The Platelet-activating Factor Receptor Protects Epidermal Cells from Tumor Necrosis Factor (TNF) α and TNF-related Apoptosis-inducing Ligand-induced Apoptosis through an NF-κB-dependent Process*

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A number of chemical mediators can induce human keratinocytes and epidermal-derived carcinomas to undergo apoptosis, or programmed cell death. Recent evidence suggests pro-inflammatory cytokines, such as interleukin-1β or transforming growth factor α, protects carcinomas from numerous pro-apoptotic stimuli. Platelet-activating factor (1-alkyl-2-acetyl-3-glycerophosphocholine; PAF) is a lipid mediator with pro-inflammatory effects on numerous cell types. Although PAF can be metabolized to other bioactive lipids, the majority of PAF effects occur through activation of a G protein-coupled receptor. Using a model system created by retroviral transduction of the PAF receptor (PAF-R) into the PAF-R-negative human epidermal cell line KB and the PAF-R-expressing keratinocyte cell line HaCaT, we now demonstrate that activation of the epidermal PAF-R results in protection from apoptosis induced by tumor necrosis factor (TNF) α or TNF-related apoptosis-inducing ligand. The PAF-mediated protection was inhibited by PAF-R antagonists, and protection did not occur in PAF-R-negative KB cells. Additionally, we show protection from TNFα- or TRAIL-induced apoptosis by PAF-R activation is dependent on the transcription factor nuclear factor (NF)-κB, because PAF-R activation-induced NF-κB and epidermal cells transduced with a super-repressor form of inhibitor κB were not protected by the PAF-R. These studies provide a mechanism whereby the epidermal PAF-R, and possibly other G protein-coupled receptors, can exert anti-apoptotic effects through an NF-κB-dependent process.

Apoptosis, or programmed cell death, is a fundamental physiological process enabling the removal of damaged or infected cells and in the control of cell populations (1). Apoptosis can occur during embryogenesis, induction, and maintenance of immune tolerance, development of the nervous system, and endocrine-dependent tissue atrophy (2). In the skin, apoptosis is involved in epidermal development and growth and deletion of UV-damaged keratinocytes (3). In addition to normal physiological conditions, essentially all chemotherapeutic agents exert their effects by induction of apoptosis (4). Thus, regulation of apoptosis can have important consequences both during development and in the treatment of cancer.

Recent evidence suggests an association between cancer and inflammation, with inflammatory mediators affecting the growth and survival of tumors (5–7). Pro-inflammatory cytokines, such as IL-1β[1] or transforming growth factor α, have been shown to protect human keratinocytes and epidermal-derived carcinomas against numerous pro-apoptotic stimuli (8–11). Thus the anti-apoptotic effects exerted by pro-inflammatory mediators may provide a mechanism for decreased effectiveness of chemotherapy. Furthermore, the protective effect of these cytokines depends upon the *de novo* synthesis of protective proteins (10, 11) implicating the activation of epidermal transcription factors.

One potential transcription factor that may mediate anti-apoptotic effects in epithelial cells is NF-κB. NF-κB proteins are sequence-specific transcription factors induced in response to inflammatory and other stressful stimuli (12, 13). Indeed, exposure to IL-1β or interferon-γ has been shown to activate NF-κB in primary cultures of human keratinocytes and in transformed keratinocytes (8, 14). These studies determined that activation of NF-κB mediated the observed anti-apoptotic activity exerted by IL-1β against TRAIL-induced apoptosis (8). Coincident to the protection from apoptosis, recent evidence indicates that activation of epidermal NF-κB enhances the expression of the inhibitor of apoptosis proteins (IAP), such as c-IAP1 and c-IAP2, which interfere with apoptosis (15, 16). Expression of IAPs and other anti-apoptotic proteins have been shown to inhibit apoptosis in various cell types (17–19).

Accumulating evidence suggests platelet-activating factor (PAF)-mediated pathways are involved in cutaneous inflammation and keratinocyte stress responses (20). PAF (1-alkyl-2-acetyl glycerophosphocholine) is a glycerophosphocholine-derived lipid mediator implicated in numerous inflammatory

[1] The abbreviations used are: IL, interleukin; PAF, platelet-activating factor; PAF-R, PAF receptor; CPAF, 1-O-hexadecyl-2-N-methylcarbamoyl-glycerophosphocholine; TNF-α, tumor necrosis factor α, TRAIL, TNF-related apoptosis-inducing ligand; GPCR, G protein-coupled receptor; NF-κB, nuclear factor-κB; AMC, 7-amino-4-methylcoumarin; IAP, inhibitor of apoptosis proteins; IκB, inhibitor κB; Z-VAD-FMK, 2-Val-Ala-Asp-FMK.

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processes. Keratinocytes synthesize PAF and related 1-acyl-PAF-like species in response to various stimuli including ionophores, growth factors, PAF agonists, the pro-oxidative stressor, *tert*-butyl hydroperoxide, ultraviolet light irradiation, or acute thermal damage (21–24). Although PAF can be metabolized to other biologically active lipids, the majority of PAF effects appear to be mediated by interaction with a G protein-coupled receptor (GPCR), the PAF receptor (PAF-R). In addition to producing PAF, keratinocytes also express the PAF-R (21), and activation of the epidermal PAF-R leads to the production and release of PAF, IL-6, IL-8, TNFα, and eicosanoids (23, 25).

