

IL-17 suppresses TNF- α -induced CCL27 production through induction of COX-2 in human keratinocytes

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Background: The chemokine CCL27 attracts skin-homing T cells. CCL27 production by keratinocytes is dependent on nuclear factor κ B (NF- κ B) activity and enhanced in lesions of patients with atopic dermatitis, psoriasis vulgaris, or allergic contact dermatitis. IL-17 is released from activated memory T cells and modulates skin inflammation.

Objective: We examined the *in vitro* effects of IL-17 on TNF- α -induced CCL27 production in human keratinocytes.

Methods: Keratinocytes were incubated with TNF- α , IL-17, or both. CCL27 secretion and mRNA levels were analyzed by means of ELISA and RT-PCR, respectively. COX-2 promoter and NF- κ B activities were analyzed by using luciferase assays. COX-2 protein levels were analyzed by means of Western blotting.

Results: IL-17 suppressed TNF- α -induced CCL27 secretion and mRNA expression and NF- κ B activity in keratinocytes. The COX-2 inhibitor NS398 counteracted the effects of IL-17, and prostaglandin E₂ prevented counteraction by NS398. IL-17 alone or synergistically with TNF- α increased prostaglandin E₂ release from keratinocytes, and the increase was suppressed by NS398. IL-17 alone or synergistically with TNF- α increased COX-2 mRNA and protein levels, promoter activity, and mRNA stability. The stimulatory effects of IL-17 on COX-2 expression were suppressed by inhibitors of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) kinase. IL-17 alone or synergistically with TNF- α induced dual phosphorylation of p38 MAPK and ERK.

Conclusion: IL-17 might suppress TNF- α -induced CCL27 production by inhibiting NF- κ B through induction of COX-2. The induction of COX-2 might be mediated by activation of p38 MAPK and ERK. T cell-derived IL-17 might alleviate T-cell skin infiltration through inhibition of CCL27 production. (J Allergy Clin Immunol 2005;116:1144-50.)

Key words: IL-17, TNF- α , CCL27, prostaglandin E₂, COX-2

Abbreviations used

ACD:	Allergic contact dermatitis
AD:	Atopic dermatitis
CRE:	Cyclic AMP response element
ERK:	Extracellular signal-regulated kinase
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
I κ B:	Inhibitory κ B
MAPK:	Mitogen-activated protein kinase
NF- κ B:	Nuclear factor κ B
PGE ₂ :	Prostaglandin E ₂
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 CCL27 production by keratinocytes is enhanced in atopic dermatitis (AD),² psoriasis vulgaris (PV), and allergic contact dermatitis (ACD)³ lesions, which might trigger T cell-mediated skin inflammation. We recently found that TNF- α promoted CCL27 production in human keratinocytes at least partially by activating nuclear factor κ B (NF- κ B) and that the chemical mediator prostaglandin E₂ (PGE₂) suppressed TNF- α -induced CCL27 production by inhibiting NF- κ B activity.⁴

The cytokine IL-17 is released from activated memory T cells.⁵ IL-17 production is increased in lesions of patients with asthma, and IL-17 might induce airway neutrophil infiltration by inducing IL-8 production in bronchial epithelial cells or promote airway remodeling by inducing production of the profibrotic cytokines IL-6 and IL-11 in fibroblasts.⁶⁻⁸ Airway hypersensitivity responses were impaired in IL-17-deficient mice, indicating involvement of IL-17 in the responses.⁹ IL-17 production is enhanced in acute AD lesions, and IL-17 might initiate dermal fibrosis in these lesions.^{10,11} IL-17 level is increased in PV and ACD skin lesions.^{12,13} IL-17 might regulate inflammation in these skin lesions; IL-17 alone or synergistically with IFN- γ or TNF- α enhances production of IL-6, IL-8, GM-CSF, or intercellular adhesion molecule 1 by keratinocytes and promotes infiltration, retention, and activation of leukocytes in the epidermis through these molecules.^{5,12-14} The induction of contact hypersensitivity is impaired in IL-17-deficient mice,⁹ supporting the proinflammatory roles of IL-17. In contrast, IL-17 shows anti-inflammatory effects in certain situations: neutralization of IL-17 aggravated dextran sulfate sodium-induced

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colitis in mice.¹⁵ IL-17 suppresses TNF- α - or IL-1-induced CCL5 production in human macrophages, corneal fibroblasts, and keratinocytes^{5,13} or suppresses TNF- α -induced CXCL10 production in corneal fibroblasts.¹⁶ Because both CCL5 and CXCL10 attract activated T cells, IL-17 might preferentially suppress production of T cell-attracting chemokines. To date, it has not been investigated whether IL-17 might upregulate or down-regulate production of CCL27, another T cell-attracting chemokine.

