The Epidermal Platelet-activating Factor Receptor Augments Chemotherapy-induced Apoptosis in Human Carcinoma Cell Lines*

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Most chemotherapeutic agents exert their cytotoxic effects in part through the induction of apoptosis. In addition, many chemotherapeutic agents are potent pro-oxidative stressors. Although the lipid mediator platelet-activating factor (PAF) is synthesized in response to oxidative stress, and many epidermal carcinomas express PAF receptors, it is not known whether PAF is involved in chemotherapeutic agent-induced apoptosis. These studies examined the role of the PAF system in chemotherapy-mediated cytotoxicity using model systems created by retroviral mediated transduction of the PAF receptor-negative human epidermal carcinoma cell line KB with the human PAF receptor (PAF-R) and ablation of the endogenous PAF-R in the carcinoma cell line HaCaT with a retroviral mediated inducible antisense PAF-R vector. The presence of the PAF-R in these models resulted in an augmentation of apoptosis induced by chemotherapeutic agents etoposide and mitomycin C but not by tumor necrosis factor-related apoptosis-inducing ligand or by C2 ceramide. Oxidative stress and the transcription factor nuclear factorκB (NF-κB) are found to be involved in this augmentative effect because it was blocked by antioxidants and inhibition of the NF-κB pathway using a super-repressor form of inhibitor B. These studies provide evidence for a novel pathway whereby the epidermal PAF-R can augment chemotherapy-induced 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 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 addition to normal physiological conditions, essentially all chemotherapeutic agents exert their effects by induction of apoptosis (3). Thus, regulation of apoptosis can have important consequences both during development and in the treatment of cancer.

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Accumulating evidence suggests platelet-activating factor (PAF1; 1-alkyl-2-acetyl glycerophosphocholine)-mediated pathways are involved in cutaneous inflammation and keratinocyte stress responses. PAF is a glycerophosphocholine-derived lipid mediator implicated in numerous inflammatory processes (for a review, see Ref. 4). 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 (5–9). 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) (for a review, see Ref. 10). In addition to producing PAF, keratinocytes and many carcinoma cell lines also express the PAF-R (11). Activation of the epidermal PAF-R leads to the production and release of PAF, IL-6, IL-8, IL-10, tumor necrosis factor-α, and eicosanoids (12–14).

In addition to a tightly coupled enzymatic pathway for PAF synthesis involving the subsequent actions of phospholipase A2 and acetyltransferase, PAF and PAF-like lipids can be produced via direct free radical-mediated cleavage of glycerophosphocholines (GPCs) containing unsaturated fatty acids (e.g. arachidonate) at the sn-2 position (15). Recent studies have suggested that the PAF-R is a target for ultraviolet B radiation in part through this non-enzymatic pathway of PAF agonist formation (7, 8, 13, 14). For example, the presence of the PAF-R augments the production of cytokines IL-8 and tumor necrosis factor-α as well as the apoptotic response of ultraviolet B radiation in carcinoma cell lines, processes inhibited by antioxidants (7, 8, 13, 16).

Inasmuch as other proapoptotic agents including chemotherapeutic agents are also potent pro-oxidative stressors, the objective of these studies was to assess whether the epidermal PAF-R could augment the cytotoxic effects of chemotherapeutic agents. Using a PAF-R-negative human carcinoma cell line transduced with the PAF-R and a novel retroviral mediated antisense strategy to ablate endogenous PAF-R expression in a PAF-R-positive epithelial carcinoma cell line, we demonstrate that the PAF-R can augment apoptosis due to etoposide and mitomycin C but not other agents such as C2 ceramide and TRAIL.

