

Prostaglandin E₂ suppresses CCL27 production through EP2 and EP3 receptors in human keratinocytes

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Background: The chemokine CCL27 attracts skin-homing T cells. CCL27 production by keratinocytes is enhanced in skin lesions from patients with atopic dermatitis or psoriasis vulgaris. It is suggested that prostaglandin E₂ (PGE₂) regulates skin inflammation.

Objective: We examined the *in vitro* effects of PGE₂ on CCL27 production in human keratinocytes.

Methods: Keratinocytes were incubated with TNF- α in the presence or absence of PGE₂. CCL27 secretion and mRNA level were analyzed by means of ELISA and RT-PCR, respectively. Nuclear factor κ B (NF- κ B)-dependent transcriptional activity was analyzed by using luciferase assays. **Results:** TNF- α increased CCL27 secretion and mRNA levels in parallel to NF- κ B activity in keratinocytes. NF- κ B p50 or p65 antisense oligonucleotides suppressed TNF- α -induced CCL27 production, indicating the requirement of NF- κ B for CCL27 production. PGE₂, EP2, or EP3 agonists reduced TNF- α -induced CCL27 secretion and mRNA levels in parallel to NF- κ B activity and CCL2, CCL5, CXCL8, and CXCL10 mRNA levels. Either EP3-specific or dual EP1-EP2 antagonist partially blocked the inhibitory effects of PGE₂ on CCL27 production and NF- κ B activity, and the addition of both completely abrogated the inhibition, whereas EP1 or EP4 antagonists were ineffective. Intracellular Ca²⁺ chelator BAPTA/AM or cyclic adenosine monophosphate (cAMP)-dependent protein kinase inhibitor H-89 partially blocked the inhibitory effects of PGE₂ on CCL27 production and NF- κ B activity, and the addition of both completely abrogated the inhibition. PGE₂ or EP3 agonist increased intracellular Ca²⁺ concentrations. PGE₂ or EP2 agonist increased intracellular cAMP concentrations.

Conclusion: PGE₂ might suppress CCL27 production by inhibiting NF- κ B activity through EP2-mediated cAMP and EP3-mediated Ca²⁺ signals. PGE₂ might terminate T cell-mediated skin inflammation by inhibiting CCL27 production. (J Allergy Clin Immunol 2004;114:1403-9.)

Key words: CCL27, prostaglandin E₂, EP2, EP3, NF- κ B, cyclic adenosine monophosphate, Ca²⁺

Abbreviations used

AD:	Atopic dermatitis
cAMP:	Cyclic adenosine monophosphate
CREB:	cAMP response element-binding protein
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
I κ B:	Inhibitory nuclear factor κ B
NF- κ B:	Nuclear factor κ B
PGE ₂ :	Prostaglandin E ₂
PKA:	Protein kinase A
PV:	Psoriasis vulgaris

The CC chemokine CCL27 is selectively produced by epidermal keratinocytes and induces skin homing of cutaneous lymphocyte-associated antigen-positive memory T cells.¹ Inflammatory cytokines, such as TNF- α , promote CCL27 production.¹ CCL27 expression by keratinocytes is enhanced in lesions of patients with atopic dermatitis (AD) and psoriasis vulgaris (PV), which might trigger T cell-mediated skin inflammation.²

The chemical mediator prostaglandin E₂ (PGE₂) is produced by a variety of cells in the skin, such as mast cells, macrophages, dendritic cells, and keratinocytes. The functions of PGE₂ in skin inflammation are complex and vary with target cell types or activation status; intradermal injection of PGE₂ induces local vasodilation and wheals.³ On the other hand, PGE₂ preferentially enhances the production of immunosuppressive type 2 cytokines, such as IL-4 or IL-10, and thus suppresses the induction of delayed-type hypersensitivity in the skin.^{4,5} It has been reported that the PGE₂ level is increased in the skin lesions of patients with AD or PV,⁶ whereas some studies have reported the decrease of PGE₂ levels in PV lesions.⁷ It is thus anticipated that PGE₂ might modulate CCL27 production by keratinocytes in AD or PV lesions. However, the effects of PGE₂ on CCL27 production have not been examined.

PGE₂ binds to 4 different G protein-coupled receptors, EP1 to EP4, on the cell surface.⁸ The activation of EP1 or EP3 induces intracellular Ca²⁺ signal, whereas the activation of EP2 or EP4 generates cyclic adenosine monophosphate (cAMP) signal.⁸ Thus PGE₂ exerts differential effects on target cells dependently on relative expression or functionality of EP subtypes.

