Activation of Cutaneous Protein Kinase Cα Induces Keratinocyte Apoptosis and Intraepidermal Inflammation by Independent Signaling Pathways

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Skin keratinocytes are major mediators of host immune responses. The skin is also a target for immunologically based inflammation in many pathological states. Activation of protein kinase C (PKC) can induce cutaneous inflammation, but the precise role of each of six cutaneous PKC isoforms (α , δ , ϵ , η , ζ , μ) that regulate normal skin homeostasis or contribute to skin pathology has not been clarified. We generated transgenic mice that overexpress PKC α in the basal layer of the epidermis and the outer root sheath of hair follicles under the regulation of the bovine keratin 5 promoter. K5-PKC α transgenic mice exhibit severe intraepidermal neutrophilic inflammation and disruption of the epidermis and upper hair follicles when treated topically with 12-O-tetradecanoylphorbol-13-acetate (TPA). Both TPA and UVB cause apoptosis in transgenic skin, but only TPA evokes intraepidermal inflammation. TPA also induces apoptosis in cultured transgenic keratinocytes, and this is prevented by an AP-1 dominant-negative construct. However, inhibiting AP-1 in vivo does not abrogate intraepidermal inflammation. Transcripts for specific cytokines and chemokines are elevated in TPA-treated cultured transgenic keratinocytes, and conditioned culture medium from these cells promotes neutrophil migration in vitro. Chemokine expression and neutrophil migration are not diminished by inhibiting AP-1. Thus, PKC α activation induces keratinocyte apoptosis via an AP-1dependent pathway and mediates chemokine induction and intraepidermal inflammation independently. This model system will be useful to define specific chemokines regulated by PKC α that promote intraepidermal neutrophilic inflammation, a condition that characterizes several human cutaneous diseases such as pustular psoriasis and acute generalized exanthematous pustulosis. The Journal of Immunology, 2003, 171: 2703-2713.

number of studies have indicated that protein kinase C (PKC)⁴ isoforms contribute to the regulation of skin homeostasis (1), and alterations in PKC signaling are fundamental to the pathogenesis of cutaneous diseases, including hyperproliferative, inflammatory, and neoplastic lesions (2–4). Analyses of both rodent and human epidermis reveal that six isoforms of PKC are expressed in keratinocytes (α , δ , ϵ , η , ζ , μ). The challenge, then, is to determine the contribution of each isoform to normal regulation or disease pathogenesis in the hope that specific agonists or antagonists will become useful pharmaceuticals in cutaneous therapy.

Several laboratories have addressed the contribution of PKC isoforms to the regulation of growth and differentiation of normal keratinocytes. PKC activity is required for expression of epidermal differentiation markers (5-8), and the expression level and intracellular distribution of individual PKC isoforms are modified during keratinocyte maturation (9). Specifically, PKC η increases during the late stages of epidermal differentiation in vitro and in vivo, and this isoform induces the expression and activation of transglutaminase 1 when overexpressed in cultured keratinocytes (10, 11). PKCδ also activates transglutaminase 1 when overexpressed, and induces a keratinocyte death pathway mediated through mitochondrial targeting (10, 11). PKC α activity is necessary for the expression of loricrin, filaggrin, surface plasmon resonance (SPR)-1, and transglutaminase 1 in cultured mouse keratinocytes and suppresses keratins 1 and 10 in the granular layer of the epidermis (5, 6). Furthermore, PKC α regulates the expression of specific AP-1 factors that are associated with particular stages of epidermal differentiation (12, 13). The relationship of PKC α to AP-1 activity in skin extends to neoplastic development where the aberrant expression of differentiation markers detected in keratinocytes transformed by a v-ras oncogene is attributed to increased activity of PKC α and activation of AP-1 transcription factors (14).

Most of the foregoing studies were conducted in vitro using intact or transfected cultured human or mouse keratinocytes induced to differentiate under varying culture conditions, sometimes in the presence of PKC inhibitors. More recently, PKC α , δ , and ϵ have been transgenically targeted to the epidermis of FVB/N mice with the keratin 14 promoter producing distinct phenotypic changes (15–17). Although undisturbed skin was normal in PKC α

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⁴ Abbreviations used in this paper: PKC, protein kinase C; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; SPR, surface plasmon resonance; COX-2, cyclooxygenase-2; BrdU, 5-bromo-2'-deoxyuridine; DAPI, 4',6'-diamidino-2-phenylindole; MIP, macrophage-inflammatory protein; RPA, RNase protection assay; TRE, TPA response element.

or δ mice, PKC ϵ mouse epidermis was slightly hyperplastic (17), suggesting this isoform contributes to keratinocyte proliferation. When the PKC activator 12-O-tetradecanoylphorbol-13-acetate (TPA) was applied to transgenic skin, sustained hyperplasia was greatest in PKC ϵ epidermis, confirming a proliferative influence of this isoform. Major differences among the three transgenic lines were detected in skin tumor induction studies using 7,12-dimethylbenz[a]anthracene as initiator and TPA as the promoter. K14-PKC8 mice developed few papillomas or carcinomas, whereas K14-PKC ϵ mice were very sensitive to carcinoma formation, developing malignant tumors even in the absence of TPA application (16, 17). K14-PKC α transgenic mice did not differ from nontransgenic mice in tumor yield (15). Wang and Smart (18) developed K5-PKC α transgenic mice on a C57B/6 background and demonstrated that skin tumor formation was not influenced by overexpression of PKC α . However, TPA treatment of the K5-PKC α mice caused severe intraepidermal and dermal inflammation, degeneration of hair follicles, and a disruption and sloughing of the epidermis (18), changes not detected in the other PKC transgenic strains. This suggested that PKC α was influencing the inflammatory response in skin, and subsequent studies suggested this was mediated, at least in part, by PG generated through activation of phospholipase A₂ and up-regulation of cyclooxygenase-2 (COX-2) (19).

We have developed FVB/N transgenic mice that overexpress PKC α in the basal layer of the epidermis and the outer-root sheath of the hair follicle under targeted expression of the keratin 5 promoter. These mice had the identical acute inflammatory response and epidermal degeneration after TPA activation as the C57BL/6 mice described by Wang and Smart. Because marked intraepidermal inflammation is an unusual response in mice, but has been seen in some human cutaneous diseases (20), we undertook a combined in vivo and in vitro analysis of transgenic skin and keratinocytes to determine which responses were primary to PKC α activity in keratinocytes, as opposed to secondary responses to the inflammatory infiltrates. We show that divergent signal transduction pathways emanating from PKC α regulate keratinocyte cell death and cutaneous inflammation, and therefore, PKC α or a downstream pathway may serve as a target for the treatment of certain inflammatory or cytotoxic skin diseases.

