Interleukin-8 Production Is Regulated by Protein Kinase C in Human Keratinocytes

Marie Chabot-Fletcher, John Breton, John Lee,* Peter Young,† and Don E. Griswold

Departments of Inflammation Pharmacology, *Cellular Biochemistry, and †Molecular Genetics, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania, U.S.A.

Interleukin-8 (IL-8) is a potent pro-inflammatory molecule present in high amounts in psoriatic skin. Here it may play an important role in the keratinocyte hyperproliferation and the neutrophil and T-lymphocyte infiltration associated with the disease. In this study the effect of protein kinase C inhibitors on IL-8 production by human keratinocytes *in vitro* was investigated. The anti-inflammatory and immunomodulatory compound auranofin ([1-thio-beta-D-glucopyranose-2,3,4,6-tetraacetato-S] [triethylphosphine] gold) is known to inhibit protein kinase C. In addition, auranofin has been shown to inhibit skin inflammation. As such, auranofin was also studied for its effect on IL-8 production. Auranofin and staurosporine, inhibitors of protein kinase C, inhibited phorbol-myristate-acetate–stimulated IL-8 production. Northern analysis of IL-8 mRNA revealed that the inhibition of IL-8 production was associated with an inhibition of IL-8 mRNA expression. In contrast, these compounds potentiated the minimal IL-8 protein and mRNA seen in response to interleukin-1β or tumor necrosis factor-α. These findings suggest that IL-8 synthesis may be either positively or negatively regulated by protein kinase C depending on the stimulus. ***Key words: auranofin/interleukin-1/phorbol ester/IL-8 mRNA. J Invest Dermatol 103:509–515, 1994***

Interleukin-8 (IL-8) is a potent pro-inflammatory polypeptide composed of 72 amino acids with a molecular weight of approximately 8 kDa [1]. Exogenous stimuli such as lipopolysaccharide (LPS), phorbol myristate acetate (PMA), tumor necrosis factor-α (TNFα), and interleukin-1β (IL-1β), will induce the production of this molecule in a variety of cells including monocytes, endothelial cells, and keratinocytes [2–8]. IL-8 acts as a chemotactic and activation factor for both neutrophils and T lymphocytes [2,9]. Stimulation of neutrophils with IL-8 is reported to result in degranulation and enzyme release in the presence of cytochalasin B [10,11], increased adherence to unstimulated endothelial cells [12], and the production and release of leukotriene B_4 (LTB_4) and 5-hydroxyeicosatetraenoic acid (5-HETE) [13,14]. In addition, IL-8 has been reported to induce the proliferation of human keratinocytes *in vitro* [15,16].

Cytokines are believed to play an important role in many inflammatory diseases of the skin. Recently, a role for IL-8 in dermatologic inflammation, particularly in psoriasis, has been suggested. Psoriasis is characterized by keratinocyte hyperproliferation and the accumulation of T lymphocytes and neutrophils in the epidermis. These hallmarks may be mediated by the activities of IL-8. In support of this, prodigious amounts of IL-8 were measured in scales taken from psoriatic lesions but not in uninvolved psoriatic and non-psoriatic skin [6,17–21]. In addition, high levels of IL-8 mRNA were demonstrated in psoriatic lesions using *in situ* hybridization [6,22]. These studies suggest that IL-8 levels are not only elevated in psoriatic skin but that keratinocytes *in situ* are capable of synthesizing IL-8.

Interleukin-8 is not found in normal skin, nor is it constitutively expressed in unstimulated keratinocytes grown in tissue culture. However, stimulation of human keratinocytes *in vitro* with IL-1β, TNF-α, or the phorbol ester PMA results in a rapid increase in IL-8 mRNA levels [6–8] and the appearance of IL-8 protein in the culture supernatants of TNFα-stimulated keratinocytes has been reported [7]. In light of the potential role of IL-8 in inflammatory skin diseases, an understanding of the regulation of its production may provide some information valuable in their treatment.

Recently, protein kinase C inhibitors have been shown to modulate a variety of IL-1β– and PMA-stimulated events [23,24]. In light of these findings, the ability of protein kinase C (PKC) inhibitors to modulate IL-8 expression was investigated. Included in the compounds tested was auranofin ([1-thio-β-D-glucopyranose-2,3,4,6-tetraacetato-S] [triethylphosphine] gold). This compound has been shown to possess various immunomodulatory and anti-inflammatory properties [25,26] and is able to inhibit protein kinase C [27]. Inhibition of protein kinase C was found to modulate IL-8 production in keratinocytes stimulated with PMA or IL-1β. Our findings suggest that IL-8 synthesis may be regulated either positively or negatively by protein kinase C, depending on the stimulus.

MATERIALS AND METHODS

Compounds and Reagents Auranofin (SK&F D-39162), triethylphosphine oxide, and 2,3,4,6-tetra-O-acetyl-1-β-thioethyl-O-glycoside were obtained from the Drug Substances and Products Registry of SmithKline Beecham Laboratories (King of Prussia, PA). Sodium aurothiomalate was purchased from Aldrich Chemical Co. (Milwaukee, WI). PMA and staurosporine were purchased from Sigma Chemical Co. (St. Louis, MO), H-7 from Biomol (Plymouth Meeting, PA), and chelerythrine from L C Services (Woburn, MA). IL-1β and TNFα were prepared at SmithKline Beecham Pharmaceuticals (King of Prussia, PA) as described previously [28,29].