In this study we investigated the *in vitro* effects of PGE₂ on the production of TNF- α -induced CCL27 and on the production of other inflammatory chemokines, CCL2,

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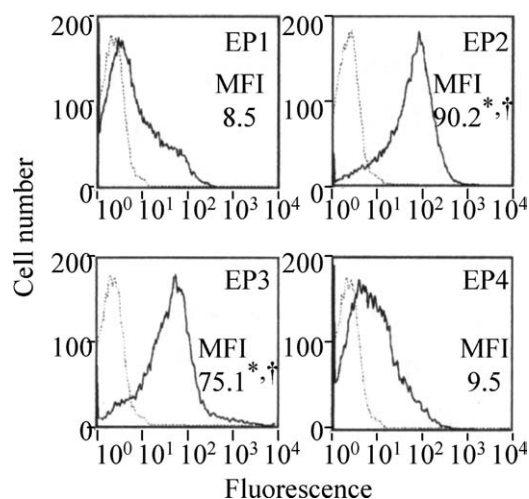


FIG 1. Flow cytometric analysis of EP1, EP2, EP3, and EP4 expression on keratinocytes. * $P < .05$ versus EP1; † $P < .05$ versus EP4. MFI, Mean fluorescence intensity.

CCL5, CXCL8, and CXCL10, by human keratinocytes. We found the inhibitory effects of PGE₂ and further analyzed EP subtypes or signals responsible for the effects by using specific EP agonists or antagonists.

METHODS

Reagents

Agonists specific to EP1 (DI-004), EP2 (AE1-259), EP3 (AE-248), and EP4 (AE1-329) and antagonists specific to EP1 (ONO-8713), EP3 (AE3-240), and EP4 (AE3-208) were donated by Ono Pharmaceutical Co (Osaka, Japan). The dual EP1-EP2 antagonist AH6809 was purchased from Cayman Chemical (Ann Arbor, Miss). Indomethacin, BAPTA/AM, H-89, and actinomycin D were purchased from Sigma Chemical Co (St Louis, Mo). Recombinant human TNF- α was from R&D Systems (Minneapolis, Minn).

Culture of keratinocytes

Human neonatal foreskin keratinocytes were cultured in serum-free KGM (Clonetics, Walkersville, Md), which consisted of the basal medium KBM supplemented with 0.5 μ g/mL hydrocortisone, 5 ng/mL epidermal growth factor, 5 μ g/mL insulin, and 0.5% bovine pituitary extract. Cells in the third passage were used.

Flow cytometry

Expression of EP1, EP2, EP3, and EP4 on keratinocytes was examined by means of flow cytometry, as previously described,⁹ with rabbit anti-EP1, anti-EP2, anti-EP3, or anti-EP4 antibody or control rabbit IgG (Cayman Chemicals) and FITC-conjugated anti-rabbit IgG (Sigma Chemical Co). Results representative of 5 separate experiments are shown in Fig 1.

CCL27 secretion

Keratinocytes (5×10^4 /well) were seeded in triplicate onto 24-well plates in 0.4 mL of KGM, adhered overnight, washed, and incubated with KBM containing 1 μ mol/L indomethacin for 24 hours. The cells were washed, pretreated with indicated concentrations of PGE₂ or EP agonists for 10 minutes, and then incubated with 10 ng/mL TNF- α in the presence or absence of PGE₂ or EP

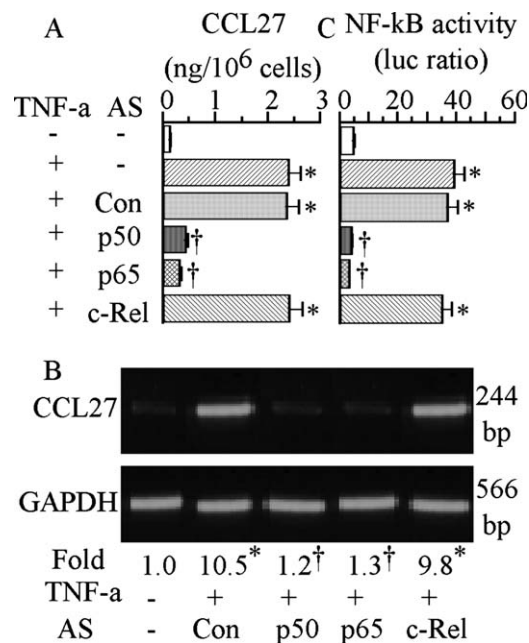


FIG 2. Inhibition by NF- κ B-induced antisense oligonucleotides on TNF- α -induced CCL27 secretion (A), mRNA expression (B), and NF- κ B transcriptional activity (C). * $P < .05$ versus control cells; † $P < .05$ versus TNF- α alone. AS, Antisense oligonucleotide; con, control; luc, luciferase.

agonists in KBM containing indomethacin for 48 hours. The supernatants were assayed for CCL27 by means of ELISA (R&D Systems). In some experiments keratinocytes were preincubated with EP antagonists (each 10 μ mol/L) or signal inhibitors for 10 minutes before the addition of PGE₂ or EP agonists (each 10 μ mol/L).