In this study we investigated the *in vitro* effects of IL-17 on TNF- α -induced CCL27 production in human keratinocytes. We found inhibitory effects of IL-17 and involvement of PGE₂ in those effects.

METHODS

Reagents

The c-Jun N-terminal kinase inhibitor SP600125 and the inhibitory κ B (I κ B) kinase inhibitor Bay 11-7082 were purchased from Biomol (Plymouth Meeting, Mass). The p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 and the MAPK/extracellular signal-regulated kinase (ERK) kinase 1 inhibitor PD98059 were from Calbiochem (La Jolla, Calif). The COX-2 inhibitor NS398, the COX-1 inhibitor SC560, and the transcriptional inhibitor actinomycin D were purchased from Sigma Chemical Co (St Louis, Mo). Recombinant human TNF- α and IL-17 were from R&D Systems (Minneapolis, Minn).

Culture of keratinocytes

Human neonatal foreskin keratinocytes were cultured in serum-free KGM (Clonetics, Walkersville, Md) consisting of 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.

CCL27 secretion

Keratinocytes (5×10^4 /well) were seeded in triplicate into 24-well plates in 0.4 mL of KGM, adhered overnight, washed, and incubated with KBM for 24 hours. The cells were washed and incubated with indicated concentrations of TNF- α , IL-17, or both in KBM for 48 hours. Culture supernatants were assayed for CCL27 by means of ELISA (R&D). In some experiments, keratinocytes were preincubated with signal inhibitors for 30 minutes before the addition of TNF- α , IL-17, or both.

RT-PCR

Keratinocytes were incubated as above for 12 hours or 2 hours to analyze CCL27 or COX-2 mRNA levels, respectively. Total cellular RNA was extracted and reverse transcribed to produce cDNA. The cDNA was thermocycled for PCR by using primers for CCL27, COX-2, COX-1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as previously described.^{14,17} PCR products were analyzed by means of electrophoresis, and densitometric analysis was performed with ATTO lane analyzer version 3 (ATTO Corp, Osaka, Japan). CCL27, COX-1, or COX-2 mRNA levels were normalized to that of GAPDH and are shown as a fold induction.

mRNA stability analysis

Keratinocytes were treated with 10 ng/mL TNF- α , 100 ng/mL IL-17, or both for 12 hours or 2 hours to analyze CCL27 or COX-2

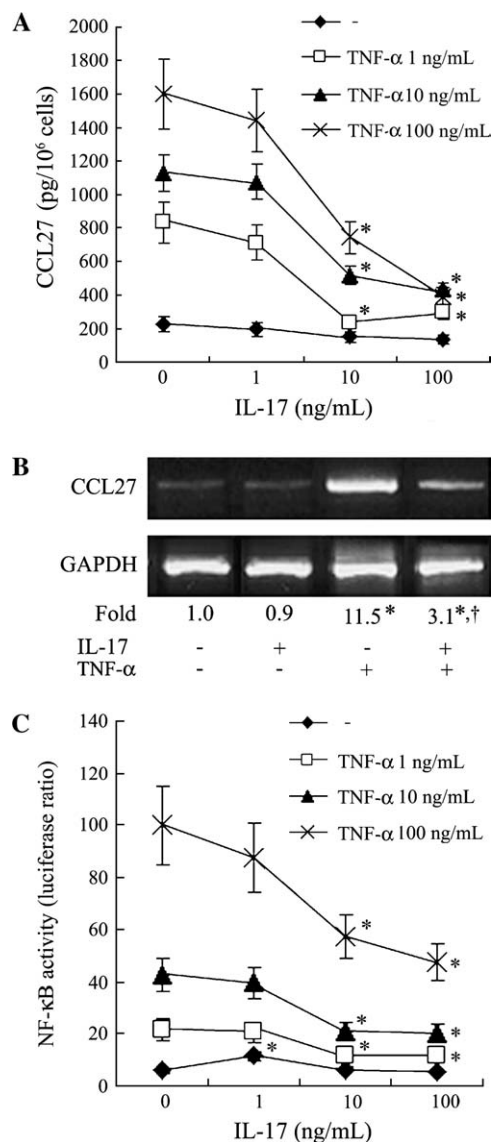


FIG 1. Inhibition by IL-17 on TNF- α -induced CCL27 secretion (A), mRNA expression (B), and NF- κ B transcriptional activity (C). * $P < .05$ versus control values; † $P < .05$ versus TNF- α alone. Data in Fig 1, A and C, are presented as means $\pm$ SD of triplicate cultures and represent 4 separate experiments. Results in Fig 1, B, represent 4 separate experiments.

mRNA stability, respectively. RNA synthesis was blocked with actinomycin D (5 μ g/mL), and RNA was isolated 0, 0.5, 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 or COX-2/GAPDH.

