Expression of the Platelet-activating Factor Receptor Results in Enhanced Ultraviolet B Radiation-induced Apoptosis in a Human Epidermal Cell Line*

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Recent studies have demonstrated that ultraviolet B radiation (UVB) damages human keratinocytes in part by inducing oxidative stress and cytokine production. Severe UVB damage to the keratinocyte can also result in apoptosis or programmed cell death. Although the lipid mediator platelet-activating factor (PAF) is synthesized in response to epidermal cell damage and epidermal cells express PAF receptors, it is not known whether PAF is involved in UVB-induced epidermal cell apoptosis. These studies examined the role of the PAF system in UVB-induced epidermal cell apoptosis using a novel model system created by retroviral-mediated transduction of the PAF receptor-negative human epidermal cell line KB with the human PAF receptor (PAF-R). Expression of the PAF-R in KB cells did not affect base-line growth or apoptosis, yet resulted in a decrease in the lag time between treatment of the cells and the induction of apoptosis following irradiation with 400 J/m² UVB. This effect was inhibited by pretreatment with the PAF-R antagonists WEB 2086 and A-85783, confirming involvement of the PAF-R in this process. At lower doses (100–200 J/m²) of UVB, only KB cells that expressed the PAF-R became apoptotic. Treatment of PAF-R-expressing KB clones with the metabolically stable PAF-R agonist 1-hexadecyl-2-N-methylcarbamoyl-3-glycerophosphocholine (CPAF) alone did not induce apoptosis but augmented the degree of apoptosis observed if CPAF was used in combination with lower doses (200 J/m²) of UVB irradiation. Interestingly, UVB irradiation was found to stimulate PAF synthesis only in PAF-R-expressing KB cell clones. The antioxidants N-acetyl cysteine, 1,1,3,3-tetramethyl-2-thiourea, and vitamin E inhibited both UVB-induced PAF biosynthesis as well as the augmentation of UVB-induced apoptosis in PAF-R-expressing KB clones, suggesting the possibility that UVB stimulates the production of oxidized lipid species with PAF-R agonistic activity in this model system. Thus, these studies indicate that a component of UVB-induced epidermal cell cytotoxicity can be modulated by PAF-R activation through the production of PAF and PAF-like species.

