Cutaneous Liver X Receptor Activation Prevents the Formation of Imiquimod-Induced Psoriatic Dermatitis

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TO THE EDITOR

Psoriasis is a chronic inflammatory skin disorder characterized by keratinocyte (KC) hyperproliferation (Nestle et al., 2009). In recent years, psoriasis is recognized as an immunometabolic disease associate with multiorgan abnormalities and dyslipidemia (Sterry et al., 2007); however, it remains unclear whether the dysregulation of lipid metabolism in the skin affects the pathogenesis of psoriasis. Liver X receptor (LXR) is a nuclear receptor composed of two isoforms: LXRα (*NR1H3*) and LXRβ (*NR1H2*) (Chawla et al., 2001), which are important regulators of cholesterol (Schulman, 2017). Previous studies showed that the topical application of LXR agonists inhibits the development of contact dermatitis (Fowler et al., 2003) and that LXRα expression is suppressed in KCs in psoriatic lesions (Gupta et al., 2010). In this study, we investigated the role of LXR signaling in psoriasis through topical application of a synthetic LXR agonist, GW3965, in an imiquimod (IMQ)-induced psoriatic murine model.

We topically applied the LXR agonist 10 mM of GW3965 or ethanol as vehicle control in combination with IMQ for 7 consecutive days. Analogous to the human psoriasis condition, LXR expression levels were lower in the IMQ-treated skin than in the control skin (Supplementary Figure S1). IMQ-induced ear swelling and epidermal hyperplasia were milder in mice treated with GW3965 than in the control mice (Figure 1a and b). We evaluated immune cell infiltration by flow cytometry on days 3 and 5 and found that the numbers of neutrophils were 2.5-fold higher in the vehicle-treated skin than in the GW3965-treated skin on day 3, but the numbers of other immune cells were comparable between the GW3965-treaded skin and control skin (Figure 1c). The mRNA expression levels of neutrophil chemoattractants (*Cxcl1, Cxcl2,* and *Ccl20*) and psoriasis-related genes (*Il1a, Il1b, Il23a,* and *Il17a*) were lower in the GW3965-treated group (Figure 1d), consistent with the lower numbers of neutrophils in the lesional skin on day 3 (Figure 1c). In contrast, the expression levels of LXR response gene *Abca1* were significantly higher in the GW3965-treated skin regardless of simultaneous IMQ treatment, showing LXR activation in the skin (Figure 1d). We also evaluated the expression levels of neutrophil activation markers such as CXCR4 and CD49b and examined the percentage of citrullinated histone H3–positive cells to check the netosis activity. No significant difference was observed in the expression of these markers between the GW3965- and the vehicle-treated group (data not shown), suggesting that neutrophil function is not influenced by LXR activation. Taken together, these results suggest that cutaneous application of the LXR agonist attenuated neutrophil recruitment in the early phase and led to blunted skin inflammation in IMQ-induced psoriatic model.

To evaluate the direct effects of LXR activation on KCs, we cultured primary murine KCs and stimulated them with IMQ with or without GW3965. The induction of mRNA expression of *Cxcl1* and *Cxcl2* but not of *Ccl20* by IMQ was significantly suppressed by GW3965 treatment (Figure 2a). Besides, the expression levels of *Abca1* were higher in the GW3965-treated group than in the vehicle-treated group. To assess human relevancy, a human KC cell line, HaCaT, was stimulated with IL-1β with or without GW3965. Similar to that in murine KCs, psoriasis-related gene expression levels were lower in the GW3965-treated group than in the control group (Supplementary Figure S2). Western blot analysis revealed that GW3965 inhibited the phosphorylation of p38 in murine KCs stimulated with IL-1β, suggesting that LXR signaling counteracts IL-1 receptor signaling in KCs (Figure 2b).