Manuscript received January 7, 1994; accepted for publication May 16, 1994.

Reprint requests to: Dr. Marie Chabot-Fletcher, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406.

Abbreviations: 5-HETE, 5-hydroxyeicosatetraeinoic acid; LTB_4, leukotriene B_4.

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Cell Culture Human foreskin keratinocytes were purchased from Clonetics Corp. (San Diego, CA) and grown as a monolayer in 24-well tissue culture plates at 37°C, 5% CO_2 in 1 ml of keratinocyte growth medium (KGM) (Clonetics Corp., San Diego, CA). These cultures are free of contaminating fibroblasts and melanocytes. KGM is based on the MCDB 153 formulation supplemented with epidermal growth factor (0.1 ng/ml), hydrocortisone (0.5 μg/ml), insulin (5.0 μg/ml), gentamicin (50 μg/ml), amphotericin-B (50 ng/ml), and bovine pituitary extract. Second-passage cultures were used on reaching 80–90% confluency, at which point they contained approximately 1×10^5 cells per well.

Interleukin-8 Production from Human Keratinocytes *in Vitro* In preliminary experiments, keratinocytes were either incubated in 1 ml of KGM containing various concentrations of PMA or IL-1β as indicated, or at a given concentration of the stimulant for various lengths of time at 37°C. In subsequent studies, the cells were incubated in KGM alone, or with the addition of drug just prior to stimulation with PMA (0.1 μM) or IL-1β (1.0 ng/ml) unless indicated. Compounds and PMA were dissolved in dimethyl sulfoxide (DMSO) and added to the samples such that the final concentration of DMSO did not exceed 0.2%. IL-1β was dissolved in KGM. After 18 h incubation the supernatants were collected and stored frozen until assayed for IL-8 content. Interleukin-8 in the culture supernatants was determined using an IL-8 immunoassay kit purchased from R&D Systems (Minneapolis, MN) following the manufacturer's instructions.

Northern Blot Analysis of Interleukin-8 mRNA Production The effect of PMA or IL-1β on keratinocyte IL-8 mRNA levels was determined by Northern blot analysis. Briefly, keratinocyte monolayers were grown in 175-cm² flasks until reaching 90% confluence as described above. The cells were stimulated with either PMA (0.1 μM) for 3 h or IL-1β (1.0 ng/ml) for 1 h in the presence or absence of drug. RNA was prepared using the guanidinium thiocyanate–cesium chloride cushion method described before [30]. RNA was electrophoresed through a 1% formaldehyde agarose gel and blotted onto 0.2-μm nitrocellulose (Schleicher & Schuell, Keene, NH) according to established methods [30]. The blot was prehybridized in 50% formamide, 5 × Denhardt's buffer [50 × = 5 g Ficoll (Type 400, Pharmacia), 5 g polyvinylpyrrolidone, 5 g bovine serum albumin (Pentex Fraction V) in 500 ml H_2O], 5 × SSPE, and 100 mg/ml denatured salmon sperm DNA for 2–4 h at 42°C. The blot was then hybridized overnight to the alkali-denatured IL-8 cDNA probe in the same solution containing 10% dextran (w/v). The filters were washed twice at room temperature in 2 × SSPE/0.1% sodium dodecylsulfate (SDS) (1 × SSPE = 150 mM NaCl, 2 mM Na H_2PO_4, 1 mM ethylenediaminetetraacetic acid [EDTA]) followed by washes in 1 × SSPE and 0.5 × SSPE at 50°C and autoradiography. After autoradiography, the blot was stripped of radioactive probe by boiling in H_2O and rehybridized as above with a cyclophillin cDNA probe. The IL-8 probe was a synthetic DNA purchased from R&D Systems and isolated from the vector by digestion with BamHI and EcoRI, followed by agarose gel purification. The cyclophilin cDNA fragment has been previously described [31]. Both fragments were labeled with α-^{32}P-dCTP using a random priming kit from Stratagene (La Jolla, CA) and purified through a Stratagene push column. Northern blots were quantitated by densitometry as described previously [31].

RESULTS

Interleukin-8 Production in Human Keratinocytes Exogenous stimuli such as PMA and IL-1β will induce the production of IL-8 in a variety of cells including monocytes and endothelial cells. Stimulation of human keratinocytes *in vitro* with PMA resulted in a dose-dependent production of IL-8 that was maximal at 0.1 μM **(Fig 1*A*)**. Interleukin-1β also stimulated the production of IL-8, with maximal stimulation observed at approximately 1.0 ng/ml **(Fig 1*B*)**. However, the extent of IL-8 production in response to this cytokine proved to be much less than it was in response to the phorbol ester PMA. That is, PMA consistently resulted in the production of 4–6 times as much IL-8 in the culture fluid as did stimulation with IL-1β.

The release of IL-8 into the culture supernatant was time dependent, increasing with time up to 48 h for both stimuli as well as for stimulation with TNFα **(Fig 2)**. The greatest release occurred during the first 8 h of stimulation with PMA, whereas the time course of IL-1β– and TNFα-stimulated IL-8 production was more protracted.

