ALK3 Is Not Required for the Embryonic Development, Homeostasis, and Repopulation of Epidermal Langerhans Cells in Steady and Inflammatory States

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**LETTERS TO THE EDITOR**

Epidermal Langerhans cells (LCs) are skin-resident dendritic cells, although recently LCs were also classified as specialized macrophages on the basis of their embryonic development shared with tissue-resident macrophages (Doebel et al., 2017; Hoeffel et al., 2012). LCs maintain skin immune surveillance and are involved in skin disorders, including psoriasis (Eidsmo and Martini, 2018; Kaplan, 2017). Adult LCs homeostasis is maintained throughout lifetime self-renewal at steady state, but the impaired LCs could arise by bone marrow-derived precursors at inflammatory state (Ginboux et al., 2006; Seré et al., 2012). Quite a few well-identified genes, including TGFβ1, are essential for epidermal LCs (Zhang et al., 2016). TGFβ1 regulates LC development, maturation, migration, and repopulation (Kaplan et al., 2007; Seré et al., 2012). In the canonical TGFβ signaling, TGFβ1 binds TGFβR1 and TGFβR2 complex, mediating the phosphorylation of SMAD2 and SMAD3 complex, which forms a heterotrimeric complex with SMAD4 and translocates into the nucleus and regulates TGFβ1-targeting genes (Derynck and Zhang, 2003). However, our genetic mouse models with deletion of Smad3 (Xu et al., 2012), Smad2, and Smad4 (Huang et al., 2020) showed no effect of SMAD pathway loss on LCs development at steady state but did show an impact on LC repopulation under inflammatory state. Nevertheless, recent studies revealed that both TGFβ family members, TGFβ1 and BMP7, can potentially signal through BMPR1A or ALK3 to induce LC differentiation in vitro (Borek et al., 2020; Yasmin et al., 2013), and BMP7-ALK3 signaling leads to the differentiation and proliferation of inflammation-associated LCs from bone marrow-derived precursors in psoriatic lesions, which promotes the psoriatic epidermal changes in patients with psoriasis as well as in a psoriatic mouse model (Borek et al., 2020). However, it remains unclear whether ALK3 is required for LC development in vivo. In this study, we reported that ALK3 is not required for LC development and repopulation in vivo and that ALK3 deletion in LCs did not significantly affect imiquimod-induced psoriatic dermatitis in mice.

To test the role of ALK3 in LC homeostasis in vivo, we first crossed ALK3<sup>−/−</sup> mice (Mishina et al., 2002) with C<sub>di11</sub>Cre<sup>+</sup> mice to generate mice with dendritic cells–specific ALK3 deletion. The expression of ALK3 in LCs from C<sub>di11</sub>Cre<sup>+</sup>ALK3<sup>−/−</sup> knockout mice was dramatically reduced compared with that of wild-type (WT) littermates (Figure 1a). As shown in Figure 1b, there was no significant alteration in the frequencies of LCs (CD45<sup>+</sup> major histocompatibility complex [MHC]II<sup>+</sup>) between C<sub>di11</sub>Cre<sup>+</sup>ALK3<sup>−/−</sup> and WT mice, suggesting that ALK3 is inessential for LC homeostasis after birth. Recent studies confirmed that LCs are derived from embryonic yolk sac and fetal liver monocyte (Kaplan, 2017), and BMP7 highly expresses in embryonic keratinocytes (Borek et al., 2020; Yasmin et al., 2013). Csf-1R Cre fate-mapper, that is, Csf-1R<i>C</i>re<sup>+</sup> mice were used to study the embryonic development of tissue-resident macrophages and LC precursors (Hoeffel et al., 2015; Schulz et al., 2012; Yao et al., 2018). Hence, we crossed Csf-1<i>RiCre</i> mice with ALK3<sup>−/−</sup> mice to generate myeloid-exclusive ALK3 deletion mice. The deletion of ALK3 on LCs was confirmed by quantitative real-time reverse transcriptase–PCR and western blot (Figure 1c). As shown in Figure 1d, the frequencies of LCs (CD45<sup>+</sup>F4/80<sup>+</sup>) at embryonic day 17.5 and postnatal day 0 were comparable between Csf-1<i>RiCre</i>ALK3<sup>−/−</sup> and WT littermates, suggesting that the embryonic generation and skin-homing programs were unaffected by ALK3 loss. Furthermore, there was no significant difference in the frequency of LCs (CD45<sup>+</sup>MHCII<sup>+</sup>) from epidermal suspension (Figure 1e) and in the number of MHCII<sup>+</sup> LC cells on the epidermal sheets (Figure 1f) between Csf-1<i>RiCre</i>ALK3<sup>−/−</sup> and WT mice at age 8 weeks. Thus, overall, ALK3 is not required for LC ontogeny during embryonic development and homeostasis of LCs after birth. A detailed description of the methods is presented in the Supplementary Materials and Methods.

