EPITHELIAL AND MESENCHYMAL CELL BIOLOGY

Local Glucocorticoid Activation by 11 β -Hydroxysteroid Dehydrogenase 1 in Keratinocytes

The Role in Hapten-Induced Dermatitis

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Over the past decade, extra-adrenal cortisol production was reported in various tissues. The enzyme that catalyzes the conversion of hormonally inactive cortisone into active cortisol in cells is 11 β -hydroxysteroid dehydrogenase 1 (11 β -HSD1). We recently reported that 11 β -HSD1 is also expressed in keratinocytes and regulates inflammation and keratinocyte proliferation. To investigate the function of 11 β -HSD1 in keratinocytes during inflammation *in vivo*, we created keratinocyte-specific 11 β -HSD1 knockout (*K5-Hsd11b1-KO*) mice and analyzed the inflammatory response in models of hapten-induced contact irritant dermatitis. *K5-Hsd11b1-KO* mice showed enhanced ear swelling in low-dose oxazolone-, 2,4,6-trinitro-1-chlorobenzene (TNCB)-, and 2,4-dinitrofluorobenzene-induced irritant dermatitis associated with increased inflammatory cell infiltration. Topical application of corticosterone dose dependently suppressed TNCB-induced ear swelling and cytokine expression. Similarly in mouse keratinocytes *in vitro*, corticosterone dose dependently suppressed 2,4,6-trinitrobenzenesulfonic acid-induced IL-1 α and IL-1 β expression. The effect of 11-dehydrocorticosterone was attenuated in TNCB-induced irritant dermatitis in *K5-Hsd11b1-KO* mice compared with wild-type mice. In human samples, 11 β -HSD1 expression was decreased in epidermis of psoriasis vulgaris compared with healthy skin. Taken together, these data suggest that corticosterone activation by 11 β -HSD1 in keratinocytes suppresses hapten-induced irritant dermatitis through suppression of expression of cytokines, such as IL-1 α and IL-1 β , in keratinocytes. (*Am J Pathol* 2016, 186: 1499–1510; <http://dx.doi.org/10.1016/j.ajpath.2016.01.014>)

Glucocorticoids (GCs) are one of the most effective classes of anti-inflammatory drugs and have been in use for more than a half century to treat acute and chronic inflammatory diseases. In dermatology, topical GCs are also often used to treat inflammatory skin diseases. GCs act by exerting an anti-inflammatory effect on keratinocytes and skin-infiltrating inflammatory cells.

Cortisol and corticosterone are the endogenous GCs in humans and rodents, respectively. GCs are released in response to various physical stressors and psychological stress. Over the past decade, research has focused on local cortisol/corticosterone production by *de novo* synthesis and by an activating enzyme.¹ Extra-adrenal production/activation of cortisol/corticosterone was reported in tissues such as the

colon, heart, and lung.^{2–8} Local cortisol/corticosterone production in the skin by a *de novo* pathway and by the cortisol-activating enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD) was also reported.^{9–23}

11 β -HSD resides in the membrane of the endoplasmic reticulum and catalyzes the interconversion of hormonally active cortisol/corticosterone and inactive cortisone/11-dehydrocorticosterone (11DHC) in cells.^{24–26} The enzyme 11 β -hydroxysteroid dehydrogenase 1 (11 β -HSD1) is widely present in the liver, lungs, adipose tissues, ovaries, and central nervous system.

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Association of 11 β -HSD1 with various diseases has been reported. In 2001, Masuzaki et al²⁷ reported that transgenic mice overexpressing 11 β -HSD1 in adipose tissue had increased adipose levels of corticosterone and developed visceral obesity that was exaggerated by a high-fat diet. A large number of findings of 11 β -HSD1 associated with obesity have been reported since then. 11 β -HSD1 is also associated with other diseases, including rheumatoid arthritis, inflammatory bowel disease, polycystic ovary syndrome, and lung disease.^{8,28–31}

Functions of 11 β -HSD1 in healthy skin physiology and disease have also been reported. Expression of 11 β -HSD1 is decreased in benign and malignant skin tumors, including seborrheic keratosis, basal cell carcinoma, and squamous cell carcinoma.³² In contrast, UVB irradiation increased expression of 11 β -HSD1.³³ In a mouse model 11 β -HSD1 negatively regulated the proliferation of keratinocytes and fibroblasts both *in vivo* and *in vitro*.¹⁶ Enhanced wound healing in 11 β -HSD1 knockout (KO) mice was also reported.³⁴

We previously initiated an investigation of the effects of 11 β -HSD1 in skin with the use of *Hsd11b1* null (*Hsd11b1*^{−/−}) mice; however, the analysis was complicated because the mice showed increased serum corticosterone concentrations, probably because of some compensatory mechanism.^{35,36} Thus, in the current study, we generated keratinocyte-specific *Hsd11b1* KO (*K5-Hsd11b1-KO*) mice to investigate the function of 11 β -HSD1 in keratinocytes and to eliminate the effect on serum corticosterone observed in conventional 11 β -HSD1 KO mice. We further investigated the expression of 11 β -HSD1 in psoriasis vulgaris.