Effect of PKC Inhibitors on IL-8 Production Cytokine production in various systems has been shown to be regulated by PKC

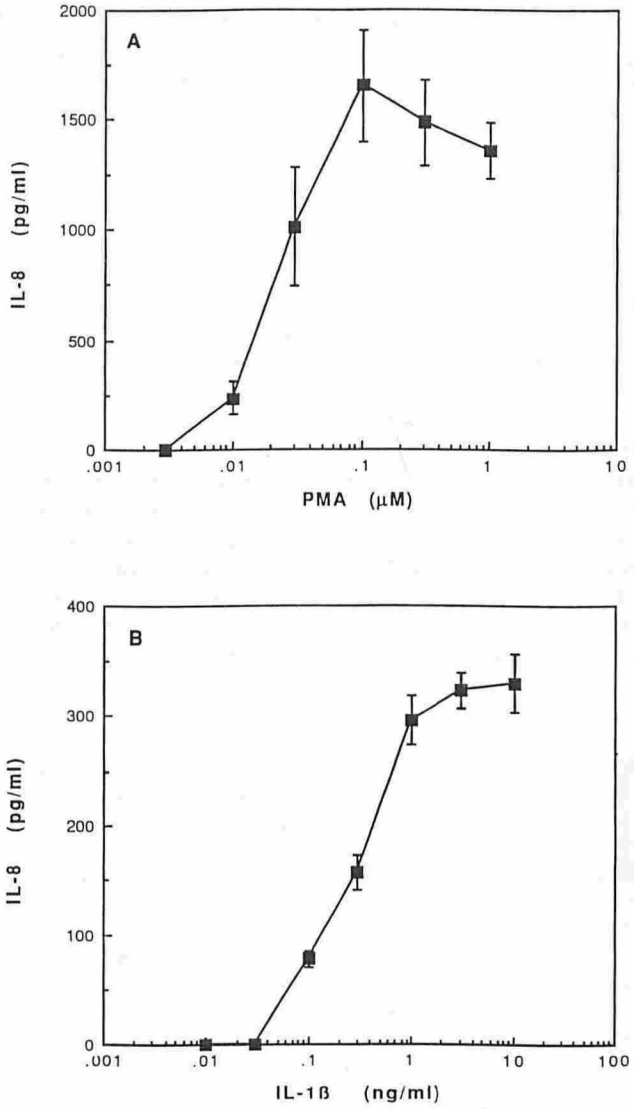


Figure 1. Effect of PMA- or IL-1β stimulation on keratinocyte IL-8 production. Human keratinocytes *in vitro* were stimulated with various concentrations of the phorbol ester PMA *(A)* or interleukin-1β *(B)* as described in *Materials and Methods*. After 18 h of incubation, the medium was collected and the IL-8 content determined by immunoassay as described. Each point represents the mean ± SD of four individual samples from a representative of at least two experiments.

[23]. Therefore, in light of the possible relation between inhibition of PKC and IL-8 synthesis, several known PKC inhibitors were studied for their ability to modulate IL-1β– and PMA-stimulated IL-8 production. The PKC inhibitor staurosporine was found to be a very potent inhibitor of IL-8 production in response to PMA **(Fig 3)**. The inhibition was dose dependent, with an IC_{50} of approximately 1.0 nM. In contrast, staurosporine potentiated IL-1β–stimulated IL-8 production in a somewhat dose-dependent manner **(Fig 3)**. Similar results were observed with the selective PKC inhibitor chelerythrine (data not shown). Thus, inhibition of PKC either inhibited or potentiated IL-8 synthesis depending on whether the keratinocytes were stimulated with PMA or IL-1β. Interestingly, staurosporine treatment resulted in a dose-dependent accumulation of IL-8 in the absence of stimulus, which was similar to its effect seen in IL-1β–stimulated cells although of lesser magnitude (data not shown).

Modulation of IL-8 Production by Auranofin To determine whether the anti-inflammatory and immunomodulatory actions of

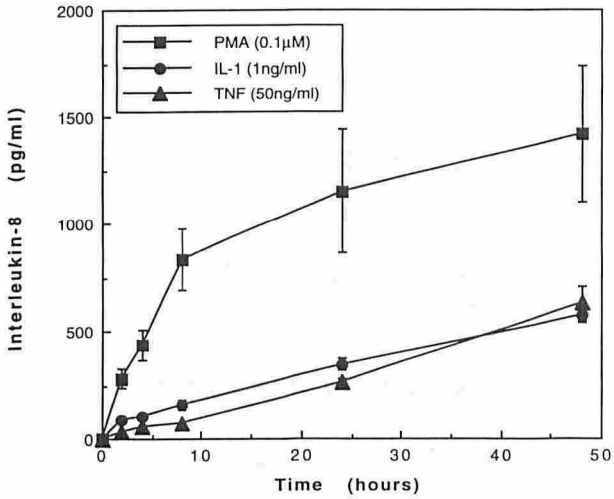


Figure 2. Timecourse of IL-8 production. Human keratinocytes *in vitro* were stimulated with either PMA (0.1 μM), IL-1β (1.0 ng/ml), or TNFα (50 ng/ml) and incubated for various lengths of time, as indicated. At the end of the incubation period, the medium was collected and the IL-8 content in each sample determined by immunoassay as described. Each point represents the mean ± SD of four individual samples from a representative of at least two experiments.