Although the ability of cytokines to protect keratinocytes from apoptosis has been established, and potential mechanisms for this protection have been suggested, it is unclear whether PAF has anti-apoptotic effects. However, several lines of evidence suggest that epidermal PAF may inhibit apoptosis. First, PAF can be produced under the same conditions that induce apoptosis and thus may have a role in the regulation of apoptosis. Exposure to chemotherapeutic agents, such as cisplatin, gentamycin, or 5-fluorouracil have been shown to induce PAF production (26, 27). Second, activation of the PAF-R expressed in Chinese hamster ovary cells has been shown to stimulate NF-κB and induce gene expression (28). Thus, activation of the keratinocyte PAF-R may induce NF-κB expression and protect keratinocytes in a manner similar to cytokines. Of significance, activation of the PAF-R has been shown to rescue B lymphocytes from apoptotic stimuli through an unknown mechanism (29).

Because PAF is produced during inflammation and oxidative stress and activates signal transduction pathways similar to the anti-apoptotic effects of growth factors and cytokines, we sought to determine whether apoptosis in epidermal cells can be modulated by PAF. In this paper, we report that activation of the epidermal PAF-R protects from apoptosis induced by either TNFα or TRAIL. This protective effect was inhibited by pretreatment with PAF-R antagonists. Stimulation of epidermal cells with a PAF receptor agonist resulted in a rapid degradation of IκB and subsequent increase in NF-κB binding. Furthermore, the protective effect induced by PAF-R activation was abolished in epidermal cells expressing a super-repressor form of IκB, indicating that the PAF-R-induced protective effect was mediated by activation of an NF-κB-dependent pathway. Thus, these results demonstrate that, in addition to the anti-apoptotic actions induced by growth factors and cytokines, activation of GPCRs such as PAF-R may also protect epithelial cells from pro-apoptotic agents.

EXPERIMENTAL PROCEDURES

Reagents—All chemicals were obtained from Sigma unless indicated otherwise. Recombinant human TRAIL/APO2L was purchased from Chemicon (Temecula, CA). Recombinant human IL-1β and TNFα were from PeproTech (Rocky Hill, NJ), the broad-spectrum caspase inhibitor 2-Val-Ala-Asp-FMK (2-VAD-FMK) was purchased from Alexis Biochemicals (San Diego, CA). The PAF-R antagonist WEB-2086 was kindly provided by Boehringer Ingelheim (Ridgefield, CT) and A-85783 was a gift from Dr. James Summers (Abbot Pharmaceuticals, Abbott Park, IL).

Cell Culture—The human epidermoid cell line KB and human keratinocyte cell line HaCaT cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) were supplemented with 10% fetal bovine serum (Intergen, Purchase, NY). A KB PAF-R model system was created by transduction of PAF-R-negative KB cells with the MSCV2.1 retrovirus encoding the human leukocyte PAF-R as described previously (25, 30). KB cells transduced with the PAF-R (KBP) or with control MSCV2.1 retrovirus (KBM) were characterized by Southern and Northern blot analysis and by radioligand binding and calcium mobilization studies to demonstrate that the PAF-R was functional (25, 30). All experiments were replicated with at least two separate KBM and KBP clones.

Generation of KBP Cells Expressing the Super-repressor IκBα (IκBM)—The IκBM containing S32A and S36A mutation of IκBα has previously been described (31), and the retroviral DNA vector MIEG3, which uses enhanced green fluorescent protein as the selectable marker, was a kind gift of Dr. David Williams (Indiana University) (32). To create KBP cells stably expressing IκBM, the IκBM cDNA was subcloned into the *Eco*RI site of MIEG3, and orientation was assessed by restriction endonuclease mapping and sequencing. Infectious amphotropic retroviruses were produced from both MIEG-IκBM and control MIEG backbone by transient transfection using standard protocols (33). Briefly, the Phoenix amphotropic packaging cell line was transfected with the DNA constructs using Fugene-6, and the supernatants collected 48 h later containing infectious virions were then used to infect KBP cells. Transduced cells were fluorescence-activated cell sorter-sorted on the basis of enhanced green fluorescent protein expression

Immunoblotting—Cells were washed twice with ice-cold phosphate-buffered saline and lysed with radioimmune precipitation buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.1% SDS, 0.5% sodium deoxycholate, 1% Nonidet P-40) containing 0.5 mM Pefabloc SC (Roche Molecular Biochemicals) and 10 mM sodium orthovanadate for 20 min on ice. IκB degradation was determined by immunoblotting with polyclonal anti-IκBα antibody (Santa Cruz Biotechnology) and enhanced chemiluminescence (Amersham Pharmacia Biotech).

Cell Viability Assay—Cells were plated at a density of 1.0×10^4 cells/well in 96-well plates and allowed to stabilize for 1 day. Cells were then treated for 24 h with fresh medium containing the indicated agents. Cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Roche Molecular Biochemicals) and analyzed using a microplate reader (Molecular Devices, Sunnyvale, CA). The effects of treatment are expressed as a percentage of viable cells using the untreated cells as the maximum cell viability.

DNA Fragmentation Analysis—Low molecular weight DNA was extracted from KB cells after TNFα or TRAIL treatment at the indicated times using the Stratagene DNA extraction kit (Stratagene, La Jolla, CA). 40 μg of total genomic DNA was separated on a 2.0% agarose gel, and the gel was stained with ethidium bromide.

Cell Death Detection Assay—Apoptosis was determined quantitatively using a cell death detection enzyme-linked immunosorbent assay (Roche Molecular Biochemicals) according to the manufacturer's instructions. The kit measures the enrichment of mono- and oligonucleosomes released into the cytoplasm of apoptotic cells as a result of DNA degradation. The absorption was measured at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). The enrichment factor was calculated using the following formula: absorbance of apoptotic cells/absorbance of control cells.

