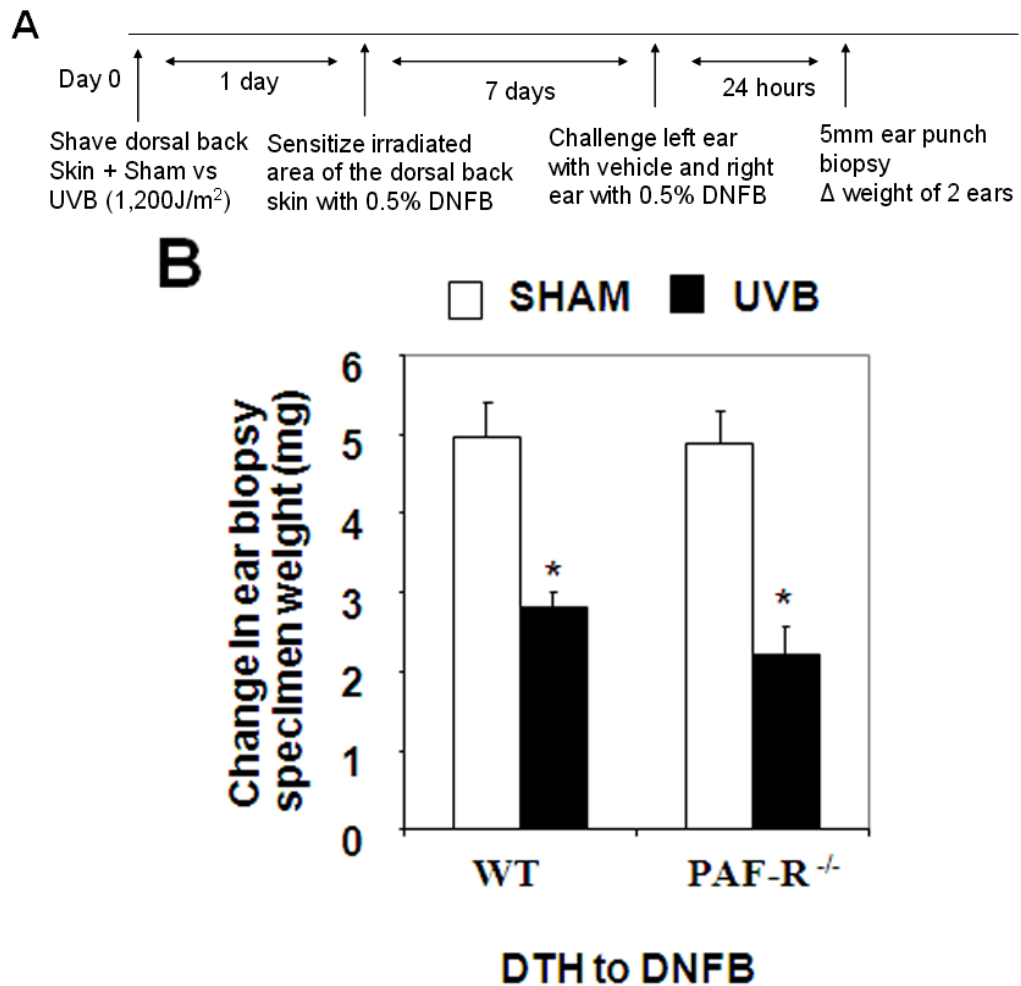
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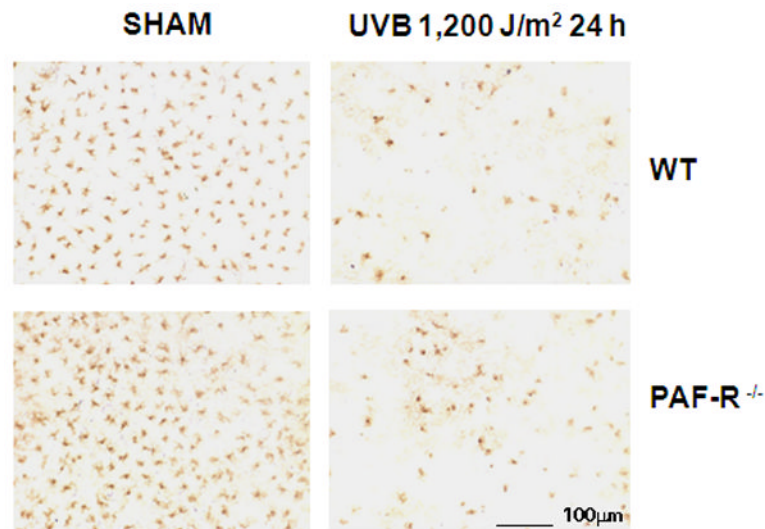
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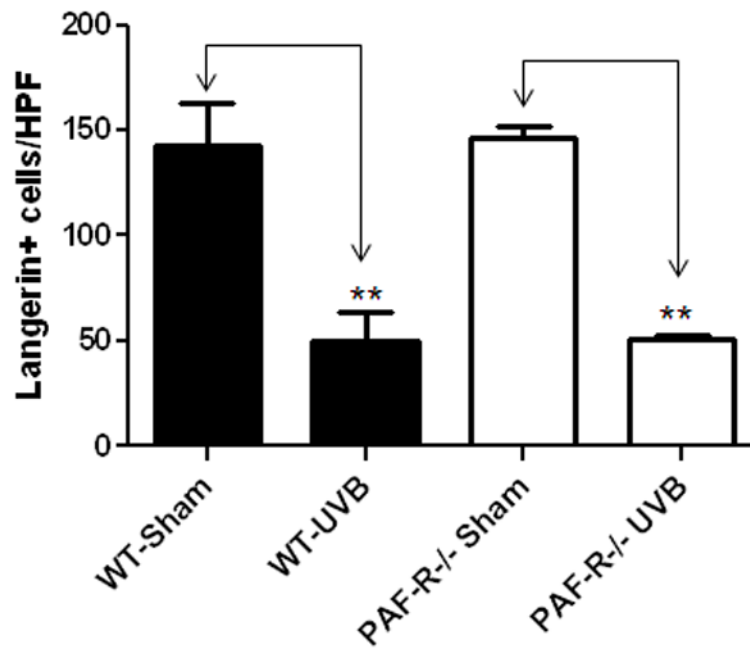


**Figure 1. Effect of local UVB-irradiation on CHS**

**A)** Schematic representation of the experimental protocol. **B)** Wild-type and *Pafr*<sup>-/-</sup> mice were treated with either local irradiation with 0 (sham) or 1,200J/m<sup>2</sup> UVB. Mice were then sensitized to DNFB and elicitation reactions performed on the dorsal ears as outlined in Materials and Methods. At 24 hours after elicitation reactions, 5 mm punch biopsies were obtained from the ears and weighed. The data listed are the mean±SE difference in ear biopsy weights from n=6 mice. \*Statistically (p<0.05) significant changes.



### Epidermal ear sheets, anti-Langerin IHC



#### Figure 2. Effect of local UVB-irradiation on Langerin expression

The dorsal ears of Wild-type and *Pafr*<sup>-/-</sup> mice were treated with either local irradiation with 0 (sham) or 1,200J/m<sup>2</sup> UVB. At 24 hours, mice were sacrificed, and the epidermal sheets from the dorsal halves of the mice ears were processed for immunostaining of langerin as outlined in Materials and Methods. **A)** Example of Langerin staining in sham versus UVB treated skin; **B).** Quantitation of Langerin-positive cells in wild-type versus PAF-R-deficient mice. \*\* Denotes statistically significant ( $p < 0.01$ ) changes. These studies are representative of at least five separate mice.