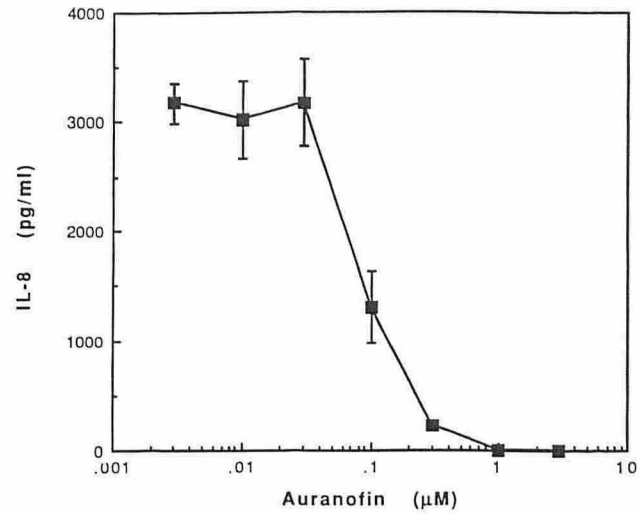


Figure 4. Effect of auranofin on PMA-stimulated IL-8 production. Human keratinocytes *in vitro* were treated with various concentrations of auranofin and then stimulated with PMA (0.1 μM) as described in *Materials and Methods*. After an 18-h incubation period, the medium was collected and the IL-8 content determined by immunoassay. IL-8 levels in control (no auranofin) cultures were 3262 ± 244 pg/ml. Each point represents the mean ± SD of four individual samples from a representative of four experiments.

auranofin might be related to cytokine production, the effect of auranofin, a known PKC inhibitor [27], on IL-8 production was investigated. Treatment of keratinocytes with auranofin just prior to stimulation with PMA resulted in a concentration-dependent inhibition of IL-8 production measured by immunoassay **(Fig 4)** or Western blot (data not shown). The IC_{50} for this inhibition was 0.1 μM.

The effect of auranofin on IL-8 production was then investigated in cells stimulated with IL-1β. In contrast to the inhibitory action of auranofin on PMA-stimulated IL-8 production, a dose-dependent potentiation of IL-8 production was observed when keratinocytes were treated with auranofin prior to stimulation with IL-1β or TNFα **(Fig 5)**. IL-8 production was increased approximately threefold over controls by 1.0 μM auranofin. This potentiation was confirmed by Western blot analysis (data not shown). Auranofin had no effect on IL-8 production in unstimulated keratinocytes (data not shown).

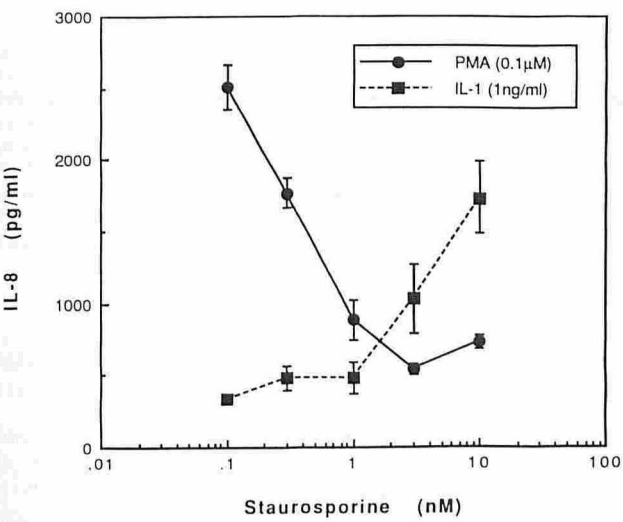


Figure 3. Effect of staurosporine on PMA- or IL-1β–stimulated IL-8 production. Human keratinocytes *in vitro* were treated with various concentrations of staurosporine and then stimulated with either PMA (0.1 μM) or IL-1β (1.0 ng/ml) as indicated. At the end of the 18-h incubation period, the medium was collected and the IL-8 content in each sample determined by immunoassay, as described. Control levels of IL-8 were 262 ± 34 pg/ml in the case of IL-1β–stimulated cells and 2506 ± 192 pg/ml in PMA-stimulated samples. Each point represents the mean ± SD of four individual samples from a representative of at least two experiments.

In an effort to further investigate auranofin's ability to modulate IL-8 production, compounds related to auranofin and another gold compound were studied for their ability to modulate IL-8 production. In contrast to the modulation of IL-8 production observed with auranofin, another gold-containing compound, sodium aurothiomalate, was inactive **(Fig 6)**. Sodium aurothiomalate has been reported to inhibit protein kinase C in Jurkat cells [32]. Its inability to modulate IL-8 production in human keratinocytes may be related to PKC isozyme selectivity. Also, the two substructures of auranofin, triethylphosphine oxide and 2,3,4,6-tetra-O-acetyl-1-β-thioethyl-O-glycoside (abbreviated triethylphosphine and glycoside, respectively, in **Fig 6**), did not alter IL-8 production in response to either PMA or IL-1β stimulation **(Fig 6)**. Thus, although the gold portion of the molecule was necessary for this biologic activity, its presence was not sufficient.

Effect of Treatment Time The mechanism whereby protein kinase C inhibitors modulate IL-8 synthesis was investigated further by examining whether the time at which the cells were treated with auranofin relative to stimulation had any effect on its ability to modulate IL-8 production. In these studies keratinocyte cultures were treated with auranofin either just prior to or 2 h after stimulation. Auranofin had no effect on PMA-induced synthesis of IL-8 when added 2 h after stimulation **(Fig 7*A*)**. Therefore, auranofin was likely to be exerting its action at a point early in the synthesis of IL-8. In contrast, auranofin was found to potentiate IL-8 production in response to IL-1β whether added just prior to or 2 h after stimulation **(Fig 7*B*)**.