Because LXR activation inhibits COX-2 expression in macrophages (Ghisletti et al., 2009, 2007; Thomas et al., 2018), we evaluated the expression levels of COX isozyme genes and their downstream lipid mediators in IMQ-stimulated murine KCs with or without GW3965 treatment. The expression of *Ptgs2* (coding COX-2), an inducible isoform in inflammatory conditions, but not of *Ptgs1* (coding COX-1), a ubiquitously expressed isoform, was induced by IMQ treatment, and this induction was significantly attenuated by GW3965 treatment in vitro (Figure 2a). To evaluate the lipid profile in the presence of an LXR agonist, we performed in vivo lipidomic analysis of the mouse ear skin treated with IMQ with or without GW3965 for 3 days (Figure 2c and d). The amount of 9-hydroxyoctadecadienoic acid and 11-hydroxyeicosatetraenoic acid, which are COX-2–mediated metabolites from linoleic acid and arachidonic acid, respectively, was significantly lower in the GW3965-treated skin than in the vehicle-treated group (Figure 2c). In addition, the levels of prostanoids, such as prostaglandin (PG)D_2, 15-keto $PGD_{2\alpha}$, 13,14-dihydro-15-keto-PGE_2, PGE_2, $PGF_{2\alpha}$, and thromboxane B_2, were lower in the LXR-treated group

Abbreviations: IMQ, imiquimod; KC, keratinocyte; LXR, liver X receptor; PG, prostaglandin

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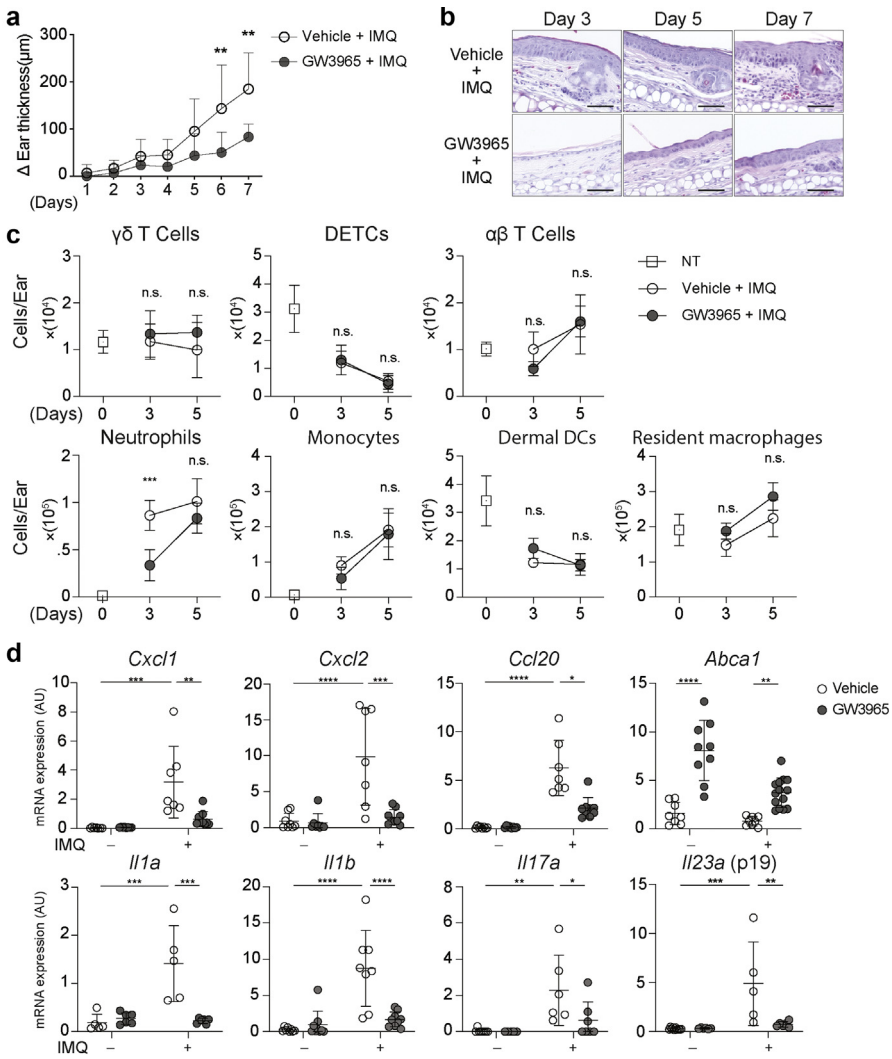