Caspase-3 Assay—The activation of the caspase proteolytic cascade was measured by the direct assay of caspase-3 enzyme activity in cell lysates using a synthetic fluorogenic substrate (caspase-3 substrate, Ac-Asp-Glu-Val-Asp-AMC (Ac-DEVD-AMC); Alexis Biochemicals, San Diego, CA) as described previously (34). Reactions were performed for 1 h at 37 °C. Release of the fluorogenic AMC moiety was measured using a Hitachi F2000 spectrophotometer (excitation, 380 nm; detection, 460 nm). The fluorescent intensity was converted to pmol of AMC released by comparison to standards of AMC (Molecular Probes, Eugene, OR). The specific activity of caspase-3 in cell lysates was determined following quantitation of the total protein in the cell lysates (NanoOrange protein quantitation reagent; Molecular Probes, Eugene, OR).

Electrophoretic Mobility Shift Assay—Whole cell extracts were prepared and subjected to electrophoretic mobility shift assay as described previously (31). Protein-DNA binding reactions were performed for 30 min at room temperature using 6 μg of whole cell extract and ^{32}P-labeled oligonucleotide probes for NF-κB consensus binding sites (Promega, Madison, WI). Complexes were separated by electrophoresis on nondenaturing 6% acrylamide gel and assayed by autoradiography.

NF-κB Reporter Assay—Cells were plated at a density of 1.5×10^6 cells in a 10-cm dish and allowed to stabilize for 1 day. Cells were then transfected using Fugene6 (Roche Molecular Biochemicals) with 10 μg of NF-κB-luciferase reporter plasmid (pNF-κB-luc) and 10 μg of pCMV-β-galactosidase as an internal control for the transfection efficiency. 24 h after transfection, cells were treated with CPAF and IL-1β or were mock-treated and then harvested in reporter lysis buffer (Promega) following an additional 6-h incubation. 20-μl aliquots of the lysates were assayed for β-galactosidase and luciferase activities using an LB9501 luminometer (Lumat). Luciferase activities were normalized for each transfection using the control β-galactosidase activities.

FIG. 1. **Effect of TNF-α (*black columns*) and TRAIL (*gray columns*) on apoptosis in KBP cells.** KBP cells were treated with 100 ng/ml TNF-α or 40 ng/ml TRAIL for 0–24 h. Following the treatment duration, the cells were collected and lysed, and (*A*) caspase-3-specific activity and (*B*) enrichment of nucleosomes into the cytoplasm were determined as an index of apoptotic activity after treatment. *C*, DNA fragmentation was examined after 24 h of treatment with TNFα or TRAIL and using UVB as a positive control (*Con*).
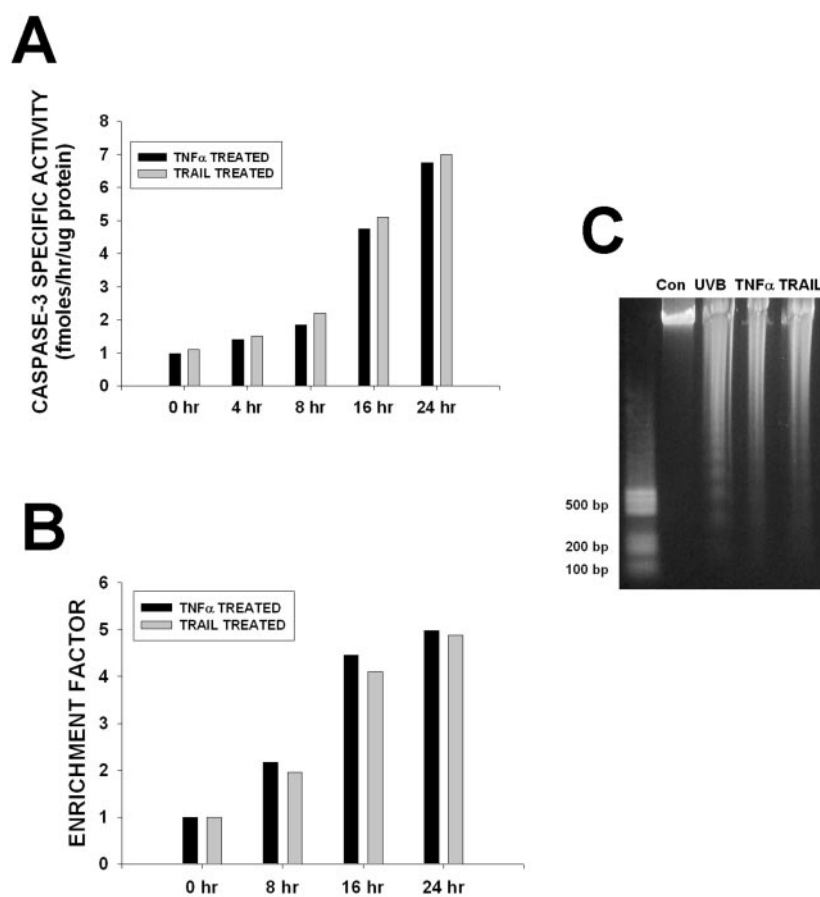
RESULTS

Because PAF may have both receptor-dependent and -independent effects (secondary to the formation of biologically active metabolites), our laboratory has previously created a model system by transduction of the PAF-R into a PAF-R-deficient epidermal cell line to study the role of the PAF-R in keratinocyte cell biology. The human epidermal cell line KB, originally obtained from a patient with an oral squamous cell carcinoma (36), does not express functional PAF-Rs, unlike normal human keratinocytes and the human keratinocyte-derived cell line HaCaT (21, 22). A PAF-R-positive KB cell line, KBP, was created by transducing KB cells with a replication-deficient MSCV2.1 retrovirus containing the human PAF-R cDNA. KB cells were also transduced with the retrovirus backbone alone to establish a vector control cell line, KBM. Expression of the PAF-R protein was verified by binding studies using radiolabeled PAF-R antagonist WEB-2086 (25). Calcium mobilization studies demonstrated that the KB PAF-R was functionally active (25, 30). Therefore, this *in vitro* epidermoid system consists of both PAF-R-negative (KBM) and -positive (KBP) cells.