Through the synthesis and release of soluble proinflammatory cytokines, chemokines, and growth factors, keratinocytes play an active role in cutaneous inflammation. Among these inflammatory and trophic compounds that can play a role in cutaneous inflammation/keratinocyte function is platelet-activating factor (1-alkyl-2-acetyl-glycero-3-phosphocholine; PAF) (reviewed in Refs. 1 and 2). Derived from glycerophosphocholine, PAF is a potent activator of many cell types including platelets, monocytes, polymorphonuclear leukocytes (PMNs), mast cells, and vascular endothelium. PAF also has trophic effects on diverse cell types (3, 4). Although PAF can be metabolized to potentially biologically active neutral lipid or phosphatidic acid species (5–7), the majority of PAF effects are thought to be mediated through a G protein-linked transmembrane receptor (PAF-R) (reviewed in Ref. 8). Consistent with the myriad of responses linked to PAF, activation of the PAF-R stimulates many signal transduction systems, including phospholipases C, A₂, and D and mitogen-activated protein kinase. PAF is the best characterized ligand for the PAF-R; yet other natural products can utilize this receptor including oxidized phospholipids derived from low density lipoproteins (9, 10), lipopolysaccharide and protein A (11), lipotechoic acid moieties on Streplococcus species (12), and 1-acyl 2-acyl GPCs (13, 14). The diversity of ligands recognized by the PAF-R could potentially allow involvement of this system in numerous pathological conditions including oxidative damage and bacterial infection. Recent evidence suggests that PAF and the PAF-R could be involved in keratinocyte biology. Keratinocytes express functional PAF-Rs (15) and synthesize PAF and 1-acyl PAF analogs in response to numerous stimuli including ionophores, growth factors, and ultraviolet radiation (16, 17). PAF is not found in normal skin but has been detected in inflammatory skin diseases including psoriasis (18) and urticaria (19). Within the epidermis, keratinocytes are chronically exposed to a powerful oxidant and DNA-damaging agent, UV light. Acute short term UVB (280–320 nm) absorption by keratinocytes results in oxidative stress and DNA damage (20). If the damage is moderate, it can be repaired (21). However, if the damage is extensive, DNA repair processes do not occur, and the keratinocytes undergo programmed cell death or apoptosis (22).
Apoptotic cells have distinct morphological characteristics; in fact, apoptosis was first recognized in cells based on the unusual morphological features of the process (23, 24). In the epidermis, apoptosis prevents keratinocytes that have extensive UVB-induced DNA damage from undergoing cell division and passing genetic mutations that might have gone unrequ- paired to any of their progeny cells, substantially lowering the risk of developing cancer. Disregulation of the apoptotic mechanism in skin can lead to erythema multiforme, lichen planus, papillomas, and skin cancer. Several features of apoptosis can be used to help define this process. For example, the condensed chromatin found in apoptotic cells can be identified by staining cells with the fluorochrome 4',6-diamidino-2-phenylindole (DAPI). The induction of apoptosis also activates a very specific proteolytic cascade. The proteases are activated collectively called caspases, and they target important cellular proteins involved in cell proliferation or DNA repair for precise cleavage (25). One of the caspase substrates is the DNA repair enzyme poly(ADP-ribose)/polymerase (PARP). Just as important proteins are targeted for organized dismantling, so is the chromatin. The induction of apoptosis leads to the activation of an endonuclease that cleaves genomic DNA between nucleosomes (26). This destruction of the genomic DNA yields the “DNA ladders” that are characteristic of the experimental proof of apoptosis.

It is not presently known whether the PAF system participates in UVB-induced apoptosis. However, several lines of evidence suggest that PAF/PAF-R could be involved in UVB-mediated keratinocyte damage. First, ultraviolet radiation has been reported to be a stimulus for PAF biosynthesis in corneal epithelial cells (17). In addition, cytokines and the oxidative stress generated in response to UVB irradiation in epidermal cells can cause PAF production in other cell types. For example, TNF-α treatment stimulates PAF synthesis in monocytes, neutrophils, and endothelial cells (27). Reactive oxygen species have also been reported to induce PAF biosynthesis in endothelial cells and myocytes (28, 29). Second, PAF has been reported to have synergistic effects in combination with known inducers of apoptosis. In immature T cells PAF has been noted to have no effect on apoptosis when administered alone; yet this lipid mediator augmented apoptosis induced by a calcium ionophore (30). Similarly, treatment of eosinophils with PAF has been found to increase FAS-induced apoptosis (31). However, PAF has also been reported to inhibit apoptosis in B cell lines (32), suggesting that modulatory effects of PAF on apoptosis may be cell type- and insult-specific.

The objective of these studies was to assess whether PAF-R activation can modulate UVB-induced apoptosis. Using a model system our laboratory has developed by retroviral-mediated gene transduction to express the human PAF-R in the model system our laboratory has developed by retroviral activation can modulate UVB-induced apoptosis. Using a may be cell type- and insult-specific.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Routine chemicals, PAF, 1-hexadecyl-2-acetyl GPC standards (2 ng each). 

**Measurement of PAF Species Following UVB Treatment**—1-Hexadecyl-2-acetyl-GPC (PAF) and 1-palmitoyl-2-acetyl-GPC (PAPC) were measured in KB cells by select ion monitoring gas chromatography mass spectrometry with deuterated internal standards with minor modifications from those previously described (16). Briefly, 10-cm dishes with >90% confluent cells were washed three times with HBSS. 1 ml of prewarmed HBSS containing 0.25% fatty acid-free bovine serum albumin (Sigma) was added to dishes, and the cells were irradiated. Immediately following irradiation, the reaction was quenched with 2 × 2 ml treatments with ice-cold ethanol, and the entire contents of the plates were scraped and placed into tubes containing deuterium-labeled 1-hexadecyl and 1-palmitoyl sn-2 acetyl GPC standards (2 ng each). Cells from similarly treated dishes were trypsinized and counted (Coulter) to derive cell numbers.