Plasmid and transfection

pNF- κ B-luc containing 4 copies of NF- κ B elements in front of TATA box upstream of firefly luciferase reporter was purchased from Clontech (San Jose, Calif). pCOX-2-luc containing COX-2 promoter (-2300/+30 bp relative to the transcriptional start site) upstream of firefly luciferase reporter was constructed as previously described.¹⁸ Transient transfection was performed with Fugene 6 (Roche, Indianapolis, Ind), as previously described.⁴ Keratinocytes were

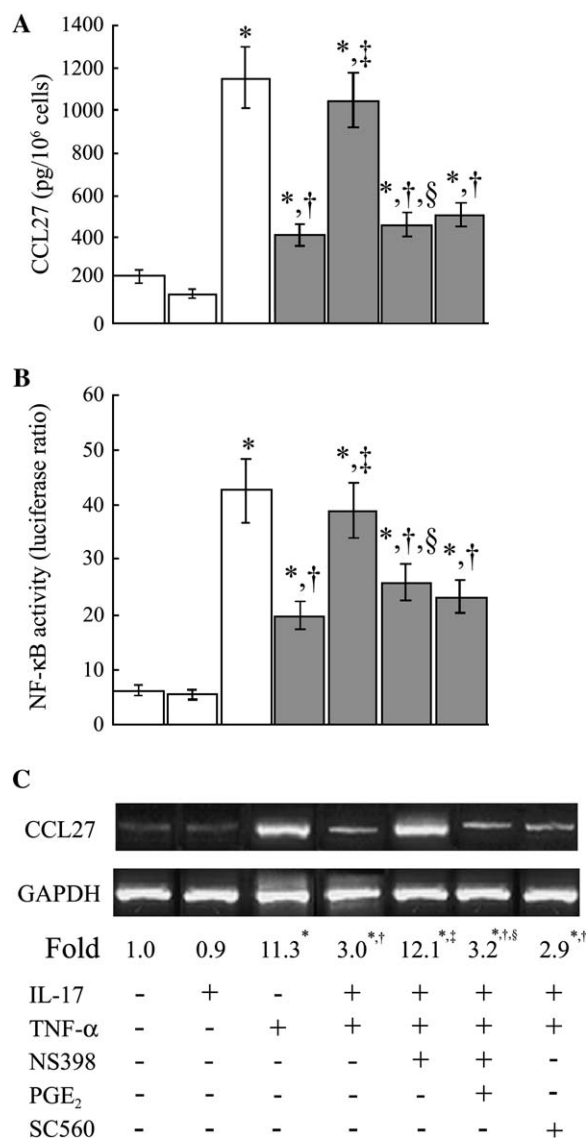


FIG 2. Effects of COX-2 inhibitor and PGE₂ on IL-17-induced inhibition of CCL27 secretion (**A**), NF-κB activity (**B**), and CCL27 mRNA expression (**C**). **P* < .05 versus control values; †*P* < .05 versus TNF-α (10 ng/mL); ‡*P* < .05 versus TNF-α plus IL-17 (100 ng/mL); §*P* < .05 versus TNF-α plus IL-17 plus NS398 (1 μmol/L). PGE₂ or SC560 were 1 or 0.1 μmol/L, respectively. Data in Fig 2, *A* and *B*, are presented as means ± SEM (*n* = 4). Results in Fig 2, *C*, represent 4 experiments.

plated in 35-mm dishes and grown to about 60% confluence. pNF-κB-luc or pCOX-2-luc and herpes simplex virus thymidine kinase promoter-linked renilla luciferase vector mixed with Eugene 6 were added to keratinocytes. After 6 hours, cells were washed and incubated in KBM for 24 hours and then treated with indicated concentrations of TNF-α, IL-17, or both. After 18 hours, firefly and renilla luciferase activities of cell extracts were quantified by using the dual luciferase assay system (Promega, Madison, Wis). NF-κB transcriptional activity and COX-2 promoter activity were expressed as ratios of firefly/renilla luciferase activity.