In initial experiments, the dose-response and time-response of TNFα- and TRAIL-induced apoptosis were determined using activation of caspase-3, a cell death detection enzyme-linked immunosorbent assay, and DNA fragmentation as three distinct markers of apoptosis in epidermal cells. Exposing KBP cells to 100 ng/ml TNFα or 40 ng/ml TRAIL for various times was found to induce caspase-3-specific activity (Fig. 1*A*), enrich the release of mono- and oligonucleosomes into the cytoplasm (Fig. 1*B*), and enhance DNA fragmentation as shown by DNA laddering (Fig. 1*C*). Inasmuch as these markers of apoptosis determined that TNFα and TRAIL treatment resulted in a maximal apoptotic effect after 24 h, subsequent experiments

therefore examined apoptosis at the 24-h time point. In addition to KBP cells, treatment with TNFα- or TRAIL-induced apoptosis in KBM and HaCaT cells was similar to that observed in KBP cells (data not shown).

To determine whether PAF-R activation affects TNF family-mediated apoptosis in epidermal cells, KB cells were pretreated with the metabolically stable PAF agonist, CPAF. Pretreatment of KBP cells for 1 h with 100 nM CPAF resulted in a decrease in the TRAIL- and TNFα-induced apoptosis (Fig. 2*A*, *black bars*), shown as a decrease in caspase-3-specific activity and decreased enrichment of nucleosomes in the cytoplasm (Fig. 2*B*); however, CPAF had no effect on apoptosis in the PAF-R-deficient KBM cells (Fig. 2*A*, *gray bars*). In contrast to the actions of CPAF, pre-treating cells for 1 h with 25 ng/ml IL-1β protected both KBP and KBM cells from TRAIL- and TNFα-induced apoptosis. These results confirm the studies of Schwartz and co-workers (8, 9) that IL-1β protects KB cells from TRAIL-induced apoptosis. Furthermore these results suggest that the anti-apoptotic effects of PAF are mediated by activation of the PAF-R, because CPAF inhibited apoptosis in KBP, but not KBM, cells.

To further confirm whether the protective effects of CPAF were mediated through a PAF-R-dependent mechanism, we determined whether the selective PAF-R antagonists A-85783 (ABT) or WEB2086 (WEB) could block the protective effects of PAF. As shown in Fig. 3*A*, exposure to WEB or ABT abolished the anti-apoptotic effects of PAF. Neither ABT nor WEB had any direct effect alone on TRAIL-induced apoptosis. In addition, pre-treatment with WEB or ABT did not affect the anti-apoptotic effects of IL-1β in KBP cells (Fig. 3*B*). Taken together, these results indicate that activation of the PAF-R mediates the protective effects of PAF on epidermal cells.

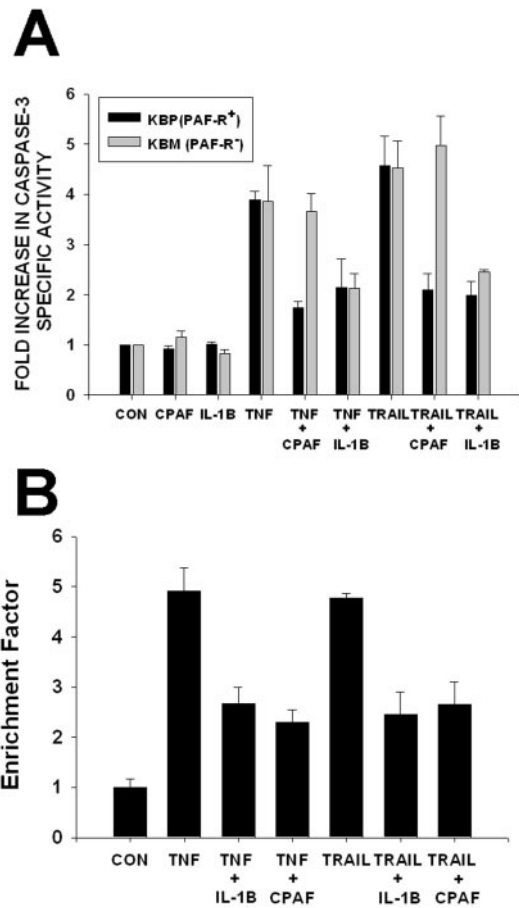To address whether the protective effects induced by CPAF

FIG. 2. **Effect of CPAF and IL-1β on TNF-α- and TRAIL-induced apoptosis in KBP (*black columns*) and KBM (*gray columns*).** The cells were treated with 100 nm CPAF or 25 ng/ml IL-1β for 1 h prior to exposure to 100 ng/ml TNF-α or 40 ng/ml TRAIL for 24 h. Following the treatment duration, the cells were collected, lysed, and (*A*) caspase-3-specific activity/mg of protein and (*B*) enrichment of nucleosomes into the cytoplasm were determined as an index of apoptotic activity in KBP cells. Data represent the -fold difference in activity or enrichment compared with untreated control (*CON*).

were secondary to the overexpression of PAF-R in KBP cells, we also examined the protective role of physiological levels of the PAF-R in the immortalized keratinocyte cell line, HaCaT. Our laboratory has demonstrated previously that HaCaT cells express functional PAF-Rs (21, 25). As illustrated in Fig. 4, treatment with 100 nM CPAF or 25 ng/ml IL-1β for 1 h prior to exposure to TRAIL or TNFα protected HaCaT cells from TNFα- and TRAIL-induced apoptosis in a manner similar to the protective effect observed with KBP cells. Taken together, these results demonstrate that activation of the PAF-R, a GPCR, protects keratinocyte and epidermal carcinoma cells from apoptosis.