Figure 1. Cutaneous LXR activation alleviates IMQ-induced psoriasis-like dermatitis and downregulates psoriasis-related gene expression. The ear thickness changes in mice treated with IMQ in combination with GW3695 or vehicle (n = 6 per group). (**b**) Representative histology with H&E staining of the ear skin from an IMQ-induced psoriasis mouse model treated with or without GW3965. A representative section of NT skin is shown as a control. Bars = 50 μm. (**c**) Kinetics of different immune cell populations from IMQ-induced psoriasis ear treated with either GW3965 or vehicle on day 3 or 5. WT was used for control (n = 5). (**d**) Quantitative RT-PCR analysis of mRNA levels in IMQ-induced psoriasis ear on day 3 with or without cutaneous GW3965 application (n ≥ 5). Results are represented as means ± SDs. $*P < 0.05$, $**P < 0.001$, $***P < 0.0001$, $****P < 0.00001$. AU, arbitrary unit; DC, dendritic cell; IMQ, imiquimod; LXR, liver X receptor; n.s., not significant; NT, nontreated; WT, wild type.

than in the controls (Figure 2d). Collectively, LXR activation was shown to inhibit COX-2 expression in IMQ-induced dermatitis and reduce the inflammatory COX-2 metabolites such as PGE_2 and thromboxane B_2. Previous studies showed that fibroblasts and KCs highly express *Ptgs2* and are considered to be the major source of inflammatory lipids during the development of psoriasis (Ikai, 1999; Ueharaguchi et al., 2018). We found that the expression of *Ptgs2* was downregulated with GW3965 treatment in cultured KCs, suggesting the involvement of KCs in prostanoids production in the IMQ-induced psoriasis model; however, we could not exclude the possible involvement of other cells. This is a remaining question for future study.

We showed that topical treatment with GW3965 attenuated *Ptgs2* expression in IMQ-treated skin (Figure 2a). Consistently, LXR activation leads to the inhibition of COX-2 expression in macrophages (Ghisletti et al., 2009). LXRs are known as important nuclear receptors controlling cellular cholesterol levels (Calkin and Tontonoz, 2012). In KCs, LXR activation increases ABCA1 expression levels that are induced by increased cholesterol levels (Jiang et al., 2006), and the reduction of total cholesterol levels using methyl-β-cyclodextrin decreases the IL-17–response gene expression levels. Of note, IL-17A induces the accumulation of intracellular cholesterol in KCs in psoriasis (Varshney et al., 2016). Taken together, previous understandings and our work suggest that cross-regulation of the LXR pathway and cholesterol metabolism in the skin might organize the chronic inflammatory loop of IL-17 in psoriasis through lipid mediators. Although our work does not identify the contribution of a specific LXR isotype, significant reduction of LXRα expression levels in psoriasis lesional skin (Gupta et al., 2010) prompt us to expect that LXRα might be a major player in this regard.

It has been reported that COX-2 is required for prostanoid synthesis in psoriasis skin lesion (Ikai, 1999) and that mechanistically, COX-derived PGs and thromboxanes promote the expression of IL-17 in psoriasis (Honda and Kabashima, 2019a, 2019b; Ueharaguchi et al., 2018). Although the effects of nonsteroidal anti-inflammatory drugs in psoriasis remain controversial (Katayama and Kawada, 1981; Kragballe and Herlin, 1983), LXR-mediated suppression of the COX-2 expression in KCs might be related to the attenuation of psoriatic inflammation.

In conclusion, we showed that cutaneous LXR activation prevents the development of psoriasis in an animal model. Our results highlight the suppression in the production of arachidonic acid metabolism in the development of psoriasis and propose LXR and its response genes as the potential therapeutic targets.

Declaration for animal use

Mice were purchased from Japan SLC (Shizuoka, Japan) and were maintained under specific pathogen-free conditions in the institute of Laboratory Animals at Kyoto University Graduate School of Medicine (Kyoto, Japan). Experiments were all performed on female C57BL/6N mice aged 6–10 weeks. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine.