Materials and Methods

Plasmid construction and generation of transgenic mice

Full-length murine PKCa cDNA was excised from PKCa-pcDNA by partial digestion with NheI and inserted in the NheI site of plasmid p368 (21), which contains the bovine keratin K5 regulatory sequence, the second intron of the rabbit β -globin gene, and polyadenylation sequences kindly provided by Dr. J. Jorcano (Centro de Investigaciones Energéticas Medioambientales, y Technológicas, Madrid, Spain) (22). From this plasmid, designated K5-PKC α , the transgene was excised by digestion with Acc65 I, purified by agarose electrophoresis and EluTip columns (Schleicher & Schuell, Keene, NH), adjusted to a final concentration of 2 µg/ml, and microinjected into the pronuclei of FVB/N mouse embryos. Founder mice were identified by Southern blotting of 10 μ g of genomic DNA from tail biopsies, digested with EcoRl, separated on a 0.8% agarose gel, and transferred to a nylon membrane. The probe was a KpnI/NheI (5-9 kb) fragment from the K5-PKC α construct that corresponds to the keratin K5 regulatory region and the β -globin intron. Subsequent offspring were identified by PCR using primers TGCATATAAATTCTGGCTGGCG and GCATGAA-CATGGTTAGCAGAGGG that span a 166-nt sequence of the β -globin intron.

K5-PKC α mice were crossed with 2× TPA response element (TRE)luciferase reporter transgenic (23) mice to generate K5-PKC α -positive and K5-PKC α -negative/2× TRE-luciferase mice. K5-PKC α mice were also crossed with CCR1^{-/-} mice to generate PKC α mice null for the chemokine receptor CCR1 (24). TPA (LC Laboratories, Woburn, MA) was dissolved in acetone, and the indicated concentrations were applied in 200 μ l.

To express the *A-FOS* transgene (a dominant negative that abolishes AP-1 DNA binding) (25) in vivo in K5-PKC α -expressing mice, a regulated

and targeted A-FOS mouse line was used. This line uses the tetracycline transactivator under regulation of the keratin 5 promoter sequences (26), which has been crossed into an A-FOS line with conditional expression controlled by the tetracycline operon (M. J. Gerdes, J. Moilia, M. R. Levy, A. Glick, S. H. Yuspa, and C. Vinson, manuscript in preparation). Mice expressing both the dominant-negative *AP-1* (A-FOS) and the *PKC* α transgenes were produced by crossing a double transgenic A-FOS (K5-tTA/tet-O-A-FOS) and K5-PKC α . At weaning, tail samples were collected and screened by three separate PCRs for each of the transgenes, and the triple transgenic mice were used for TPA treatment.

Cell culture

Primary mouse keratinocytes and hair follicle buds were isolated from newborn transgenic and wild-type littermate epidermis as described (27). Primary keratinocytes were seeded at a density of 5×10^6 cells per 60-mm dish (or equivalent concentrations) in Ca²⁺- and Mg²⁺-free MEM (Life Technologies, Rockville, MD) supplemented with 8% Chelex (Bio-Rad, Richmond, CA), treated FBS (Gemini Bio-Products, Woodland, CA), and 0.2 mM Ca²⁺. After 24 h, cultures were switched to the same medium with 0.05 mM Ca²⁺ to select for basal cells. TPA was reconstituted in DMSO, and primary keratinocytes were treated with TPA at varying concentrations and for various times as indicated in individual experiments.

Chemotaxis assays

Chemotaxis assays were performed using 48-well chemotaxis chambers (NeuroProbe, Cabin John, MD) as described previously (24). A total of 26–28 μ l of conditioned medium collected at different time points after TPA treatment of cultured keratinocytes was placed in the wells of the lower compartment of the chamber, and 50 µl of mouse peritoneal neutrophils (1.5×10^5) were placed in the wells of the upper compartment. The upper and lower compartments were separated by a polyvinylpyrolidone-free polycarbonate membrane (3-µm pore size; NeuroProbe). The pore size was chosen to allow the migration of neutrophils, but not monocytes. After incubation at 37°C for 1 h, the filters were removed, washed on the upper side, and stained. Cells migrating across the filters were counted under light microscopy after the samples were coded. The results were expressed as a chemotaxis index, which represents the fold increase in the number of migrated cells in six high-powered fields in response to TPA stimulated supernatants over the spontaneous cell migration in response to control medium.

In vivo UVB irradiation of mice

Age-matched adult mice were irradiated with 2000, 3000, or 4000 J/m² UVB generated from four Westinghouse FS20 Sunlamp bulbs (270–385 nM emission spectrum with peak at 313 nM). The irradiated area was confined to a dorsal region by placement of a template that shielded unirradiated skin. The energy emitted by the lamps was measured with a model PMA 2100 meter (Solar Light, Philadelphia, PA) and a model PMA 2106 UV radiation detector calibrated to register the energy from 282 to 326 nm. Irradiated skin was harvested at 24 or 48 h, fixed in neutral formalin, and stained with H&E or assayed for apoptotic cells.

Immunohistochemistry and immunofluorescence

Shaven transgenic and wild-type animals were treated topically with a single dose (2 μ g) of TPA in 200 μ l of acetone. Skin was excised at various times after treatment and fixed in zinc fixative (BD PharMingen, San Diego, CA) or 10% formalin solution (Sigma-Aldrich, St. Louis, MO), paraffin embedded, sectioned, and stained with H&E. Serial sections were incubated with rabbit anti-human T cell CD3 affinity purified Ab (DAKO, Carpenteria, CA), rat anti-mouse F4/80 mAb to identify murine macrophages (Serotec, Oxford, UK), or biotinylated rat mAb Ly-6G to detect peripheral neutrophils (Caltag Laboratories, Burlingame, CA). PKC α was detected in ethanol-fixed skin sections and methanol:acetone-fixed cultured cells using a mouse mAb against human PKC α (Sigma-Aldrich) and an anti-mouse IgG coupled to FITC (Vector Laboratories, Burlingame, CA). Cultured keratinocytes were pulsed for 1 h with 25 µM 5-bromo-2'-deoxyuridine (BrdU) and fixed in methanol:acetone (1:1). Cultures were incubated with a mouse monoclonal anti-BrdU Ab (BD Bioscienses, San Jose, CA) followed by a secondary biotinylated anti-mouse Ab (Jackson ImmunoResearch Laboratories, West Grove, PA). Immunoreactivity was detected using the ABC and DAB kits from Vector Laboratories. BrdU incorporation was quantitated by counting BrdU-labeled nuclei in 100 cells from three randomly chosen areas. Experiments were repeated three times. For 4',6'-diamidino-2-phenylindole (DAPI) staining, a mounting medium with DAPI (Vector Laboratories) was used under coverslips overlying fixed cells in situ. Fluorescence was detected with a Leitz DMRB microscope.