To determine whether the PAF-R-mediated protection from apoptosis would affect the survival of epidermal cells, we examined the viability of cells treated with either TRAIL or TNFα in the presence or absence of CPAF, IL-1β, or the nonspecific caspase inhibitor z-VAD-fmk. TRAIL and TNFα treatment decreased the number of viable KBP cells by 41.6 ± 1.8 and 49.5 ± 8.3%, respectively (Fig. 5). Pre-treatment of KBP cells for 1 h with 100 nM CPAF or 25 ng/ml IL-1β protected cells, shown as a 25–30% increase in cell viability. Treatment with 10 μM of the caspase inhibitor z-VAD-fmk also blocked the decrease in cell viability induced by TRAIL and TNFα. Thus, the cytoprotective actions induced by the PAF-R exerted a survival effect on TRAIL- and TNFα-treated epidermal cells, presumably by inhibiting apoptosis.
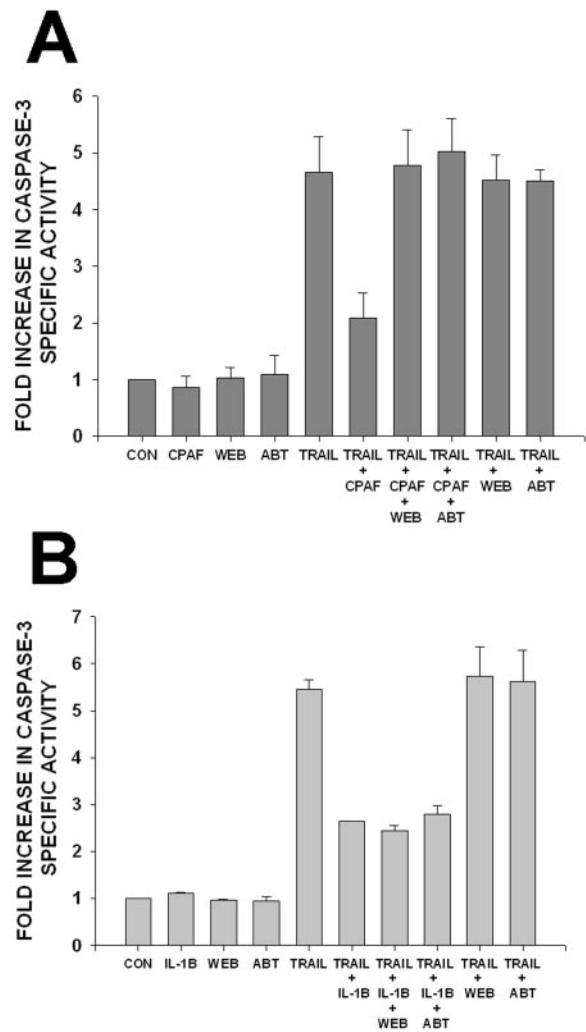

FIG. 3. **Effect of PAF-R antagonists, WEB or ABT, on the protective effect of (*A*) CPAF or (*B*) IL-1β.** KBP cells were exposed to 10 μm WEB or ABT 30 min prior to pretreatment with CPAF or IL-1β. Following pretreatment, the cells were exposed to 40 ng/ml TRAIL for 24 h. The cells were collected and lysed, and caspase-3-specific activity/mg of protein was determined as an index of apoptotic activity. Data represent the -fold difference in activity compared with untreated control (*CON*).

Because pro-inflammatory cytokines and growth factors can induce anti-apoptotic effects through activation of the transcription factor NF-κB, we next examined whether the PAF-R protective effects were similarly mediated through an NF-κB-dependent pathway. Exposure to CPAF or IL-1β induced a time-dependent degradation of IκBα in KBP cells resulting in the disappearance of immunoreactive IκB within 15–20 min (Fig. 6*A*). Secondary to the degradation of IκBα, NF-κB binding activity was increased and reached maximal activity ~1 h after treatment (Fig. 6*B*). However, CPAF did not induce IκB degradation nor increase NF-κB binding activity in control KBM cells unlike IL-1β, which induced NF-κB in both KBP and KBM cells (data not shown). To establish that the PAF-R is functionally coupled to a pathway leading to activation of NF-κB, KBP cells were transfected with a luciferase reporter containing NF-κB consensus binding sites and were then stimulated with CPAF or IL-1β. Exposure to CPAF or IL-1β resulted in a 6.2 ± 1.0 (*n* = 6)- or 5.1 ± 0.5 (*n* = 6)-fold increase in NF-κB-dependent gene transcription over unstimulated cells, respectively (Fig. 6*C*). Thus, activation of the PAF-R is functionally coupled to a biochemical pathway that results in NF-κB activation and gene transcription.
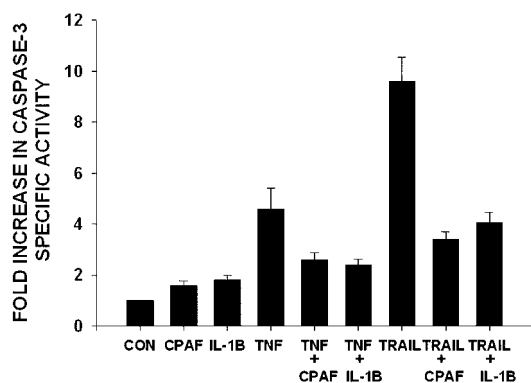
PAF-R-induced Anti-apoptotic Activity