RT-PCR

Keratinocytes were incubated as above for 12 hours, and then total cellular RNA was extracted and reverse transcribed to produce cDNA.¹⁰ The cDNA was thermocycled for PCR by using primers for CCL27 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as previously described.^{1,10} The PCR products were analyzed by means of electrophoresis, and densitometric analysis was performed with National Institutes of Health Image Software. The CCL27 mRNA level was normalized to that of GAPDH. Results representative of 5 separate experiments are shown in Figs 2, B, and 3, B.

For the analyses of CCL2, CCL5, CXCL8, and CXCL10 mRNAs, real-time RT-PCR was performed, as previously described,¹¹ with SYBRGreen PCR core reagents mix (Applied Biosystems, Foster City, Calif).

mRNA stability analysis

Keratinocytes were pretreated with PGE₂ or EP agonists (each 10 μ mol/L) for 10 minutes and then treated with 10 ng/mL TNF- α for 12 hours. RNA synthesis was blocked with actinomycin D (5 μ g/mL), and RNA was isolated 0, 1, 2, 4, 8, 16, and 32 hours later. RT-PCR was performed as described above, and the decay of mRNA was determined from band density ratios of CCL27/GAPDH.

Plasmid and transfection

pNF- κ B-luciferase containing 4 copies of nuclear factor κ B (NF- κ B) enhancer elements in front of the TATA box upstream of firefly luciferase reporter was purchased from Clontech (San Jose, Calif). Transient transfection was performed with Fugene 6 (Roche,

Indianapolis, Ind), as previously described.¹² Keratinocytes were plated in 35-mm dishes and grown to about 60% confluence. pNF- κ B-luciferase and herpes simplex virus thymidine kinase promoter-linked renilla luciferase vector mixed with Fugene 6 was added to keratinocytes. After 6 hours, the cells were washed and incubated in KBM containing indomethacin for 24 hours and then pretreated with PGE₂ or EP agonists and treated with TNF- α . After 18 hours, firefly and renilla luciferase activities of the cell extracts were quantified by means of dual luciferase assay system (Promega, Madison, Wis). NF- κ B transcriptional activity was expressed as a ratio of firefly/renilla luciferase activity.

Treatment with antisense oligodeoxynucleotides

Antisense oligonucleotides against NF- κ B p50, p65, c-Rel, or control scrambled oligonucleotide were synthesized by using phosphoramidite chemistry, as previously described.¹³ Keratinocytes were transfected with the indicated oligonucleotides (0.5 μ mol/L) premixed with Fugene6 in KBM for 4 hours. The cells were then incubated with TNF- α .

Measurement of cAMP

Keratinocytes were incubated with TNF- α plus PGE₂ or EP agonists for 5 minutes and were analyzed for intracellular cAMP levels by using ELISA (R&D Systems).

Measurement of intracellular Ca²⁺ concentration

Keratinocytes were cultured with KBM containing indomethacin for 24 hours. The intracellular Ca²⁺ concentration was measured by means of spectrofluorometry, as previously described.¹³ Keratinocytes were incubated with 3 μ mol/L Fura 2-AM (Calbiochem, La Jolla, Calif) for 30 minutes; put on the stage of the inverted fluorescence microscope; preincubated with TMB-8 (10 μ mol/L), SK&F96365 (10 μ mol/L), or U73122 (1 μ mol/L, all from Calbiochem) for 10 minutes; and then exposed to TNF- α (10 ng/mL), PGE₂, or EP agonists (each 10 μ mol/L).

Measurement of PGE₂ release

Keratinocytes were incubated with KBM alone or TNF- α in the presence or absence of indomethacin for 48 hours. The supernatant PGE₂ level was measured by means of ELISA (R&D Systems).

Statistical analyses

One-way ANOVA with the Scheffe multiple comparison test was used in Figs 1; 2, 3, B and C; 4; 5, B; and 6 and Tables I and II. One-way ANOVA with the Dunnet multiple comparison test was used in Fig 3, A. A *P* value of less than .05 was considered significant. Data in figures and tables are presented as means $\pm$ SEM of 5 separate experiments.

RESULTS

Expression of EP subtypes on keratinocytes

Keratinocytes expressed EP1, EP2, EP3, and EP4 on their surface (Fig 1). Expression levels of EP2 or EP3 were higher than those of EP1 or EP4.