RT-PCR

RNA was isolated from cultured keratinocytes with TRIzol (Invitrogen, San Diego, CA). For cDNA synthesis, 2 μ g of total RNA were reversetranscribed using Superscript II Reverse Transcriptase (Invitrogen). PCR amplifications were performed in a volume of 20 μ l using Platinum PCR SuperMix (Invitrogen). The primers used for this analysis were as follows: GAPDH forward sequence, ATG GTG AAG GTC GGT GTG AAC G; GAPDH reverse sequence, ACC TGG TCC TCA GTG TAGC CCA; macrophage-inflammatory protein (MIP)-2 forward sequence, CTG CCG GCT CCT CAG TGC TGC ACT G; MIP-2 reverse sequence, GCC TTG CCT TTG TTC AGT ATC TTT TGG; CCR1 forward sequence, GAC CAG CAT CTA CCR GTT CA; CCR1 reverse sequence, GCA GAA ACA AAT ACA CTC AG; COX-2 forward sequence, CAC AGT ACA CTA CAT CCT GAC C; COX-2 reverse sequence TCC TCG CTT CTG ATT CTG TCT TG; MIP-3α forward sequence, AGC CAG GCA GAA GCA AGC AAC TAC; MIP-3 α reverse sequence, CTG TGT CCA ATT CCA TCC CAA AAA; TNF- α forward sequence, ATG AGC ACA GAA AGC ATG ATC CG; TNF- α reverse sequence GCA ATG ACT CCA AAG TAG ACC TGC CC. To avoid saturation or the plateau effect of amplification, PCR was limited to a total of 20 cycles for GAPDH and MIP-3 α , 28 cycles for MIP-2, COX-2, and TNF- α , and 30 cycles for CCR1. Each reaction was performed from three independent experiments.

RNase protection assay (RPA)

RPAs were performed using Riboquant Transcription and RPA kit (BD PharMingen) according to manufacturer's instructions with the following modifications. The labeled Multiprobe templates (BD PharMingen) and Mig 416 probes were purified using a g50 spin column (Amersham Pharmacia Biotech, Piscataway, NJ). RNase inactivation and precipitation were performed with a master mixture containing 200 µl of Ambion RNase inactivation reagent, 50 µl of ethanol, and 1 µl of Ambion GycoBlue per RNA sample. After the individual RNase-treated samples were added to 250 μ l of the inactivation/precipitation mixture, the samples were mixed well, placed at -20° C for 30 min, and centrifuged at $20,800 \times g$ for 15 min. The supernatants were decanted, a sterile cotton swap was used to remove excess liquid, and the pellet was resuspended in 5 µl of BD PharMingen sample buffer. For all RPAs, the protected bands were separated on SequaGel-6 (National Diagnostics, Atlanta, GA). Gels were dried under vacuum at 80°C for ~1 h and placed into storage phosphor cassettes and exposed for 16-24 h. The images were visualized and quantitated using a Typhoon 8600 PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Protein immunoblot analysis

For immunoblot analysis of proteins isolated from skin, samples were frozen in liquid nitrogen and homogenized in lysis buffer containing 20 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1.5 mM MgCl₂, 10% glycerol, 1% Triton X-100, 5 mM EDTA, 1 mM PMSF, 2 μ g/ml aprotinin, and 20 μ M leupeptin. Cultured cells were lysed in the same buffer with the addition of 200 μ M NaVO₃ and 10 mM NaF for detecting PKC isoforms. Proteins were quantified by the Bradford method (Bio-Rad) and separated on 7.5% SDS-PAGE for immunoblotting. PKC α mAb was used at 1:50,000 (Sigma-Aldrich), PKC δ polyclonal Ab at 1:30,000 (R&D Systems, Minneapolis, MN), PKC ϵ polyclonal Ab at 1:1000 (Life Technologies), and PKC ζ polyclonal Ab at 1:500 (Sigma-Aldrich). The ECL SuperSignal (Pierce, Rockford, IL) detection system was used.

Cell viability assays

For detecting apoptotic cells in vivo, formalin-fixed skin sections were analyzed using an in situ apoptosis detection kit DermaTACS (Trevigen, Gaithersburg, Maryland). Briefly, this method uses a brominated nucleotide (BrdU) incorporated by the TdT at the sites of DNA fragmentation. The incorporated BrdU is detected using a biotinylated anti-BrdU Ab in combination with a streptavidin-peroxidase conjugate. Labeled cells were visualized with the TACS Blue Label (Trevigen), and sections were counterstained with eosin. Sections were visualized by light microscopy.

For in vitro analyses, primary transgenic and wild-type keratinocytes were seeded in 12-well dishes at a concentration of 1.5×10^6 cells per well, cultured for 3 days, and treated in triplicate with various doses of TPA for 6 h. To determine cell number, keratinocytes were trypsinized and counted using a Z series Coulter counter. Cell viability was determined after 18 h of TPA treatment by MTT assay using a cell proliferation assay kit, the CellTiter 96 (Promega, Madison, WI). For MTT assays with the PKC α inhibitor Go6976, cells were treated for 1 h with 1 μ M and 0.5 μ M of the inhibitor before TPA treatment. The results represent the mean of three independent experiments.

Annexin V and caspase activity assays

PKCα transgenic and wild-type keratinocytes were treated with TPA, trypsinized, and incubated with annexin V-biotin/propidium iodide solution (Annexin V Apoptosis Detection kit; BD PharMingen) for 20 min at room temperature. The reaction was then incubated with streptavidin-APC and analyzed by flow cytometry using a FACSCalibur (BD Biosciences). Caspase activity was analyzed using the CaspaTag Caspase Activity kit (Intergen, Purchase, NY), and keratinocytes were treated with TPA for 3 h. Cells were incubated for 1 h with $60 \times$ FAM-VAD-FMK working dilution in medium with TPA and washed three times with 1× working dilution wash buffer; coverslips were mounted with DAPI solution. Caspase activity was detected by fluorescence microscopy because of binding of the fluorescein derivative of Z-VAD.