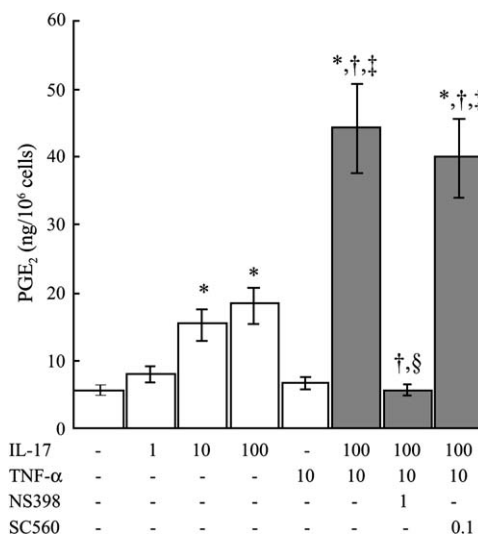


FIG 3. IL-17- and/or TNF-α-induced release of PGE₂. **P* < .05 versus control values; †*P* < .05 versus IL-17 (100 ng/mL); ‡*P* < .05 versus TNF-α; §*P* < .05 versus TNF-α plus IL-17 (100 ng/mL). The concentration units were nanograms per milliliter for IL-17 and TNF-α and micromoles per liter for NS398 and SC560, respectively. Data are presented as means ± SEM (*n* = 4).

Measurement of PGE₂ release

Keratinocytes were incubated with indicated concentrations of IL-17, TNF-α, or both for 48 hours. The supernatant PGE₂ amount was measured by means of ELISA (R&D).

Western blotting

Western blotting was performed as previously described.¹⁹ Keratinocytes were preincubated with signal inhibitors for 30 minutes and then incubated with 100 ng/mL IL-17, 10 ng/mL TNF-α, or both for 15 minutes or 2 hours to analyze phosphorylation of kinases or expression of COX-2 proteins, respectively. Cellular proteins extracted in lysis buffer (50 mmol/L Tris-HCl [pH 7.4]; 150 mmol/L NaCl; 2 mmol/L EDTA; 1 mmol/L phenylmethylsulfonyl fluoride; 10 μg/mL each of aprotinin, leupeptin, and pepstatin; 1% Nonidet P-40; 1 mmol/L Na₃VO₄; and 1 mmol/L NaF) were subjected to SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membrane was blocked and incubated with primary antibody and then with horseradish peroxidase-conjugated secondary antibody, washed, and developed with enhanced chemiluminescence (Amersham, Arlington Heights, Ill). Primary antibodies used were anti-COX-2, COX-1 (Cayman Chemical Co, Ann Arbor, Miss), anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, Calif), anti-total and phospho-p38 MAPK, and anti-total and phospho-ERK1/2 (New England Biolabs, Beverly, Mass). COX-1 or COX-2 protein levels were normalized to those of GAPDH and are shown as a fold induction. The ratios of phosphorylated kinase/total kinase levels are shown as a fold induction.

Statistical analyses

Data in Fig 1, *A* and *C*, are presented as means ± SD of triplicate cultures and represent 4 separate experiments. One-way ANOVA with the Dunnett multiple comparison test was used in these figures. Data in Figs 1, *B*, 2, 3, 4, 5, and 6 are presented as means ± SEM of 4 separate experiments. One-way ANOVA with the Scheffe multiple comparison test was used in these figures. A *P* value of less than .05 was considered significant.