Involvement of NF- κ B in TNF- α -induced CCL27 production

Constitutive PGE₂ release from keratinocytes was 4.09 $\pm$ 0.41 pmol/10⁶ cells (mean $\pm$ SEM, *n* = 5), which was

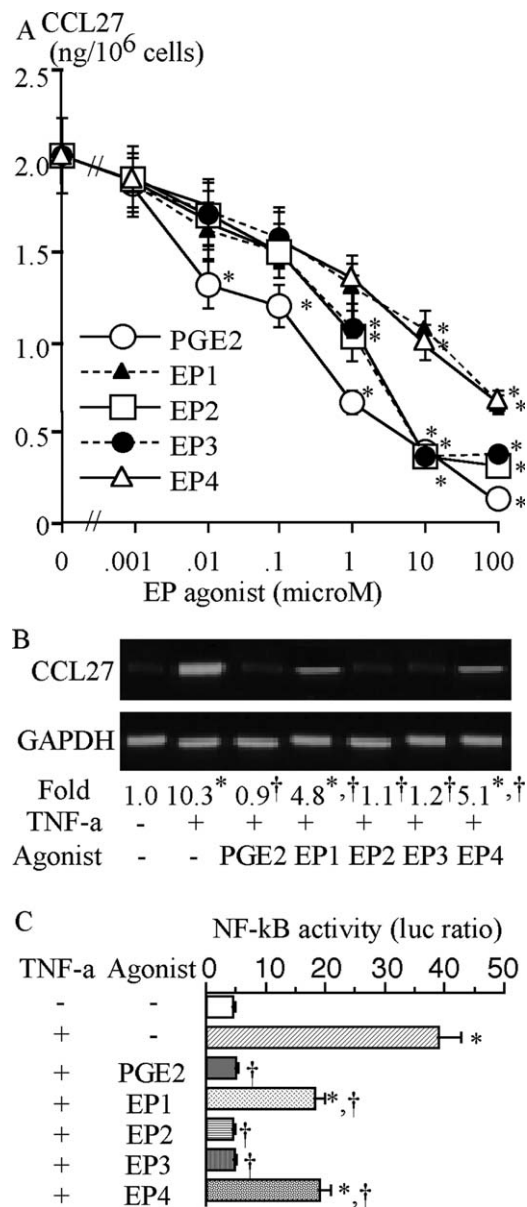


FIG 3. Inhibition by PGE₂ or EP agonists on TNF- α -induced CCL27 secretion (A), mRNA expression (B), and NF- κ B transcriptional activity (C). CCL27 secretion in control cells was 0.21 $\pm$ 0.03 ng/10⁶ cells. Fig 3, A: **P* < .05 versus TNF- α alone. Fig 3, B and C: **P* < .05 versus control cells; †*P* < 0.05 versus TNF- α alone.

increased to 26.01 $\pm$ 2.32 pmol/10⁶ cells by using 10 ng/mL TNF- α . The cyclooxygenase inhibitor indomethacin, 1 μ mol/L, reduced PGE₂ release to an undetectable level (<0.36 pmol/10⁶ cells) in the presence or absence of TNF- α but did not reduce cell viability (>95% viable). To avoid the influence of endogenous PGE₂ from keratinocytes, further experiments were performed in the presence of indomethacin.

Keratinocytes constitutively secreted a marginal amount of CCL27 (mean $\pm$ SEM, 0.21 $\pm$ 0.03 ng/10⁶ cells; *n* = 5), which was increased 11.2-fold by TNF- α (Fig 2, A). In parallel, TNF- α increased the steady-state

TABLE I. Real-time RT-PCR analysis of the effects of PGE₂ or EP agonists on TNF- α -induced chemokine expression

	CCL2	CCL5	CXCL8	CXCL10
TNF- α	5.5 $\pm$ 0.6	6.8 $\pm$ 0.7	7.5 $\pm$ 0.7	3.1 $\pm$ 0.3
TNF- α + PGE ₂	1.2 $\pm$ 0.2*	1.3 $\pm$ 0.2*	1.1 $\pm$ 0.1*	0.9 $\pm$ 0.1*
TNF- α + EP1	3.1 $\pm$ 0.3*	3.8 $\pm$ 0.4*	4.5 $\pm$ 0.5*	1.9 $\pm$ 0.2*
TNF- α + EP2	1.4 $\pm$ 0.2*	1.6 $\pm$ 0.3*	1.2 $\pm$ 0.2*	1.1 $\pm$ 0.2*
TNF- α + EP3	1.3 $\pm$ 0.2*	1.4 $\pm$ 0.2*	1.3 $\pm$ 0.3*	1.0 $\pm$ 0.2*
TNF- α + EP4	3.2 $\pm$ 0.4*	3.7 $\pm$ 0.4*	4.7 $\pm$ 0.4*	1.8 $\pm$ 0.2*

Each chemokine mRNA level was normalized to the GAPDH level. Results are expressed as relative expression versus control cells. The concentration of each agent is as follows: TNF- α , 10 ng/mL; PGE₂ or EP agonists, 10 μ mol/L. **P* < .05 versus TNF- α alone.