Assays of TRE-luciferase activity

TRE-luciferase activity was measured in primary keratinocytes isolated from K5-PKC $\alpha/2 \times$ TRE-Luc and wild-type/2× TRE-Luc bigenic mice. Cells were treated with TPA or DMSO for the indicated period of time; then, total cell extracts were prepared according to manufacturer's protocol (Clontech Laboratories, Palo Alto, CA). Relative light units were normalized to protein determined by the Bradford protein assay (Bio-Rad). Results are expressed as fold increase of luciferase activity in TPA treated over respective control.

EMSA

Nuclear extracts (4 μ g) were incubated with a ³²P-labeled double-stranded probe containing the AP-1 consensus sequence (Promega) as described previously (28).

Adenovirus infection

A dominant-negative AP-1 vector, A-FOS, was introduced into keratinocytes using an adenoviral construct driven by a CMV promoter (25, 29), and empty adenovirus was used as control. The cells were infected for 30 min in serum-free medium with a multiplicity of infection of five viral particles per cell and 2.5 μ g/ml Polybrene (Sigma-Aldrich) to enhance uptake. Serum containing medium was added to the cells for the next 48 h after the infection.

Results

$PKC\alpha$ mediates massive intraepidermal inflammation and sloughing in vivo

To generate a skin-targeted PKC α transgenic mouse, murine PKC α cDNA was cloned under the regulation of the keratin 5 promoter (Fig. 1A). Transgenic mice were generated using the FVB/N mouse strain, and several founder lines were developed expressing the transgene at high (10-fold) or moderate (3- to 5-fold) levels over the endogenous expression as shown by Southern and Western blot analysis. The plasmid construct used to generate PKC α transgenic mice contains the rabbit β -globin intron that can be detected in transgenic DNA by Southern analysis (Fig. 1B) and by PCR (Fig. 1C). Specific overexpression of the PKC α isoform could be seen in the skin and cultured keratinocytes by Western analysis and did not alter the level of other PKC isoforms in keratinocytes (Fig. 1, D and E). Localization of the $PKC\alpha$ transgene appeared to be targeted to the basal layer of the epidermis and the outer root sheath of the hair follicle as expected by keratin 5 expression (Fig. 2A). In cultured transgenic keratinocytes, PKC α was detected in the cytoplasm and more intensely in the perinuclear area as determined by immunofluorescence (Fig. 2B).

There were no abnormalities in the development of the transgenic animals compared with wild-type littermates, and the untreated epidermis appeared normal. However, the high-expressing PKC α transgenic animals were very sensitive to a single topical treatment of TPA to the skin. The moderate expressing lines were also TPA sensitive, but the changes described below were more focal than general. Subsequent experiments were performed on the high expressing line. As early as 12 h after TPA treatment, there was a severe inflammatory response in the skin of PKC α transgenic mice that was associated with a disruption of the epidermis



FIGURE 1. Generation of transgenic mice overexpressing PKC α under the regulation of the keratin 5 promoter. *A*, For generating the transgene construct for skin-targeted PKC α transgenic mice, PKC α cDNA was microinjected into the pronuclei of FVB/N mouse embryos. *B*, DNA isolated from the tails of several founder transgenic and wild-type mice was digested with *Eco*RI and analyzed by Southern blotting with a probe for the bovine K5 promoter regulatory region. *C*, The transgene can be detected by PCR using primers to the β -globin intron of the transgene construct. WT = wild type; MW = molecular weight. *D*, Western analysis for PKC α protein expression can be detected from the skin of several different transgenic founder mice as well as nontransgenic mice. *E*, Primary keratinocytes isolated from a high expresser line of transgenic mice specifically overexpress PKC α in vitro, but not other isoforms as determined by Western blots of cell lysates.

(Fig. 3A). A time course indicated that the inflammatory cells migrate rapidly out of dermal vessels and into the upper dermis around hair follicles within 6 h. By 12 h there is marked inflammation in the follicles and epidermis with the formation of microabscesses. Destruction of the epidermis and the upper hair follicles is observed by 12-18 h. The region of the follicle below the sebaceous gland is largely spared. By 24-48 h, the epidermal compartment is composed of inflammatory cells encapsulated by a stratum corneum. This inflammatory response is very similar to the phenotype described by Wang and Smart (18) for K5 PKC α transgenic mice on a C57BL/6 background. A series of leukocyte selective Abs was used for immunohistochemistry on serial sections (Fig. 3B), revealing that neutrophils (Ly-6G-positive cells) are the predominant inflammatory component of the acute reaction in the follicles and epidermis of transgenic mice. Neutrophils can also be seen trafficking through the dermis of both wild-type and transgenic animals, but significant epithelial targeting is confined to the transgenic skin. Immunohistochemical staining for macrophages (F4/80-positive cells) or T cells (CD-3⁺ cells) in serial sections did not reveal an accumulation of these cell types (data not shown) within the first 48 h.

Remarkably, the massive and uniform destructive consequences of a single low dose TPA exposure on transgenic skin did not



FIGURE 2. Detection of PKC α in the skin and isolated keratinocytes of K5-PKC α transgenic mice. WT = wild type. *A*, PKC α is overexpressed in the basal layer of the epidermis and in the outer root sheath of hair follicles in transgenic skin detected by immunofluorescence with a PKC α -specific Ab. *B*, Cultured keratinocytes isolated from transgenic mice express abundant PKC α in the cytoplasm with perinuclear accentuation.

cause any apparent adverse effect on the host. By 3 days after treatment, there is almost complete regeneration of the epidermis, and over the next several days there is a marked hyperplastic response in the epidermis and regeneration of the hair follicles (Fig. 3*A*). Thus, a protected population of regenerative, multipotential cells below the level of the sebaceous gland must respond rapidly to the destruction of the superficial epithelial components of the skin.