1 The abbreviations used are: PAF, platelet-activating factor; PAF-R, PAF receptor; CPAF, 1-hexadecyl-2-N-methylcarbamoyl-glycerophosphocholine; IL, interleukin; GPC, glycerophosphocholine; GPCR, G protein-coupled receptor; fMLP, N-formyl-methionyl-leucyl-phenylalanine; fMLP-R, fMLP receptor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; NF-κB, nuclear factorκB; as-PAFR, antisense PAF-R; Tet, tetracycline; AMC, 7-amino-4-methylcoumarin; CM-DCFDA, 5,6-chloromethyl-2’,7’-dichlorodihydrofluorescein diacetate.
The mechanism of how PAF-R activation could augment chemotherapy-induced apoptosis was also examined in these studies, and it was found to be dependent upon activation of NF-κB proteins, sequence-specific transcription factors induced in response to inflammatory and other stressor stimuli (for a review, see Ref. 17). Of note, activation of NF-κB has been shown to protect epithelial cells against the apoptotic effects of Tumor necrosis factor-α-enhanced expression of the antiapoptotic protein survivin, the stimulant that can induce this apoptotic response include IL-1β, γ interferon, and PAF. Although associated with a protective response in this setting, activation of the NF-κB pathway has also been shown to augment proapoptotic responses (20–22). The current findings describe a putative mechanism by which this G protein-coupled receptor can augment pro-oxidative and proapoptotic stressors in epithelial cells and could provide a novel pathway by which carcinomas can be more susceptible to chemotherapy-induced cytotoxicity.

MATERIALS AND METHODS

Reagents—All chemicals were obtained from Sigma unless indicated otherwise. Recombinant human TRAIL/APO2L was purchased from Chemicon (Temecula, CA). IL-1β was purchased from Peprotek (Rocky Hill, NJ).

Cell Culture—The human epidermoid cell line KB and human keratinocyte cell line HaCaT were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) and were supplemented with 10% fetal bovine serum (HyClone, Logan, UT). 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 (12). KB cells transduced with the PAF-R (KBP) or with control MSCV2.1 retrovirus (KB) were characterized by Southern and Northern blot analysis and by radioligand binding and calcium mobilization studies to demonstrate that the PAF-R was functional (12). Similarly, to create a KB-mu cell line expressing the fMLP receptor (KBF), the IMLP-R cDNA was cloned into the MSCV2.1 retroviral vector. The presence of a functional IMLP-R in KBF cells was confirmed by Northern blotting to demonstrate fMLP-R mRNA and by a positive intracellular calcium mobilization response to exogenous fMLP using the fluorescent dye Indo-1 as described previously (12). All experiments were replicated with at least two separate KBF, KBP, and KBF clones.

Generation of HaCaT Cells Expressing an Inducible PAF R Antisense HaCaT as-PAFR System—The HaCaT as-PAFR model system was established using the RevTet-on system (Clontech). PAF receptor cDNA was cloned in a reversed orientation into the HindIII site of the response retroviral vector pRevTRE. The insert orientation was confirmed by restriction mapping and sequencing. To generate the retroviruses, amphotrophic packaging cell line Phoenix 293 was transfected with either the retroviral Tet-on regulator, the pRevTRE-as-PAFR, or the pRevTRE backbone using FuGENE 6 (Roche Applied Science), and transient supernatants containing infectious amphotropic retrovirus were collected 48 h later. In the first round of infection, the infectious supernatants containing infectious amphotropic retrovirus were collected 48 h later. The second round of infection involved transduction of PAF-R-negative KB cells with the MSCV2.1 retrovirus encoding the human leukocyte PAF-R as described previously (12). KB cells transduced with the PAF-R (KBP) or with control MSCV2.1 retrovirus (KB) were subjected to infection with the infectious supernatant made from transient supernatants containing infectious amphotropic retrovirus (KBM), KBP, and KBF clones. To generate the retroviral vector pRevTRE-as-PAFR, the insert orientation was assessed by restriction endonuclease mapping and sequencing. Infectious amphotropic retroviruses were produced from both KBM and KBP. The infectious supernatant was collected 48 h later containing infectious viruses, and the supernatants collected 48 h later containing infectious viruses were then used to infect KBM/KBP cells. Transduced cells were sorted by a fluorescence-activated cell sorter on the basis of enhanced green fluorescent protein expression.

Cell Death Detection Assay—Apoptosis was determined quantitatively using a cell death detection enzyme-linked immunosorbent assay (Roche Applied Science) 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; Alexis Biochemicals, San Diego, CA) as described previously (25). Reactions were performed for 1 h at 37°C. Release of the fluorogenic AMC moiety was measured using a Hitachi F2000 spectrophotofluorometer (excitation, 380 nm; detection, 460 nm). The fluorescence 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 (Nanorange protein quantitation reagent, Molecular Probes).