IL-8 Gene Expression in Cultured Keratinocytes Barker and co-workers have previously demonstrated rapid and dramatic IL-8 mRNA production in keratinocytes stimulated with either IL-1β or PMA [7]. Peaks in the expression of IL-8 mRNA were reported at 1 h and 3 h, respectively [7]. Therefore, these timepoints were chosen to investigate the effect of PKC inhibitors, including auranofin,

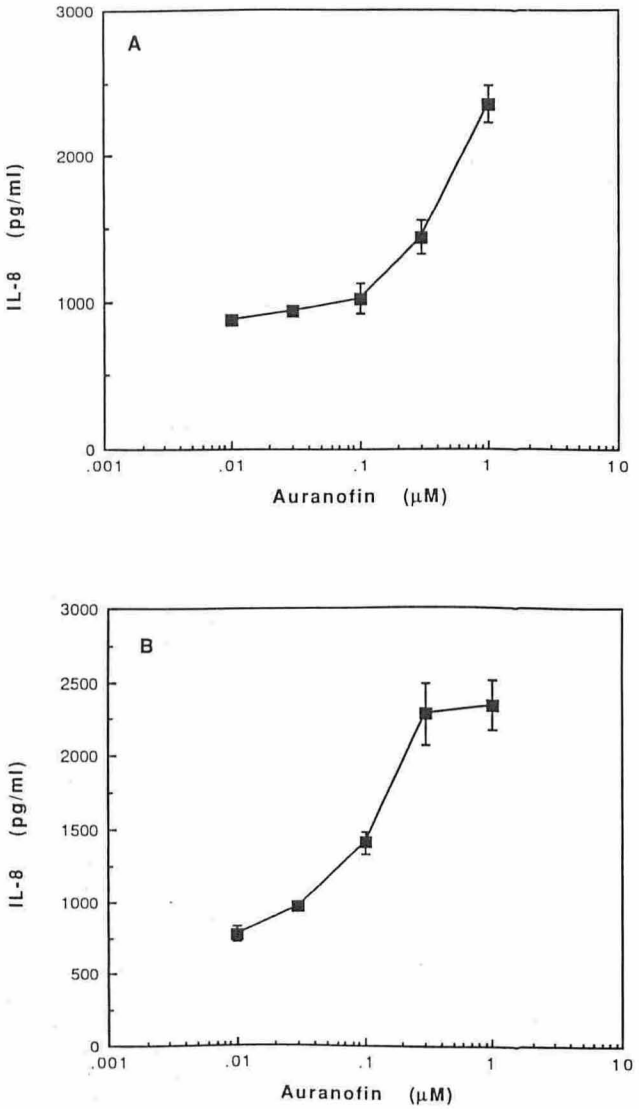


Figure 5. Effect of auranofin on IL-1β– and TNFα-stimulated IL-8 production. Human keratinocytes *in vitro* were treated with various concentrations of auranofin and then stimulated with either IL-1β (1 ng/ml) *(A)* or TNFα (50 ng/ml) *(B)*, as described in *Materials and Methods*. After an 18-h incubation period the medium was collected and the IL-8 content determined by immunoassay. IL-8 levels in control (no auranofin) cultures were 966 ± 21 pg/ml in IL-1–stimulated samples and 821 ± 38 pg/ml in TNF-stimulated samples. Each point represents the mean ± SD of four individual samples from a representative of at least two experiments.