Loss of keratinocyte viability is a consequence of activation of $PKC\alpha$ independent of the inflammatory infiltrate

TUNEL assays revealed that apoptosis of the epidermis and upper hair follicle could be detected as early as 6 h after TPA application to transgenic skin, before the major infiltration of neutrophils was seen (Fig. 4D). A greater number of keratinocytes were positively stained by 12 h, but the infiltration of neutrophils at that time made it difficult to distinguish cell types definitively. Apoptotic keratinocytes were mostly absent from TPA-treated wild-type epidermis (Fig. 4C). To determine whether the intraepidermal inflammatory infiltrate is a nonspecific consequence of the apoptosis, transgenic mice were irradiated with either 2000, 3000, or 4000 J/M² UVB, and their skin was examined for apoptotic cells (Fig. 4, A and B). Although a substantial number of apoptotic cells were detected at 24 h, the inflammatory response was confined to the dermal compartment (Fig. 4, E and F). This suggests that the intraepidermal inflammatory response to TPA is a specific consequence of PKC α activation, and not a general response to cell killing, but it does not exclude the possibility that apoptosis is secondary to inflammation.

To distinguish inflammation-independent effects of PKC α activation on the viability of epidermal and upper follicle cells, keratinocytes and hair follicle buds were isolated from wild-type and transgenic skin and cultured in 0.05 mM Ca²⁺ medium that selects for the proliferative K5-expressing population. Transgenic keratinocytes were very sensitive to small concentrations of TPA in vitro, assuming a dendritic morphology that persisted for 6–12 h before cells became rounded and detached from the substrate (Fig. 5A). In contrast, wild-type keratinocytes developed a dendritic shape only transiently and at higher TPA concentrations before reassuming an epithelial morphology. By 6 h, TPA exposure resulted in a dose-dependent decrease in cell number and BrdU incorporation in transgenic keratinocytes (Fig. 5, *B* and *C*). At this

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FIGURE 3. Sensitivity to TPA is enhanced in skin of K5-PKCa transgenic mice. WT = wild type. A, A single dose of TPA (2 μ g) in acetone was applied to the shaved backs of PKC α transgenic and wild-type littermates, and samples of treated skin were collected at various times and stained with H&E. B, Sections adjacent to the H&E samples were immunostained using Ly-6G Ab that specifically recognizes neutrophils. The dark brown staining indicates positively stained cells. Inflammatory cells infiltrate into the hair follicle and epidermis in the transgenic mice, while wild-type littermates have minimal dermal inflammation.



time point, the number of cells incorporating BrdU was reduced by >80% in transgenic keratinocytes but <20% in wild-type keratinocytes at the highest TPA dose tested. Together, these results suggested that activation of over-expressed PKC α in keratinocytes resulted in a rapid cell cycle block and cell loss consistent with the in vivo damage to epidermal cells.

In vivo data suggested that before the inflammatory response epidermal and upper hair follicle cells became apoptotic when exposed to TPA. This response was tested directly on transgenic keratinocytes in the in vitro model. MTT assays revealed a TPA dose-dependent loss of cell viability in transgenic, but not wildtype, keratinocytes within 6 h (Fig. 5D). Pretreatment with a PKC α inhibitor, Go6976, prevented cell death in TPA-treated PKC α keratinocytes, confirming that the PKC α activity was responsible for the decrease in cell viability (Fig. 5E). Additional analyses suggested that PKC α activation initiated an apoptotic death pathway (Fig. 6, A and B). By FACS analysis, the number of annexin Vpositive cells increased from $\sim 10-40\%$ in TPA-treated transgenic, but not wild-type, keratinocytes. Furthermore, there was a 5-fold increase in the number of attached cells that stained for activated caspases in PKC α keratinocytes treated with TPA. These results are consistent with the analysis of apoptotic transgenic keratinocytes detected in vivo, and indicate that the observed loss of keratinocytes in vivo is likely to be a direct consequence of PKC α activation independent of the inflammatory response.

PKC α mediates expression of multiple cytokines and chemokines in keratinocytes

In the previous description of the TPA-exposed K5PKC α transgenic skin phenotype by Wang and Smart (18), enhanced expres-

sion of transcripts for MIP-2 and TNF- α were detected, as well as elevated levels of COX-2 protein. We have confirmed the up-regulation of MIP-2 and COX-2 transcripts by RT-PCR in TPAtreated cultured transgenic keratinocytes (Fig. 7A), and in addition detected strong induction of MIP-3 α . We have used the in vitro model to examine expression of other cytokines and chemokines that may contribute to the inflammatory response. RPAs were used to screen for cytokine and chemokine transcripts that might be differentially regulated by PKC α activation in the first 3 h after TPA treatment (Fig. 7*B*). The detection of TNF- α by this method is consistent with the results of Wang and Smart (18) assayed by Northern blot. In addition, specific up-regulation of MIP-1 α and β and IL-12p40, but not IL-18, were detected. In fact, TPA caused a reduction of IL-18 transcripts in transgenic keratinocytes. Of particular interest is the detection, both by RPA (data not shown) and RT-PCR analysis, of CCR1 that is up-regulated by TPA treatment in transgenic keratinocytes. The expression of this receptor and CXCR2 on keratinocytes in an environment where ligands such as MIP-1 α and MIP-2 are also up-regulated suggests that an autocrine mechanism may be functioning in the entirety of the keratinocyte response to PKC α activation.

In vitro chemotaxis assays were used to test whether culture supernatant from TPA-treated K5-PKC α keratinocytes contains chemotactic activity. Supernatants from TPA-treated transgenic cells were able to attract neutrophils to a greater extent than supernatants from treated wild-type keratinocytes (Fig. 7*C*), confirming that functional chemokines are released from keratinocytes after activation of PKC α .

Both CCR1 and CCR5 are the major receptors for MIP-1 α , but CCR1 is the dominant receptor for MIP-1 α in mouse neutrophils.



FIGURE 4. PKC α activation elicits apoptosis in the skin of K5-PKC α mice. Apoptosis was evaluated by nick-end labeling of DNA in skin from wild-type (*A*, *C*, *E*) or K5-PKC α (*B*, *D*, *F*) mice after TPA or UV light treatment. Mice were treated with a single dose of TPA on the shaved back, and 6 h later skin samples were taken. Skins from UVB exposed mice were collected 24 and 48 h after irradiation (2000 J). Skin sections (magnification, ×400) from wild-type (*A*) and transgenic (*B*) skin 24 h after UVB irradiation, wild-type (*C*) and transgenic (*D*) skin 6 h after TPA; H&E staining of wild-type (*E*) and transgenic (*F*) skin 48 h after UVB irradiation. Arrows point to apoptotic nuclei.