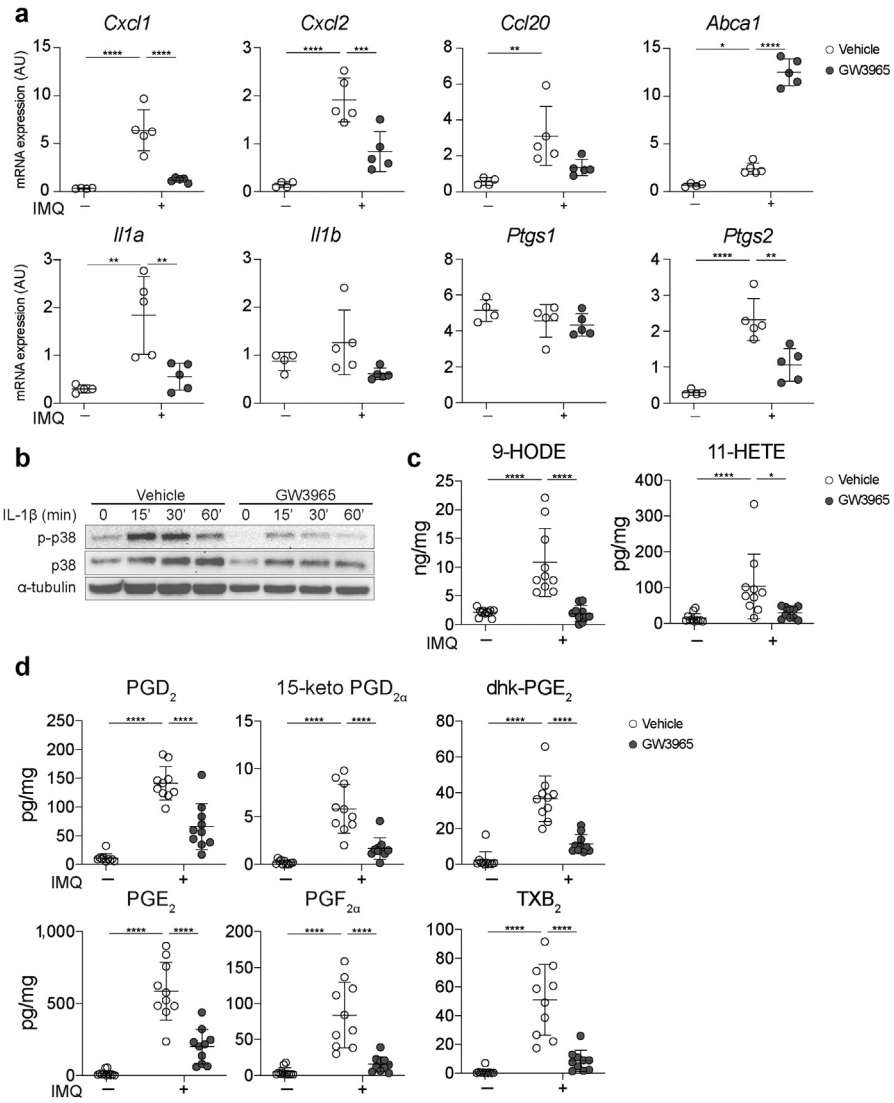

Figure 2. LXR activation leads to downregulation of inflammatory gene expression and inhibition of COX-2 expression in keratinocytes and downregulation of COX-2 products in the skin of IMQ-induced dermatitis. (**a**) Quantitative RT-PCR analysis of mRNA levels 3 hours after IMQ stimulation in murine keratinocytes with or without overnight stimulation with GW3965 (n = 5 per group). (**b**) Representative western blot analysis of murine keratinocytes treated with or without GW3965 stimulated with recombinant IL-1β at the indicated time points. Total cell lysates were resolved by SDS-PAGE followed by immunoblotting. (**c, d**) Lipidomic analysis of COX-2 products of linoleic and arachidonic acid metabolism. Murine ears at 3 days of IMQ-induced psoriasis with or without GW3965 treatment were analyzed. (**c**) COX-2–mediated metabolites from linoleic acid and arachidonic acid. (**d**) COX-2 metabolite derived from arachidonic acid (n = 10 per group). Results are shown as means ± SDs. $*P < 0.05$, $**P < 0.001$, $***P < 0.0001$, $****P < 0.00001$. 9-HODE, 9-hydroxyoctadecadienoic acid; 11-HETE, 11-hydroxyeicosatetraenoic acid; AU, arbitrary unit; dhk-PGE_2, 13,14-dihydro-15-keto-PGE_2; IMQ, imiquimod; LXR, liver X receptor; min, minute; PG, prostaglandin; TX, thromboxane.