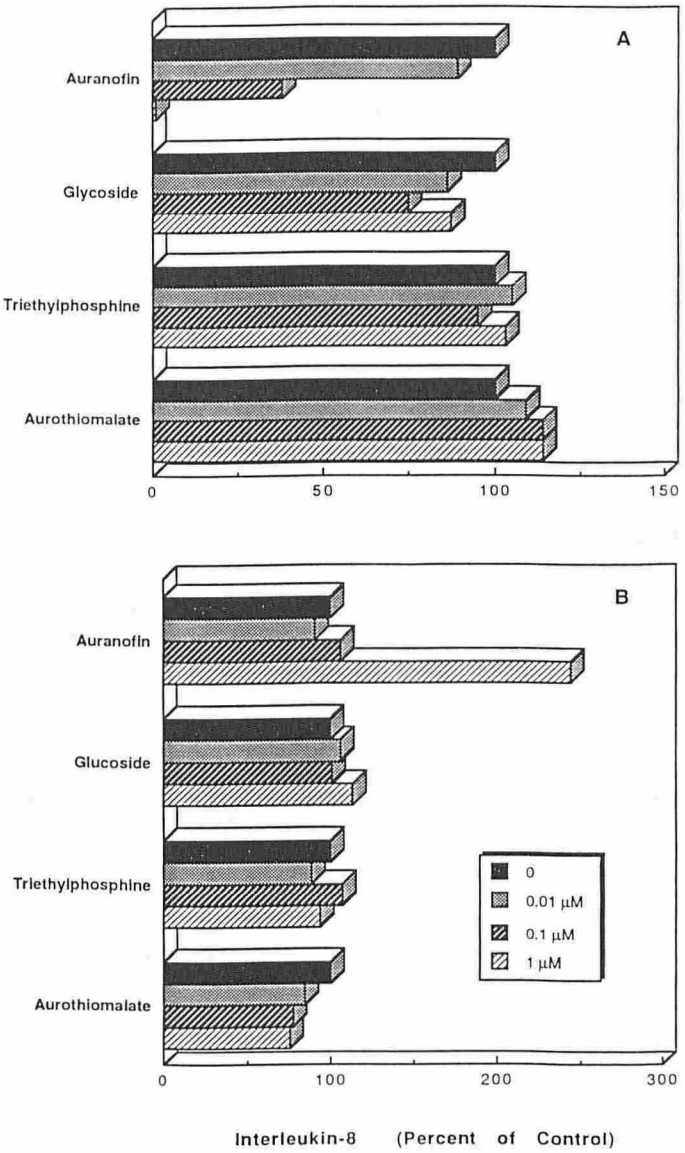


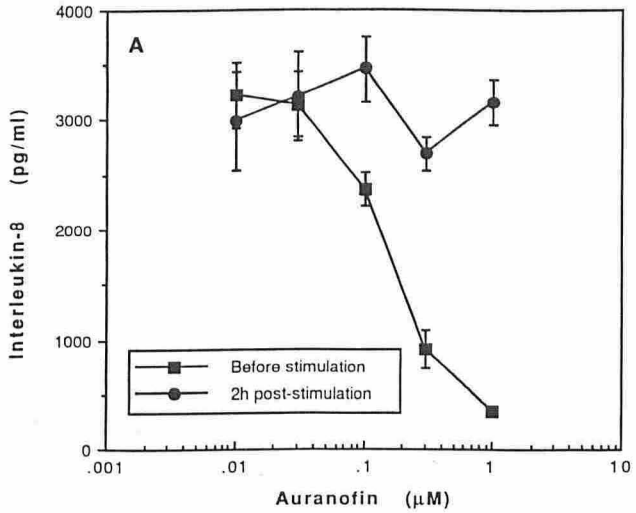
Figure 6. Effect of the auranofin substructures and sodium aurothiomalate on IL-8 production. Human keratinocytes were treated with either auranofin, its substructures, or sodium aurothiomalate and then stimulated with PMA (0.1 μM) *(A)* or IL-1β (1.0 ng/ml) *(B)* as described. The cultures were incubated for 18 h, after which the medium was collected and the levels of IL-8 in the medium determined by immunoassay as described in *Materials and Methods*. Each point represents the mean ± SD of four individual samples from a representative of at least two experiments.

on IL-8 mRNA expression. IL-8 mRNA was not detectable in unstimulated keratinocytes, indicating that the gene is not constitutively expressed in these cells **(Fig 8)**. However, stimulation of keratinocytes with PMA (0.1 μM) for 3 h resulted in the expression of a 1.8-kb product corresponding to IL-8 mRNA **(Table I, Fig 8)**. IL-8 gene expression was 34% and 2% of control in samples treated with 0.05 μM and 0.5 μM auranofin, respectively **(Table I, Fig 8)**. Consistent with its effects on IL-8 protein production, staurosporine also inhibited IL-8 mRNA expression in PMA-stimulated keratinocytes by 89% at a dose of 10 nM **(Table I)**.

Treatment of human keratinocytes with IL-1β also increased the expression of IL-8 mRNA after 1 h of stimulation **(Table I)**. The effects of auranofin and staurosporine observed with respect to IL-8 protein levels were reflected in a potentiation of IL-8 gene expression measured 1 h after IL-1β stimulation **(Table I)**. Interleukin-8 mRNA was 113% of control with 0.05 μM auranofin and 325% of control at the 0.5-μM dose. Thus, the ability of PKC inhibitors to modulate IL-8 production appears to occur at the messenger RNA level.

Co-stimulation of Keratinocytes with IL-1β and PMA Interleukin-β and PMA have been reported to work in concert in the stimulation of increased amounts of IL-2 produced from activated EL4-6.1 cells [23]. Similarly, these two stimuli were found to have a synergistic effect on the production of IL-8 from cultured keratinocytes **(Fig 9)**. Whereas both stimuli were effective by themselves, a combination of suboptimal concentrations of IL-1β and PMA resulted in approximately threefold as much IL-8 compared to stimulation with PMA only and greater than the sum of the IL-8 produced in response to the two stimuli independently.

Considering the magnitude of the increase in IL-8 production, the ability of auranofin to modulate IL-8 production under such co-stimulation conditions was investigated. As was seen in kerati-



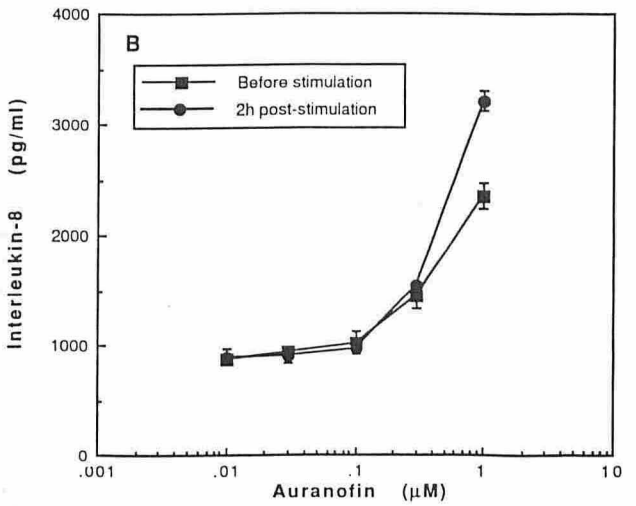


Figure 7. Effect of time of treatment on auranofin-mediated modulation of IL-8 production. Human keratinocytes were either stimulated with PMA (0.1 μM) *(A)* or IL-1β (1.0 ng/ml) *(B)* in the presence of various concentrations of auranofin or stimulated and then incubated for 2 h at 37°C prior to treatment with auranofin. The cultures were incubated for a total of 18 h. At the end of the incubation, the medium was collected and the IL-8 content determined by immunoassay. Each point represents the mean ± SD of four individual samples from a representative of two experiments.