Intracellular Hydrogen Peroxide Measurements—To measure intracellular hydrogen peroxide levels, cells were plated on coverslips were loaded with a 10 μM concentration of the fluorescent dye CM-DCFDA (Molecular Probes) for 6 h at 37°C in the dark. CM-DCFDA fluorescence was measured at an excitation wavelength of 480 nm and an emission wavelength of 520 nm using a Hitachi F2000 spectrophotofluorometer (excitation, 480 nm; detection, 520 nm).

NF-κB Reporter Assay—Cells were plated at a density of 1.5 × 10⁴ cells in a 10-cm dish and allowed to stabilize for 1 day. Cells were then transfected using FuGENE 6 (Roche Applied Science) with 10 μg of NF-κB reporter plasmid (pNF-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, IL-1β, or chemotherapeutic agents 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 lumimeter (Lumat). Luciferase activities were normalized for each transfaction using the control β-galactosidase activities.

RESULTS

The KB PAF-R Model System—Since 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 epithelial cell biology. The human epidermal carcinoma cell line KB does not express functional PAF-Rs unlike normal human keratinocytes and the human keratinocyte-derived carcinoma cell line HaCaT (11, 12). A PAF-R-positive KB cell line, KBF, 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, KBF. Expression of the PAF-R protein was verified by binding studies using radiolabeled PAF-R antagonist WEB 2066 (12). Calcium mobilization studies demonstrated that the KB PAF-R was functionally active (12). Therefore, this in vitro epidermoid system consists of both PAF-R-negative (KBM) and -positive (KBF) cells.

Cytotoxic Effects of Chemotherapeutic Agents in KB Cells—In initial experiments, the dose-response (Fig. 1) and time-response (Fig. 2) effects of etoposide, mitomycin C, C์ ceramide, and...
and TRAIL on apoptosis in KBM versus KBP cells were determined by measurement of caspase-3 enzyme activity. Exposing KB cells to both chemotherapeutic agents etoposide and mitomycin C as well as C2 ceramide and TRAIL resulted in increased caspase-3 enzyme activity levels. The levels of caspase-3 enzyme induction were enhanced in PAF-R-expressing KBP over control KBM cells in response to etoposide and mitomycin C. However, C2 ceramide and TRAIL treatment resulted in similar levels of apoptosis in KBP and KBM cells.

We next examined the effect of chemotherapeutic agents on KB cells using a cell death detection enzyme-linked immunosorbent assay as a second distinct marker of apoptosis in carcinoma cells. As shown in Fig. 3, treatment with etoposide and mitomycin C resulted in an enhanced release of mono- and oligonucleosomes into the cytoplasm in KBP over KBM cells. However, C2 ceramide and TRAIL treatment resulted in similar levels of apoptosis in KBP and KBM cells. We next examined the effect of chemotherapeutic agents on KB cells using a cell death detection enzyme-linked immunosorbent assay as a second distinct marker of apoptosis in carcinoma cells. As shown in Fig. 3, treatment with etoposide and mitomycin C resulted in an enhanced release of mono- and oligonucleosomes into the cytoplasm in KBP over KBM cells. However, C2 ceramide and TRAIL treatment resulted in similar levels of apoptosis in KBP and KBM cells.

Effects of Ablation of the Epidermal PAF-R on Chemotherapeutic Agent-induced Cytotoxicity—The next studies were designed to assess whether endogenous levels of the epidermal PAF-R could affect responsiveness of the cells to chemotherapeutic agents. To address this question HaCaT keratinocytes that express native PAF-Rs were transduced with a retrovirus from which an antisense RNA corresponding to the PAF-R mRNA could be induced (HaCaTpRevTRE-as-PAFR). Radioligand binding studies with the PAF-R antagonist [3H]WEB 2086 were used to assess the ability of the antisense system to ablate PAF-R expression in HaCaT cells. Binding studies using 10 nM [3H]WEB 2086 revealed 56 ± 13 (mean ± S.E., n = 3) fmol of [3H]WEB 2086 specifically bound/10^6 untreated HaCaTpRevTRE-as-PAFR cells. In contrast, KBP cells specifically bound 357 ± 73 fmol of [3H]WEB 2086/10^6 cells,