Deletion of CCR1 in mice resulted in impaired neutrophil infiltration in several mouse models (24, 30–32). Because MIP-1 α is significantly up-regulated in TPA-treated transgenic keratinocytes, we tested whether neutrophil infiltration in the PKC α transgenic mice is mediated by MIP-1 α and its receptor CCR1. We crossed K5-PKC α mice with CCR1^{-/-} mice, treated bigenic K5-PKC α / CCR1^{-/-} mice topically with TPA, and compared the inflammatory response with K5-PKC α /CCR1^{+/-} littermates. In both genotypes, intraepidermal inflammation was massive and equivalent (Fig. 8A), suggesting that CCR1 is not a major mediator of neutrophil infiltration in response to PKC α activation. Confirmatory results were obtained using supernatants from TPA-treated K5-PKC α keratinocytes in chemotaxis assays with CCR1^{-/-} or CCR1^{+/+} neutrophils (Fig. 8*B*). Whereas recombinant MIP-1 α confirmed the requirement for CCR1 to induce chemotaxis for this ligand, and fMet-Leu-Phe confirmed the capability for chemotaxis in neutrophils of both genotypes, conditioned medium from TPAtreated K5-PKC α keratinocytes was equally chemotactic in the presence or absence of CCR1 on the neutrophilic target. Thus, CCR1 is not required for either in vivo or in vitro chemotaxis, and MIP-1 α is not likely to be a critical mediator of intraepidermal inflammation in this model.

AP-1 is an effector of PKC α -mediated apoptosis in keratinocytes

Previous data from keratinocytes suggested that PKC α regulation of gene expression in differentiating keratinocytes occurs through a common signal transduction pathway that involves AP-1 activity (12). Similarly, PKC-mediated apoptosis has been linked to AP-1 activation through p38 and Jun kinases (33, 34). To test this link in



FIGURE 5. TPA treatment of K5-PKC α transgenic keratinocytes inhibits DNA synthesis and causes cell death. *A*, Primary keratinocytes from transgenic and nontransgenic mice were cultured in 0.05 mM Ca²⁺ and treated with 5 ng/ml TPA. After 6 h, transgenic keratinocytes developed a dendritic morphology, and cells detached from the culture substrate. WT = wild type. *B*, After treatment of transgenic and wild-type cultures with varying doses of TPA, dishes were washed with PBS, and attached cells were counted and expressed as a percent of total cells. *D*, Similarly treated dishes were assayed for cell viability by MTT assay. *E*, Keratinocytes were pretreated for 1 h with the PKC inhibitor Go6976 before the addition of TPA to the culture medium and tested for viability by MTT assay after 6 h. WT = wild type.

the PKC α transgenic model, primary keratinocytes from PKC $\alpha/2 \times$ TRE-luciferase bitransgenic mice and nontransgenic mice were treated with TPA, and reporter activity was quantified in total cell extracts. TPA treatment induces a strong and sustained increase of AP-1-dependent reporter activity only in the PKC α transgenic keratinocytes (Fig. 9). To test whether this increased AP-1 activity was associated with the TPA-induced apoptotic response, primary keratinocytes from PKC α transgenic mice and nontransgenic mice were transduced with an adenovirus (A-FOS) that expresses a dominant-negative construct for the AP-1 family of transcription factors. The A-FOS mutant dimerizes with Jun family members and prevents DNA binding, as the DNA binding domain of the dominant-negative mutant is replaced with an amphipathic region that is unable to bind to DNA (25, 35). Infection with the A-FOS adenovirus prevented the increase in AP-1-specific DNA binding activity in TPA-treated transgenic keratinocytes (Fig. 9B) and modified the biological effects of PKC α overexpression and activation. Expression of A-FOS prevented cell killing by TPA in PKC α transgenic cells as determined by MTT assay (Fig. 9C). Although an AP-1 binding site is present in the promoter region of the keratin 5 gene (36), A-FOS did not interfere with $PKC\alpha$ transgene expression as determined by immunoblots in A-FOS- or A-CMV-infected transgenic keratinocytes (data not shown).



FIGURE 6. TPA induces apoptosis in K5-PKC α transgenic keratinocytes. *A*, PKC α transgenic and wild-type (WT) keratinocytes were treated with 5 ng/ml TPA for 6 h, and attached and floating cells were combined and analyzed for annexin V binding by FACS. The data shown are representative of two independent experiments and represent the percentage of annexin V⁺ cells in the total population. *B*, PKC α transgenic and nontransgenic keratinocytes were exposed to TPA for 6 h and then incubated with a fluorescein-labeled caspase inhibitor, FAM-VAD-FMK, using the CaspaTag kit (Intergen). Cells with activated caspases were detected with fluorescence microscopy and quantitated as a percent of total cells stained with DAPI. Both DAPI staining and FAM-VAD-FMK labeling were repeated three times.

PKCα-mediated inflammation is independent of AP-1

We generated triple transgenic mice by crossing bigenic mice that carry a tetracycline-regulated, K5-targeted A-FOS construct with the K5-PKC α mice. The triple transgenic mice were TPA treated under conditions of A-FOS expression (without doxycycline), and skin was collected at various time points. Expression of A-FOS did not prevent the intraepidermal inflammatory infiltrate seen in K5-PKC α mice (Fig. 10A). Transgenic A-FOS also did not prevent the destruction of the epidermis or hair follicles that occur as a consequence of the inflammatory infiltrate or the extensive hyperplasia that results during the recovery process (Fig. 10A). Furthermore, A-FOS transduction in transgenic keratinocytes in vitro did not influence chemotactic activity of culture supernatants after TPA treatment (Fig. 10B). This was consistent with the even higher expression of specific chemokines detected by RT-PCR or RPA analysis in A-FOS-transduced, TPA-treated K5-PKCα keratinocytes (Fig. 10, C and D). In contrast, A-FOS did reduce expression of COX-2 and CCR1 in TPA-treated transgenic cells (Fig. 10, C and D).