FIG. 4. **Effect of CPAF and IL-1β on TNF-α- and TRAIL-induced apoptosis in the human keratinocyte cell line, HaCaT.** The cells were treated with 100 nm CPAF or 25 ng/ml IL-1β for 1 h prior to exposure to 100 ng/ml TNF-α or 40 ng/ml TRAIL for 24 h. Following the treatment duration, the cells were collected and lysed, and caspase-3-specific activity/mg of protein was determined as an index of apoptotic activity. Data represent the -fold difference in activity compared with untreated control (*CON*).
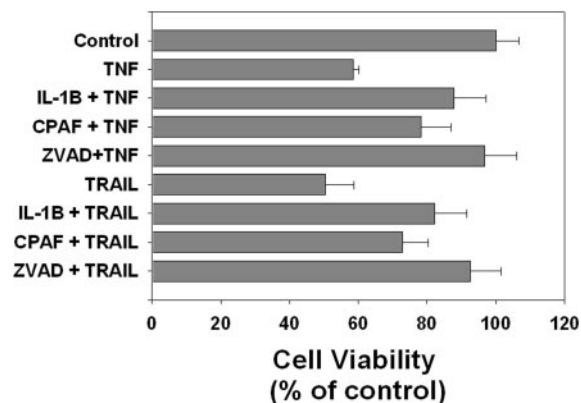

FIG. 5. **Effect of CPAF, IL-1β, or the caspase inhibitor z-VAD-fmk on viability of KBP cells treated with TNF-α or TRAIL.** The cells were treated with 100 nm CPAF, 25 ng/ml IL-1β, or 10 μm z-VAD-fmk for 1 h prior to exposure to 100 ng/ml TNF-α or 40 ng/ml TRAIL for 24 h. Following the treatment duration, the cell viability was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell growth/death assay. Data represent the percent of viable cells compared with untreated control.

To determine whether the PAF-R protective effects were dependent on the NF-κB pathway, KBP cells were transduced by retroviral infection to express a super-repressor IκB protein (KBP-MIEG-IκBM). Whereas both CPAF and IL-1β induced IκB degradation and increased NF-κB binding activity in KBP cells transfected with the vector backbone (KBP-MIEG), the stimulated IκB degradation (Fig. 7*A*) and NF-κB binding activity (Fig. 7*B*) were abolished in KBP-MIEG-IκBM cells. Furthermore, the CPAF- and IL-1β-induced protection from TNFα- and TRAIL-induced apoptosis were decreased in KBP-MIEG-IκBM cells (Fig. 7, *C* and *D*), indicating that the anti-apoptotic activity induced by PAF-R is mediated by an NF-κB-dependent pathway. In addition to abolishing the protective effects, treatment with TNFα resulted in an augmentation of apoptosis in KBP-MIEG-IκBM cells compared with KBP-MIEG cells; however, TRAIL-induced apoptosis was similar in KBP-MIEG-IκBM and KBP-MIEG cells. These findings are supported by previous studies that demonstrated that the inhibition of the NF-κB pathway sensitizes various cells types to TNFα-induced apoptosis (37–39). Taken together, these findings support the hypothesis that activation of the epidermal PAF-R results in an NF-κB-dependent protection from the pro-apoptotic stimulation induced by TNFα and TRAIL.
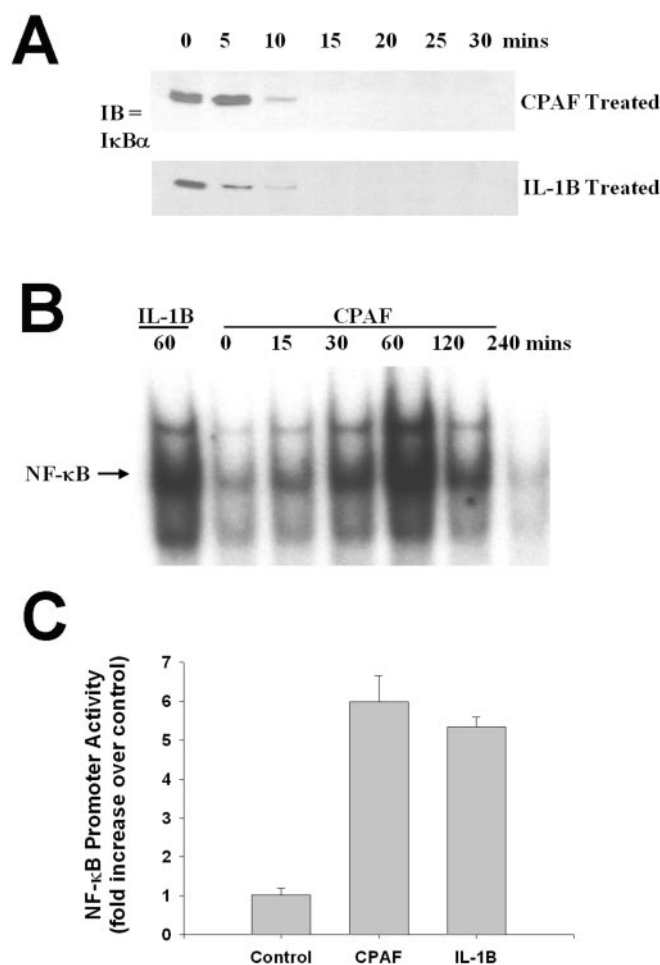