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To assess whether this proapoptotic effect of the PAF-R is a general characteristic of GPCRs, KB cells were transduced with the fMLP-R. This GPCR was chosen as a control since epithelial cells, unlike bacteria, do not produce the peptide ligand fMLP. Calcium mobilization assays with Indo-1-loaded KBF cells resulted in intracellular calcium responses to 100 nM fMLP confirming that the fMLP-R was functional (data not shown). Consistent with the notion that chemotherapeutic agents are not activating GPCRs in a ligand-independent fashion, transducing KB cells with the fMLP-R did not affect responses to etoposide or mitomycin C (Fig. 4).

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Fig. 3. Effects of etoposide, mitomycin C, C₂ ceramide, and TRAIL on nucleosome enrichment in KBM versus KBP cells. KB cells were treated with 6 μg/ml etoposide, 5 μg/ml mitomycin C, 200 μM C₂ ceramide, or 40 ng/ml TRAIL for 16 h. Following the treatment duration, the cells were collected and lysed, and enrichment of nucleosomes into the cytoplasm was determined as an index of apoptotic activity in KBP cells. Data represent the fold difference in enrichment compared with untreated control (CON). The values are the mean ± S.D. of enhancement from a typical experiment from three to five separate experiments. ETOP, etoposide; MMC, mitomycin C; CER, C₂ ceramide.

Fig. 4. Effects of etoposide, mitomycin C, C₂ ceramide, and TRAIL on caspase-3 enzyme activation in KBM versus KBP cells. KB cells were treated with 6 μg/ml etoposide, 5 μg/ml mitomycin C, 200 μM C₂ ceramide, or 40 ng/ml TRAIL for 16 h. Following the treatment duration, the cells were collected and lysed, and caspase-3-specific activity was determined as an index of apoptotic activity after treatment. The values are mean ± S.D. of duplicate samples from a typical experiment from three to four separate experiments. CON, control.
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Ablation of the endogenous PAF-R in HaCaT cells resulted in a diminishment of caspase-3 induction due to chemotherapeutic agents mitomycin C and etoposide, yet it did not affect responses to C2 ceramide and TRAIL (Fig. 5C). Similar findings were obtained when apoptosis was measured by release of mono- and oligonucleosomes by enzyme-linked immunosorbent assay (Fig. 5D). These studies indicate that endogenous levels of PAF-R expression in carcinomas are adequate to modulate

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Chemotherapy activates PAF receptor.

The role of oxidative stress on PAF-R augmentation of chemotherapeutic agent-induced cytotoxicity—The finding that the epidermal PAF-R could augment apoptosis due to etoposide and mitomycin C led to further studies defining whether this was occurring secondarily to the pro-oxidative characteristics of these agents. To test this, KB cells were loaded with the H$_2$O$_2$-sensitive fluorescent dye CM-DCFDA, and levels of intracellular H$_2$O$_2$ were measured. As has been reported previously (26), activation of the epidermal PAF-R with CPAF resulted in increased intracellular H$_2$O$_2$ (Fig. 6). Consistent with their reported ability to induce a pro-oxidative stress (27, 28), both etoposide and mitomycin C treatment resulted in increased intracellular H$_2$O$_2$ levels in KB cells. The levels of intracellular H$_2$O$_2$ generated in response to chemotherapeutic agents were greater in KBP than in KBM cells, which is consistent with the hypothesis that treatment of cells with etoposide or mitomycin C can activate the PAF-R. Treatment with TRAIL (Fig. 6) or C$_2$ ceramide (not shown) did not significantly affect intracellular H$_2$O$_2$ levels.

The next studies used antioxidants to confirm the involvement of oxidative stress in the ability of chemotherapeutic agents to activate the epidermal PAF-R and thus modulate apoptosis. Pretreatment of KB cells with the antioxidants trolox and resveratrol (29) blunted etoposide-mediated caspase-3 induction selectively in KBP cells (Fig. 7). These antioxidants similarly blocked caspase-3 induction by mitomycin C by ~50% in KBP cells (not shown). However, pretreatment with these two antioxidants did not affect apoptosis induced by etoposide or mitomycin C in KBM cells nor did it alter the effects of TRAIL or C$_2$ ceramide in either KBM or KBP cells (not shown). Altogether these studies provide solid support for the hypothesis that oxidative stress induced by chemotherapeutic agents results in PAF-R activation, which can augment the proapoptotic effects of these agents.