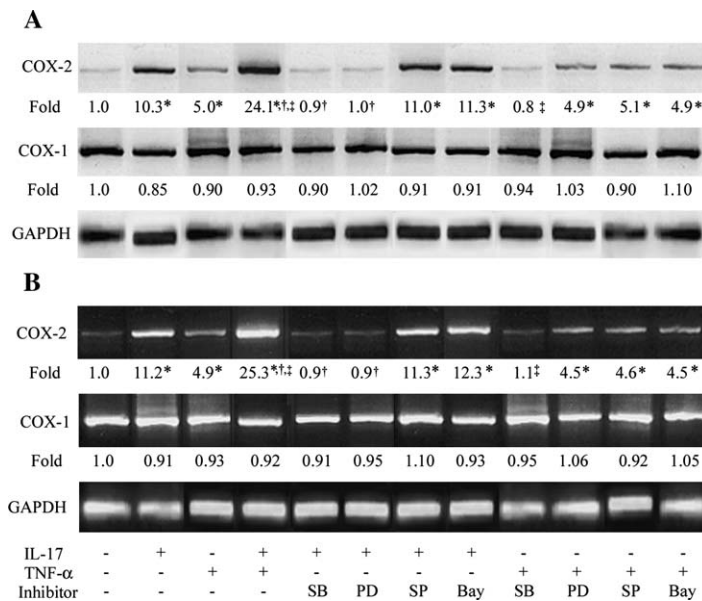


FIG 4. IL-17- and/or TNF- α -induced expression of COX-2 protein (A) and mRNA (B). * $P < .05$ versus control values; † $P < .05$ versus IL-17; ‡ $P < .05$ versus TNF- α . The concentration of each agent is as follows: TNF- α , 10 ng/mL; IL-17, 100 ng/mL; SB203580 (SB), 0.1 μ mol/L; PD98059 (PD), 1 μ mol/L; SP600125 (SP), 1 μ mol/L; Bay 11-7082 (Bay), 1 μ mol/L. Results represent 4 experiments.

RESULTS

IL-17 inhibits TNF- α -induced CCL27 production

We examined the effects of IL-17 on TNF- α -induced CCL27 production. IL-17 did not significantly alter basal CCL27 secretion; however, it did significantly reduce TNF- α -induced CCL27 secretion. IL-17 (100 ng/mL) suppressed TNF- α (1, 10, or 100 ng/mL)-induced CCL27 secretion by 63%, 62%, or 76% compared with control values, respectively (Fig 1, A). IL-17 did not alter the viability of keratinocytes (>96% viable). In parallel to protein secretion, IL-17 suppressed TNF- α -induced CCL27 mRNA expression (Fig 1, B). We next examined whether IL-17 reduced the stability of CCL27 mRNA. The estimated half-life of CCL27 mRNA was presented as a mean $\pm$ SEM ($n = 4$) of 8.2 ± 0.8 , 8.1 ± 0.9 , 8.3 ± 0.9 , or 8.2 ± 0.8 hours in TNF- α (10 ng/mL)-treated and TNF- α plus IL-17 (1, 10, or 100 ng/mL)-treated keratinocytes, respectively, and there were no significant differences in the comparison of either group ($P > .05$, 1-way ANOVA with the Scheffe multiple comparison test). Thus IL-17 did not reduce the stability of CCL27 mRNA, indicating the suppression of CCL27 production at the transcriptional level.

In parallel to CCL27 production, IL-17 dose-dependently reduced TNF- α -induced NF- κ B transcriptional activity (Fig 1, C). IL-17 (1 ng/mL) alone modestly increased NF- κ B activity (1.97-fold of control values); however, higher concentrations of IL-17 reduced TNF- α -induced NF- κ B activity. IL-17 (100 ng/mL) suppressed TNF- α (1, 10, or 100 ng/mL)-induced NF- κ B activity by 46%, 52%, and 52% compared with control values, respectively. These results suggest that IL-17 might

suppress TNF- α -induced CCL27 production at least partially through suppression of NF- κ B activity.

Suppression of CCL27 synthesis by IL-17 is mediated by COX-2 and PGE₂

In our previous study PGE₂ suppressed TNF- α -induced CCL27 production through suppression of NF- κ B activity.⁴ PGE₂ synthesis is regulated by the activity of COX, which converts arachidonic acid to PGH₂, a precursor of PGE₂. There are 2 distinct isoforms of COX, COX-1 and COX-2. COX-1 is constitutively expressed, whereas COX-2 is induced by inflammatory or mitogenic stimuli.¹⁹ It is reported that IL-17 alone or synergistically with TNF- α or LPS induces COX-2 expression and PGE₂ release in human chondrocytes, synovocytes, and myofibroblasts.²⁰⁻²² We thus examined the involvement of PGE₂ and COX-2 in the suppression of TNF- α -induced CCL27 production by IL-17. The COX-2 inhibitor NS398 counteracted the suppressive effects of IL-17 on TNF- α -induced CCL27 secretion (Fig 2, A), mRNA expression (Fig 2, C), and NF- κ B activity (Fig 2, B), and the effects of NS398 were prevented by PGE₂. The COX-1 inhibitor SC560 did not counteract the suppressive effects of IL-17. These results indicate that IL-17 might suppress TNF- α -induced NF- κ B activity and CCL27 production through COX-2 and PGE₂.