Materials and Methods

Materials

2,4,6-Trinitrobenzenesulfonic acid (TNBS) and 2,4,6-trinitro-1-chlorobenzene (TNCB) were purchased from Nakarai (Kyoto, Japan) and Tokyo Chemical Industry (Tokyo, Japan), respectively. 2,4-Dinitrofluorobenzene (DNFB), oxazolone (OXA), corticosterone, and 11DHC were purchased from Sigma-Aldrich (St. Louis, MO).

Cell Culture

Isolation and culture of mouse keratinocytes were performed as previously described.¹⁶ Full-thickness skin harvested from 12-week-old mouse ear and tail was treated with 4 mg/mL dispase (Gibco; Invitrogen, Paisley, UK) for 1 hour at 37°C. Next, the epidermis was peeled from the dermis. The epidermis was trypsinized to prepare single cells. The cells were then incubated in EpiLife (Life Technologies, Carlsbad, CA) for 6 hours at 37°C in an atmosphere of 5% CO₂ in culture dishes precoated with type 1 collagen (Iwaki Glass, Chiba, Japan). Nonadherent cells were washed away with phosphate-buffered saline twice, and adherent cells were

cultured for 2 to 3 days in EpiLife. One day before use in experiments culture medium was replaced with cortisol- and phenol red-free EpiLife to eliminate the effect of cortisol.

Mice

Animal care was in strict accordance with the institutional guidelines of Osaka University. All animal experiments were performed with the approval of the Animal Experiments Committee of Osaka University (no. 20-003-0). *Hsd11b1*^{tm1a/tm1a} mice equivalent to *Hsd11b1*^{−/−} mice were obtained as previously described.³⁵ To create *Hsd11b1*^{flox/flox} mice, *Hsd11b1*^{tm1a/tm1a} mice were crossed with B6.Cg-Tg (ACTFLPe)9205Dym/J mice purchased from The Jackson Laboratory (Bar Harbor, ME) to remove the en2SA-IRES- β gal-pA-loxP- β act::neo-pA cassette. Generation of K5-Cre transgenic mice was previously described.³⁷ K5-Cre transgenic mice were bred with *Hsd11b1*^{flox/flox} mice to generate mice that carried the K5-Cre transgene and a floxed *Hsd11b1* allele (*K5*^{Cre/0}; *Hsd11b1*^{flox/+}). These mice were then mated with each other. Offspring that carried *K5*^{Cre/0}; *Hsd11b1*^{flox/flox} and *K5*^{Cre/0}; *Hsd11b1*^{+/+} were used for further analyses as *K5-Hsd11b1-KO* and wild-type, respectively. Generation of *Hsd11b1*^{−/−} mice was described in detail in our previous study.³⁵

Histopathologic Analysis

Samples of normal skin from healthy volunteers were taken with written informed consent. All studies were approved by the ethical committee of Osaka University. Detailed patient demographic characteristics (age, sex, and biopsy site) are described in [Supplemental Table S1](#). Human samples and mouse skin were fixed in 10% formaldehyde for 24 hours, followed by embedding in paraffin and microtome sectioning. Slides were stained with hematoxylin and eosin. For immunofluorescence analysis, sections were hydrated by passage through xylene and graded ethanols. After antigen retrieval for 10 minutes at 115°C in citrate buffer, pH 6.0 (for 11 β -HSD1) for 7 minutes at room temperature in Proteinase K (for CD45, Gr-1, F4/80), the slides were blocked with serum-free protein block (Dako-Cytomation, Carpinteria, CA) for 30 minutes, then incubated with primary antibody overnight at 4°C (rabbit anti-11 β -HSD1 antibody ab109554; dilution 1:100; Abcam, Cambridge, UK; mouse anti-Keratin 14 antibody ab49806; dilution 1:100; Abcam), followed by secondary antibody (anti-rabbit Alexa Fluor 555, A21428; anti-mouse Alexa Fluor 488, A11001; Invitrogen) and nuclear staining (Hoechst 33342; Invitrogen). For immunohistochemical analysis, sections were hydrated by passage through xylene and graded ethanols. After antigen retrieval for 10 minutes at 130°C in citrate buffer, pH 6.0, the slides were incubated in 0.3% H₂O₂ to block endogenous peroxidase activity. The slides were blocked with serum-free protein block (Dako-Cytomation) for 30 minutes and then incubated with rabbit anti-chemokine ligand 8 antibody (Bioss, Woburn,