Involvement of the NF-κB system in PAF-R-mediated augmentation of chemotherapeutic agent-induced cytotoxicity—The mechanism by which the PAF-R could augment the cytotoxic effects of chemotherapeutic agents is not known. Indeed, activation of the epidermal PAF-R is linked to numerous signal transduction pathways that could be responsible for the augmented apoptotic effects (10). It should be noted that like a diverse group of stimuli including pro-oxidative stressors the epidermal PAF-R induces the transcription factor NF-κB, and activation of this same pathway has also been reported to have potent proapoptotic effects in various cell types (20–22).

To test whether PAF-R-mediated activation of the NF-κB pathway was involved in the augmentation of chemotherapy-mediated apoptosis, we examined the ability of etoposide and mitomycin C to activate the NF-κB system in KB cells as well as whether a dominant-negative inhibitor of NF-κB could affect PAF-R-mediated augmentation of apoptosis in response to these chemotherapeutic agents. First, gel shift studies were used to examine levels of NF-κB in KBM and KBP cells. ETOP, etoposide; TRO, trolox; RES, resveratrol; CON, control.

![Figure 7](http://www.jbc.org/)

**Figure 7.** Effect of antioxidants on etoposide-induced caspase-3 enzyme activation in KB cells. KB cells were pretreated for 30 min with a 10 μM concentration of antioxidants trolox and resveratrol or Me$_2$SO vehicle before addition of 6 μg/ml etoposide. Following a further 16-h treatment duration, the cells were collected and lysed, and caspase-3-specific activity was determined as an index of apoptosis. The values are mean ± S.D. of a typical experiment from three separate experiments. ETOP, etoposide; TRO, trolox; RES, resveratrol; CON, control.

![Figure 8](http://www.jbc.org/)

**Figure 8.** Effect of chemotherapeutic agents on NF-κB binding and activation in KB cells. A, following a 1-h treatment with 100 nM CPAF, 100 nM fMLP, 6 μg/ml etoposide, 5 μg/ml mitomycin C, or 25 ng/ml IL-1β, KB cells were harvested and lysed. 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 or OCT-1 control and electrophoretically separated. B, KBM or KBP cells transfected with NF-κB luciferase reporter gene were stimulated with 6 μg/ml etoposide, 5 μg/ml mitomycin C, 100 nM CPAF, or 25 ng/ml IL-1β for 6 h, and relative luciferase activity was normalized to β-galactosidase. * denotes statistically significant differences between KBP and KBM cells. ETOP, etoposide; MMC, mitomycin C; CON, control; no tx, no treatment.
Recent studies have shown that oxidative stress can trigger the production of lipids with PAF-R agonist activity. For example, chemical oxidation of low density lipoproteins results in the production of a PAF-R activity that has been shown to consist of fragmented alkyl GPCs including 1-hexadecyl-2-butanoyl-GPC and 1-hexadecyl-2-butenoyl-GPC along with trace levels of authentic PAF (15). Of significance, systemic exposure to the strong oxidative stress of tobacco smoke has been shown to induce the production of these PAF-R agonists in hamsters in vivo (32). Other pro-oxidative stressors such as UVB radiation can also stimulate the PAF-R through production of PAF and PAF-like lipids (7, 13). Several lines of evidence suggest that chemotherapeutic agents etoposide and mitomycin C are activating the PAF-R through the production of PAF/PAF-like species. First, these chemotherapeutic agents are known pro-oxidative stressors (27, 28); this is confirmed in the present studies using the fluorescent dye CM-DROP to measure intracellular H2O2 (Fig. 6). Other proapoptotic stimuli that do not induce an oxidative stress (C2 ceramide and TRAIL) did not have differential effects on KBM versus KBP cells. Second, pretreatment with antioxidants blocked the augmentation of chemotherapy-mediated apoptosis found in PAF-R-expressing KBP yet did not affect that in KBM cells. The finding that expression of the GPCR for fMLP in KB cells (KBF) did not affect chemotherapy-induced apoptosis suggests that these agents were not nonspecifically activating the GPCR in a ligand-independent fashion. Thus, the data presented in these studies support the hypothesis that these chemotherapeutic agents have the ability to induce the production of PAF/PAF-like species. Ongoing studies are attempting to define the structural identity of chemotherapy-induced PAF-R agonistic activity.