level of CCL27 mRNA (Fig 2, B). Because TNF- α potentially increased NF- κ B transcriptional activity (Fig 2, C) and NF- κ B is responsible for the expression of a variety of TNF- α -inducible chemokines,¹⁴ we examined the involvement of NF- κ B in CCL27 production. Antisense oligonucleotides to NF- κ B p50 or p65 suppressed TNF- α -induced CCL27 secretion (Fig 2, A) and mRNA expression (Fig 2, B) in parallel to the inhibition of NF- κ B activity (Fig 2, C), whereas these were not altered by antisense oligonucleotides to c-Rel. These results suggest that NF- κ B p50 and p65 might be responsible for TNF- α -induced CCL27 production.

Effects of PGE₂ or EP agonists on TNF- α -induced CCL27 production

We then examined the effects of PGE₂ or EP agonists on TNF- α -induced CCL27 production. PGE₂ and agonists for EP1 to EP4 dose dependently decreased TNF- α -induced CCL27 secretion (Fig 3, A). The order of inhibitory capacity was as follows: PGE₂ > EP2 = EP3 > EP4 = EP1; the inhibitory concentration of 50% was 0.25, 2, 2.4, 12, or 15 μ mol/L, respectively. PGE₂ or EP agonists at less than 100 μ mol/L did not alter the viability of keratinocytes (>96% viable). PGE₂ or individual EP agonists (each 10 μ mol/L) suppressed TNF- α -induced CCL27 mRNA expression (Fig 3, B), and inhibitory capacities of PGE₂, EP2, or EP3 agonists were higher than those of EP1 or EP4 agonists.

We next examined whether PGE₂ or EP agonists reduced the stability of CCL27 mRNA. The estimated half-life of CCL27 mRNA was (mean $\pm$ EM, *n* = 5) 8.1 $\pm$ 0.8, 8.3 $\pm$ 0.9, 8.0 $\pm$ 0.9, 8.2 $\pm$ 0.8, 8.1 $\pm$ 0.9, or 8.2 $\pm$ 0.9 hours in TNF- α , TNF- α plus PGE₂, EP1, EP2, EP3, or EP4 agonist-treated keratinocytes, respectively, and there were no significant differences in the comparison of any 2 groups (*P* > .05, ANOVA with the Scheffe multiple comparison test). Thus PGE₂ or EP agonists did not reduce the stability of CCL27 mRNA, indicating that these might suppress CCL27 production at the transcriptional level.

In parallel to CCL27 production, PGE₂ or EP agonists suppressed TNF- α -induced NF- κ B activity (Fig 3, C), and inhibitory capacities of PGE₂, EP2, or EP3 agonists were higher than those of EP1 or EP4 agonists. These results suggest that PGE₂ or EP agonists might suppress

TABLE II. Effects of BAPTA/AM or H-89 on PGE₂, EP2, or EP agonist-induced inhibition of CCL27 secretion and NF- κ B activity

	CCL27 (ng/10 ⁶ cells)	NF- κ B (luciferase ratio)
—	27 $\pm$ 3	4.8 $\pm$ 0.5
TNF- α	236 $\pm$ 29*	38.5 $\pm$ 4.1*
TNF- α + PGE ₂	28 $\pm$ 3†	5.1 $\pm$ 0.6†
TNF- α + PGE ₂ + BAPTA/AM	132 $\pm$ 29†‡	22.5 $\pm$ 2.9†‡
TNF- α + PGE ₂ + H-89	130 $\pm$ 31†‡	23.1 $\pm$ 3.0†‡
TNF- α + PGE ₂ + BAPTA/AM + H-89	225 $\pm$ 21‡	37.4 $\pm$ 4.0‡
TNF- α + EP2	35 $\pm$ 4†	5.6 $\pm$ 0.6†
TNF- α + EP2 + BAPTA/AM	39 $\pm$ 4†	6.0 $\pm$ 0.7†
TNF- α + EP2 + H-89	220 $\pm$ 21§	33.1 $\pm$ 3.0§
TNF- α + EP2 + BAPTA/AM + H-89	223 $\pm$ 26§	35.4 $\pm$ 4.0§
TNF- α + EP3	32 $\pm$ 3†	5.9 $\pm$ 0.7†
TNF- α + EP3 + BAPTA/AM	232 $\pm$ 29	37.5 $\pm$ 3.9
TNF- α + EP3 + H-89	30 $\pm$ 3†	6.1 $\pm$ 0.6†
TNF- α + EP3 + BAPTA/AM + H-89	225 $\pm$ 21	37.4 $\pm$ 4.0

The concentration of each agent is as follows: TNF- α , 10 ng/mL; PGE₂, EP2, or EP3 agonists, 10 μ mol/L; BAPTA/AM or H-89, 1 μ mol/L.