MA) overnight at 4°C, followed by incubation with rabbit anti-11 β -HSD1 antibody, rat anti-CD45 antibody (550539; dilution 1:100; BD Biosciences, San Jose, CA), rat anti-Gr-1 antibody (14593181; dilution 1:100; eBioscience, San Diego, CA), or rat anti-F4/80 antibody (MCA497R; dilution 1:100; AbD Serotec, Oxford, UK) overnight at 4°C. After washing with TBS-T (50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, and 0.1% v/v Tween-20), slides were developed with the use of the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and counterstained with hematoxylin. Rabbit and rat IgG served as isotype controls.

Staining Quantification

Epidermal staining intensity and extent were blindly evaluated by two individuals (M.T. and S.M.). For each specimen, staining index was assessed by multiplying the staining intensity score by the extent of staining score as previously reported.³² Staining intensity was graded as follows: 1, none; 2, weak; 3, moderate; 4, strong. Staining extent was graded as follows: 1, 0% to 24%; 2, 25% to 49%; 3, 50% to 74%; and 4, 75% to 100%. On the basis of our formula, the index ranges from 1 to 16.

Hapten-Induced Irritant Dermatitis Model

Both surfaces of the right ear of each mouse was painted with 0.2% OXA, 0.2% TNCB, or 0.3% DNFB in 1:4 oil/acetone. Left ear was painted with 1:4 olive oil/acetone as vehicle control. Ear skin was harvested 24 hours after treatment.

RNA Isolation and Quantitative Real-Time PCR

For collection of samples, full-thickness ear skin, liver, and adrenal gland were harvested and crushed in liquid nitrogen. Total RNA was isolated from cells or tissues with the use of the SV Total RNA Isolation System (Promega, Madison, WI). The product was reverse-transcribed into first-strand cDNA. The expression of IL-1 α , IL-1 β , CXCL1, IL-6, and Hsd11b1 were measured with the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Relative mRNA levels were normalized to that of hypoxanthine phosphoribosyltransferase (HPRT). Sequence-specific primers were designed as follows: IL-1 α , sense 5'-TCAAGATGGCCAAAGTTCCT-3', antisense 5'-CTTCAGAATCTTCCCGTTGC-3'; IL-1 β , sense 5'-ACCCTGCAGCTGGAGAGTGT-3', antisense 5'-CAAA-CCGTTTTTCCATCTTCTTCTT-3'; CXCL1, sense 5'-GCC-TATCGCCAATGAGCTG-3', antisense 5'-TCTCCGTTA-CTTGGGGACAC-3'; IL-6, sense 5'-CTGATGCTGGTGA-CAACCAC-3', antisense 5'-CAGAATTGCCATTGCAC-AAC-3'; Hsd11b1, sense 5'-AAAATTACCTCTCCCGA-TCCT-3', antisense 5'-GGCAGCGAGACACTACCTTC-3'; and HPRT, sense 5'-CACAGGACTAGAACACCTGC-3', antisense 5'-GCTGGTGAAGAGGACCTCT-3'. Quantitative real-time PCR (40 cycles of denaturation at 92°C for 15

seconds and annealing at 60°C for 60 seconds) was run on an ABI 7000 Prism (Applied Biosystems).

Western Blot Analysis

Mouse skin samples were crushed in liquid nitrogen and solubilized at 4°C in lysis buffer (0.5% sodium deoxycholate, 1% Nonidet P40, 0.1% sodium dodecyl sulfate, 100 μ g/mL phenylmethylsulfonyl fluoride, 1 mmol/L sodium orthovanadate, and protease inhibitor cocktail). Human skin samples were treated with 4 mg/mL dispase (Gibco/Invitrogen) for 1 hour at 37°C, and the epidermis was peeled from the dermis. Protein (30 μ g) was separated on SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA). Nonspecific protein binding was blocked by incubating the membranes in 5% w/v nonfat milk powder in TBS-T. The membranes were incubated with mouse anti-IL-1 β antibody MAB201 (R&D Systems, Minneapolis MN) diluted 1:1000, sheep anti-11 β -HSD1 antibody diluted 1:200 (The Binding Site, Birmingham, UK), or mouse anti- β -actin antibody A5441 (Sigma-Aldrich) diluted 1:10,000 for 60 minutes at room temperature. Then, the membranes were washed three times in TBS-T for 5 minutes. Finally, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse or anti-sheep antibody at a dilution of 1:10,000 for 60 minutes at room temperature. Protein bands were detected with the ECL Plus kit (GE Healthcare, Buckinghamshire, UK). The intensity of the bands was quantified with ImageJ software version 1.48V (NIH, Bethesda, MD; <https://imagej.nih.gov/ij/download.html>).