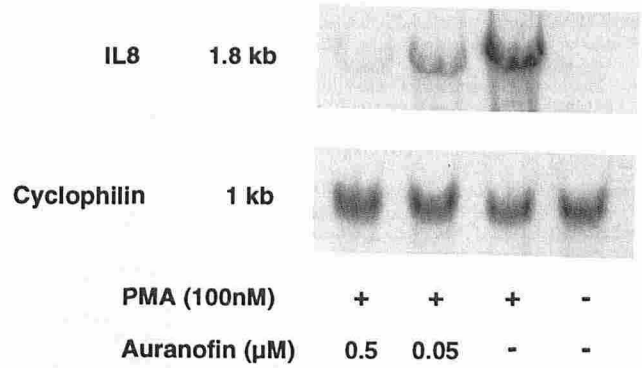


Figure 8. Northern blot of auranofin-treated keratinocytes. Human keratinocytes were treated with or without PMA (0.1 μM for 3 h) in the presence or absence of auranofin at the concentration indicated. At that time the RNA was extracted and the IL-8 mRNA subjected to Northern blot analysis, as described in *Materials and Methods.* Shown is a representative of two experiments.

nocytes stimulated with PMA only, auranofin treatment resulted in a dose-dependent inhibition of IL-8 production (IC_{50} = 0.3 μM) **(Fig 10)**.

DISCUSSION

Interleukin-8 is a potent pro-inflammatory cytokine present in prodigious amounts in psoriatic lesions [6,16–18]. Here it may contribute to the neutrophil and T-lymphocyte infiltration and keratinocyte hyperproliferation characteristic of this disease. Although much is known about the biologic properties of IL-8, little is known concerning the regulation of its synthesis. As such, an understanding of the mechanisms by which the synthesis of IL-8 can be regulated would prove beneficial in the design of compounds to modulate IL-8 levels. These, in turn, may prove beneficial in the treatment of inflammatory diseases, including those of the skin.

Consistent with previous findings [6,7], human keratinocytes in culture were found to respond to stimulation with PMA or IL-1β by increasing IL-8 mRNA. Accompanying this increase in mRNA was an increase in IL-8 protein levels released into the medium. As such, these stimuli increased both the transcription and translation of the IL-8 gene product. Furthermore, the protein kinase C inhibitors staurosporine and auranofin were shown to differentially modulate both mRNA and protein levels depending on whether IL-8 synthesis was stimulated with IL-1β or PMA. Although the correlation between IL-8 mRNA and protein levels clearly suggests that the effect takes place at the pretranslational level, it is not possible to conclude from these studies whether the modulation of IL-8 mRNA levels is related to gene transcription or to the stability of the mRNAs.

In contrast to auranofin, staurosporine was found to upregulate the production of IL-8 in the absence of stimulation. A similar phenomenon has been reported by Cassatella and co-workers [33] in staurosporine-treated human neutrophils. These authors reported that staurosporine induced the expression of IL-8 mRNA as well as protein in the neutrophil. It is likely that staurosporine also upregulates the expression of IL-8 mRNA in human keratinocytes because no IL-8 mRNA is detectable in the absence of stimulation. Whether this modulation is related to inhibition of basal PKC activity is unclear at present. However, the ability of chelerythrine, a more selective PKC inhibitor [34], to exert a similar effect on the production of IL-8 suggests that PKC is involved. Thus, inhibition of

Table I. Effect of PKC Inhibitors on Interleukin-8 mRNA Expression[a]

Treatment	Area Under Curve	Percent Control
PMA (0.1 μM)		
Control	6.7	100
Auranofin (0.05 μM)	2.3	34
Auranofin (0.5 μM)	0.119	2
Control	3.3	100
Staurosporine (10 nM)	0.37	11.2
IL-1β (1.0 ng/ml)		
Control	0.43	100
Auranofin (0.05 μM)	0.49	113
Auranofin (0.5 μM)	1.4	325
Staurosporine (10 nM)	0.64	149

[a] Human keratinocytes were stimulated with either PMA (0.1 μM for 3 h) or IL-1β (1.0 ng/ml for 1 h) in the presence or absence of the compounds indicated. At that time RNA was extracted and the IL-8 mRNA quantitated as described in *Materials and Methods.* Each value represents a single determination from a representative of at least two experiments.