FIG. 6. **Effect of CPAF and IL-1β on IκB degradation and NF-κB binding and activity in KBP cells.** *A*, following the treatment with 100 nm CPAF or 25 ng/ml IL-1β, the cells were harvested and lysed with radioimmune precipitation buffer. Approximately 40 μg of protein was separated on a 10% SDS-polyacrylamide gel electrophoreses, and IκBα immunoreactivity was determined using a polyclonal antibody. *IB*, immunoblot. *B*, for NF-κB binding whole cell extracts were obtained through three cycles of freeze/thaw and diluted to a concentration of 2 μg/ml in lysis buffer. Whole-cell extracts were incubated with radiolabeled NF-κB probe and electrophoretically separated. *C*, KBP cells transfected with NF-κB luciferase reporter gene were stimulated with CPAF or IL-1β for 6 h, and relative luciferase activity was normalized to β-galactosidase.

DISCUSSION

These studies provide evidence that in epidermal cells, the PAF-R can protect against apoptosis induced by TNFα receptor superfamily members via an NF-κB-dependent process. The protective effect of CPAF, but not IL-1β, occurred through activation of the PAF-R, because the protection from apoptosis was only seen in PAF-R-expressing KBP and HaCaT cells, and the protection was inhibited by using PAF-R antagonists. Our results describe a novel function for a G protein-coupled receptor, namely the induction of an NF-κB-dependent, anti-apoptotic pathway by which the PAF-R receptor can protect carcinomas from chemotherapy-induced apoptosis and describes a putative mechanism in tumors for the pro-survival effects associated with inflammation and inflammatory mediators (5–7).

Several lines of evidence suggest that the PAF-R-induced protection from apoptosis involves an NF-κB-dependant transduction cascade. Activation of the PAF-R in KBP and HaCaT cells resulted in a rapid, time-dependent degradation of IκB and corresponding increase in NF-κB binding activity, which was not observed in PAF-R-deficient KBM cells (data not

FIG. 7. **Effect of CPAF and IL-1β on NF-κB induction and PAF-R-mediated anti-apoptotic effects in KBP-MIEG (control cells) and KBP-MIEG-IκBM (expressing super-repressor IκB) cells.** Following treatment with 100 nm CPAF or 25 ng/ml IL-1β, cells were collected, and (*A*) IκBα-immunoreactivity and (*B*) NF-κB binding activity was determined. *C* and *D*, KBP-MIEG (*gray columns*) and KBP-MIEG-IκBM (*black columns*) cells were treated with CPAF or IL-1β for 1 h prior to exposure to 100 ng/ml TNF-α or 40 ng/ml TRAIL for 24 h. Following the treatment duration, the cells were collected and lysed, and (*C*) caspase-3-specific activity/mg of protein or (*D*) enrichment of nucleosomes into the cytoplasm was determined as an index of apoptotic activity. Data represent the -fold difference in activity or enrichment compared with untreated control (*CON*). *IB*, immunoblot.
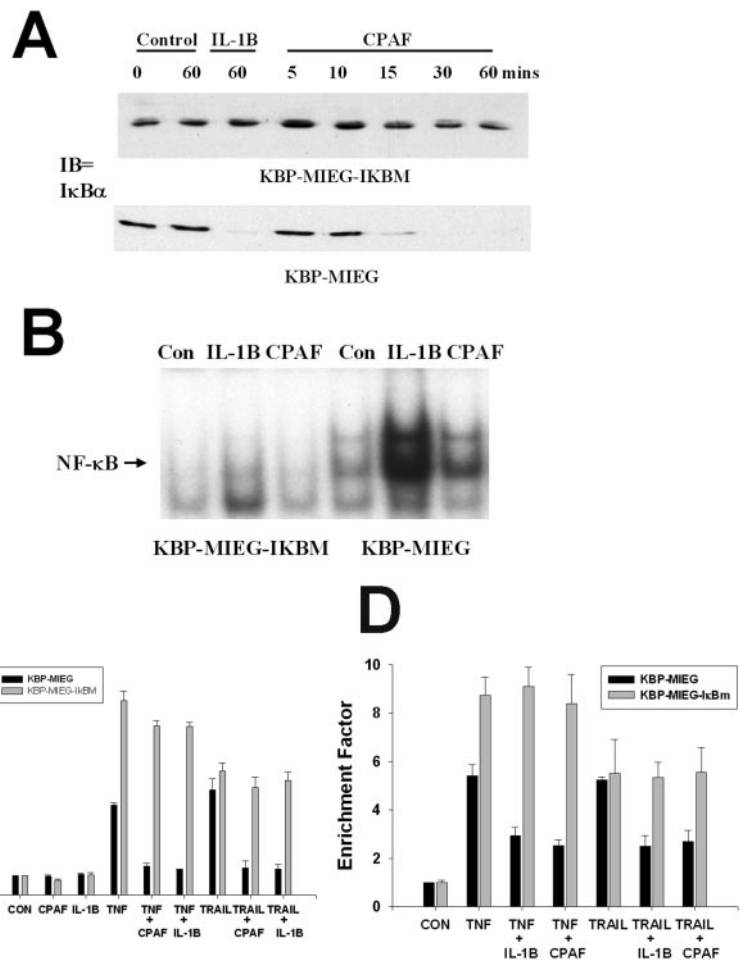
shown). The kinetics of PAF-induced NF-κB activation in epidermal cells are similar to results with PAF-R transfected into Chinese hamster ovary cells (28). In addition, both the IκB degradation and increased NF-κB binding activity were abolished in KBP cells expressing a super-repressor form of IκB. The inhibition of PAF-stimulated NF-κB activation effectively blocked the PAF-induced protection from apoptosis in KBP cells. These results confirm the findings of Schwarz and co-workers (8, 9) that the IL-1β-induced protection is mediated by the NF-κB pathway and thus establish that stimulation of the NF-κB pathway either by cytokines or by PAF can protect against TRAIL-induced apoptosis. Also similar to the results with IL-1β, we found that PAF-mediated protection occurred when the PAF-R was stimulated prior to or concurrent with apoptotic stimulation, as the addition of PAF following TRAIL or TNFα treatment did not protect cells from apoptosis. The time course of protection suggests that activation of NF-κB is necessary prior to induction of apoptosis for any protective effects. Our results are also supported by observations in other cell types that activation of NF-κB protects from apoptosis induced by members of the TNF receptor superfamily (39–42). Taken together, our results establish that in addition to growth factors and cytokines, G protein-coupled receptor agonists that activate the NF-κB pathway can provide anti-apoptotic effects.