The current studies also begin to define mechanistically how PAF-R activation can augment apoptosis by chemotherapeutic agents. GPCRs including the PAF-R have been shown to activate the NF-κB system (19). Consistent with the involvement of NF-κB in the augmentation of chemotherapy-induced apoptosis, etoposide and mitomycin C treatment of KBP cells resulted in a much greater level of NF-κB binding activity than that in KBM cells. Blocking NF-κB activation with a super-repressor IκB mutant decreased the augmentative effect of PAF-R expression on chemotherapy-induced apoptosis. Of note, preliminary studies in our laboratory indicate that the augmentation of UVB radiation-induced apoptosis in KBP cells is also blocked by ablation of NF-κB activation (data not shown).

Although expression of the fMLP-R in KB cells did not affect chemotherapy-induced apoptosis, this receptor was found to have in common with the PAF-R the ability to activate the NF-κB pathway. Indeed fMLP treatment of KBF cells resulted in NF-κB binding by gel shift assays (Fig. 8B) and induced IκBα degradation (data not shown). Transfection of KBF cells with an NF-κB-luciferase reporter plasmid to measure NF-κB activity also revealed increased levels in KBF cells in response to fMLP. In fact, the effects of 100 nM fMLP on KBF cells were similar to those induced by 100 nM CPAF in KBF cells (data not shown). The ability of the fMLP-R to stimulate the NF-κB system yet not to affect chemotherapy-induced responses of NF-κB activation or apoptosis supports the contention that these pro-oxidative chemotherapeutic agents are activating the epidermal PAF-R via production of endogenous ligands.

The mechanism(s) by which the NF-κB pathway can potentially promote or protect against apoptosis in epithelial carcinomas is unclear at this time and is an active area of study. Although NF-κB activation by the PAF-R appears to be responsible for promoting apoptosis in response to chemotherapeutic agents or UVB radiation, we have previously demonstrated...
that PAF-R activation of NF-κB protects epithelial cells against apoptosis induced by TRAIL and tumor necrosis factor-α (19). That PAF-R-mediated activation of the NF-κB system could yield such dissimilar outcomes as either promoting or protecting against proapoptotic stimuli is consistent with data from several recent studies from a variety of cell types (18–22). For example, in rat primary forebrain cultures N-methyl-D-aspartate-induced apoptosis has been shown to be dependent upon superoxide-mediated NF-κB activation (20). Similar findings have been reported in glutamate-induced cytotoxicity (21). One example, in rat primary forebrain cultures 

Along with the effects of NF-κB translocation on apoptotic defenses, another possible explanation for the differences in PAF-R-mediated NF-κB-dependent protection against or augmentation of apoptosis lies in the nature of the proapoptotic stimuli. Chemotherapeutic agents and UVB radiation are potent DNA-damaging agents, and thus concomitant activation of the NF-κB system in the presence of significant DNA damage might not allow antiapoptotic proteins such as inhibitor of apoptosis proteins to be produced. Thus, other potential NF-κB-linked proapoptotic pathways could predominate.

In summary, the present studies demonstrate that the presence of the epidermal PAF-R results in augmentation of the cytoprotective effects of etoposide and mitomycin C. We describe a novel pathway by which these pro-oxidative stressors induce PAF/PAF-like species that then activate the NF-κB pathway through the PAF-R. The biological significance of this pathway is not clear, but it could be the impetus for further studies to define whether the presence of the PAF-R could modulate chemotherapeutic responses \textit{in vivo}. Finding that this novel pathway is an important determinant in the effectiveness of certain chemotherapeutic agents would be useful for planning chemotherapeutic strategies in PAF-R-expressing tumor cells.

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