**Statistics**—Data are presented as the means ± S.D. Statistical significance is assessed by the Student’s t test, and significance is set at p < 0.05.

**RESULTS**

**The KB PAF-R Model System**—Because PAF may have both receptor-dependent and -independent effects (secondary to the formation of biologically active metabolites), a model system was developed to study the role of the PAF-R in epidermal cell function. This system utilizes the human epidermal cell line KB, which, unlike normal human keratinocytes, does not express functional PAF-Rs (15, 16). A PAF-R-positive KB cell line was created by transducing KB cells with the replication-de-
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Cytotoxic Effects of UVB on KB Cells—In our first studies, KBM and KBP clones were irradiated with 400 J/m² UVB, and the cells were examined for gross morphological changes 6 h following irradiation. Expression of the PAF-R alone did not affect the morphology of the cells. However, upon exposure to UVB, an increased cytotoxic response was noted in KBP cells in comparison with KBM cells. To assess whether the increased cell death induced by UVB irradiation of KBP cells was because of apoptosis, we examined the cells for the following three hallmarks of apoptosis: nuclear condensation, internucleosomal DNA cleavage, and caspase activation. KBM and KBP cells were irradiated with either 0 or 400 J/m² UVB, and all of the cells in the culture dish were harvested 6 h later. The cells were then stained with DAPI and examined by fluorescence microscopy. Unirradiated KBM (Fig. 1A) or KBP (Fig. 1C) cells demonstrated primarily round, homogeneously stained nuclei, indicating no degradation of nuclear material. A small percentage of the nuclei from KBM cells irradiated with 400 J/m² UVB (Fig. 1B) displayed evidence of apoptosis, identified by the smaller, more intensely stained nuclear blebs. In contrast, KBP cells irradiated with 400 J/m² UVB contained predominantly apoptotic nuclei. The induction of apoptosis in these cells was confirmed by extracting genomic DNA from irradiated and unirradiated KBM and KBP cells. Again, KBM and KBP cells were irradiated with 0 or 400 J/m² UVB, and genomic DNA was extracted at 4 and 8 h post-irradiation. Unirradiated KBM or KBP cells did not exhibit the characteristic DNA ladders found in apoptotic cells (Fig. 2A). KBM cells irradiated with 400 J/m² and harvested at 8 h post-irradiation demonstrated faint nucleosome-sized DNA fragments. However, KBP cells (two separate clones are shown in Fig. 2B) irradiated with 400 J/m² UVB displayed extensive DNA ladders 8 h after UVB irradiation. The presence of apoptotic cells was confirmed by a third set of experiments that assayed the activation of caspase proteolytic cascade. KBM and KBP cells were irradiated as described previously, and the activation of caspase enzymes was monitored by the specific cleavage of the PARP protein. A small percentage of caspase-cleaved PARP protein was detected in KBM cells 8 h following UVB irradiation (Fig. 2B). In KBP cells, all of the detectable PARP protein was cleaved by 8 h after 400 J/m² of UVB (Fig. 2B). Together, these three distinct assays for apoptosis demonstrate that the presence of the PAF-R increased the sensitivity of KB cells to undergo UVB-induced apoptosis.