LCs could be derived from bone marrow under inflammatory conditions, and we found that SMAD2 and/or SMAD4 is required for LC repopulation from bone marrow in UVC-treated skin (Huang et al., 2020). To explore the role of ALK3 in LC repopulation, the embryonal LCs (CD45<sup>+</sup>MHCII<sup>+</sup>C<sub>di207</sub>hi) in Csf-1<i>RiCre</i>ALK3<sup>−/−</sup> and WT littermates were analyzed at 2 weeks after UVC treatment and were found comparable (Figure 1g), indicating that ALK3 is not required for LCs repopulation. This observation is in line with the recent study demonstrating that BMP7 fails to induce the differentiation of LC-like cells in mouse bone marrow

**Abbreviations:** LC, Langerhans cell; MHC, major histocompatibility complex; WT, wild type

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**ALK3 Is Not Required for the Embryonic Development, Homeostasis, and Repopulation of Epidermal Langerhans Cells in Steady and Inflammatory States**

TO THE EDITOR
Figure 1. ALK3 is not required for LC development, homeostasis, and repopulation. (a) Relative mRNA expression of ALK3 in sorted LCs from CD11cCreALK3fl/fl (KO) and WT mice by QRT-PCR (n = 3, ***P < 0.001). (b) Flow cytometry analysis showing the frequencies of epidermal LCs (CD45+MHCI+) in CD11cCreALK3fl/fl (KO) and WT mice aged 8 weeks (n = 3–4, ns). (c) Relative mRNA and protein expression of ALK3 in sorted LCs from Csf-1RcreALK3fl/fl (KO) and WT mice by QRT-PCR and western blot, respectively (n = 3, ***P < 0.001). (d) Flow cytometry analysis showing the frequencies of epidermal LCs (CD45+F4/80+) at E17.5 and at birth (P0) in Csf-1RcreALK3fl/fl (KO) and WT littermate mice (n = 3–14). (e) Flow cytometry analysis showing the frequencies of epidermal LCs (CD45+MHCI+) in Csf-1RcreALK3fl/fl (KO) and WT mice aged 8 weeks (n = 3–4). (f) Immunostaining of MHCI (green) on epidermal sheets. Bar = 100 μm, original magnification ×10. (g) An LCs repopulation study showing the frequencies of epidermal LCs (CD45+MHCI+) in the upper panel and the frequencies of bone marrow–derived LCs (MHCI+CD1207hi) gated on CD45+MHCI+ LCs in the lower panel between Csf-1RcreALK3fl/fl (KO) and WT mice after UVC treatment (n = 3). E17.5, embryonic day 17.5; KO, knockout; LC, Langerhans cell; MHC, major histocompatibility complex; ns, not significant; QRT-PCR, quantitative real-time reverse transcriptase–PCR; WT, wild type.
cultures and fetal liver cells (Capucha et al., 2018).

The main functions of LCs include their phagocytosis and upregulating their CD80/86 expression on stimulation. We next tested the role of ALK3 in LC function. Freshly isolated epidermal LCs from Csf-1RcreALK3fl/fl and WT mice were incubated with FITC-dextran isomer for 45 minutes at 37 °C or 4 °C (control), respectively. The LCs labeled with FITC-dextran (CD45^+ MHCII^+ FITC^+) were defined to have effective phagocytosis. No significant differences were identified in LCs phagocytosis on the basis of dextran uptake (Figure 2a). In addition, spontaneous maturation was evaluated after 72 hours of in vitro culture, and mature LCs based on the percentages of CD80^hiLCs and CD86^hiLCs after in vitro incubation were comparable between Csf1RcreALK3fl/fl and WT mice (Figure 2b). Thus, these data suggest that ALK3 is dispensable for LC phagocytosis and maturation.