**P* < .05 versus control cells.

†*P* < .05 versus TNF- α alone.

‡*P* < .05 versus TNF- α plus PGE₂.

§*P* < .05 versus TNF- α plus EP2 agonist.

||*P* < .05 versus TNF- α plus EP3 agonist.

TNF- α -induced CCL27 production by inhibiting NF- κ B activity. In addition to CCL27, the expression of CCL2, CCL5, CXCL8, and CXCL10 in keratinocytes is enhanced by TNF- α and dependent on NF- κ B.^{13,14} The TNF- α -induced mRNA expression for these cytokines was also suppressed by PGE₂ or EP agonists, and inhibitory capacities of PGE₂, EP2, or EP3 agonists were higher than those of EP1 or EP4 agonists (Table I). These results support that PGE₂ or EP agonists might suppress NF- κ B-dependent chemokine expression.

We then analyzed the contribution of each receptor to PGE₂-induced inhibitory effects. EP3-specific or dual EP1-EP2 antagonist partially blocked PGE₂-induced inhibition of CCL27 secretion (Fig 4, A) or NF- κ B activity (Fig 4, B), and the addition of both agents completely abrogated the inhibition. On the other hand, EP1 or EP4 antagonists did not significantly counteract the PGE₂-induced inhibition of CCL27 secretion or NF- κ B activity. Similar results were obtained with CCL27 mRNA levels (data not shown). These results suggest that EP2 and EP3 might be mainly responsible for PGE₂-induced inhibition of CCL27 production and NF- κ B activity.

Involvement of Ca²⁺ or cAMP in PGE₂-induced inhibition of CCL27 production

We next examined the involvement of Ca²⁺ or cAMP in PGE₂-induced inhibition of CCL27 production and NF- κ B activity. The inhibitory effects of PGE₂ were partially blocked by the intracellular Ca²⁺ chelator BAPTA/AM or

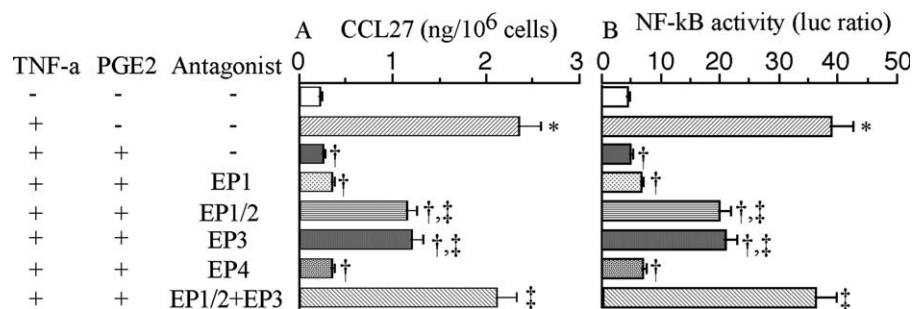


FIG 4. Blocking effects of EP antagonists on PGE₂-induced inhibition of CCL27 secretion (A) and NF-κB transcriptional activity (B). **P* < .05 versus control cells; †*P* < .05 versus TNF-α alone; ‡*P* < .05 versus TNF-α plus PGE₂.

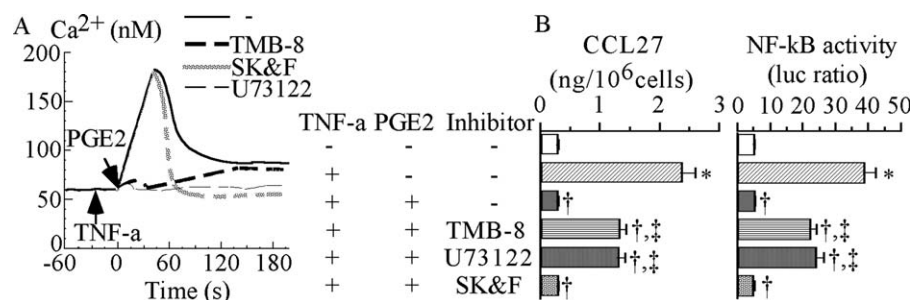


FIG 5. A, PGE₂-induced increase of intracellular Ca²⁺ concentration. B, Blocking effects of TMB-8 or U73122 on PGE₂-induced inhibition of CCL27 secretion or NF-κB activity. **P* < .05 versus control cells; †*P* < .05 versus TNF-α alone; ‡*P* < .05 versus TNF-α plus PGE₂. SK&F, SK&F96365; luc, luciferase.