Our next studies examined the dose and time dependence of UVB-induced apoptosis in KB cells. Fig. 3A depicts a dose-response curve for UVB-induced apoptosis (as assessed by DAPI staining) in treated KB cells at 6 h. UVB treatment caused a significant increase in apoptosis in KBP cells compared with KBM cells at each dose of UVB radiation tested. The kinetics of UVB-induced apoptosis in KBM versus KBP cells following irradiation with 400 J/m² was next examined. As shown in Fig. 3B, apoptosis was seen in response to UVB irradiation at earlier time points in KBP cells than in KBM cells. In KBP cells treated with 400 J/m², 50% apoptosis was achieved at less than 4 h versus 8 h in the KBM cells. By 16 h, there was no difference in the amount of apoptosis between KBP and KBM cells (Fig. 3B). Similarly, the amounts of PARP cleavage and DNA laddering at 24 h after treatment with 400 J/m² UVB were similar in KBM and KBP clones (data not shown). These experiments suggested that the presence of the PAF-R accelerated the induction of apoptosis in response to a lethal dose of UVB radiation.

Effects of PAF-R Antagonists on UVB-induced Apoptosis—To confirm that the differences in UVB-induced apoptosis between KBM and KBP cells were because of PAF-R activation, the ability of the competitive PAF-R antagonists A-85783 (35) or WEB 2086 (36) to inhibit the accelerated induction of apoptosis seen in PAF-R expressing KB cells was evaluated. Pretreat-

FIG. 1. DAPI staining of KB cells before and after UVB irradiation. KBM (A and B) or KBP (C and D) cells were untreated (A and C) or irradiated with 400 J/m² (B and D) and stained with DAPI at 6 h post-treatment.

FIG. 2. UVB-induced DNA laddering and PARP cleavage in KBP cells. A, DNA laddering. Total cellular DNA was extracted from a KBM or two KBP clones 0, 4, or 8 h after irradiation with 400 J/m² UVB. Extracted DNA was separated on a 2% agarose gel and stained with ethidium bromide. B, PARP cleavage. Protein was extracted from a KBM or two KBP clones 0, 4, or 8 h after irradiation with 400 J/m² UVB and separated by gel electrophoresis. Immunoblotting was performed using an antibody that binds both cleaved and uncleaved PARP, which were detected as two separate bands. Densitometry was performed to determine the amount of PARP cleavage.
The ability of exogenous PAF to modulate apoptosis in response to a sublethal dose of UVB was next investigated. Because PAF is rapidly degraded by acetylhydrolases found in serum (37), the metabolically stable PAF-R agonist CPAF (38) was used in these experiments. Treatment of KBM or KBP cells with CPAF alone (1 nM to 1 \( \mu \)M) did not induce apoptosis (data not shown). However, high (2–10 \( \mu \)M) doses of CPAF exerted cytotoxic effects on both KBP and KBM clones, suggesting a PAF-R-independent process (data not shown). As shown in Fig. 5, a combination of 500 nM CPAF and irradiation with a sublethal (200 J/m\(^2\)) dose of UVB resulted in a further increase in the amount of apoptosis in KBP cells over UVB irradiation alone. Altogether, these findings indicate that the difference in UVB responsiveness between KBP and KBM clones is because of the presence of the PAF-R.

As shown in Fig. 3B, irradiation of KBM and KBP cells with 400 J/m\(^2\) UVB resulted in essentially equal amounts of apoptosis at 16 and 24 h. Further experiments were performed to assess whether sublethal UVB doses at these later time points have a differential effect between KBP and KBM cells. In these experiments, the amount of apoptosis induced by low dose (100 and 200 J/m\(^2\)) UVB on KBP and KBM cells at 24 h were compared. Treatment of KBP cells with these lower doses of UVB resulted in increased cytotoxicity over similarly treated KBM cells (Fig. 6). In particular, irradiation of KB cells with 100 J/m\(^2\) UVB induced apoptosis selectively in KBP cells at 24 h.

**Effects of UVB on KB PAF Biosynthesis**—Our finding that the expression of the PAF-R in an epidermal cell line aug-

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**FIG. 3.** The kinetics of UVB-induced apoptosis in KB cells. A, dose response curve for UVB-induced apoptosis in KB cells. Cells were irradiated with various doses of UVB as indicated and stained with DAPI 6 h after irradiation. B, time course of UVB-induced apoptosis in KB cells. Cells were exposed to 400 J/m\(^2\) UVB and stained with DAPI at the indicated times. Each point represents the percentage of apoptosis ± S.D. from at least four separate experiments with representative KBM and KBP clones. Similar results were obtained in one other KBM and two other KBP clones. An asterisk denotes statistically (\( p < 0.05 \)) significant difference in UVB-induced apoptosis between KBM and KBP cells.