Enzyme-Linked Immunosorbent Assay

Blood was collected from each mouse in the evening, and the plasma corticosterone concentration was measured with a corticosterone electroimmunoassay kit (Cayman Chemical Company, Ann Arbor, MI). For IL-1 α , culture media was collected, and IL-1 α concentration was measured with an IL-1 α mouse enzyme-linked immunosorbent assay kit (R&D Systems). For organ culture analysis, full-thickness skin harvested from 12-week-old mouse ear was treated with 4 mg/mL dispase (Gibco/Invitrogen) for 1 hour at 37°C, and the epidermis was peeled from the dermis. The epidermis (25 cm²) was cultured in cortisol- and phenol red-free EpiLife for 24 hours with or without 1 nmol/L 11DHc, followed by harvesting of culture medium.

Statistical Analysis

The data are expressed as means $\pm$ SD. The Student's *t*-test was used to determine the level of significance of differences between the two groups. Analysis of variance for the groups was performed by analysis of variance followed by the Bonferroni-Dunn for multiple comparisons to allow

Figure 1 Generation of *K5-Hsd11b1-KO* mice. **A:** Construction of *Hsd11b1*^{tm1a/+} ES cells (left). Genotyping of mice shows WT band at 317 bp and K-KO band at 550 bp. **B:** Hematoxylin and eosin staining (upper panel) and immunohistochemical staining of 11β-HSD1 (lower panel) of 8-week-old WT and *K5-Hsd11b1-KO* mouse ear skin. Rabbit IgG was used as an isotype control. **C:** Quantitative real-time PCR analysis of relative expression of 11β-HSD1 in keratinocytes, adrenal gland, and liver of WT and K-KO mice. HPRT was used as an internal control. **D:** ELISA analysis of serum corticosterone concentration of WT and K-KO mice. **E:** ELISA of corticosterone concentration in organ cultures of epidermis derived from WT and K-KO mice treated with or without 1 nmol/L 11DHC. **F:** Immunohistochemical staining of 11β-HSD1 in low-dose OXA-induced CHS in WT mouse ear. **G:** Immunohistochemical staining of 11β-HSD1 in low-dose OXA-induced irritant dermatitis model in WT mouse ear. Data are expressed as means ± SD. *n* = 4 to 6. **P* < 0.05, based on one-way analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons; ****P* < 0.001, based on Student's *t*-test. Scale bar = 25 μm. CHS, contact hypersensitivity; DNFB, 2,4-dinitrofluorobenzene; ELISA, enzyme-linked immunosorbent assay; ES, embryonic stem; HPRT, hypoxanthine phosphoribosyltransferase; HT, heterozygous knock out; K-KO, homozygous knockout; OXA, oxazolone; WT, wild-type; 11DHC, 11-dehydrocorticosterone; 11β-HSD1, 11β-hydroxysteroid dehydrogenase 1.

pairwise testing for significant differences between groups. Statistical significance was defined as $P < 0.05$.

Results

Generation of *K5-Hsd11b1-KO* Mice

To investigate the function of 11 β -HSD1 in keratinocytes, we generated *K5-Hsd11b1-KO* mice. A conditional *Hsd11b1* allele was generated by flanking exons 3 (ENSMUSE00001341737) and 4 (ENSMUSE00001337115) with loxP sites (Figure 1A). Expression of 11 β -HSD1 was significantly decreased in *K5-Hsd11b1-KO* keratinocytes (Figure 1B). However, *K5-Hsd11b1-KO* mouse skin at steady state did not show a significant skin phenotype macroscopically or microscopically (Figure 1B). Expression of 11 β -HSD1 was decreased in keratinocytes but not in adrenal gland or liver, indicating targeted KO in keratinocytes (Figure 1C). Serum corticosterone concentration did not differ in wild-type and *K5-Hsd11b1-KO* mice (Figure 1D), which is in contrast to the findings of our previous study in *Hsd11b1*^{-/-} mice.³⁵ Corticosterone concentration in organ cultures of epidermis derived from *K5-Hsd11b1-KO* mice was significantly lower than in those from wild-type mice treated with 11DHC (Figure 1E).