Discussion

The K5-PKC α transgenic mouse provides a useful model to explore the influence of this PKC isoform on cutaneous physiology and pathology. We now show that transgenic keratinocytes in vitro are a valid model to explore downstream signaling pathways at the cellular level. As first reported by Wang and Smart (18) for the



FIGURE 7. PKC α regulates the expression of inflammatory intermediates and causes the release of neutrophil chemoattractants. *A*, Agarose gel stained with ethidium bromide showing RT-PCR product for MIP-2 (281 bp), MIP-3 α (260 bp), COX-2 (400 bp), CCR1 (581 bp), and GAPDH (835 bp). RNA was extracted from wild-type (WT) and K5-PKC α cells 3 h after TPA treatment. DMSO was used as control. *B*, RPA was performed as described under *Materials and Methods*, with RNA extracted from WT and K5-PKC α cells 3 h after TPA treatment. The mRNA expression of the housekeeping gene *L32* was included to normalize for gel loading. *C*, Chemotactic activity of culture supernatant collected from WT and K5-PKC α keratinocytes after TPA treatment over the period indicated. Fold increase (chemotaxis index) of neutrophil migration in response to TPA was compared with supernatant collected after DMSO treatment. Bars represent the mean \pm SEM of two triplicate determinations.

C57BL/6 background, and now confirmed for the FVB/N background, keratinocyte PKC α is a major mediator of neutrophilic inflammation both in the dermal and epidermal compartments. Wang and Smart demonstrated a role for phospholipase A₂ and mitogen-activated protein kinase kinase signaling in the generation of PG (19) that may contribute to inflammation in this model. We now show that activation of PKC α increases expression of multiple cytokines and chemokines and increases chemokine receptor CCR1 transcripts in the keratinocytes themselves. We further show that activation of PKC α in the FVB/N strain produces keratinocyte growth arrest and apoptosis that is dependent on intact AP-1 transcriptional activation, but independent of the inflammatory infiltrate. Likewise, the apoptotic response is not likely to be the direct signal for the inflammatory infiltrate, because substantial apoptosis induced by UVB does not result in intraepidermal inflammation in transgenic skin. Together, it would appear that PKC α controls two distinct responses in keratinocytes, one related to growth and viability that is AP-1-dependent and the other related to chemotaxis and inflammation that appears to be independent of AP-1.

Growth arrest mediated by PKC α overexpression has been previously demonstrated in epithelial cells, including those derived



FIGURE 8. Ablation of CCR1 does not prevent the cutaneous inflammatory response. K5-PKC α mice were crossed with CCR1^{-/-} mice, and offspring were genotyped. *A*, Six-week-old K5-PKC α mice either heterozygous or null for CCR1 were treated with a single dose of TPA (2 µg) in acetone, and samples of treated skin were collected at 24 h and H&E stained. *B*, Neutrophils chemotaxis, bars represent the mean ± SEM of triplicate determination using neutrophils from CCR1^{+/+} and CCR1^{-/-} mice in response to MIP1- α (10 nM), fMet-Leu-Phe (1 µM), or conditioned medium (CM) from TPA-treated K5-PKC α -cultured keratinocytes.

from intestine and mammary gland (37–39). However, in transgenic mouse skin, overexpression of PKC α was not sufficient to alter growth until it was activated by phorbol ester. This implies that only a low level of endogenous activator is present in resting skin keratinocytes. Because activated PKC α regulates the state of calcium dependence of desmosomal connections (40), strict regulation of this isoform in epidermis is likely to be necessary for proper cell-cell adhesion in the formation of the skin barrier. In pathological states associated with epidermal damage, endogenous PKC activators may be elevated, and PKC α activation may reduce cell-cell adhesion, allowing for keratinocyte migration and sloughing of damaged cells. Our studies suggest another function for cutaneous PKC α . Under pathological conditions, activation of this isoform could also contribute to both inflammation and cell killing that is often required before the healing process.

Considerable evidence has accumulated implicating PKC δ in keratinocyte apoptosis (11, 41). Those studies indicated a direct role for the δ isoform translocating to mitochondria and acting on a mitochondrial apoptotic pathway to reduce keratinocyte viability. In contrast, PKC α is frequently anti-apoptotic by virtue of its ability to phosphorylate and activate BCL2 in mitochondria (42). However, PKC α is proapoptotic for prostate and gastric cancer cells (43, 44), suggesting that the cell type, physiological state of the target cell, or subcellular distribution of the enzyme determines the response to PKC α activation. In skin, activation of overexpressed PKC α in keratinocytes is proapoptotic, but paradoxically, high constitutive activity of PKC α is characteristic of neoplastic epidermal cells transformed by the *ras* oncogene (45). Such cells also have increased levels of diacylglycerol, the endogenous activator of PKC α (46). Because the proapoptotic activity of cutane-



FIGURE 9. Increased AP-1 activity is associated with PKC α activation and keratinocyte apoptosis. Keratinocytes were isolated from the skin of bigenic mice and cultured for 3 days. A, AP-1-dependent luciferase activity in wild-type/2X TRE-Luc (WT-TRE Luc) and K5-PKC $\alpha/2 \times$ TRE-Luc (K5-PKCα-TRE-Luc) in response to TPA was compared at various time points. Bars represent mean ± SEM of quadruplicate determinations. Values are expressed as fold increase over respective control and are representative of three independent experiments. B, Nuclear extracts from A-CMV (control) or A-FOS (dominant-negative) adenovirus-transduced primary keratinocytes were reacted with a ³²P-labeled AP-1 binding sequence and evaluated for levels of AP-1 DNA binding activity using EMSA. Keratinocytes were cultured for 4 days, infected with adenovirus for 48 h, and treated with DMSO (-) or TPA (+). C, The viability of K5-PKC α primary keratinocytes transduced with the A-CMV or A-FOS adenoviruses at a multiplicity of five viral particles per cell was analyzed by MTT assay. Cells were treated with TPA for 18 h. Experiments were confirmed three times independently.

ous PKC α is prevented by blocking the AP-1 pathway, the proximal affectors of apoptosis are likely to be AP-1-regulated genes. The expression of AP-1 factors is altered in neoplastic keratinocytes (14), and this could contribute to the differences in response to PKC α activation between normal and neoplastic keratinocytes. Elevated PKC α activity in neoplastic cells could also contribute to the inflammatory response in cutaneous neoplasms. We cannot exclude a role for TNF- α as a mediator of the apoptosis observed in this model. TNF- α is PKC inducible and proapoptotic when overexpressed in mouse keratinocytes (47), and is highly induced in PKC α transgenic skin. However, TNF- α is not suppressed by A-FOS in PKC α transgenic keratinocytes (Fig. 10). Exogenous TNF- α cannot induce apoptosis in cultured PKC α keratinocytes (C. Cataisson and S. H. Yuspa, unpublished data). Together, these results suggest that TNF- α is not the mediator of the apoptotic response, but further studies will be required to elucidate the AP-1-dependent pathways altering keratinocyte viability.