**FIG. 4.** The effect of PAF-R antagonists on UVB-induced apoptosis in KB cells. Cells were pretreated with indicated concentrations (\( \mu \)M) of A-87573 (A) or WEB 2086 (B) or Me\(_2\)SO vehicle (0.5%) 30 min prior to UVB irradiation (400 J/m\(^2\)). Each point represents the mean percentage of apoptosis ± S.D. at 6 h by use of DAPI staining from at least three separate experiments with representative KBM and KBP clones. Similar results were obtained in one other KBM and two other KBP clones. An asterisk denotes statistically (\( p < 0.05 \)) significant inhibition of UVB-induced apoptosis in KBP cells by PAF-R antagonist.
mented UVB-induced apoptosis and was inhibited by two structurally dissimilar competitive PAF-R antagonists implied that UVB could induce the biosynthesis of PAF-R agonist(s) in these cells. To test whether this UVB-induced agonistic activity was because of PAF, KBM and KPB clones were treated with UVB (320–2650 J/m²), and PAF (1-hexadecyl-2-acetyl-GPC) and the PAF-R agonist 1-palmitoyl-2-acetyl-GPC were measured by mass spectrometry (16). As shown in Fig. 7, UVB treatment induced the biosynthesis of both PAF and PAPC in KPB cells, with significant levels of PAF and PAPC measured following irradiation of all doses of UVB tested. However, UVB treatment of KPB clones did not result in significant amounts of PAF or PAPC biosynthesis over untreated cells (Fig. 7). Inasmuch as PAF-R activation is a known stimulus for PAF biosynthesis in PMNs (37) and epidermal cells (16), our finding that UVB stimulated PAF biosynthesis only in PAF-R-expressing KPB (but not KBM) cells. Similar results were seen in one other KPB and two other KPB clones. Irradiation of KPB cells resulted in statistically (p < 0.05) significant increases in PAF and PAPC over unstimulated cells at all UVB dosages tested.

**Fig. 5.** The effect of CPAF on UVB-induced apoptosis in KB cells. Immediately following UVB irradiation (200 J/m²), 500 nM CPAF or ethanol vehicle (0.1%) was added to cells. Each point represents the mean percentage of apoptosis ± S.D. at 6 h by use of DAPI staining from at least three experiments with representative KBM and KPB clones. Similar results were obtained in one other KBM and two other KPB clones. One asterisk denotes that UVB treatment of KPB cells resulted in a statistically significant (p < 0.05) increase in apoptosis compared with similarly treated KBM cells. Two asterisks denotes that UVB + CPAF treatment of KPB cells resulted in a significant (p < 0.05) increase in apoptosis over KPB cells treated with UVB radiation alone.

**Fig. 6.** The effect of low dose UVB treatment on KB cell apoptosis at 24 h. Cells were irradiated with various doses of UVB as indicated and stained with DAPI 24 h after irradiation. Each point represents the percentage of apoptosis ± S.D. from at least four separate experiments from representative KBM and KPB clones. An asterisk denotes statistically (p < 0.05) significant difference in UVB-induced apoptosis between KBM and KPB cells in response to both 100 and 200 J/m².