Expression of 11 β -HSD1 Increases in Low-Dose OXA-Induced CHS and Irritant Dermatitis in Mouse Skin

To examine the dynamics of 11 β -HSD1 expression after contact hypersensitivity (CHS) and irritant dermatitis, wild-type mice were sensitized with 5% OXA and elicited with 0.2% OXA to the ear (CHS model) or treated with single application of 0.2% OXA to the ear (irritant dermatitis model). Expression of 11 β -HSD1 in epidermis increased after exposure to 0.2% OXA in both the CHS and irritant dermatitis models (Figure 1, F and G).

Low-Dose OXA-Induced Irritant Dermatitis Is Exacerbated in *K5-Hsd11b1-KO* Mice

To determine the role of 11 β -HSD1 in hapten-induced irritant dermatitis, ear swelling induced by a single application of OXA was evaluated. Ear swelling induced by low-dose (0.2%) OXA was significantly greater in *K5-Hsd11b1-KO* than in wild-type mice (Figure 2A). Accordingly, IL-1 β expression was significantly elevated after 24, 72, and 168 hours in ears with 0.2% OXA-induced irritant dermatitis in *K5-Hsd11b1-KO* mice compared with wild-type mice (Figure 2, B–D). CXCL1 expression trended to increase after

Figure 2 Low-dose OXA-induced irritant dermatitis is exacerbated in *K5-Hsd11b1-KO* mice. **A:** Ear swelling in models of irritant dermatitis induced by 0.2% OXA. **B–D:** Quantitative real-time PCR analysis of relative expression of IL-1 β and CXCL1 in vehicle-treated and 0.2% OXA-induced irritant dermatitis in skin of WT and K-KO mice at 24 (**B**), 72 (**C**), and 168 (**D**) hours. HPRT was used as an internal control. Data are expressed as means $\pm$ SD. $n = 6$. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, based on one-way analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons. HPRT, hypoxanthine phosphoribosyltransferase; K-KO, homozygous knockout; OXA, oxazolone; WT, wild-type.

Figure 3 Low-dose DNFB- and TNCB-induced irritant dermatitis is exacerbated in *K5-Hsd11b1-KO* mice. **A**: Ear swelling in vehicle-treated mice and in 0.3% DNFB- and 0.2% TNCB-induced models of irritant dermatitis (24 hours). **B**: Hematoxylin and eosin staining of ear skin of vehicle-treated mice and in models of 0.3% DNFB- and 0.2% TNCB-induced irritant dermatitis (30 hours). **C–E**: Representative DAB staining of CD45, F4/80, and Gr-1. Rat IgG was used as isotype control. **F–H**: The number of CD45⁺, F4/80⁺, and Gr-1⁺ cells per high-power field. Three sections from each mouse were evaluated. Data are expressed as means $\pm$ SD. $n = 6$. * $P < 0.05$, ** $P < 0.01$, based on one-way analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons (**A**); * $P < 0.05$, *** $P < 0.001$, based on two-way analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons (**F–H**). DAB, diaminobenzidine; DNFB, 2,4-dinitrofluorobenzene; K-KO, homozygous knockout; TNCB, 2,4,6-trinitro-1-chlorobenzene; WT, wild-type.

Figure 4 Low-dose DNFB- and TNCB-induced irritant proinflammatory cytokine expression is exacerbated in *K5-Hsd11b1-KO* mice. **A–F:** Quantitative real-time PCR analysis of relative expression of IL-1 β (**A** and **D**), IL-6 (**B** and **E**), and CXCL1 (**C** and **F**) in 0.3% DNFB-induced (**A–C**) and 0.2% TNCB-induced (**D–F**) irritant dermatitis (30 hours) in WT and K-KO mouse skin. HPRT was used as an internal control. **G:** Western blot analysis of IL-1 β expression in ear skin of 0.3% DNFB- and 0.2% TNCB-induced irritant dermatitis (30 hours). β -Actin was used as an internal control. Data are expressed as means $\pm$ SD. $n = 6$. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, based on one-way analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons. DNFB, 2,4-dinitrofluorobenzene; HPRT, hypoxanthine phosphoribosyltransferase; K-KO, homozygous knockout; TNCB, 2,4,6-trinitro-1-chlorobenzene; WT, wild-type.

24 and 168 hours and was significantly elevated after 72 hours in ears with 0.2% OXA-induced dermatitis in *K5-Hsd11b1-KO* mice compared with wild-type mice.