The massive intraepidermal inflammatory infiltrate seen after PKC α activation is an unusual finding in a mouse model. Because



FIGURE 10. Inhibiting AP-1 activity in K5-PKC α transgenic skin does not prevent the cutaneous inflammatory response. K5-PKC α mice were crossed with K5-A-FOS mice, and offspring were genotyped. *A*, Six-weekold mice expressing both transgenes and K5-PKC α littermates were treated with a single dose of TPA (2 μ g) in acetone applied to the shaved backs. Samples of treated skin were collected at 24 h and 4 days, and formalinfixed sections were stained with H&E. *B*, Conditioned medium from TPAtreated cultured K5-PKC α keratinocytes transduced with A-FOS or CMV adenovirus was assayed for chemotaxis activity as described in Fig. 7. *C* and *D*, Keratinocytes were cultured for 4 days, infected with A-CMV or A-FOS adenoviruses for 48 h, and treated with DMSO (–) or TPA (+) for 3 h. mRNA expression was analyzed by RPA (*C*) or RT-PCR (*D*) as described in Fig. 7 and *Materials and Methods*.

this was detected in both C57BL/6 and FVB/N strains, it is unlikely to be a function of unique genetic modifiers as has been reported for other inflammatory responses (48). PKC activation in mouse skin is commonly associated with a mixed leukocyte dermal inflammation (49), but only occasional leukocytes are detected intraepidermally. Thus, PKC α activation must have a unique role in directing neutrophils into the epidermis. We have used an in vitro model to assay for cytokines and chemokines that may distinguish the cytokine/chemokine profile of PKC α overexpressing keratinocytes from wild-type controls. The up-regulated expression of COX-2 combined with the previous report of phospholipase A activity under the control of PKC α (18, 19) suggested the PG might contribute to this phenotype. However, targeting of COX-2 to transgenic mouse skin caused epidermal hyperplasia, but did not produce intraepidermal inflammation (50). PG may contribute in other ways, perhaps by increasing vascular permeability to allow easier migration of neutrophils.

We then performed an initial screen for cytokines/chemokines using RPA on TPA-treated wild-type and transgenic keratinocytes. This revealed substantial differences in the expression of a number of inflammatory mediators, which will need further exploration.

Epidermal targeting of IL-1 α or IL-1R produced a mixed dermal inflammatory response that does not resemble the inflammatory response seen in PKC α mice (51, 52). It is unlikely that MIP-1 α is responsible for the phenotype, because deletion of its dominant receptor in neutrophils, CCR1, did not reduce the intraepidermal inflammatory infiltrate. MIP-1 β is predominantly chemotactic for monocytes, and this was not a predominant cell type in the inflammatory lesions. Two cytokines, IL-12p40 and TNF- α , could contribute to the phenotype by stimulating specific downstream chemokines. IL-12 is known to induce IFN-y-inducible protein-10 as well as monocyte chemoattractant protein-1 and lymphotactin, and is predominantly a T cell cytokine. Interestingly, IL-12p40 is induced by TNF- α , and conversely, IL-12 suppresses TNF- α in the skin (53, 54). The TNF- α pathway is an interesting candidate to participate in the PKC α -mediated inflammatory response. TNF- α is up-regulated early in induced cutaneous neutrophilic inflammatory responses (55). Genetic deletion of $I\kappa B\alpha$ produced a dermatitis characterized by intraepidermal neutrophilic abscesses and elevation of TNF- α levels in the skin (56, 57). Although targeting TNF- α to the epidermis produced only a mild dermal infiltrate (58) that did not reproduce the I κ B α null phenotype, this may reflect an extraordinarily elevated TNF- α level not achieved as a physiological response. The TNF- α /NF- κ B pathway then must be considered in the future evaluation of the inflammatory arm of the PKC α response. Semiquantitative RT-PCR analysis suggested two other chemokines for further study. In particular, expression of MIP-2, a mouse homologue of IL-8, was substantially increased in transgenic keratinocytes. IL-8 has been associated with the intraepidermal pustules in psoriasis (59). The receptor for MIP-2, CXCR2, is a major chemokine receptor expressed in neutrophils and mediates neutrophilic inflammation. CXCR2^{-/-} mice have defects in neutrophil recruitment in an acute inflammatory response in several mouse models. Although MIP-2 per se has not been tested by targeting to murine epidermis, skin targeting the homologous chemokine KC, which shares the CXCR2, produced only a mild dermal infiltrate in a transgenic model (60). Nevertheless, MIP-2 elevation is detected as an early response to induced neutrophilic inflammation in mouse models and requires further analysis (55). Similarly, MIP-3 α , a ligand for CCR6, is elevated in psoriasis and is induced by TNF- α (61). Although MIP-3 α is thought to be chemotactic for dendritic cells, its potential role in the acute inflammatory response in this model deserves further study.

The PKC α transgenic mouse model is promising for discovering the underlying trophic interactions that produce intraepidermal inflammation and for testing potential new targets for therapy. In addition to exploring the role of cytokines and chemokines, other studies can address the contribution of chemokine receptors such as CCR1 and others. In the case of CCR1, the bigenic model we developed should be useful to test a potential role for keratinocyte CCR1 in apoptosis or growth regulation, particularly because the microenvironment also contains elevated MIP-1 α , a ligand for this receptor. However, present data indicate that CCR1 ligands are not responsible for intraepidermal inflammation. If the K5-PKC α mouse is to serve as a model for human disease or therapy of cutaneous inflammation, there should be pathological conditions where PKC α is activated in the skin. The similarity of the inflammatory skin lesions in the PKC α mouse to aspects of human pustular psoriasis has been noted (18), and similar lesions are detected in the spontaneous mouse mutant "flaky skin," a putative animal model for human psoriasis (62). Additionally, MIP-3 α and IL-8/ MIP-2 are elevated in some psoriatic lesions, suggesting further similarities (59, 61). Several reports have suggested that PKC signaling is altered in psoriasis (2, 63), and the endogenous PKC

ligand diacylglycerol is elevated in psoriatic lesions but not uninvolved skin (64). Further studies to address this issue seem warranted. Even in the absence of a specific human pathological homologue, K5-PKC α mice and keratinocytes derived from them will advance our understanding of the important contribution that PKC signaling makes in mediating chemokine expression and cutaneous inflammation. Such understanding is essential to develop rational therapies for cutaneous inflammation.

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