IL-17 enhances COX-2 expression and PGE₂ release

We then examined whether IL-17 might increase PGE₂ release from keratinocytes (Fig 3). IL-17 alone dose-dependently increased PGE₂ release from keratinocytes,

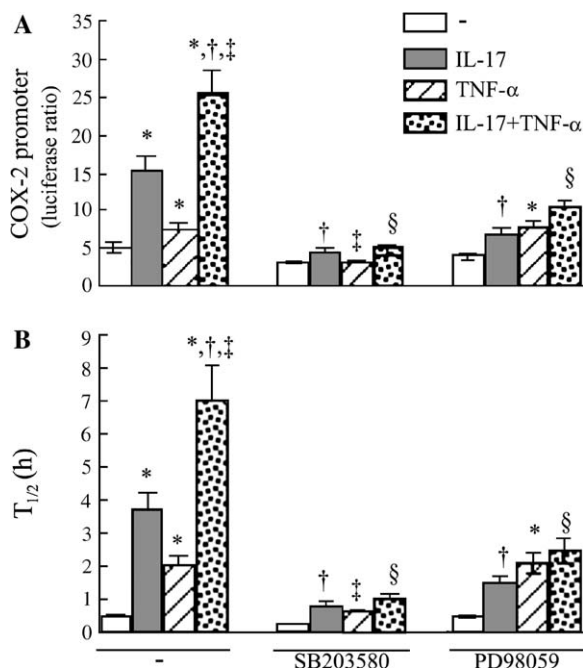


FIG 5. IL-17- and/or TNF- α -induced increases of COX-2 promoter activity (**A**) and mRNA half-life ($T_{1/2}$, **B**). * $P < .05$ versus control values; † $P < .05$ versus IL-17; ‡ $P < .05$ versus TNF- α ; § $P < .05$ versus IL-17 plus TNF- α . The concentration of each agent is as follows: TNF- α , 10 ng/mL; IL-17, 100 ng/mL; SB203580, 0.1 μ mol/L; PD98059, 1 μ mol/L. Data are presented as means $\pm$ SEM ($n = 4$).

and the increase at 100 ng/mL was 3.28-fold of control values, whereas TNF- α only modestly increased the release (1.20-fold or 1.19-fold at 10 or 100 ng/mL, respectively). IL-17 (100 ng/mL) plus TNF- α (10 ng/mL) synergistically increased PGE₂ release 7.98-fold of control values, and NS398 reduced the release by 87%, whereas the reduction by SC560 was much lower (10%), indicating that the main source of PGE₂ was COX-2.

We then examined whether IL-17 alone or together with TNF- α enhanced COX-2 expression in keratinocytes. Basal COX-2 protein (Fig 4, A) and mRNA (Fig 4, B) levels were low, and these were markedly increased by IL-17. TNF- α increased COX-2 protein and mRNA levels less potently than IL-17. IL-17 plus TNF- α synergistically increased the COX-2 expression. IL-17-induced increases of COX-2 protein and mRNA levels were suppressed by p38 MAPK inhibitor SB203580 and MAPK/ERK kinase 1 inhibitor PD98059, which inhibits activation of ERK. The c-Jun N-terminal kinase inhibitor SP600125 or the I κ B kinase inhibitor Bay 11-7082 did not suppress the effects of IL-17. TNF- α -induced COX-2 mRNA and protein expression were suppressed by SB203580 but not by PD98059, SP600125, or Bay 11-7082. Compared with COX-2, basal COX-1 protein and mRNA levels were higher, and these were not altered by IL-17, TNF- α , or both. These results suggest that p38 MAPK might be involved in IL-17- and TNF- α -induced COX-2 expression and that ERK might be involved in IL-17-induced, but not TNF- α -induced, COX-2 expression.

It is reported that COX-2 expression is regulated at both the transcriptional and posttranscriptional levels.²⁰ IL-17 increased COX-2 promoter activity 3.0-fold of control values, whereas TNF- α less potently increased the activity (1.47-fold), and IL-17 plus TNF- α synergistically increased the activity 4.98-fold (Fig 5, A), indicating that IL-17 alone or synergistically with TNF- α enhanced COX-2 transcription. IL-17 increased the half-life of COX-2 mRNA 7.4-fold of control values, whereas TNF- α less potently increased the half-life 4.0-fold, and IL-17 plus TNF- α synergistically increased the half-life 14.0-fold (Fig 5, B), indicating that IL-17 alone or synergistically with TNF- α enhanced COX-2 mRNA stability. SB203580 suppressed IL-17-, TNF- α -, or IL-17 plus TNF- α -induced increases of COX-2 promoter activity and mRNA half-life. PD98059 suppressed the effects of IL-17 or IL-17 plus TNF- α on COX-2 promoter activity and mRNA half-life; however, it did not suppress those of TNF- α alone. These results suggest that IL-17 alone or synergistically with TNF- α enhanced COX-2 transcription and mRNA stability through p38 MAPK and ERK.