Low-Dose DNFB- and TNCB-Induced Irritant Dermatitis Is Exacerbated in *K5-Hsd11b1-KO* Mice

We further evaluated irritant dermatitis induced by the haptens DNFB and TNCB in *K5-Hsd11b1-KO* mice. As in OXA-induced irritant dermatitis, a single application of 0.3% DNFB or 0.2% TNCB to the mouse ear significantly enhanced ear swelling in *K5-Hsd11b1-KO* mice compared with wild-type mice (Figure 3A). Hematoxylin and eosin staining found enhanced edema with an increase in infiltrating cells in *K5-Hsd11b1-KO* mice (Figure 3B). Accordingly, the number of infiltrating leukocytes (CD45 $^{+}$ cells) (Figure 3, C and F), macrophages (F4/80 $^{+}$ cells) (Figure 3, D and G), and neutrophils (Gr-1 $^{+}$ cells) (Figure 3, E and H) was significantly higher in both DNFB- and TNCB-induced irritant dermatitis in *K5-Hsd11b1-KO* mice. No apparent difference was observed in expression of glucocorticoid receptor in the wild-type and *K5-Hsd11b1-KO* mice (Supplemental Figure S1). Next, expression of the proinflammatory cytokines IL-1 β , IL-6, and CXCL1 in harvested full-thickness skin was evaluated. Expression of all three cytokines was significantly elevated in *K5-Hsd11b1-KO* mice compared with wild-type mice in both DNFB-induced irritant dermatitis (Figure 4, A–C) and TNCB-induced irritant dermatitis (Figure 4, D–F). In addition, increased IL-1 β expression was also found by Western blot analysis (relative intensity of IL-1 β / β -actin bands) (Figure 4G).

Topical Corticosterone Application Dose Dependently Suppresses TNCB-Induced Irritant Dermatitis

Because the data suggest that the activation of corticosterone by 11 β -HSD1 in keratinocytes suppressed hapten-induced dermatitis, we next evaluated the effect of topical corticosterone application in the model of TNCB-induced irritant dermatitis. Corticosterone at doses of 10^{-2} to 10^{-4} mol/L was topically applied after application of 0.2% TNCB to the mouse ear. Topical application of corticosterone suppressed TNCB-induced ear swelling in a dose-dependent manner (Figure 5A). Consistent with this observation, IL-1 β , and CXCL1 expression was also dose dependently suppressed by topical corticosterone application (Figure 5, B–D).

Corticosterone Dose Dependently Suppresses TNBS-Induced Inflammation in Keratinocytes in Culture

Keratinocytes play an important role in hapten-induced dermatitis by producing cytokines, such as IL-1 β , IL-1 α , tumor necrosis factor- α , and IL-18, with hapten stimulation.^{38,39} We next evaluated the effect of corticosterone at various doses *in vitro* with the use of mouse keratinocytes. TNBS was used in the *in vitro* study because it is water soluble. Addition of TNBS to the medium dose dependently induced the expression of 11 β -HSD1, IL-1 α , and IL-1 β (Figure 5, E–G). Corticosterone at a physiologic dose of 10^{-10} to 10^{-6} mol/L was added to culture medium after stimulation of cells with TNBS. TNBS-induced IL-1 α mRNA expression was significantly suppressed by 10^{-6} mol/L corticosterone (Figure 5H), and TNBS-induced IL-1 β mRNA

Figure 5 Topical corticosteroid application dose dependently suppresses TNBC-induced irritant dermatitis *in vivo* and TNBC-induced inflammation in keratinocytes *in vitro*. **A:** Ear swelling in mice treated with vehicle and TNBC with or without indicated dose of corticosteroid (24 hours). **B–D:** qPCR analysis of relative expression of IL-1β (**B**), IL-6 (**C**), and CXCL1 (**D**) in mouse ear skin treated with vehicle and 0.2% TNBC with or without indicated dose of corticosteroid (30 hours). HPRT was used as an internal control. **E–G:** qPCR analysis of relative expression of 11β-HSD1 (**E**), IL-1α (**F**), and IL-1β (**G**) in cultured mouse keratinocytes treated with vehicle or indicated doses of TNBS for 24 hours. HPRT was used as an internal control. **H** and **I:** qPCR analysis of relative expression of IL-1α (**H**) and IL-1β (**I**) in cultured WT mouse keratinocytes treated with vehicle or TNBS with or without indicated dose of cortisol for 24 hours. HPRT was used as an internal control. **J:** Enzyme-linked immunosorbent assay analysis of IL-1α in medium of cultured WT mouse keratinocytes treated with vehicle and TNBS with or without the indicated dose of cortisol for 24 hours. Data are expressed as means ± SD. $n = 6$ (**B–D**); $n = 4$ (**E–I**). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, based on one-way analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons. HPRT, hypoxanthine phosphoribosyltransferase; qPCR, quantitative real-time PCR; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNBC, 2,4,6-trinitro-1-chlorobenzene; WT, wild-type; 11β-HSD1, 11β-hydroxysteroid dehydrogenase 1.