H-89, an inhibitor of cAMP-dependent protein kinase (protein kinase A [PKA]), and were completely abrogated by the addition of both agents (Table II). These results suggest that both Ca²⁺ and cAMP might independently contribute to PGE₂-induced inhibition of CCL27 production and NF-κB activity. On the other hand, the inhibitory effects of EP2 agonist were counteracted by H-89 but not by BAPTA/AM, whereas those of EP3 agonist were counteracted by BAPTA/AM but not by H-89. The results suggest that cAMP or Ca²⁺ might be responsible for EP2- or EP3-mediated inhibition, respectively. The results with the EP1 agonist were similar to those with the EP3 agonist, whereas the results with the EP4 agonist were similar to those with the EP2 agonist (data not shown).

We then examined whether PGE₂ or EP agonists induce Ca²⁺ or cAMP signals in keratinocytes. PGE₂ evoked an initial increase in the intracellular Ca²⁺ level at 30 seconds, followed by a decrease to a plateau higher than basal level at 90 seconds, and this level was maintained for 5 minutes (Fig 5, A). The initial Ca²⁺ increase was inhibited by TMB-8, an inhibitor of Ca²⁺ release from the intracellular store, whereas the late phase of increase was inhibited by SK&F96365, which inhibits Ca²⁺ entry. These results indicate that the initial increase was mediated by intracellular Ca²⁺ mobilization, whereas the late phase was mediated by Ca²⁺ influx. Phospholipase C generates inositol 1,4,5-triphosphate, which induces in-

tracellular Ca²⁺ mobilization.¹⁵ Inositol 1,4,5-triphosphate, its metabolite, or both also induce Ca²⁺ influx through the membrane-bound Ca²⁺ channel.¹⁵ The phospholipase C inhibitor U73122 suppressed both PGE₂-induced initial and the late phases of Ca²⁺ increase, indicating that PGE₂-induced inositol 1,4,5-triphosphate, its metabolite, or both might trigger both events. TMB-8 and U73122 partially blocked the inhibitory effects of PGE₂ on TNF-α-induced CCL27 secretion or NF-κB activity, whereas SK&F96365 did not (Fig 5, B), indicating that only the initial Ca²⁺ increase is responsible for the inhibitory effects of PGE₂. EP3 agonist generated the initial Ca²⁺ increase, as well as PGE₂, and EP1 agonist less potently but significantly increased the Ca²⁺ level, whereas EP2 or EP4 agonists showed only marginal effects (Fig 6, A). These results suggest that the activation of EP3 or EP1 might generate Ca²⁺ signal, which might suppress NF-κB activity and NF-κB-dependent CCL27 production.

PGE₂ also increased the intracellular cAMP level by 10.1-fold over that of control cells (Fig 6, B). EP2 increased the cAMP level, as well as the PGE₂ level, and EP4 agonist less potently but significantly increased the cAMP level, whereas EP1 or EP3 agonists were ineffective. These results suggest that the activation of EP2 or EP4 might generate cAMP signal, which might suppress NF-κB activity and NF-κB-dependent CCL27 production.

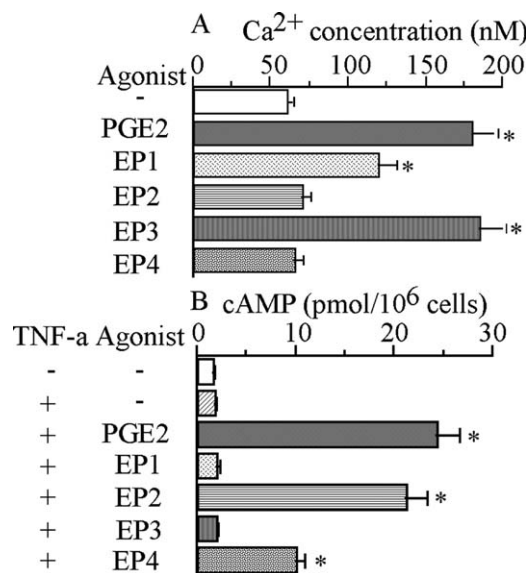


FIG 6. Maximal increase of intracellular Ca^{2+} concentration (**A**) and increase of intracellular cAMP level (**B**) by PGE₂ or EP agonists (each 10 $\mu\text{mol/L}$). * $P < .05$ versus control cells.

DISCUSSION

PGE₂ inhibited TNF- α -induced CCL27 production in keratinocytes. The effects mainly depended on EP2-induced cAMP and EP3-induced Ca^{2+} signals, both of which suppressed the NF- κB activity responsible for CCL27 production. Human CCL27 promoter has not been completely characterized; however, it might have NF- κB elements because NF- κB p50 or p65 antisense oligonucleotides suppressed CCL27 expression. Further studies should identify the NF- κB elements responsible for CCL27 transcription.