In addition to the present finding that PAF-R activation protects from apoptosis induced by members of the TNFα receptor superfamily, PAF has also been shown to protect B-lymphocytes from apoptosis that occurs during immune cell maturation (29). In a similar manner, growth factors and cytokines such as tumor growth factor α and IL-1β can protect keratinocytes from TNFα- and TRAIL-induced apoptosis (8,

10). Our findings that PAF can exert protective effects from apoptosis appear to contradict previous studies suggesting that PAF acts to enhance apoptosis. Indeed PAF has been shown to augment ionophore-induced apoptosis in T cells (43). Furthermore, studies from our group have demonstrated that PAF can augment the UV radiation induced-apoptosis in epidermal cells (44). Thus, the ability of PAF or cytokines to exert protective effects may be dependent upon the specific type of pro-apoptotic stimulation. For example, PAF protects keratinocytes from TNFα-induced apoptosis but enhances UV-induced apoptosis in keratinocytes. In support of this hypothesis, it should be noted that the protective effects of the PAF-R on TRAIL-induced apoptosis required new RNA and protein synthesis, whereas the pro-apoptotic effects of the PAF-R following UV were not affected by RNA and protein synthesis inhibitors (44). The PAF-R is not unique in this respect as IL-1β had been shown to protect keratinocytes from Fas- and TRAIL-induced apoptosis but enhances the apoptotic response to UV (8, 9).

Although our results indicate that activation of the PAF-R induces anti-apoptotic actions through an NF-κB-dependent pathway, the precise mechanisms of PAF-R-mediated protection remains unknown. Studies in epidermal cells have suggested that the IAP mediate the IL-1β protective effect from TRAIL-induced apoptosis (9). IAPs block numerous apoptotic stimuli, including cell death induced by irradiation, growth factor withdrawal, and exposure to chemotherapeutic agents including agents acting on death receptors (45–47) by directly binding and inhibiting caspase proteins, including caspase-3 (48, 49). Thus, it is interesting to speculate that the PAF-R-induced protection might be mediated by IAPs in a manner similar to IL-1β. However, protection from apoptosis may re-

sult from the induction of proteins other than IAPs or may involve the actions of multiple anti-apoptotic proteins. Recently, the NF-κB-induced expression of Bcl-x$_L$ and the TRAIL decoy receptor DcR1 were reported to protect cells from TRAIL-induced apoptosis (39, 42, 50). Whereas Bcl-x$_L$ family members can block TRAIL-induced apoptosis by preventing the release of cytochrome *c* (51, 52), TRAIL decoy receptors have been proposed to act as functional antagonists by acting to attenuate the apoptotic actions of TRAIL (53). PAF has been shown to enhance the expression of Bcl-x$_L$ (54) and thus may modulate apoptosis through this pathway; however, the expression of TRAIL decoy receptors on primary keratinocytes has been reported to not affect the sensitivity to TRAIL-induced apoptosis compared with immortalized keratinocytes (55), suggesting that the PAF-R protection does not likely result from the production of decoy TRAIL receptors. Additional studies are underway in our laboratory to determine the downstream effect(s) of PAF-R-mediated anti-apoptotic actions.

Recent studies have suggested that chronic inflammatory conditions are associated with an increased pre-disposition to cancer (5–7) putatively by enhancing the survival of tumor cells. Inasmuch as the PAF system appears to play an important role in keratinocyte function and stress responses, this lipid mediator could also be an important cellular survival agent. Although human keratinocytes and epidermal cell lines do not synthesize significant amounts of PAF under resting conditions, numerous diverse stimuli associated with inflammation including reactive oxygen species, cytokines, and physical damage, in addition to UV radiation and chemotherapy, as well as PAF-R activation itself, all result in significant levels of PAF biosynthesis in these cell types. Keratinocytes also express functional PAF-Rs, and activation of the epidermal PAF-R leads to the biosynthesis and release of numerous pro-inflammatory mediators including IL-6, -8, and TNF, as well as release of PAF (21–23, 25, 30). Thus, the ability of the PAF-R to activate NF-κB and thereby protect epidermal cells from apoptosis appears to provide one putative mechanism for the pro-inflammatory-mediated decrease in tumor susceptibility to chemotherapy. Further studies are warranted to determine whether other GPCRs may be involved in the protection of cancer cells from apoptosis that occurs during inflammation.

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The Platelet-activating Factor Receptor Protects Epidermal Cells from Tumor Necrosis Factor (TNF) α and TNF-related Apoptosis-inducing Ligand-induced Apoptosis through an NF- κB-dependent Process

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