expression was significantly suppressed by 10^{-7} and 10^{-6} mol/L corticosteroid (Figure 5I). In addition, the results of the enzyme-linked immunosorbent assay found that TNBS-induced IL-1α production was significantly suppressed by 10^{-7} and 10^{-6} mol/L corticosteroid (Figure 5J). TNBS-induced IL-1β was undetectable in culture medium (data not shown).

Response to 11DHC Application in TNBC-Induced Irritant Dermatitis Is Attenuated in *K5-Hsd11b1-KO* Mice

Finally, we evaluated the effect of topical application of 11DHC in TNBC-induced irritant dermatitis. Suppression of TNBC-induced ear swelling was milder in the 11DHC group than in the corticosteroid group of *K5-Hsd11b1-KO* mice (Figure 6A).

Figure 6 Response to 11DHC application in TNCB-induced irritant dermatitis is attenuated in *K5-Hsd11b1-KO* mice. **A** and **B**: Ear swelling in mice treated with 0.2% TNCB with or without 10^{-3} mol/L corticosterone or 10^{-3} mol/L 11DHC for 24 hours. **B–D**: Quantitative real-time PCR analysis of relative expression of IL-1 β (**B**), CXCL1 (**C**), and IL-6 (**D**) in mouse ear skin treated with 0.2% TNCB with or without 10^{-3} mol/L corticosterone or 10^{-3} mol/L 11DHC for 26 hours. HPRT was used as an internal control. Data are expressed as means $\pm$ SD. $n = 6$. * $P < 0.05$, ** $P < 0.01$, based on one-way analysis of variance, followed by the Bonferroni-Dunn test for multiple comparisons. HPRT, hypoxanthine phosphoribosyltransferase; K-KO, homozygous knockout; TNCB, 2,4,6-trinitro-1-chlorobenzene; WT, wild-type; 11DHC, 11-dehydrocorticosterone; 11 β -HSD1, 11 β -hydroxysteroid dehydrogenase 1.

Consistent with this observation, IL-1 β and IL-6 expression was also only mildly suppressed by 11DHC compared with corticosterone in *K5-Hsd11b1-KO* mice (Figure 6, B–D).

Expression of 11 β -HSD1 Decreases in Psoriasis Vulgaris Epidermis

We previously reported decreased expression of 11 β -HSD1 in skin tumors, such as basal cell carcinoma and squamous cell carcinoma and in seborrheic keratosis, and the association of 11 β -HSD1 expression level with cell proliferation.³² The expression of 11 β -HSD1 was also decreased in epidermis in psoriasis vulgaris skin (Figure 7, A–C). In addition, 11 β -HSD1 expression was lower in lesional psoriasis vulgaris skin than in marginal skin (Figure 7D). The 11 β -HSD1 staining score was significantly lower in psoriasis vulgaris than in healthy skin and correlated negatively with epidermal thickness in psoriasis vulgaris (Figure 7E). Expression of 11 β -HSD1 was also significantly decreased by Western blot analysis in epidermis in psoriasis vulgaris (Figure 7, F and G).

Discussion

Skin is exposed daily to various forms of mechanical and chemical stimulation. We hypothesized that a local stress-regulatory mechanism might exist in keratinocytes to counterbalance this repeated stimulation. To address this, we focused on local corticosterone activation by 11 β -HSD1 in keratinocytes. In this study, we found that low-dose

OXA-induced irritant dermatitis was exacerbated in *K5-Hsd11b1-KO* mice. *K5-Hsd11b1-KO* mice also showed an enhanced inflammatory reaction to single applications of low-dose TNCB and DNFB. These results suggest that irritant dermatitis induced by haptens is exacerbated in *K5-Hsd11b1-KO* mice, probably through absence of corticosterone activation by 11 β -HSD1 in keratinocytes. Because topical cortisol application dose dependently suppressed TNCB-induced irritant dermatitis, and because suppression of TNCB-induced irritant dermatitis was attenuated in *K5-Hsd11b1-KO* mice treated with 11DHC compared with 11DHC-treated wild-type mice, local cortisol activation is likely important to suppress skin inflammation.