**Fig. 7.** UVB treatment results in PAF and PAPC biosynthesis in KPB but not KBM cells. KB cells were irradiated with various doses of UVB followed by measurement of 1-hexadecyl-2-acetyl GPC (PAF; A) or PAPC (B) by gas chromatography mass spectrometry. Each point represents the amount of PAF or PAPC in ng/10⁶ cells ± S.D. from at least four separate experiments using representative KBM and KPB clones. Similar results were seen in one other KBM and two other KPB clones. Irradiation of KPB cells resulted in statistically (p < 0.05) significant increases in PAF and PAPC over unstimulated cells at all UVB dosages tested.
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DISCUSSION

These studies provide the first evidence that the epidermal PAF-R may be involved in UVB-induced apoptosis. The study of PAF/PAF-R is limited by the rapid metabolism of PAF and the fact that PAF metabolites can exert biological activity independent of the PAF-R (5–7). In addition, structurally similar lysophosphatidylcholines can signal various cell types, again, independent of the PAF-R (39). The model system used in these studies was developed to overcome some of the current limitations in the study of PAF/PAF-R and to account for the diverse ligands recognized by the PAF-R (9–14).

KB cells are a human epidermal cell line originally derived from a patient with a nasopharyngeal carcinoma (34). KB cells are often used as a model of keratinocytes because they synthesize many of the same cytokines including interleukins 1, 6, and 8 and TNF-α. KB cells also respond to TNF-α, and UVB treatment of these cells induces prostaglandin and TNF-α synthesis similar to primary cultures of human keratinocytes (40, 41). In contrast to normal human keratinocytes, KB cells do not express PAF-R mRNA (33) and lack PAF-R protein by radioligand binding studies (15, 33) and immunohistochemical studies using a specific PAF-R polyclonal antibody (15). In addition, treatment of these cells with PAF or PAF-R agonists does not trigger intracellular calcium mobilization, arachidonic acid release, or PAF production (16, 33). However, KB cells stimulated with the calcium ionophore A23187 synthesize PAF (16), indicating intact, intracellular mechanisms for PAF biosynthesis in this PAF-R-negative cell line. As described previously, KB cells were transduced with the human leukocyte PAF-R cDNA using a replication-defective retrovirus (33). To control for possible effects of integration of a retrovirus into genomic DNA, KB cells were also transduced with the empty MSCV 2.1 retrovirus alone (KBM). With both PAF-R-positive (KBP) and -negative (KBM) cells, this model system can help to discern PAF-R-dependent versus PAF-R-independent effects of PAF. In particular, this model system can account for non-PAF PAF-R agonists such as sn-2 short chain phosphocholines, which have been shown to be produced in response to lipid peroxidation (reviewed in Refs. 42 and 43).

Expression of the PAF-R in the PAF-R-negative human epidermal cell line KB resulted in an increased susceptibility to UVB-induced apoptosis. This heightened effect of UVB on KBP over control KBM cells took two forms. High dose (400 J/m²) UVB treatment of KBP cells decreased the lag time between treatment of the cells and induction of apoptosis (Fig. 3B). Low dose (100 J/m²) UVB resulted in selective apoptosis in KBP cells (Fig. 6). Because KBP cells undergo apoptosis in response to doses of UVB that do not affect KBM cells, we conclude that activation of the PAF-R lowers the threshold of a cell to undergo apoptosis in response to UVB in this model system.

That stimulation of the epidermal PAF-R can prime these cells for UVB-induced apoptosis is consistent with previous reports that PAF increases apoptosis induced by a calcium ionophore in immature T cells (30) and by FAS in human eosinophils (31). In these disparate model systems as well as in the current studies, PAF-R activation alone was not an adequate stimulus to induce apoptosis. This priming effect of the PAF-R on apoptosis resembles PAF effects on PMN superoxide production. PAF alone does not induce detectable superoxide production in PMNs unless they are pretreated with cytochalasin B or propranolol; yet pretreatment with PAF can augment PMN superoxide production in response to FMLP or CSA (44).