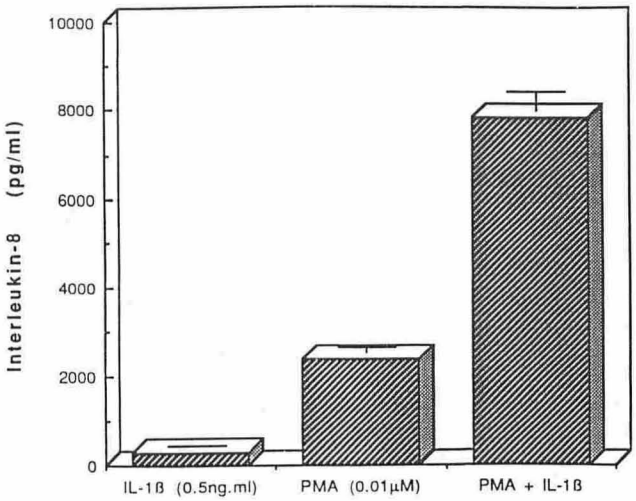


Figure 9. Effect of co-stimulation with PMA and IL-1β on keratinocyte IL-8 production. Human keratinocytes were stimulated with either IL-1β (0.5 ng/ml), PMA (0.01 μM), or a combination of the two stimuli and incubated for 18 h at 37°C. At the end of the incubation, the medium was collected and the IL-8 content determined by immunoassay. Each point represents the mean ± SD of four individual samples from a representative of two experiments.

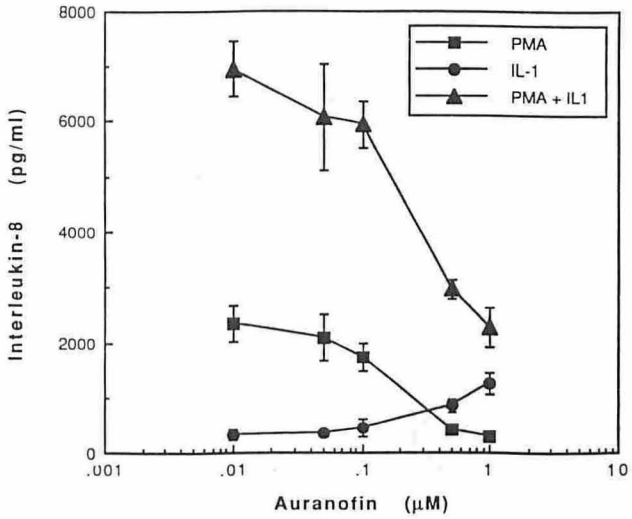


Figure 10. Effect of auranofin on co-stimulated IL-8 production. Human keratinocytes were stimulated with a combination of PMA (0.01 μM) and IL-1β (0.5 ng/ml) in the presence of various concentrations of auranofin. After an 18-h incubation the medium was collected and the IL-8 content determined by immunoassay. Each point represents the mean ± SD of four individual samples from a representative of two experiments.

staurosporine-sensitive enzymes is likely to be related to the increase of IL-8 gene expression.

The ability of PKC inhibitors to differentially modulate IL-1β- and PMA-stimulated events has been reported in other systems. A similar phenomenon has been observed for the production of fructose 2,6-bisphosphate and PGE_2 in cultured rheumatoid synovial cells and human dermal fibroblasts [24], as well as in the EL4-6.1 thymoma cell line with respect to IL-2 and IL-4 synthesis [23]. Dornand *et al* [23] reported that nanomolar concentrations of staurosporine strongly potentiated the production of IL-2 or IL-4 when EL4-6.1 cells were stimulated by IL-1. However, these investigators found that staurosporine dose-dependently inhibited the production of IL-2 and IL-4 resulting from stimulation with PMA. A strong correlation between the effect of staurosporine on IL-2 and IL-4 protein secretion and mRNA levels was reported and interpreted as an indication that the differential regulation of cytokine production by inhibitors of PKC took place at the pretranslational level [23].

The 5′-flanking region of the IL-8 gene contains several potential binding sites for known nuclear factors whose activity may be regulated by PKC. Included among these is NF-κB [1]. The PKC inhibitor H-7 has been reported to block NF-κB activation by the phorbol ester PMA [35], whereas activation of NF-κB by IL-1/TNF was reported to be insensitive to PKC inhibitors [30,31]. Thus, PMA and IL-1/TNF may be exerting their effects through different signal transdution pathways wherein IL-1- and TNF-mediated responses may not directly involve the activation of PKC. However, PKC may play a role in the modulation of the IL-1/TNF signal transduction pathway. Alternatively, one may speculate that isozymes of PKC that are not sensitive to the commonly utilized inhibitors may play a role.

Additional evidence for the potential regulation by PKC at the pretranslational level has been observed in the activation of NF-κB by TNFα. Hohmann and co-workers [36] have reported that HL-60 and El-4 cells incubated with TNFα in the presence of staurosporine showed a twofold increase in the levels of NF-κB activity compared to with TNFα alone. A similar phenomenon may also be involved in the potentiation of IL-1-mediated events by inhibitors of PKC. Interestingly, staurosporine has been reported to increase the activity of a set of novel serine/threonine kinases [37]. As such, PKC may function as a negative regulator of other protein kinases. Thus, the synthesis of IL-8 in response to IL-1β, TNFα, or PMA may result from the activation of a responsive enhancer in the 5′-flanking region of the IL-8 gene by a nuclear factor whose activity is regulated by a member of the protein kinase C family or another kinase, which in turn is regulated by PKC. Differential regulation of nuclear factor activity by PKC depending on the stimulus may underlie the results described herein.

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