We then examined whether IL-17, TNF- α , or both might activate p38 MAPK or ERK. p38 MAPK and ERK are activated by dual phosphorylation at threonine and tyrosine residues. IL-17 potently enhanced dual phosphorylation of p38 MAPK (Fig 6, A). TNF- α less potently enhanced the phosphorylation of p38 MAPK, and IL-17 plus TNF- α synergistically enhanced the phosphorylation. SB203580 suppressed p38 MAPK phosphorylation induced by IL-17, TNF- α , or both. IL-17 enhanced the dual phosphorylation of ERK1/2, whereas TNF- α did not enhance basal and IL-17-induced ERK1/2 phosphorylation (Fig 6, B). PD98059 suppressed the IL-17-induced phosphorylation of ERK1/2. These results suggest that IL-17 alone or synergistically with TNF- α activates p38 MAPK and ERK1/2, which might promote COX-2 expression and PGE₂ release.

DISCUSSION

IL-17 suppressed TNF- α -induced CCL27 production in keratinocytes. IL-17 induced COX-2 expression and increased PGE₂ release, and released PGE₂ suppressed TNF- α -induced CCL27 production. We recently found that PGE₂ induced cyclic AMP or Ca²⁺ signals through EP2 or EP3 receptors on keratinocytes, respectively, and both signals suppressed TNF- α -induced NF- κ B activity and CCL27 production.⁴ When PGE₂ is released from keratinocytes in autocrine and paracrine manners, PGE₂ concentrations on the surface of keratinocytes might be much higher than those of cultured supernatants. Thus endogenously produced PGE₂ might more effectively act on keratinocytes and thus more potently suppress CCL27 production than exogenously added PGE₂.

It is known that IL-17 binding to its receptors activates ERK and p38 MAPK through activation of *src* tyrosine kinase in human renal epithelial cells.²³ Both ERK and p38 MAPK mediated IL-17-induced COX-2 transcription in human keratinocytes. It is reported that cyclic AMP

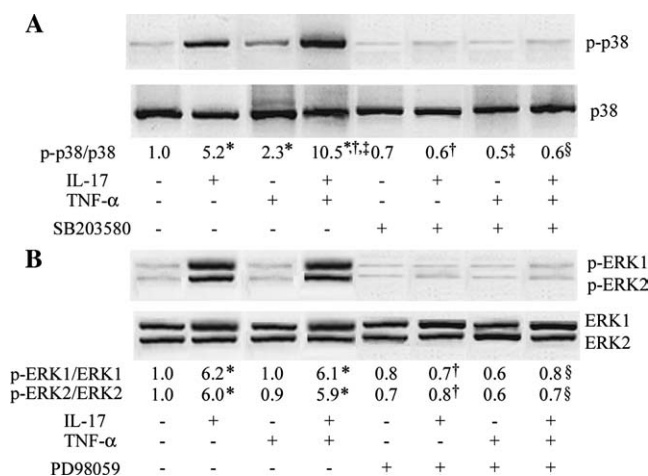


FIG 6. IL-17- and/or TNF- α -induced activation of p38 MAPK (**A**) and ERK1/2 (**B**). * $P < .05$ versus control values; † $P < .05$ versus IL-17; ‡ $P < .05$ versus TNF- α ; § $P < .05$ versus TNF- α plus IL-17. The concentration of each agent is as follows: TNF- α , 10 ng/mL; IL-17, 100 ng/mL; SB203580, 0.1 μ M; PD98059, 1 μ M. *p-p38*, Phosphorylated p38 MAPK; *p38*, total p38 MAPK; *p-ERK*, phosphorylated ERK; *ERK*, total ERK. Results represent 4 experiments.