Data availability statement

No datasets were generated or analyzed during this study.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: MO; Data Curation: TO, TY; Formal Analysis: MO, YI; Funding Acquisition: KK; Investigation: MO; Methodology: MO, GE, TD; Project Administration: KK; Resources: TO, YI, TY; Supervision: GE, TD, KK; Validation: ZC, RA, TM; Visualization: KK; Writing - Original Draft Preparation: MO; Writing - Review and Editing: GE, TD, TN, AK

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.08.432.

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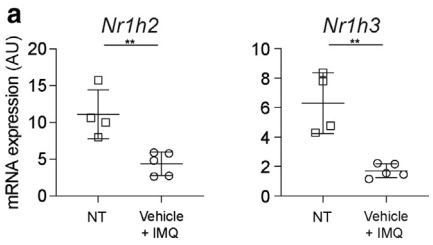
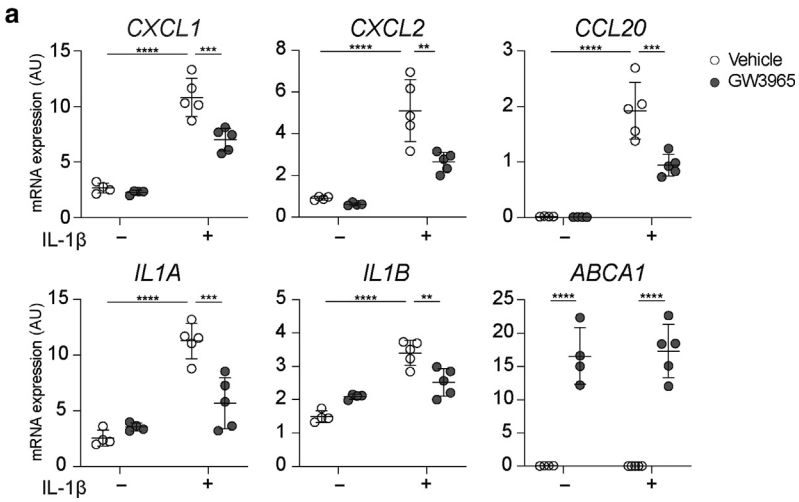
SUPPLEMENTARY MATERIALS



Supplementary Figure S1. Murine IMQ-induced psoriasis-like dermatitis has lower LXRα expression than the NT group. (**a**) Murine ears were collected after 7 days of treatment with IMQ or vehicle (NT) topically, and the mRNA expression of *Nr1h2* (LXRβ) and *Nr1h3* (LXRα) was quantified using quantitative RT-PCR. Expression was normalized to *Gapdh* expression. (n = 5 per group). Results are shown as means ± SDs. $^{**}P < 0.001$. AU, arbitrary unit; IMQ, imiquimod; LXR, liver X receptor; NT, nontreated.



Supplementary Figure S2. LXR application reduced psoriasis-related genes in human keratinocytes, and lower *NR1H3* gene expression levels were observed in psoriasis lesional skin. (**a**) Quantitative RT-PCR analysis of human keratinocyte cell line (HaCaT) stimulated with recombinant IL-1β for 24 hours with or without overnight GW3965 treatment. Samples were collected 24 hours after stimulation (n = 5 per group). Results are shown as means ± SDs. $^{**}P < 0.001$, $^{***}P < 0.0001$, $^{****}P < 0.00001$. AU, arbitrary unit; LXR, liver X receptor.