Finally, we investigated the role of ALK3 in LCs in the imiquimod-induced psoriasis-like dermatitis mouse model. By monitoring the scores of erythema, scales, and thickness of imiquimod-treated mouse skin, we found no significantly altered inflammation on the basis of PASI score between Csf1RcreALK3fl/fl and WT mice (Figure 2c). Furthermore, histopathological analysis of ear sections showed no significant changes in imiquimod-induced epidermal thickening (acanthosis) and infiltrating cells (Figure 2d).

Overall, our results highly suggest that BMP7- or TGFβ1-induced ALK3 signaling is not required for the LC embryonic development, homeostasis after birth in steady state, and repopulation in inflamed state in mice. ALK3 is dispensable for phagocytosis and maturation of LCs, and lack of ALK3 in LCs does not significantly affect imiquimod-induced psoriatic dermatitis in mice. These results oppose recent studies demonstrating that BMP7- or TGFβ1-induced ALK3 signaling is required for LC differentiation and function. This discrepancy may be related to the differences between in vitro and in vivo studies or between humans and mice as well as due to the nonspecific ALK2/3/6 inhibitor dorsomorphin used. Therefore, further investigation is warranted to clarify the potential involvement of BMP7-ALK2 or ALK6 in mouse LCs and BMP7/ALK3 signaling pathway in human LCs.

**ETHICS STATEMENT**

All animal experiments were approved by the Institutional Animal Care and Use Committee of Henry Ford Health System and performed in accordance with the National Institutes of Health guidelines.

**Data availability statement**

Data related to this article are available on request.

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**Figure 2.** ALK3 is dispensable for LC phagocytosis and maturation, and the lack of ALK3 does not affect IMQ-induced psoriatic dermatitis in mice. (a) Flow cytometry analysis showing the frequencies of epidermal LCs (CD45^+ MHCII^+ FITC-dextran^+ ) after 37 °C or 4 °C (as control) incubation for 45 minutes between Csf-1RcreALK3fl/fl (KO) and WT mice (n = 3). (b) Flow cytometry analysis showing the ratio of mature LCs (CD45^+ MHCII^+ CD80^hi and CD45^+ MHCII^+ CD86^hi) from Csf-1RcreALK3fl/fl (KO) and WT mice after 72 h of in vitro culture (n = 3). (c) The back skin and ears of mice were treated with 5% IMQ for 5 consecutive days. PASI (cumulative scores of erythema, scaling, and thickness) of their back skin was scored daily for 5 days. (d) The thickness of ear skin sections on day 6 (bar = 100 μm). The epidermal thickness was measured with the assistance of ImageJ software (National Institutes of Health, Bethesda, MD). h, hour; IMQ, imiquimod; KO, knockout; LC, Langerhans cell; MHC, major histocompatibility complex; ns, not significant; WT, wild type.
ALK3 Is Not Required for Epidermal Langerhans Cell

Q Yu et al.

**AUTHOR CONTRIBUTIONS**

Conceptualization: QSM, LZ; Data Curation: QYu, NP, LZ, QSM; Formal Analysis: QYu, NP, LZ, QSM; Funding Acquisition: QSM, LZ, JTE; Investigation: QYu, NP, LZ, QSM; Methodology: QYu, NP, QYi, JTE, LZ, QSM; Project Administration: LZ, QSM; Resources: YM; Validation: QYu, NP, LZ, QSM; Visualization: QYu, NP, LZ, QSM; Writing - Original Draft Preparation: QYu, NP, QYi, JTE, YM, LZ, QSM; Writing - Review and Editing: QYu, NP, QYi, JTE, YM, LZ, QSM

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Qijun Yi et al.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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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.2020.10.028.