Previous studies suggest several mechanisms for cAMP/PKA-mediated inhibition of NF- κB activity, and which mechanism works depends on cell types, promoter context, or sources of cAMP-increasing stimuli: (1) PKA induces the phosphorylation of cAMP response element-binding protein (CREB) and the phosphorylated CREB competes with NF- κB p65 for limiting amounts of transcriptional coactivator CREB-binding protein¹⁶; (2) cAMP induces the transcription of C/CAAT/enhancer-binding protein β , which forms a complex with p65 and suppresses its transactivation potential¹⁷; (3) PKA suppresses the phosphorylation of TATA-binding protein, a transcriptional cofactor for NF- κB ¹⁸; or (4) cAMP stabilizes inhibitory NF- κB (I κB), which constitutively binds and inactivates NF- κB p50/p65.¹⁹ Further studies should define which mechanism or mechanisms might work in PGE₂-mediated inhibition of CCL27 expression in keratinocytes.

Ca^{2+} signal differentially regulates NF- κB activity dependently on cell types; ionomycin enhances phorbol ester- or TNF- α -induced NF- κB transcriptional activity in Jurkat T cells.²⁰ In T cells the Ca^{2+} -dependent

phosphatase calcineurin might accelerate phosphorylation, degradation, or both of I $\kappa\text{B}\alpha$. Contrarily, calcineurin prevents the phosphorylation and inactivation of I κB in chick embryo forebrain.²¹ In bronchial epithelial 16HBE cells, ionomycin suppresses phorbol ester-induced DNA binding and transcriptional activity of NF- κB through calcineurin, which might stimulate the synthesis of an unidentified repressor of NF- κB . In keratinocytes of neuroectodermal origin, mechanisms similar to those of epithelial or neural cells might work in Ca^{2+} -induced suppression of NF- κB activity.

Although EP1 or EP4 agonists partially suppressed CCL27 production, EP1 or EP4 were not involved in PGE₂-induced inhibition of CCL27 production (Fig 4, A). This is possibly due to the relatively lower expression, affinity, or both of EP1 and EP4 compared with that of EP2 and EP3 in keratinocytes, as reported previously.²² EP4 is more susceptible to short-term (seconds to minutes) agonist-induced desensitization,²³ whereas EP2 expression is enhanced by PGE₂,²⁴ indicating that EP4-mediated signals might wane more rapidly compared with EP2-mediated signals.

PGE₂-mediated suppression of CCL27 expression is paradoxical because the PGE₂ level is increased in AD or PV lesions associated with CCL27 overexpression. CCL27 overexpression might be mediated by other inflammatory mediators, such as leukotriene B₄, substance P, or histamine, the levels of which are increased in AD or PV lesions.^{3,6,10,25} Because these mediators enhance NF- κB activity,^{10,26} they might promote NF- κB -dependent CCL27 expression. The stimulatory effects of these mediators might exceed the inhibitory effect of PGE₂ and result in the overexpression of CCL27 in AD or PV lesions.

The level of PGE₂ released from TNF- α -incubated keratinocytes (26.01 pmol/ 10^6 cells, corresponding to approximately 3 nmol/L) was less than threshold (10 nmol/L) for the inhibition of CCL27 production. This indicates that endogenous PGE₂ from keratinocytes might be insufficient for the inhibition of CCL27 production. However, in lesions with AD or PV, more than threshold levels of PGE₂ might be released from other neighboring cells, such as macrophages, dendritic cells, or mast cells, which abundantly infiltrate in AD or PV lesions.²⁷⁻²⁹ In these cells TNF- α enhances PGE₂ release by inducing the expression of cyclooxygenase 2.¹⁴ Thus PGE₂ from TNF- α -stimulated cells neighboring keratinocytes might inhibit TNF- α -induced CCL27 secretion from keratinocytes and thus suppress the skin homing of T cells, indicating negative feedback control by PGE₂. PGE₂ suppresses TNF- α production in macrophages³⁰ or inhibits the expression of cutaneous lymphocyte-associated antigen on T cells and thus suppresses their skin homing.³¹ In addition to CCL27, PGE₂ suppressed the expression of other TNF- α -inducible chemokines (eg, CCL2, CCL5, CXCL8, or CXCL10), which recruit neutrophils, macrophages, or type 1 T cells. PGE₂ might thus terminate ongoing inflammatory infiltration by inhibiting NF- κB -dependent chemokine expression. Topical administration of PGE₂ might be therapeutically useful for the lesions of

patients with AD or PV. It is also reported that indomethacin, which inhibits PGE₂ production, exacerbated PV,³² while topical PGE₂ gel improved PV.³³ These reports may be related to the PGE₂-induced inhibition of NF- κ B-dependent chemokine expression. We should further examine whether topical PGE₂ might reduce such chemokine expression in AD or PV lesions.

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