Consistent with the ability of the PAF-R to modulate UVB-induced apoptosis was our finding that UVB treatment of KBP cells pretreated with the antioxidants N-acetyl cysteine, TMTU, and α-tocopherol (vitamin E) inhibited subsequent UVB-induced PAF biosynthesis as shown in Fig. 8A. These antioxidants had similar inhibitory effects on UVB-induced PAF biosynthesis in KBP cells. In two separate experiments using two different KBP clones, TMTU and vitamin E had no effect on PAF (or PACP) biosynthesis induced by a 5-min treatment with 250 nM CPAF, suggesting that antioxidants did not exert their effects in this system through direct inhibition of PAF-R-mediated PAF biosynthesis. Pretreatment of KBP cells with these antioxidants also resulted in diminished apoptosis in response to 400 J/m² UVB at 6 h (Fig. 8B). Altogether, these findings suggest the possibility that PAF-R augmentation of UVB-induced apoptosis is mediated in part by UVB-induced production of PAF-R-agonistic oxidized lipid species.
cells resulted in PAF and PAPC biosynthesis. Interestingly, UVB irradiation did not stimulate PAF/PAPC production in KBM cells. One possible explanation for this disparity could be that UVB treatment does not trigger PAF biosynthesis directly but instead stimulates production of non-PAF-R agonists like short chain sn-2 oxidized phospholipid, and the PAF/PAPC measured in KBP cells are subsequent to PAF-R stimulation. This notion is supported by the recent report that cigarette smoke, a known inducer of oxidative stress, triggers the production of non-PAF-R agonists in rodents in vitro (45). That PAF or structurally similar compounds synthesized in response to oxidative damage could be involved in apoptosis is suggested by the recent report that overexpression of PAF acetylhydrolase II (which can inactivate short chain sn-2 phospholipids) in Chinese hamster ovary cells is protective against oxidative stress-induced apoptosis (46). Our finding that antioxidants inhibited both UVB-induced (but not CPAF-induced) PAF biosynthesis as well as the augmentation of apoptosis in KBP cells (Fig. 8, A and B) is consistent with a role for PAF-like lipids formed by phospholipid oxidation in these processes. The nature of this UVB-induced PAF-like activity synthesized by KB cells is unknown. Ongoing studies have found that lipid extracts from both UVB-treated KB and KBM cells (but not unstimulated cells) have PAF-like activity (by measurement of intracellular calcium mobilization using Indo-1-loaded KBP versus KBM cells). We are currently in the process of characterizing this UVB-induced PAF agonist activity.

In addition to the ability of oxygen radicals to induce the production of PAF and oxidatively fragmentedGPCs with PAF-like activity, these reactive chemical species have also been reported to irreversibly inactivate human plasma PAF-acetylhydrolase (47). Thus, oxidative stress could generate exaggerated PAF-R-mediated responses. It is possible that a portion of the PAF we measure in response to UVB stimulation could be because of an inhibition of its metabolism.

Human keratinocytes undergo programmed cell death in response to numerous stimuli in vitro, and epidermal apoptosis is seen in a wide range of cutaneous diseases. Current studies in our laboratory are characterizing the types of cytotoxic stimuli that can be modulated by PAF-R activation. In addition to UVB, we find that TNF-α-induced apoptosis is also increased in KBP over KBM cells, suggesting that the PAF-R can modulate other cytotoxic stimuli in epidermal cells. Of note, TNF-α and UVB share the ability to induce reactive oxygen species (48) and PAF biosynthesis (27). Future studies are planned to define the types of cytotoxic stimuli that are augmented by expression of the PAF-R, the nature of the PAF-like species involved, as well as the mechanism(s) by which PAF-R activation increases epidermal cell cytotoxicity. An understanding of the process by which PAF-R stimulation can amplify epidermal cell cytotoxicity may provide insights into the priming phenomenon, as well as an understanding of the regulation of apoptosis in keratinocytes.

Although human keratinocytes synthesize PAF (16) and express functional PAF-Rs (15), the role of the PAF-R in epidermal cell function is not clear. These studies suggest that one of the functions of the epidermal PAF-R could be to augment the cytotoxic damage in response to noxious agents like UVB radiation. These findings may have clinical implications because certain populations are potentially more susceptible to PAF effects because of deficiency of the PAF metabolizing enzyme acetyl hydrolase (49). A better understanding of the functions of the PAF system in keratinocyte biology and cutaneous inflammation may lead to therapeutic interventions designed around this lipid mediator.