response element (CRE; -59/-53 bp) on COX-2 promoter confers basal and inducible COX-2 transcription in human keratinocytes.²⁴ ERK and p38 MAPK might further activate downstream kinases, such as mitogen- and stress-activated protein kinase 1, which could phosphorylate and activate transcription factors, CRE-binding protein, and/or activating transcription factor 1 and induce CRE-dependent COX-2 transcription by these factors.²⁵ COX-2 promoter contains CCAAT/enhancer-binding protein β element (-132/-124 bp), and this element might also confer p38 MAPK-mediated, ERK-mediated, or both mediated COX-2 transcription; phosphorylation of CCAAT/enhancer-binding protein β by these kinases might promote its transcriptional activity.^{26,27} Although NF- κ B elements (-445/-427 bp and -223/-214 bp) exist on human COX-2 promoter,²⁶ the NF- κ B inhibitor Bay 11-7082 did not suppress COX-2 expression induced by IL-17 or TNF- α , indicating that the NF- κ B elements might not mediate transcriptional responses to these cytokines in keratinocytes. Consistent with keratinocytes, IL-17-induced COX-2 transcription in human chondrocytes was independent of NF- κ B,²⁰ whereas that seen in human intestinal myofibroblasts was dependent on NF- κ B,²² indicating cell-type specificity. In human embryonic fibroblasts or macrophages, IL-17 increased NF- κ B activity by activating I κ B kinase through the adapter protein TNF receptor-associated factor 6.^{5,28} However, the NF- κ B-potentiating effect of IL-17 is generally lower than those of other inflammatory cytokines, such as TNF- α or IL-1 β , and is not significant in human chondrocytes²⁰ or murine MC-3T3-E1 cells.²⁹ In human keratinocytes low concentration of IL-17 (1 ng/mL) weakly enhanced NF- κ B activity; however, higher concentrations of IL-17 (10 or 100 ng/mL) suppressed TNF- α -induced NF- κ B activity through induction of PGE₂. Thus regulation of NF- κ B activity by IL-17 might vary with cell types, concentrations, or associated stimuli.

Both ERK and p38 MAPK mediated IL-17-induced COX-2 mRNA stabilization. The 3' untranslated region of COX-2 mRNA contains AU-rich elements that regulate destabilization of mRNA.³⁰ These AU-rich elements are bound by mRNA-destabilizing proteins like tristetraprolin and stabilizing proteins like HuR, and the balance of these proteins might regulate mRNA stability. Phosphorylation by p38 MAPK and ERK or their downstream kinases might reduce binding of destabilizers, augment binding of stabilizers, or both and thus suppress the decay of COX-2 mRNA. As well as IL-17, angiotensin II stabilizes COX-2 mRNA through ERK and p38 MAPK in rat vascular smooth muscle cells.³¹ It is also reported that p38 MAPK stabilizes COX-2 mRNA by inhibiting deadenylation of poly (A) tail.³⁰

It is surprising that the magnitude of COX-2 induction by TNF- α was much lower than that by IL-17 because TNF- α generally induces the expression of inflammatory molecules (CCL2, CXCL8, or intercellular adhesion molecule 1) more potently than IL-17.¹³ However, COX-2 is not always a proinflammatory enzyme because the COX-2 product PGE₂ can play anti-inflammatory roles; PGE₂ suppresses production of inflammatory IFN- γ or enhances production of anti-inflammatory IL-10 in T cells or macrophages.^{32,33} Possibly, COX-2 expression in keratinocytes might be hierarchically regulated and might be more inducible by IL-17 containing both proinflammatory and anti-inflammatory properties than by prototypical proinflammatory TNF- α .

Both IL-17 and TNF- α levels are increased in lesions of patients with acute AD,^{10,34} with PV,^{12,35} and in the elicitation phase of ACD.¹³ It is reported that *in vivo* injection of CCL27 induced recruitment of T cells to the skin, and conversely, injection of anti-CCL27 antibody impaired T-cell recruitment to ACD skin lesions in mice,³ indicating a pivotal role of CCL27 in T-cell skin infiltration. Thus our results *in vitro* indicate that T cell-derived IL-17 might

reduce TNF- α -induced CCL27 secretion in keratinocytes and thus at least partially alleviate T-cell infiltration into AD, PV, or ACD skin lesions. The inhibition of CCL27 secretion by IL-17 was mediated by induction of COX-2, which might be a defensive mechanism. COX-2 inhibitor counteracted the IL-17-induced inhibition of CCL27 secretion. Consistent with this observation, COX-2 inhibitor exacerbated chemically induced colitis in mice, and COX-2 knockout mice were more susceptible to such colitis.³⁶ It is thus indicated that COX-2 inhibitors might exacerbate skin inflammation. We should further examine whether CCL27 expression and T-cell infiltration in AD, PV, or ACD lesions might be reduced by topical IL-17 or enhanced by COX-2 inhibitor or anti-IL-17 antibody.

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