Keratinocytes play an important role in CHS, as do immune cells, such as T cells and dendritic cells. Haptens directly stimulate keratinocytes to produce proinflammatory cytokines, such as IL-1 β , IL-18, and tumor necrosis factor- α .³⁸ Neutrophil-recruiting chemokines, such as CXCL1 and CXCL2, are also produced by activated keratinocytes.³⁹ In addition, IFN- γ and tumor necrosis factor- α produced by recruited T helper type 1 cells in CHS stimulate keratinocytes to produce chemokines, such as chemokine ligand 8, CXCL9, CXCL10, and CXCL11.⁴⁰ In our study, the addition of TNBS to culture medium dose dependently induced IL-1 α and IL-1 β production, suggesting the importance of these cytokines in keratinocytes in hapten-induced dermatitis. IL-1 β was not detected in culture medium of cells treated with TNBS, possibly because activation of caspase-1 through inflammasome activation is essential to process IL-1 β in keratinocytes, and TNBS does not activate this mechanism.³⁸

Figure 7 11β-HSD1 expression decreases in psoriasis vulgaris epidermis. **A:** Representative immunofluorescence staining of 11β-HSD1 (red) and keratin 14 (green) in healthy and psoriasis vulgaris skin. Rabbit and mouse IgG were used as isotype controls for 11β-HSD1 and keratin 14, respectively. **B:** Representative DAB staining of 11β-HSD1 in psoriasis vulgaris. **C:** 11β-HSD1 staining score in healthy and psoriasis vulgaris skin. **D:** DAB staining of 11β-HSD1 in psoriasis vulgaris, including marginal skin and lesional skin. **E:** Correlation between epidermal thickness and 11β-HSD1 staining score in psoriasis vulgaris. **F:** Western blot analysis of 11β-HSD1 expression in healthy and psoriasis vulgaris skin. β-Actin was used as an internal control. **G:** Intensity of 11β-HSD1 band relative to that of β-actin. Data are expressed as means ± SD. $n = 19$ healthy skin samples; $n = 15$ psoriasis vulgaris skin samples. Scale bars: 100 μm (**A** and **B**); 250 μm (**D**). *** $P < 0.001$, based on Student's t -test. DAB, diaminobenzidine; 11β-HSD1, 11β-hydroxysteroid dehydrogenase 1.

Although GCs are strong immunosuppressive drugs, the endogenous GC, cortisol, is immunomodulatory rather than immunosuppressive. The action of GCs is concentration dependent.⁴¹ In our study, addition of cortisol at 10^{-7} or 10^{-6} mol/L suppressed the expression of IL-1α and IL-1β. In human keratinocytes, IL-1β-induced IL-6 production was stimulated by low-dose (10^{-10} mol/L) cortisol but suppressed with high-dose (10^{-5} mol/L) cortisol,⁴² similar to the current results. Because 11β-HSD1 regulates cortisol

concentration between these physiologic doses, 11β-HSD1 in keratinocytes may be an important molecule in suppressing irritant dermatitis induced by low-dose haptens in our study. Note that CHS and irritant dermatitis induced by high-dose haptens were apparently no different in wild-type and *K5-Hsd11b1-KO* mice (data not shown). These data suggest that a local immunosuppressive effect through the action of 11β-HSD1 in keratinocytes is only limited to mild inflammation. Although the cortisol production pathway is

reported to be a minor one in keratinocytes compared with those of other steroids,⁴³ we believe it is important in skin inflammation.

Finally, we evaluated 11 β -HSD1 expression in psoriasis vulgaris. Expression of 11 β -HSD1 was decreased and negatively correlated with epidermal thickness. Expression of 11 β -HSD1 might be decreased in psoriasis vulgaris due to epidermal hyperproliferation, because the 11 β -HSD1 expression level is negatively correlated with keratinocyte proliferation.^{16,32} Considering the results in *K5-Hsd11b1-KO* mice, reduced 11 β -HSD1 expression in keratinocytes might attenuate the local immunosuppressive effect in these cells and result in a hyperinflammatory response.

On the basis of our findings, we propose that 11 β -HSD1 in keratinocytes suppresses mild hapten-induced irritant dermatitis, possibly through reduction of IL-1 α and IL-1 β production in these cells. This is the first study to show the importance of local corticosterone activation in keratinocytes *in vivo* in suppressing local skin inflammation. Targeting the local cortisol activation system in keratinocytes might be a novel pharmaceutical strategy in the future.

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Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2016.01.014>.

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